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ORIGINAL ARTICLE

# Effect of Propolis on the Isolated Bacterial Infection on Covid-19 patient in Erbil City-Iraq

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## ABSTRACT

**Background:** Propolis, a resin-like substance collected by honeybees, has long been known for its antimicrobial capabilities.
**Aim:** To explore the antibacterial potential of ethanolic propolis extracts (EEP) against bacterial isolates obtained from COVID-19-infected patients in Erbil, Iraq.
**Methods:** Clinical samples were obtained from 50 confirmed COVID-19 patients admitted to the Emergency Management Center (EMC) in Erbil. Standard microbiological methods were used for bacterial isolation and identification. Propolis extracts at a concentration of 1500 µg/mL were applied in wells on culture plates, and antibacterial activity was assessed by measuring zones of inhibition.
**Results:** Out of 50 samples, 39 yielded positive bacterial cultures while 11 showed no bacterial growth. Among the isolates, 7 were Gram-positive and 23 were Gram-negative. Klebsiella species were the most frequently isolated Gram-negative pathogens, followed by E. coli and Pseudomonas spp. Staphylococcus aureus was the dominant Gram-positive isolate. No methicillin-resistant S. aureus (MRSA) strains were detected. The study demonstrated variable inhibition zones in response to different EEP concentrations.
**Conclusion:** The ethanolic extract of propolis exhibited significant antibacterial effects against both Gram-positive and Gram-negative bacteria isolated from COVID-19 patients. The inhibition varied depending on bacterial species and EEP concentration.
**Keywords:** COVID-19, Klebsiella spp., Staphylococcus aureus, Pseudomonas spp., E. coli, Propolis, Antibacterial activity.

## INTRODUCTION

SARS-CoV-2 emerged in Wuhan, China in December 2019, has caused a global health crisis resulting in millions of cases and widespread transmission. By March 2020, the World Health Organization declared COVID 19 a pandemic[3]. Serious public health concerns were fueled as the disease rapidly spread across continents. Common with viral respiratory illnesses and often worsen clinical outcomes, secondary bacterial infections are commonly associated with viral respiratory illnesses. During the past pandemics and seasonal outbreaks bacterial co infection was a major contributor to patient mortality and morbidity[7].

Staphylococcus aureus, Klebsiella pneumoniae, E. coli, and Pseudomonas aeruginosa are frequently encountered pathogens that can cause infections of skin, soft tissue, wound, bloodstream and pulmonary[6]. There is growing scientific interest in propolis, which has been traditionally used and whose antimicrobial properties have been verified. Its activity against bacteria, viruses, fungi and protozoa has been confirmed by studies. Although Grange and Davey (1990) specifically referred to its effectiveness primarily against Gram positive bacteria, there were further investigations which identified its limited activity against Gram negative bacteria and dermatophytes[5].

## MATERIALS AND METHODS

**Study design:**
Fifty patients who were hospitalized to Erbil City's Emergency Management Center (EMC for COVID-19) provided samples for the study.

**Collection of specimens:**
Using sterile disposable containers, specimens were taken from several COVID-19 patient sources. For additional examination, these specimens were sent right away to Laboratory-Erbil.

**Propolis concentrations from ethanol extraction:**
The Ethanolic Extraction propolis (EEP) was applied at several doses to combat both Gram-positive and Gram-negative germs.

**Kirby-Bauer sensitivity testing method:**
By connecting the loop to the top of four or five well-isolated colonies of the organism (of comparable appearance), the inoculum was created from the primary cultures and then moved to a saline tube. For 18 to 24 hours, the plates were kept in an incubator set at 37ºC. Every inhibitory zone's diameter, including the disc's diameter, was measured. Clinical and Laboratory Standards Institute recommendations (CLSI, 2016) were followed in the interpretation of the data.

**Methicillin disk diffusion method:**
Detection of MRS Amethicillin disk diffusion method was carried according to BSAC, 2008. Medium: Mueller-Hintonagar was prepared with 2% sodium chloride. After autoclaving, the media was mixed well and poured into sterilized plates at a depth of 4mm(±0.5mm), the plates were allowed to dry then stored.

