Indian Journal of Natural Products and Resources
Vol. 15(1), March 2024, pp. 113-121
DOI: 10.56042/ijnpr.v15i1.2829

# Antimicrobial and antihelminthic properties of different extracts of propolis

Anita Rana[1]* and Neelima R Kumar[2]

[1]Department of Biosciences (UIBT), Chandigarh University, Mohali 140413, India
[2]Department of Zoology, Panjab University, Chandigarh 160014, India

*Received 18 June 2023; revised received 14 January 2024; accepted 30 January 2024*

This study aimed to evaluate the antimicrobial potential of honey bee product propolis against Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus pneumoniae*) and Gram-negative bacteria (*Escherichia Coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*) as well as against yeasts by disc diffusion assay and broth dilution method and also against amphistome by bioassay method under *in vitro* conditions. For this, ethanolic, methanolic and water extracts of propolis were prepared and tested for antimicrobial and antihelminthic activity. Ampicillin (antibacterial), Amphotericin B (antifungal) and Albendazole (anti-helminthic) were used as positive controls. Antibacterial and antifungal activities were determined by reading inhibition zone diameters (mm) after 24 hours of incubation at 37°C for bacteria and 28°C for fungi and broth dilution methods. Ethanolic extract of propolis was found to be most effective against antimicrobial properties. Results also demonstrated that propolis was more effective towards Gram-positive bacteria. Positive controls showed an efficient action against all the organisms used in present studies, but propolis was not found to be effective against amphistome.

**Keywords:** Amphistome, Amphotericin, Ampicillin, Antihelminthes, Antimicrobial, Propolis

**IPC code; Int. cl. (2021.01)**– A61K 35/00, A61K 35/644, A61P 31/00, A61P 33/10

## Introduction

Honey bees are regarded as an additional source of income for agriculturists; bees collect various substances from plants, add their secretions, process them in the hive, and finally allow them to ripen. These ripened substances serve as commercial bee hive products. The bee products derived from plants and modified by bees are honey, pollen and propolis, while the bee products synthesized by bees themselves are beeswax, royal jelly and bee venom. Apitherapy is an alternative line of treatment based on the use of these products. The term was initially limited to the applications of bee venom for therapeutic purposes. Lately, with the accumulation of information regarding the potential of almost all bee products in curative traditional remedies, the term 'Apitherapy' has come to encompass alternative therapeutics. These products have been used in traditional medicine since ancient times in many countries for their various biological and pharmacological properties. There is increasing interest in their bioactivity and implementation in alternative medicine and Apitherapy. Literature about honey bee product propolis selected for the present studies is reviewed in this section.

The term propolis, also known as 'bee glue', comes from two Greek words, i.e., 'pro'- which means in defence of and 'polis'-which means the city; thus, propolis means in defence of the city or the beehive. Propolis, a natural bee product, is a complex dark brown resinous fluid collected by worker honey bees from plant exudates and is mixed with bee wax, bee pollen and hypo-pharyngeal glands secretions for use in the hive as a sealant[1,2]. Honey bees use it to cover surfaces; seal cracks and gaps, and thus maintain internal temperature and weather conditions in the hive[3-5]. That is why it is also called bee glue. Moreover, using propolis, bees provide an aseptic environment in the hive and protect the larvae from microbes and spore-producing organisms like fungi and moulds[6,7]. Using propolis by bee colonies might be considered an agent of social immunity[8]. Bees use it as a potent chemical weapon against several pathogenic microorganisms and an embalming substance to mummify large invaders like insects and lizards that have already been killed and are too heavy to be removed by small bees from the beehive[3,9]. Propolis exhibits various biological properties, including antibacterial, antifungal, and antiviral, as

*Correspondent author
Email: anitakadian23@gmail.com

well as antioxidant, anti-inflammatory and anti-cancer properties[10-13].

Though literature about the therapeutic properties of propolis is abundant, however, there are some gaps in its background knowledge such as; the diversity of propolis types, its relation with pathogenic microbe's especially multidrug resistant ones remains to be under investigation so far. Moreover, several types of propolis from active apicultural centres in India have been poorly studied regarding their antimicrobial potential. Despite interesting results obtained from Indian propolis the testing of its efficacy, its synergistic behaviour with commercial antimicrobial drugs and its utilization for human welfare little or nothing has been done.

Increasing antibiotic resistances among microorganisms necessitate the present study which embodies the results of investigations undertaken to evaluate honey bee product propolis for its biological activities. The antimicrobial and antihelminthic properties of propolis were studied using *in vitro* methods. Determination of the antimicrobial activity of propolis was done using the disc diffusion method and broth dilution method against pathogenic and non-pathogenic bacteria and yeasts. In the disc diffusion method, the organisms were screened for their susceptibility towards propolis (extracted in ethanol, methanol and water), and applied to the disc of an agar plate at the concentration range of 1.562-300 mg/disc. Ethanolic extract of propolis was used to see antihelminthic activity.

