Effect of Propolis on the Isolated Bacterial Infection on Covid-19 Patients in Erbil city-Iraq

Ahmed Othman Pirdawid[1], Shler Qasim hussien[2], Zubaidah H. Mahmood Agha[3], Saeedah H Aljadani[4]

Department of Biology, Collage of Science, Duhok University, Kurdistan Region, Iraq

[2] Department of Medical Laboratory Technology, Collage of Health Technology, Erbil Polytechnic University, Kurdistan Region, Iraq

[3] Department of Medical Biochemical Analysis, Cihan University-Erbil, Kurdistan Region, Iraq.

[4] Department of Medical Laboratory Technology, Collage of Health Technology, Erbil Polytechnic University, Kurdistan Region, Iraq

Abstract—**Background: One of the valuable products which produced by honey bees from plant resinous materials is Propolis, it has been widely used in traditional herbal medicine as immune system proponent, and health aid. Recently, interest in the Propolis products has been noted worldwide, especially after COVID-19 pandemic.**

Materials and Methods: Samples were collected from (50) of different source of COVID-19 patients by using sterile disposable container who were admitted to Emergency Management Center (EMC for covid19) in Erbil city. The identification of bacterial isolates through microbiological method standards were performed after clinical samples had been cultured. Different concentration started from forty µl of propolis ethanol extract (1500, µg/ml) had been aseptically casted into each well on inoculated plates.

Results: From the total isolates, 39 of sample where confirmed as positive culture, while 11 samples where confirmed as negative culture. Out of 39 bacterial isolates, gram positive bacteria were seven isolates while gram negative bacteria were thirty two isolates. The most frequent etiological agents isolate as gram negative-bacteria from covid 19 patients was Klebsiella spp followed E. coli and Pseudomonas spp. The most common gram-positive pathogen bacteria species were S. aureus. The results showed that there was no methicillin resistant Staphylococci in our study, while (ESBL) production in enterobacterace family was notably high. However, propolis extract used for each species of isolates high concentration of propolis gave the most effective antimicrobial results.

Conclusion: Statistically analysis showed that there are differences between different concentrations of Propolis extract used for each species of isolates.

Index Terms— **Covid-19, Klebsiella spp, S. aureus, Pseudomonas spp, Staphylococcus, Propolis.**

I. INTRODUCTION

When for the first time in Wuhan (China) in 2019, the first case of (SARS-CoV-2) severe acute respiratory syndrome coronavirus had been reported, millions of the same case worldwide were noted as the confirmatory situation. Thus, (WHO) the World Health Organization stated the pandemic was real on March 11, 2020, which is still continuing. As the novel coronavirus has spread too fast all around China, this resulted in an epidemic, which turned into a pandemic that lead to an increasing number of cases in different countries all around the world. Since the first reports of COVID-19, the infection has dispersed to contain more than 81.552 cases in China and growing cases (>1.400.000) worldwide, that lead to encouragement of the World Health Organization (WHO) to proclaim a public health emergency in late January 2020 and describe it as a pandemic in March 2020.

Clinical impairment maybe caused by complication of viral infection to respiratory system, which resulted from secondary bacterial infections. During influenza pandemics as well as seasonal influenza, secondary bacterial infections have remarkable role of morbidity and mortality of patients, they also have role in other respiratory diseases. Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, and Escherichia coli were the major bacterial pathogens that commonly cause superficial skin and soft tissue infections, surgical wound infections, and sometimes-fatal blood stream infections and pneumonia (Morris et al., 2017; Makky et al, 2019).

Recently, scientists are attracted to propolis due to its beneficial substances, that are used in cosmetics as well as in medicine. Furthermore, it has been used by people in medicine for a long time. Riaz (2019). There are different investigations about its antimicrobial property, as they have confirmed its antiprotozoal, antifungal, antiviral, and antibacterial activities (Thanoon et al.,2022).

The bactericidal effect of propolis in vitro toward upper respiratory tract infection factors was observed by Focht et al., (1993). Propolis activity against Gram-positive bacteria was reported by Grange and Davey (1990). On the other hand, it has restricted activity toward Gram-negative ones. These discoveries were confirmed by Dobrowolski et al., (1991) too, who noticed its fungicidal activity, particularly against dermatomycoses. propolis fungicidal and its fungistatic

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property were reported by Lilenbaum and Barbosa (1994), mainly the yeasts susceptibility for this product.