**Diffusion method to determine the antibacterial activity of propolis (ethanol extracts):**
Forty µl of different concentrations of propolise than olextracts (1500,µg/ml) were poured into each well on inoculated plates aseptically. All plates were incubated at 37ºC for 24h. They were inspectedforthezone0.8.

**Statistical analysis:**
Analysis of data will perform by using Excel program. Results will express as Histogram and Graphical presentation.

## RESULTS

Fifty samples were taken from COVID-19 patients who were brought to Erbil City's Emergency Management Center (EMC). Table 1 shows that 39 samples had a verified positive culture and 11 samples had a confirmed negative culture. Seven gram-positive and twenty-three gram-negative bacteria were identified from the 39 total bacterial isolates (Table 2).

According to the Table 3 the most common bacterial isolated from patients with COVID-19 were: 17 isolates of *Klebsiella* spp*.,* 10 isolates of *E.coli,* followed by 7 isolates for *Staphylococcus aureus* and 5 isolates for *Pseudomonas* spp.

Klebsiella species were the most common etiological agents isolated as gram-negative bacteria from COVID-19 patients, followed by E. coli and Pseudomonas species. S. aureus species were the most prevalent gram-positive pathogen bacterium. The gram-positive bacterial isolates were examined for antibiotic resistance. These isolates' responses to antibiotics varied, according to the data (Table 4). Methicillin-resistant S. aureus MRSA was not found in any of the Staphylococcus isolates that were tested for methicillin resistance.

The results in Table 5 revealed that *Klebsiellaspp.* were 100% sensetive to ciprofloxacin, while 80% and 20% for *E.coli* and *Pseudomonas spp.* respectively. All gram-negative bacteria showed high resistance to impenem.

**Table 1:** Frequency of type of cultures.

| Type of growth cultures | No. of isolates |
|---|---|
| Positive culture | 39 |
| Negative culture | 11 |
| Total | 50 |

**Table 2:** Number and percentage of type of bacteria causing secondary lung infectionincovid19.

| Type of growth cultures | No. of isolates |
|---|---|
| Gram +ve | 7 |
| Gram–ve | 23 |
| Total | 39 |

**Table 3:** Identity of isolated bacteria

| Identity of isolated bacteria | |
|---|---|
| **Bacterial isolate** | **No. of isolates** |
| Staphylococcus aureus | 7 |
| Klebsiellaspp. | 17 |
| E.coli | 10 |
| Pseudomonas spp. | 5 |
| Total | 39 |

**Table 4:** Pattern of susceptibility of gram-negative bacteria to various antibiotics.

| NO | Isolated Bacteria | Types of Antibiotics % | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | CX | KF | APX | L | CN | VA | P | AK | CIP | Methecilin Resistance |
| 17 | *Staphylococcus aureus* | R | 100% | 100% | 71% | 57% | 0 | 0 | 43% | 0 | 43% | 0 |
| | | S | 0 | 0 | 29 | 43% | 100% | 100% | 57% | 100% | 57% | 100% |

**Note:** CX(Cloxacillin), KF (Cephalexin), APX(Ampiclox), L(Levofloxacin), CN(Gentamycin), VA(Vancomycin), P(Penicillin), AK(Amikacin), and CIP (Ciprofloxacin).