## Material and Methods

#### Collection and sample preparation of Propolis

This was collected by scrapping with a clean hive tool from the top bars of the combs in a honey bee colony located near Chandigarh, and the analysis was done in February and March 2023. In the present study, three different extracts of propolis (EP), *viz*. ethanolic (EEP), methanolic (MEP) and water (WEP), were prepared by the method of Orsi[14] and Kumar[15]. For this, crude propolis (30 g each) was added to different flasks containing 100 mL of 70% ethanol, methanol, and water, respectively. The components were dissolved by occasional shaking at room temperature. It was kept in the dark for 8 to 10 days. The solutions were filtered through Whatman filter paper and evaporated to dryness. Final concentrations were made based on dry weight in water and kept at -5°C till use. The solutions were filtered through 0.22 µ Millipore membrane in a sterile tube and stored at -5°C.

#### *Helminths (Amphistomes)*

*Gastrothylax crumenifer*, were obtained from the large intestine of sheep/goat procured from the local slaughterhouse.

#### Procurement of Microorganisms

Microorganisms such as bacteria (*Staphylococcus aureus:* MTCC No- 1144, *Staphylococcus epidermidis:* MTCC No-9040, *Streptococcus pneumoniae:* MTCC No- 2672, *Salmonella enterica:* MTCC No-3231, *Escherichia coli:* MTCC No-2314, *Bacillus subtilis*: MTCC No-2435, *Pseudomonas aeruginosa* MTCC No-3465 and fungi (*Candida albicans* (Yeast): MTCC No- 4748, *Saccharomyces cerevisiae* (Yeast): MTCC No- 3090) were procured from IMTECH (Institute of Microbial Technology), Chandigarh, India. The organisms were maintained in the suitable media (agar plates at 4°C). The strains were checked biochemically prior to usage.

#### *In vitro* antihelminthic activity of bee products

A worm motility inhibition assay was employed to evaluate the antihelminthic activity of propolis under *in vitro* conditions. The *in vitro* antihelminthic activity was conducted at three different concentrations (100, 300, and 500 mg/mL) to determine the inhibitory effect of bee product extracts on amphistome worms. Mature amphistome worms (*G. crumenifer*) were collected from the large intestine of sheep/goat procured from a local slaughterhouse (Fig. 1). The worms were washed in phosphate-buffered saline (PBS pH 7.2) and then suspended in PBS. Albendazole dissolved in 1% DMSO and diluted in PBS at concentrations of 5, 10, and 15 µg/mL, and PBS alone served as positive and negative control, respectively. There were three

Fig. 1 — Amphistomes (*Gastrothylax crumenifer*) from the stomach of sheep/goat.

replicates for each treatment concentration. Ten vigorously motile worms were placed in each petri dish containing test solutions, and observations were made at 15, 30, 60, and 120 minute intervals for cessation of motility by gross visual motility of worms as an index for antihelminthic activity. After exposure to different treatments, the worms were put in lukewarm PBS for 30 minutes to confirm mortality.

## Results and Discussion

A thorough literature survey revealed that most second-generation antibiotics are ineffective in controlling the disease due to emerging drug resistance in pathogenic organisms. Therefore, the drug has to be given at a higher dosage and for a long duration, which leads to drug-induced toxicity and side effects. Numerous natural products are being reported to ameliorate the toxicity and oxidative stress caused by antibiotics. Therefore, a systematic study was carried out to evaluate the antimicrobial potential of honey bee product propolis against a range of Gram-positive and Gram-negative bacteria as well as in yeasts by disc diffusion assay and broth dilution method and against amphistome by the bioassay method under *in vitro* conditions.

### *In vitro* antimicrobial activity of propolis

#### *Disc diffusion method*

The antimicrobial activity of propolis was evaluated for three types of extracts, *viz.* ethanolic, methanolic, and water. Organisms initially selected for antimicrobial studies with propolis were nonpathogenic Gram (+ve) and Gram (-ve) *viz. B. subtilis, S. pneumonia, E. coli, P. aeruginosa.* After that, putative pathogenic Gram (+ve) bacteria *viz. S. aureus, S. epidermidis* and Gram (-ve) bacteria *viz. S. enterica* was screened for the inhibitory activity of propolis using the disc diffusion method and broth dilution method. The stock solution was made at a concentration of 300 mg/mL. These were serially diluted to obtain the concentration of 300, 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.562 mg/mL. Agar plates were made, and 25–50 µL of each organism was uniformly spread on the plates. The inoculum was always prepared for fresh 24–48 hours before the start of the experiment. Then, 25 µL of all the above-mentioned concentrations of each product was applied on separate agar plates and incubated at their respective growth conditions. After 24–48 hours, clear zones of inhibitions of culture growth around the discs with propolis were measured. The results obtained are shown in (Tables 1–9). The effectiveness of bee products was also compared with standard antibiotics as positive controls, such as ampicillin (antibacterial), Amphotericin B (antifungal) and Albendazole (antihelminth).