II. MATERIALS AND METHODS

A. Study Design

Samples were collected from (50) patients who were admitted to Emergency Management Center (EMC for covid19) in Erbil city.

B. Specimen Collection

Specimens were collected from different source of COVID-19 patients by using sterile disposable container. These specimens were immediately transported to Laboratory-Erbil for further analysis.

C. Concentrations of Ethanolic Extraction Propolis

Different concentrations of the Ethanolic Extraction propolis (EEP) were used against both Gram-negative and Gram-positive bacteria.

D. Kirby-Bauer Procedure for Sensitivity Testing

The inoculum was prepared from the primary cultures, by touching the loop to the top of each four or five well isolated colonies (of similar appearance) of the organism then transferred to a tube of saline. The plates were placed in an incubator at 37ºCfor (18-24h). Mueller-Hinton agar was prepared for susceptibility testing and the diameter of each inhibition zone was measured (including the diameter of the disc). The results were interpreted according to Clinical and Laboratory Standards Institute guidelines (CLSI.,2016).

E. Methicillin Disk Diffusion Method

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

F. Diffusion Method to Determine The Antibacterial Activity of Propolis (Ethanol Extracts)

Forty µl of different concentrations of propolis ethanol extracts (1500, µg/ml) were poured into each well on inoculated plates aseptically. All plates were incubated at37ºC for 24 h. They were inspected for the zone 0.8.

G. Statistical Analysis

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

III. RESULTS

A. Frequency of Type of Cultures

Fifty samples were collected from patients infected with covid-19 who were admitted to Emergency Management Center (EMC) in Erbil city. 39 of sample where confirmed as positive culture, while 11 samples where confirmed as negative culture Table (1).

Table (1): Frequency of type of cultures

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

B. Number and Percentage of Type of Bacteria Causing Secondary Infection in Covid19

Table (2) shows the number of type of bacteria (gram positive and gram bacteria) causing infection in covid19.Gram positive represent only 7 (14%) while gram negative were 32 isolates (46%).

Type of bacteria	No. of isolates
a	7
Gram –ve	32
Total	**39**

C. Identity of Isolated Bacteria

The identity, number and percentages of the most common organism isolated from the causing secondary infection in covid19 patient are shown in table (3). The results showed that the highest percent of the isolates belonged to the genus Staphylococci representing 7(14%) isolates, followed by Klebsiella spp. (34%). While, Pseudomonas spp. showed the lowest isolate (10%).

Table (3): Identify of isolated bacteria

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

D. Antimicrobial Susceptibility Patterns Against Bacterial Isolated from the Causing Secondary Infection in Covid19 Patient

Table (4) shows the antimicrobial susceptibility patterns of the isolated bacteria. The result showed that vancomycin was the most effective drug against gram positive (100%) and the least effective were Cloxacillin and AK (Amikacin).Table (5) shows that IMP(Impenem)was the most effective agent against all gram-negative bacterial isolate. While, the least effective antibiotic against gram negative bacteria was Amoxicillin clavulanic acid and Amikacin.

Table (4): Pattern of susceptibility of gram-positive bacteria to various antibiotics.

NO	Isolated Bacteria	Types of Antibiotics %										
			CX	KF	APX	L	CN	VA	P	AK	CIP	Methicillin Resistance
7	*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

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(Ciprofloxacin).

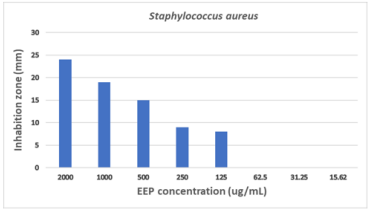
Table(5): Pattern of susceptibility of gram-negative bacteria to various antibiotics.