**Table 5:** Pattern of susceptibility of gram-negative bacteria to various antibiotics.

| NO | Isolated Bacteria | Types of Antibiotics % | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | CTX | IMP | CIP | MEM | AK | AMC |
| 17 | *Klebsiellaspp.* | R | 41% | 41% | 0 | 24% | 59% | 52% |
| | | S | 59% | 59% | 100% | 76% | 41% | 48% |
| 10 | *E.coli* | R | 50% | 0 | 20% | 0 | 50% | 100% |
| | | S | 50% | 100% | 80% | 100% | 50% | 0 |
| 5 | *Pseudomonas spp.* | R | 100% | 20% | 80% | 80% | 60% | 90% |
| | | S | 0 | 80% | 20% | 20% | 30% | 10% |

**Note:** CTX(Cefotaxim), IMP (Impenem), MEM (Meropenem), AMC(Amoxicillin-clavulanic acid)

**Figure 1:** EEP (Ethanolic Extraction propolis) effect on *Staph. aureus*.

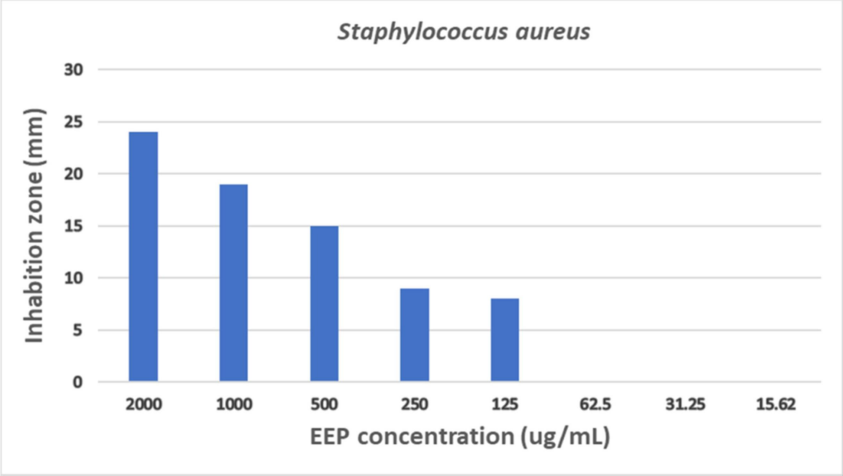



**Figure 2:** EEP (Ethanolic Extraction propolis) effect on*E. coli*.

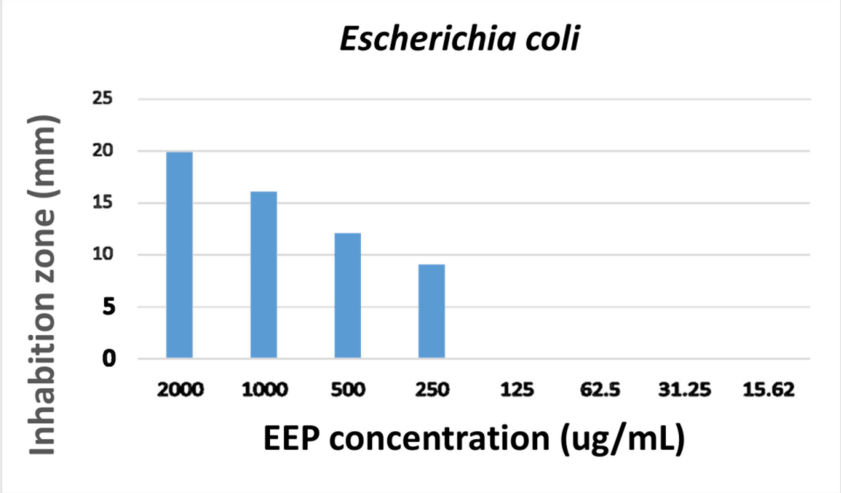


**Figure 3:** EEP (Ethanolic Extraction propolis) effect on *Klebsiella pneumoniae*.

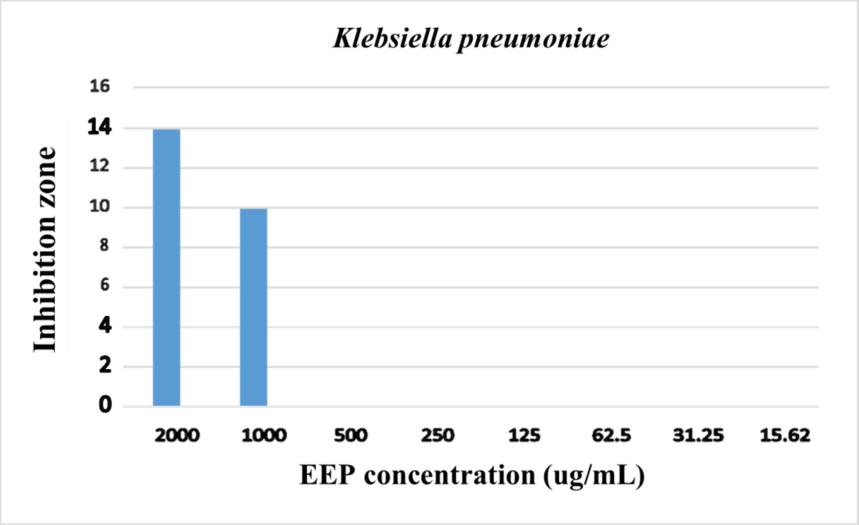
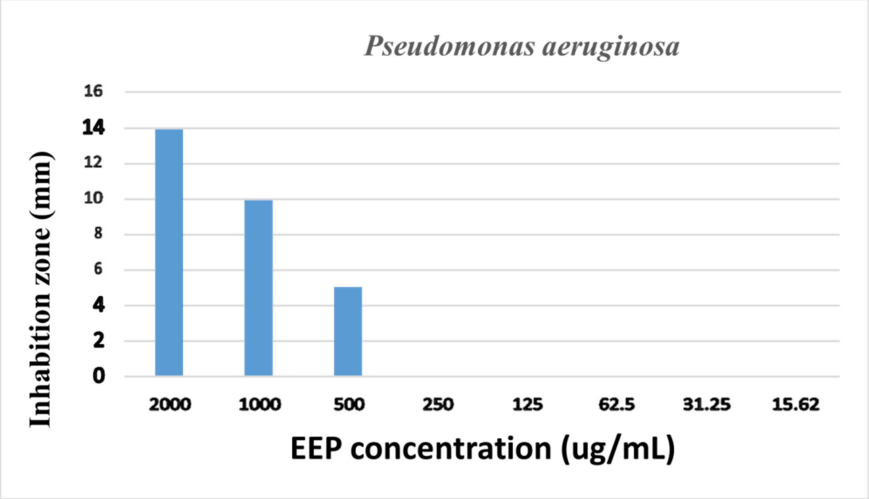



**Figure 4:** EEP (Ethanolic Extraction propolis) effect on *pseudomonasaeruginosa*.




## DISCUSSION

High rates of morbidity and death are linked to secondary bacterial infections, which occur in patients during or after an initial infection with an infectious pathogen, frequently a virus (Morris et al., 2017; Wang et al., 2018).

During viral epidemics, co-infections, secondary infections, or "super infections" can happen. The morbidity and death rates of individuals who first get ill with lung viral illnesses are significantly impacted by secondary bacterial infections (Manohar et al., 2020).

Eleven out of the fifty samples showed no signs of growth. This may be because preventative medicines such azithromycin, moxifloxacin, ceftriaxone, vancomycin, or cefepime are frequently given to patients with lung viral infections, including COVID-19, in order to lower the risk of subsequent infections. (Holshue and colleagues, 2020; Wang and colleagues, 2020; Manohar and colleagues, 2020).

Our findings indicate that the majority of the bacteria found in secondary infections were Gram-negative, including Enterobacteriaceae, P aeruginosa, and S. aureus. This conclusion is consistent with the findings of Russell et al., 2021. A number of variables, such as the usage of antibiotics and cross-transmission via infection control gaps, contribute to the establishment of multidrug-resistant organisms and their dissemination throughout healthcare settings. Hygiene and infection control protocols were significantly improved during the COVID-19 pandemic. In 2022, Ngoula et al. In contrast to prior research by Habib et al., 2022, the findings of this study showed that there was no methicillin-resistant S. aureus (MRSA).

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