#### Propolis

##### *Ethanolic extract*

For ethanolic extract of propolis, the zones of inhibition observed against Gram-positive bacteria *S. epidermidis* varied from 6.73±0.75–19.59±2.12 mm, *B. subtilis* 11.75±0.75–16.91±0.42 mm, *S. aureus* 9.28±0.57–17.58±0.62 mm and for *S. pneumoniae* 10.08±0.46–17.008±0.98 mm at concentrations ranging from 1.562 to 300 mg/mL as shown in Table 1. The zones of inhibition observed against Gram-negative bacteria such as *E. coli* were 8.35±0.66–15.25±0.35 mm, *P. aeruginosa* from 6.5±0.98–10.2±0.96 mm at concentrations ranging from 1.562–300 mg/mL. In the case of *S. enterica,* no inhibition was observed for concentrations between 1.562–12.5 mg/mL, while between 25–300 mg/mL, the ZOI varied from 9.5±1.29–14.0±1.65 mm (Table 2). The results

Table 1 — Antimicrobial activity of ethanolic extract of propolis (EEP) against Gram (+ve) bacteria

| Ethanolic extract of propolis | | *Bacillus subtilis* | *Staphylococcus epidermidis* | *Staphylococcus aureus* | *Streptococcus pneumoniae* |
|---|---|---|---|---|---|
| S. No | (mg/mL) | Zones of inhibitions (mm) | | | |
| 1. | 1.562 | 11.75±0.75* | 6.73±0.75 | 9.28±0.57 | 10.08±0.46 |
| 2. | 3.125 | 12.41±0.64 | 7.06±0.72 | 8.00±4.69 | 11.00±2.69 |
| 3. | 6.25 | 12.59±0.12 | 8.06±0.31 | 10.08±1.07 | 11.25±2.07 |
| 4. | 12.5 | 13.00±0.77 | 9.18±0.12 | 10.68±0.85 | 12.50±0.77 |
| 5. | 25 | 13.64±0.84 | 11.91±0.92 | 11.63±0.31 | 13.60±0.81 |
| 6. | 50 | 14.41±0.83 | 14.91±1.18 | 12.58±0.56 | 14.50±0.96 |
| 7. | 100 | 15.15±0.59 | 15.91±0.83 | 13.95±0.53 | 14.95±0.41 |
| 8. | 200 | 16.06±0.83 | 17.56±1.16 | 16.23±0.57 | 16.00±0.42 |
| 9. | 300 | 16.91±0.42 | 19.59±2.12 | 17.58±0.62 | 17.008±0.98 |

*All the values are expressed as mean±S.D (n=5)

Table 2 — Antimicrobial activity of ethanolic extract of propolis (EEP) against Gram (-ve) bacteria

| Ethanolic extract of propolis S. No | Ethanolic extract of propolis (mg/mL) | *Escherichia coli* | *Pseudomonas aeruginosa* | *Salmonella enteric* |
|---|---|---|---|---|
| | | Zones of inhibitions (mm) | | |
| 1. | 1.562 | 8.35±0.66* | 6.5±0.98 | NI |
| 2. | 3.125 | 9.065±0.65 | 7.2±0.86 | NI |
| 3. | 6.25 | 9.825±0.32 | 7.8±1.02 | NI |
| 4. | 12.5 | 10.75±0.67 | 8.0±1.00 | NI |
| 5. | 25 | 11.687±0.96 | 8.0±.021 | 9.5±1.29 |
| 6. | 50 | 12.75±0.35 | 8.5±0.810 | 10.8±1.06 |
| 7. | 100 | 13.68±0.37 | 9.8±0.37 | 11.5±1.20 |
| 8. | 200 | 14.75±0.29 | 10.8±0.36 | 13.6±2.20 |
| 9. | 300 | 15.25±0.35 | 10.2±0.96 | 14.0±1.65 |

*All the values are expressed as mean±S.D (n=5)

Table 3 — Antimicrobial activity of ethanolic, methanolic and water extracts of propolis against yeast

| Propolis extracts | | *Candida albicans* | | | *Saccharomyces cerevisiae* | | |
|---|---|---|---|---|---|---|---|
| | | EEP | MEP | WEP | EEP | MEP | WEP |
| S. No | mg/mL | Zones of inhibitions (mm) | | | | | |
| 1. | 1.562 | NI | NI | NI | NI | NI | NI |
| 2. | 3.125 | 8.23±0.68* | NI | NI | 6.43±0.40 | NI | NI |
| 3. | 6.25 | 12.28±0.75 | NI | NI | 7.30±1.28 | NI | NI |
| 4. | 12.5 | 14.68±0.59 | NI | NI | 9.78±0.74 | NI | NI |
| 5. | 25 | 16.68±0.51 | 7.88±0.78 | NI | 11.88±0.62 | NI | NI |
| 6. | 50 | 17.68±0.97 | 10.63±1.65 | NI | 14.93±0.85 | 8.20±0.54 | NI |
| 7. | 100 | 19.80±0.94 | 15.68±0.95 | NI | 16.95±0.73 | 10.40±1.27 | 8.68±0.83 |
| 8. | 200 | 21.33±0.81 | 18.83±1.93 | 9.75±1.05 | 18.40±0.43 | 13.43±0.61 | 11.30±0.48 |
| 9. | 300 | 23.70±0.74 | 21.50±0.80 | 13.18±0.94 | 20.70±1.25 | 16.38±0.92 | 13.78±0.87 |

*All the values are expressed as mean±S.D (n=5)