NO	Isolated Bacteria	Types of Antibiotics %						
			CTX	IMP	CIP	MEM	AK	AMC
17	*Klebsiella spp.*	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%

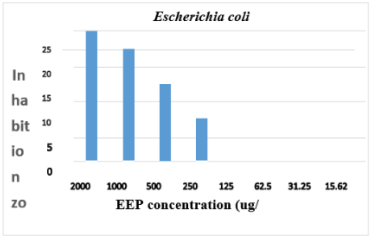
Note: CTX(Cefotaxim),IMP (Impenem),MEM (Meropenem), AMC(Amoxicillin-clavulanic acid)

Antimicrobial Activity Patterns of EEP (Ethanolic Extraction Propolis µg/ml) Against Isolated Strains from the COVIED 19 Patients

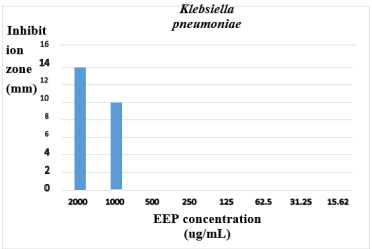
The in vitro activity of the ethanolic extract of PBP is against S. aureus shown in Figure (1). Five different concentrations of the ethanolic extract of PBP (125, 250, 500,1000, and 2000 µg/ml) were used in determining the in vitro activity in triplicates. The 2000 µg/ml, the extract had the greatest antibacterial activity, followed by 1000 and 500 µg/ml, with 125, and 250 µg/ml having the least inhibition of the bacterial activity against S. aureus. again the 2000 µg/ml extract of ethanolic extract of PBP had the greatest antibacterial activity against gram Negative bacteria shown in Figure (2,3,4).



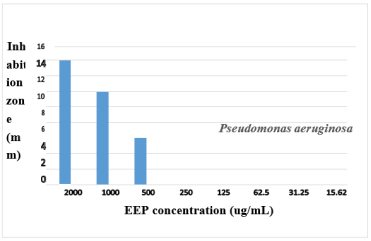
Figure(1): EEP (Ethanolic Extraction propolis) effect on Staph. aureus.



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 pseudomonas aeruginosa.

IV. DISCUSSION

During or after initial infection with an infective pathogen, which is often a virus, secondary bacterial infections will grow (Morris et al.,2017; Wang et al.,2018) as they are related with high death rates and morbidity rates. Co-infections, secondary infections, or "super infections" occur in the time of viral epidemics. It has been reported that secondary bacterial infections has important role in the death rate and morbidity of patients whom were initially infected by pulmonary viral diseases (Manohar et al.,2020; Thanoon et al.,2021)

No growth was observed in 11 samples from the total of 50 samples. This may be attribute to prophylactic antibiotics such as: moxifloxacin, cefepime, vancomycin, or azithromycin for those patients whom suffered from pulmonary viral infection, in order to protect them from secondary infections (Holshue et al., 2020; Wang et al., 2020; Manohar et al.,2020).

According to our results bacteria that identified in secondary infections were predominantly Gram-negative organisms, including Enterobacteriaceae and P.aeruginosa, in addition to S.aureus this finding is in line with result of Russell et al.,2021. The emergence of multidrug-resistant organisms and their spread across healthcare settings are caused by multiple factors, including antibiotics use and cross-transmission due to gaps in infection control (Kamela et al., 2022; Ali et al., 2022). During the COVID-19 pandemic, infection control and hygiene measures were drastically upgraded (Thanoon et al.,2020; Ngoula et al., 2022). However, the results showed that there was no methicillin resistant S.aureus, it is differ from other study conducted by Habib et al., 2022. However, (ESBL) production in enterobacterace family was notably high this is in line with result of Lontsi et al., 2022.We found that high concentration of propolis have the most effect on gram positive and gram negative bacteria.

CONCLUSION

1-The most common pathogenic bacterial secondary infections in covid 19 patient were Klebsiella spp. Followed by E. coli

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and Pseudomonas spp. was the least frequent among gram negative bacteria.

2-S. aureus most common pathogenic bacterial secondary infections in covid 19 among gram positive bacteria.

3. Gram-negative bacteria were the most common microorganisms isolated as a secondary infection in covid 19 patients due to hospital acquired infections.

4. The results showed that there was no methicillin resistant S.aureus.

5. (ESBL) production in enterobacterace family was notably high.

6. Statistically analysis showed that there are differences between different concentration of Propolis extract used for each species of isolates high concentration of propolis gave the most effective antimicrobial results

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