Table 4 — Antimicrobial activity of methanolic extract of propolis (MEP) against Gram (+ve) bacteria

| Methanolic extract of propolis | | *Bacillus subtilis* | *Staphylococcus epidermidis* | *Staphylococcus aureus* | *Streptococcus pneumoniae* |
|---|---|---|---|---|---|
| S. No | (mg/mL) | Zones of inhibitions (mm) | | | |
| 1. | 1.562-3.125 | NI | NI | NI | NI |
| 2. | 6.25 | 7.00±0.41* | 7.31±0.75 | 8.55±0.26 | NI |
| 3. | 12.5 | 7.91±0.43 | 7.59±0.42 | 9.33±1.12 | 8.13±0.12 |
| 4. | 25 | 10.00±0.35 | 8.41±0.72 | 10.78±1.09 | 9.72±2.09 |
| 5. | 50 | 11.15±0.12 | 9.91±1.20 | 11.80±0.59 | 10.60±0.99 |
| 6. | 100 | 12.50±0.41 | 12.00±1.24 | 12.25±0.38 | 11.45±0.88 |
| 7. | 200 | 13.41±0.62 | 13.41±0.66 | 13.33±0.46 | 12.93±0.66 |
| 8. | 300 | 14.56±0.13 | 15.50±0.94 | 14.25±0.41 | 13.55±0.22 |

*All the values are expressed as mean ± S.D (n=5)

Table 5 — Antimicrobial activity of methanolic extract of propolis (MEP) against Gram (-ve) bacteria

| Methanolic extract of propolis | | *Escherichia coli* | *Pseudomonas aeruginosa* | *Salmonella enteric* |
|---|---|---|---|---|
| 1. | 1.562-6.25 | | NI | |
| 2. | 12.5 | 6.83±0.24* | NI | NI |
| 3. | 25 | 7.50±0.02 | NI | NI |
| 4. | 50 | 8.41±0.59 | NI | NI |
| 5. | 100 | 9.65±0.59 | 8.5±1.02 | 11.6±1.60 |
| 6. | 200 | 11.33±0.51 | 10.5±1.08 | 13.2±1.22 |
| 7. | 300 | 12.18±0.77 | 11.5±1.96 | 18.2±.988 |

*All the values are expressed as mean± S.D (n=5)

Table 6 — Antimicrobial activity of water extract of propolis (WEP) against Gram (+ve) bacteria

| Water extract of propolis | | *Bacillus subtilis* | *Staphylococcus epidermidis* | *Staphylococcus aureus* | *Streptococcus pneumoniae* |
|---|---|---|---|---|---|
| S. No | (mg/mL) | Zones of inhibitions (mm) | | | |
| 1. | 1.562-12.50 | NI | NI | NI | NI |
| 2. | 25 | 9.31±0.75* | 10.18±0.31 | NI | NI |
| 3. | 50 | 10.56±0.31 | 11.83±0.83 | 7.40±0.64 | NI |
| 4. | 100 | 11.65±0.83 | 13.59±1.12 | 9.15±0.60 | 9.15±0.34 |
| 5. | 200 | 14.33±0.47 | 16.74±1.14 | 10.75±0.44 | 11.55±0.22 |
| 6. | 300 | 16.00±1.24 | 19.74±1.59 | 13.05±0.82 | 12.55±0.26 |

*All the values are expressed as mean ± S.D (n=5)

Table 7 — Antimicrobial activity of water extract of propolis (WEP) against Gram (-ve) bacteria

| Water extract of propolis | | *Escherichia coli* | *Pseudomonas aeruginosa* | *Salmonella enteric* |
|---|---|---|---|---|
| S. No | (mg/mL) | Zones of inhibitions (mm) | | |
| 1 | 1.562-25 | | NI | |
| 2 | 50 | 8.65±0.66* | NI | NI |
| 3 | 100 | 9.83±0.47 | NI | NI |
| 4 | 200 | 12.06±0.72 | 7.2±1.6 | 7.5±0.92 |
| 5 | 300 | 14.06±1.16 | 9.5±0.89 | 9.2±0.86 |

*All the values are expressed as mean±S.D (n=5)

Table 8 — Optical density observed against Gram (+ve) and Gram (-ve) bacteria with ethanolic extract of propolis

| Propolis | (O.D) for Gram (+ve) Bacteria | | | | (O.D) for Gram (-ve) Bacteria | | |
|---|---|---|---|---|---|---|---|
| EEP (mg/mL) | *Bacillus subtilis* | *Staphylococcus epidermidis* | *Staphylococcus aureus* | *Streptococcus pneumoniae* | *Escherichia coli* | *Pseudomonas aeruginosa* | *Salmonella enterica* |
| Control | 0.99 | 1.23 | 1.32 | 1.12 | 1.46 | 1.22 | 1.24 |
| 3 mg/ml | 0.790 | 0.92 | 0.980 | 0.92 | 1.200 | 0.980 | 1.02 |
| 7.5 mg/ml | 0.62 | 0.76 | 0.705 | 0.7600 | 0.98 | 0.820 | 0.927 |
| 15 mg/ml | 0.50 | 0.62 | 0.68 | 0.62 | 0.80 | 0.780 | 0.790 |
| 30 mg/ml | 0.42 | 0.50 | 0.48 | 0.420 | 0.69 | 0.620 | 0.620 |
| 60 mg/ml | 0.31 | 0.38 | 0.31 | 0.290 | 0.48 | 0.590 | 0.40 |

Table 9 — Antihelminthic activities of propolis, positive control (Albendazole) and negative control (Normal saline)

| Propolis | Concentrations | 15 | 30 | 60 | 120 |
|---|---|---|---|---|---|
| | mg/mL | | min | | |
| Ethanolic extract of propolis in (mg/mL) | 100 | 9 | 9 | 7 | 4 |
| | 300 | 8 | 7 | 6 | 4 |
| | 500 | 7 | 5 | 6 | 3 |
| Positive control in (µg/mL) (Albendazole) | 5 | 8 | 6 | 2 | 0 |
| | 10 | 8 | 5 | 2 | 1 |
| | 15 | 8 | 5 | 3 | 0 |
| Negative control | (Normal saline only) | 9 | 8 | 6 | 4 |

obtained for the zone of inhibition for *C. albicans* ranged from 8.23±0.68–23.70±0.74 mm for an ethanolic extract of propolis at concentrations of 3.125 to 300 mg/mL. No inhibition zones were observed for the concentration of 1.562 mg/mL (Table 3). Similar results have been reported previously[16], where the comparative study of *in vitro* methods was done to analyse the activity of propolis extracts with different compositions against species of *C. albicans* and observed that the agar dilution in plates showed the clearest results. The results obtained against *S. cerevisiae* ranged from 6.43±0.40–20.70±1.25 mm for concentrations between 3.125 to 300 mg/mL of ethanolic extract of propolis. Here, as in the case of *Candida*, no inhibition was observed at 1.562 mg/mL of propolis's ethanolic extract (Table 3).

***Methanolic extract***

The zones of inhibition observed with methanolic extract of propolis at range of concentrations 6.25-300 mg/mL for *B. subtilis* varied from 7.00±0.41–14.56±0.13 mm, for *S. epidermidis* it varied from 7.31±0.75–15.50±0.94 mm and for *S. aureus* it varied from 8.55±0.26– 4.25±0.41 mm. No inhibition zones were observed at concentrations between 1.562–3.125 mg/mL methanolic extract of propolis. For *S. pneumoniae*, the zones of inhibition varied from 8.13±0.12–13.55±0.22 mm at concentrations from 12.50–300 mg/mL. No inhibition was observed at concentrations 1.562–6.25 mg/mL (Table 4). There were no zones of inhibition observed at concentrations ranging from 1.562–6.25 mg/mL of methanolic extract of propolis against Gram-negative bacteria, and values observed for *E. coli* varied from 6.83±0.24–12.18±0.77 at higher concentrations ranging from 12.50–300 mg/mL. In the case of *S. enterica*, zones of inhibition were in the range of 11.6±1.60–18.2±.988 mm at concentrations varying between 100–300 mg/mL and no inhibition zones were observed at concentrations lower than this. The values observed against *P. aeruginosa* varied from 8.5±1.02–11.5±1.96 mm for 100–300 mg/mL of methanolic extract of propolis (Table 5). Results obtained for *C. albicans* varied from 7.88±0.78–21.50±0.80 mm at concentrations between 25 to 300 mg/mL. No inhibition zones were observed for concentrations between 1.562 and 12.5 mg/mL. The inhibition zones against *S. cerevisiae* were from 8.20±0.54–16.38±0.92 mm at concentrations from 50–300 mg/mL with no inhibitions between concentrations of 1.562–25 mg/mL (Table 3).

***Water extract***

The zones of inhibition observed for the water extract of propolis for *B. subtilis* varied from 9.31±0.75–16.00±1.24 mm and for *S. epidermidis* from 10.18±0.31–19.74±1.59 mm at concentrations ranging from 25–300 mg/mL, no inhibition zones were observed against both *B. subtilis* and *S. epidermidis* at concentrations between 1.562–12.5 mg/mL water extract of propolis. The values for *S. aureus* were observed to vary from 7.40±0.64–13.05±0.82 mm at concentrations ranging from 50–300 mg/mL, and no inhibition was observed at lower concentrations. The values observed for *S. pneumoniae* varied from 9.15±0.34–12.55±0.26 mm at concentrations ranging from 100–300 mg/mL (Table 6). The results indicated that the highest inhibition was found against *S. epidermidis* with water extract from propolis. The Gram-positive bacteria showed no inhibition zones at concentrations ranging from 1.562–25 mg/mL of propolis water extract. The most sensitive Gram-negative bacteria was found to be *E. coli*, and the values observed varied from 8.65±0.66–14.06±1.16 mm at concentrations ranging from 50–300 mg/mL. The zones of inhibition observed for *S. enterica* were 7.5±0.92–9.2±0.86 mm at concentrations varying from 200–300 mg/mL. The values observed for *P. aeruginosa* were from 7.2±1.6-9.5±0.89 mm for 200–300 mg/mL of water extract of propolis, and no inhibition zones were observed for both *S. enterica* and *P. aeruginosa* for concentrations between 1.562–100 mg/mL (Table 7). The highest zone of inhibition observed with the water extract of propolis was against *E. coli*, followed by *S. enterica*, and the least against *P. aeruginosa* (Table 7). The results obtained in the present studies were in agreement with results observed in the *in vitro* antimicrobial activity of propolis, collected during the four seasons, on bacterial strains[17]. In the case of *C. albicans*, the zones of inhibition ranged from 9.75±1.05–13.18±0.94 mm at concentrations between 200 to 300 mg/mL. No inhibition zones were observed for the concentration from 1.562–100 mg/mL. *S. cerevisiae* was inhibited to the extent of 8.68±0.83–13.78±0.87 mm at concentrations from 100-300 mg/mL, and there was no inhibition with concentrations between 1.562–50 mg/mL (Table 3).

A perusal of the data revealed that the ethanolic extract of propolis was the most effective against all the microorganisms used in the present study. This might be because of the better solubility of its phytoconstituents in ethanol, as observed by Kalia[18]. It was further observed that Gram-positive bacteria were more susceptible to the inhibitory activity of propolis than Gram-negative bacteria. Among yeast, *C. albicans* were more susceptible to propolis than *S. cerevisiae*. The results are in agreement with Garedewa[19], where the antibacterial action of three different types of propolis extracts, water-extracted propolis (WEP), propolis volatiles (PV) and ethanol-extracted propolis (EEP), were carried out. The observed results proved that the water-extracted propolis solution had the weakest antibacterial and antifungal action compared to the other two extracts, which showed similar effects. The therapeutic use of propolis is also corroborated from earlier studies of

Souza[20] who investigated the antibacterial activity of propolis produced throughout the year using different methods. Results revealed that the antibacterial activity of propolis depends upon the season and collection methods. Regueira[21] also investigated the effect of the dry and rainy seasons on the antibacterial activity and chemical composition of Brazilian red propolis. For this, the samples were collected in rainy and dry seasons and analyzed by HPLC-DAD. The extracts were tested alone and in association with antibiotics against *E. coli*, *P. aeruginosa* and *S. aureus*. Results obtained showed that the MIC values against *E. coli* ranged from 128 mg/mL to 512 mg/mL. The red propolis showed MIC values of 512 mg/mL against both strains of *P. aeruginosa* and 64 to 1024 mg/mL against *S. aureus*. A synergistic effect was also observed when propolis was combined with gentamicin against all tested strains. On the basis of the observed synergistic activity, it was suggested that using red propolis collected in the drier periods could prove a good adjuvant against multi-resistant bacterial infections. In studies of Taher[22], the synergistic effect between ethanolic extracts of propolis and 12 different antibiotic drugs was evaluated against multi-resistant *K. pneumoniae* isolated from infected wounds. The evaluation was done using the agar well diffusion method and the disc diffusion method. Results showed that the isolated bacteria were resistant to most antibiotics used in the experiment, especially those containing $\beta$-lactam ring. Combination with alcoholic extract of propolis increased the inhibition zones of all antibiotics and the sensitivity of bacteria increased in direct correlation to the increase in the concentration of alcoholic extract of propolis. The antibacterial activity against Gram-positive, Gram-negative bacteria and fungus culture was also investigated in studies of Kartal[23] by disc diffusion method at four different concentrations of ethanolic extracts (30, 50, 70, and 96% ethanol) of each sample from Turkey. The results obtained proved that the antibacterial activity was due to caffeic acid and its esters.

The antibacterial activity of honey and propolis against *S. aureus* and *E. coli* was also studied previously[24] by using the disc diffusion method, MIC, MBC and gradient-plate techniques. The combined results obtained from the disc diffusion test, MIC, MBC and gradient-plate techniques suggested that propolis at concentrations of 2.74 to 3.5 mg/mL was effective against *S. aureus* and *E. coli*, respectively. On the contrary, honey was effective in inhibiting *S. aureus* at the concentration of 375.0 mg/mL but failed to inhibit *E. coli* growth at the same concentration. The combined results from all methods proved the antibacterial activity of propolis and honey against *S. aureus*. Their studies also suggested that *S. aureus* is more susceptible to the effect of propolis than *E. coli*. The antimicrobial activity of propolis, bee pollen and beeswax against pathogenic bacteria, microscopic fungi and yeasts was also supported by earlier studies[25] using the agar well diffusion method. Santana[26] evaluated the efficacy of ethanolic extracts of propolis (EEP) against *S. aureus* cultivated in complex media or milk. Results obtained showed that EEP decreased the growth of *S. aureus* in BHI media and showed bactericidal activity.

The antimicrobial activities observed for propolis are highly attributed to its phenolic compounds, such as flavonoids. Among them, the most potent microbicidal compounds in propolis are flavanone pinocembrin (5,7-dihydroxyflavanone) and its 3-OH analogue flavonol galangin (3,5,7-trihydroxyflavon). Caffeic acid (3, 4-dihydroxycinnamic acid) and its esters, volatile fractions with phenols and/or terpenoids and chrysin (5,7-dihydroxyflavone) also possess notable antimicrobial activities[27].

**Broth dilution method**

To determine inhibitory concentrations of the honey bee product propolis against the organisms listed previously and to study the effect of a range of concentrations of different extracts on the growth of an organism, experiments were done with the broth dilution method. Organisms were grown in the presence of honey bee product propolis at concentrations ranging from 3 mg/mL - 60 mg/mL. Growth of Gram-positive and Gram-negative non-pathogenic bacteria *viz. B. subtilis*, *E. coli*, *P. aeruginosa* and *S. pneumoniae* were measured at the late log phase. Then pathogenic Gram-positive (+ve) bacteria *viz. S. aureus, S. epidermidis* and Gram-negative bacteria *viz. S. enterica* was screened separately for the inhibitory activity of propolis by broth dilution assay. The growth of each organism was measured at a late log phase by taking O.D. at 600 nm (Table 8). Determination of antimicrobial activity by broth dilution method for propolis revealed that there is a concentration-dependent decline in the growth of organisms under study. Therefore, this concludes the antimicrobial properties of propolis. The antimicrobial properties observed for propolis

could be due to cell wall lyses and plasma membrane degradation, which leads to a loss of potassium ions and the damage caused by provoking cell autolysis[28].

***In vitro* antihelminthic activity**

Parasitic infection is a major health problem worldwide and is responsible for considerable economic losses in the livestock industry. Amongst helminths, an infection caused by amphistome is more serious than that caused by roundworm. Medicinal plant research continues to be considered a fruitful approach to searching for safer, cheaper and eco-friendly antihelminths drugs[29]. Honey bee products have multiple medicinal properties[30,31]. The present study was undertaken to evaluate antihelminthic activity of propolis by the Petri dish method, in comparison with a standard drug Albendazole, against amphistome (*G. crumenifer)* parasite in the large intestine of sheep/goat through *in vitro* studies by the worm motility inhibition assay.

Ethanolic extract of propolis was used for this study, as this was observed to be the most effective for microorganisms tested during the *in vitro* study. Mortality was observed after every 15, 30, 60 and 120 minutes in the entire test group. The data obtained is presented in Table 9. A perusal of the results obtained revealed that propolis at the highest concentration tested (500 mg/mL) after completion of 120 minutes of the experiment did not give any more mortality than the negative control (3 and 4 live amphistome, respectively) and was, therefore not effective in controlling the parasite. The positive control using Albendazole, however, at a much lower concentration (5, 10, 15 µg/mL), was able to arrest the parasite almost cent per cent at the end of the experiment. Results, therefore, suggested that propolis was not a potent helminthic agent and is not suitable for application against amphistome *G. crumenifer*.

## Conclusion

The results obtained revealed that propolis is a significant antimicrobial bee product. It acts against both Gram-positive as well as Gram-negative bacteria and showed higher inhibitory activity against Gram positive bacteria. As per observation, *S. epidermidis* followed by *S. aureus* and *S. pneumonia* were found to be most susceptible to ethanolic extracts of propolis and among yeast; *C. albicans* was more susceptible as compared to *S. cerevisiae* with both ethanolic as well as methanolic extracts of propolis. The entire range of bee products at all concentrations used in the present study did not show any effect against amphistome. For future direction it is concluded that despite interesting results obtained from Indian propolis the testing of its efficacy, its synergistic behaviour with commercial antimicrobial drugs and its utilization for human welfare require knowledge and more literature should be searched and added to this in future.

## Conflict of interest

The authors declare no conflict of interest.

## References


1 Aggarwal R, Suri M and Bagai U, *In vitro* anthelmintic effect of ethanolic and aqueous extracts of calotropis procera, *azadirachta indica* and *punica granatum* on *trichuris globulosa*, an intestinal nematode of sheep, *European J Biomed Pharm Sci*, 2016, **3**, 575.

2 Anjum S I, Ullah A, Khan K A, Attaulah M, Khan H, *et al.*, Composition and functional properties of propolis (bee glue): A review, *Saudi J Biol Sci*, 2019, **26**, 1695

3 Rana A, Kumar N R and Kaur J, Therapeutic effect of propolis on *Staphylococcus aureus* induced oxidative stress in spleen of BALB/c mice. A biochemical and histopathological study, *Indian J Nat Prod Resour*, 2022, **13**(3), 1-10, doi:10.56042./ijnpr.v13i3.51887.

4 Rana A, Kumar N R and Kaur J, Therapeutic effect of propolis on *Staphylococcus aureus* induced oxidative stress in kidney of BALB/c mice. A biochemical and histopathological study, *Indian J Exp Biol*, 2022, **60**, 597-606.

5 Rana A and Kumar N R, Antioxidative potential of pollen, propolis and bee bread against damage caused by *Staphylococcus aureus* in liver and kidney of BALB/c Mice: A biochemical study, *J Sci Ind Res*, 2023, **82**, 1-9.

6 Rana A and Bajwa H K, Therapeutics of bioactive compounds from medicinal plants and honeybees product against cancer, *J Sci Ind Res*, 2023, **27**, 1-15.

7 Dabori K and Rana A, Characterization of chemical composition of ethanolic extract of bee pollen in India, *J Environ Biol*, 2023, **44**(6), 811-817.

8 Didaras N A, Karatasou K, Dimitriou T G, Amoutzias G D and Mossialos D, Antimicrobial activity of bee-collected pollen and beebread: State of the art and future perspectives, *Antibiotics* (*Basel*), 2020, **9**, 811, doi:10.3390/antibiotics 9110811.

9 Garedewa A, Schmolza E and Lamprech I, Microbiological and calorimetric investigations on the antimicrobial actions of different propolis extracts: An *in vitro* approach, *Thermochimica Acta*, 2004, **422**, 115.

10 Ghangale G R, Tushar M and Jadhav N D, *In vitro* antihelminthic activity of alcoholic extract of *Allivum Sativum* against rumen amphistome, *Vet Boukra World*, 2009, **2**, 385.

11 Kacanio M, Vukovic N, Chlebo R, Katarína J, Dżugan M, *et al.*, The antimicrobial activity of honey, bee pollen loads and beeswax from Slovakia, *Arch Biol Sci*, 2012, **64**, 927.

12 Rana A and Kumar N R, Antioxidative potential of propolis on *Staphylococcus aureus* infected BALB/c mice: A biochemical study, *Indian J Biochem Biophys*, 2022, **59**, 1006-1015, doi:10.56042/ijbb.v59i10.58820.

13 Kartal M S, Kayaa S, Kurucu S and Topcu G, Antimicrobial and antifungal activity of propolis samples from two different regions of Anatolia, *J Ethnopharmacol*, 2003, **86**, 69-73.

14 Kocot J, Kiełczykowska M, Luchowska-Kocot D, Kurzepa J and Musik I, Antioxidant potential of propolis, bee pollen and royal jelly: Possible medical application, *Oxid Med Cell Longev*, 2018, **2**, 7074209.

15 Rana A and Parmar A S, Re-exploring silver nanoparticles and its potential applications, *Nanotechnol Environ Eng*, 2022, **8**, 789-804, doi:org/10.1007/s41204-022-00301-w.

16 Martinello M and Mutinelli F, Antioxidant activity in bee products: A review, *Antioxidants*, 2021, **10**, 71, doi:org/ 10.3390/antiox 10010071.

17 Massaro C F, Simpson J B, Powell D and Brooks P, Chemical composition and antimicrobial activity of honeybee (*Apis mellifera ligustica*) propolis from subtropical eastern Australia, *Naturwissenschaften*, 2015, **102**, 68, doi:10.1007/s00114-015-1318-z.

18 Orsi R O, Fernandes A, Bankova V and Sforcin J M, Antibacterial effects of Brazilian and Bulgarian propolis and synergistic effects with antibiotics acting on the bacterial DNA and folic acid, *Nat Prod Res*, 2007, **26**, 344-349.

19 Pasupuleti V R, Sammugam L, Ramesh N and Gan S H, Honey, propolis and royal jelly: A comprehensive review of their biological actions and health benefits, *Oxid Med Cell Longev*, 2017, **2017**, 21, doi:10.1155/2017/1259510. 1259510.

20 Rahman M M, Richardson A and Sofian-Azirun M, Antibacterial activity of propolis and honey against *Staphylococcus aureus* and *Escherichia coli*, *Afr J Microbiol Res*, 2010, **4**, 1872-1878.

21 Rana A, Antibacterial, antifungal and antihelminthic properties of ethanolic, methanolic and water extracts of pollen, *J Pharm Res Int*, 2021, **33**(53B), 78-88, doi:10.9734/jpri/2021/v33i53B33682.

22 Neto M S R, Tintino S R, da Silva A R P, Costa M D S, Boligon A A, *et al.*, Seasonal variation of Brazilian red propolis: Antibacterial activity, synergistic effect and phytochemical screening, *Food Chem Toxicol*, 2017, **107**, 572-580.

23 Santana H F, Barbosa A A T, Ferreira S O and Mantovani H C, Bactericidal activity of ethanolic extracts of propolis against *Staphylococcus aureus* isolated from mastitic cows, *World J Microbiol Biotechnol*, 2012, **28**, 485, doi:10.1007/s11274-011-0839-7.

24 Sawaya A C H F, Palma A M, Caetano F M, Marcucci M C, Cunha S, *et al.*, Comparative study of *in vitro* methods used to analyze the activity of propolis extracts with different compositions against species of Candida, *Lett Appl Microbiol*, 2002, **35**, 203-207.

25 Sforcin E L and Funari S R C, Seasonal effect of Brazilian propolis on biochemical variables, *J Venom Anim Toxins Including Trop Dis*, 2000, **8**, 244-254.

26 Siheri W, Alenezi S, Tusiimire J and Watson D G, The chemical and biological properties of propolis, In *Bee Products-Chemical and Biological Properties*, Edited by J M Alvarez-Suarez, (Springer International Publishing: Cham, Switzerland), 2017, 137.

27 Simone-Finstrom M and Spivak M, Propolis and bee health: The natural history and significance of resin use by honey bees, *Apidologi*, 2010, **41**, 295-311.

28 Souza E A, Inoue H T, Junior A F, Veiga N and Orsi R O, Influence of seasonality and production method on the antibacterial activity of propolis, *Acta Sci Anim Sci*, 2014, **36**, 49-53.

29 Sun C, Wu Z, Wang Z and Zhang H, Effect of ethanol/water solvents on phenolic profiles and antioxidant properties of Beijing propolis extracts, *Evid-Based Complement Alternat Med*, 2015, **2015**, 1-9, doi: 10.1155/2015/595393.595393.

30 Taher N M, Synergistic effect of propolis extract and antibiotics on multi-resist *Klebsiella pneumoniae* strain isolated from wound, *Adv Life Sci Tech*, 2016, **43**, 1-28.

31 Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J and Pérez-Alvarez J A, Functional properties of honey, propolis and royal jelly, *J Food Sci*, 2008, **73**, R117-R124.