The Egyptian Journal of Hospital Medicine (July 2023) Vol. 92, Page 6246- 6255

Antimicrobial Activity of Some Honeybee Products on Multidrug-Resistant Secondary Microbial Infection from COVID-19 Patients

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ABSTRACT

Background: COVID-19 patients are vulnerable to develop secondary microbial infections that are frequently multidrug-resistant (MDR) and increase the severity of the disease and mortality risk, which has created an urgent need for the use of natural products as antimicrobial agents.

Objective: This study aimed to evaluate and compare the polyphenol content of some honeybee products from different origins as well as their antimicrobial activity against some MDR secondary microbial infections in COVID-19 patients.

Methods: During the winter of 2021, sixteen clinical microbial isolates were collected from sputum and chest swabs of COVID-19 patients from some hospitals in Cairo, Egypt, and their antimicrobial susceptibility testing was performed. The total phenolic content (TPC) and total flavonoid content (TFC) of eight honeybee products from different origins were evaluated, and their antimicrobial activity was determined by recording the inhibition zone diameter (IZD) and minimum inhibitory concentration (MIC).

Results: According to results, Turkish propolis, Egyptian royal jelly sample1, and Egyptian honey contained the highest polyphenol content and consequently showed significant antimicrobial activity compared to other bee products under study. Turkish propolis contained elevated polyphenol contents (TPC= 322.33 mg Gallic acid (GAE)/100 g and TFC= 88.974 mg Quercetin (QUE)/100 g) and expressed its antimicrobial activity with IZD ranging 15.33–28.33 mm and MIC value of 0.105–7.5 mg/ml. Also, Egyptian royal jelly1 and Egyptian honey contained high polyphenol contents (TPC= 134.737 mg GAE/100 g and TFC= 78.162 mg QUE/100 g) and (TPC= 98.571 mg GAE/100 g and TFC= 44.487 mg QUE/100 g), respectively, and showed antimicrobial activity with IZD of 0.00–21.66 mm and 0.00–22.00 mm, respectively, as well as MIC values of 1.150–4.69% and 6.25–37.50%, respectively. Indeed, all honey and royal jelly samples showed no activity against *Candida* spp., while propolis exhibited good action against it.

Conclusion: Honeybee products are promising natural products that possess unique antimicrobial activities that help to fight MDR secondary microbial infections in COVID-19 patients, in which their antimicrobial activity is largely affected by polyphenol contents and geographical origins.

Keywords: Honey, MDR, MIC, Polyphenols, Propolis, and Royal Jelly.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a serious viral infectious disease caused by the recently identified severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2). It was declared in March 2020 by the World Health Organization (WHO) as a worldwide pandemic causing millions of morbidities and death [(1)]. Several studies on COVID-19 patients have reported the prevalence of secondary microbial (bacterial and fungal) infections, with an increasing predominance of multidrug-resistant (MDR) Gram-negative bacteria and *Candida* species [(2, 3)]. The increased prevalence of MDR microbial infections may be due to the empiric prescription of antibiotics for the majority of patients suspected or diagnosed with COVID-19 [(2)]. Therefore, there is an increasing demand for the development of different choices other than communal antibiotics, such as apitherapy, to correct immunological deficiencies in COVID-19 patients as well as to prevent antimicrobial resistance [(4)].

Apitherapy or bee therapy involves the use of bee products for therapeutic purposes. Honey, propolis, and royal jelly are important honeybee products that have been used since ancient times owing to their health benefits and pharmacological activities [(4)]. These products possess pharmacological and biological properties in varying degrees owing to their content of antioxidant compounds, such as phenolic acids, flavonoids, and/or terpenoids. Variations in the active contents of each bee product were strongly influenced by their floral sources, geographical location, weather, season, and extraction methods [(5)].

Honey is one of the oldest bee products and has been produced by bees from the nectar of many flowers. It is an aqueous supersaturated mixture of sugars with trace amounts of organic acids, proteins, minerals, vitamins, and polyphenols (flavonoids and phenolic compounds) that are important for the biological properties of honey [(6)]. Nearly all types of honey, including monofloral and multi-floral honey, have antimicrobial activity in different ranges [(7)].

Propolis (bee glue) is a natural sticky resinous substance collected by bees from the resinous secretion of buds of different plant species and, which then combines

Received: 19/02/2023
Accepted: 19/04/2023

with bee saliva and wax to fill cracks in beehives and provide a protective barrier against invading microbes [8]. Propolis, unlike honey and royal jelly, has no nutritional value and was used in folk medicine at 300 B.C. It contains more than 300 biologically active compounds, including phenolic acids and their esters, flavonoids, steroids, terpenoids, amino acids, and inorganic compounds [9]. Propolis possesses strong antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, antidiabetic, antiallergic, and anticancer properties [4, 9].

Royal jelly (RJ) is a viscous, yellowish, milky bee product secreted from the salivary glands of worker bees and is considered a special nutriment for feeding larvae and queen bees (*Apis mellifera*) [5]. It is a highly nutritive bee product that is rich in proteins, amino acids, carbohydrates, lipids, mineral salts, vitamins, and polyphenols. It has been used worldwide as a dietary supplement as well as a therapeutic agent owing to its excellent bioactivities, including antibacterial, antitumor, immunomodulatory, antioxidant, anti-inflammatory, anti-aging, and fertility enhancing effects [10].

Hence, this study was a trial to study, evaluate, and compare the polyphenol content of honey, propolis, and royal jelly from different origins, as well as the antimicrobial activity of these bee products on MDR secondary microbial infections in COVID-19 patients.

MATERIAL AND METHODS

Honeybee products:

Eight honeybee products were collected from different regions: three propolis samples (two Egyptian and one Turkish), three honey samples from different countries (Egypt, Turkey, and Saudi Arabia), and two Egyptian royal jelly samples from different regions. Once honeybee samples were collected in a sterile dark glass container, honey samples were stored at laboratory room temperature, while royal jelly and propolis samples were stored in the freezer and refrigerator until extraction, respectively. Propolis samples were extracted according to **El-Guendouz *et al.*** [8] with some modifications: fifty grams of propolis were cut into small pieces and extracted with 500 ml of 70% ethanol (1:10, w/v) at 37 °C for 7 days under agitation, protected from light, and then centrifuged for 10 min. The supernatant was evaporated under vacuum at 50 °C until dryness to obtain pure propolis extract in powder form, and the product obtained was referred to as the ethanolic extract of propolis (EEP).

Estimation of polyphenol content:

The total phenolic contents (TPC) of honey, propolis, and royal jelly samples were estimated using Folin Ciocalteu reagent according to **Hegazi *et al.*** [11] with slight modifications. Briefly, 0.5 ml of the propolis solution (10 mg/ml), honey solution (10%), or royal jelly solution (10%) was mixed with 2.5 ml of Folin-Ciocalteu's reagent (0.2 N) for 5 min. Then, 2 ml of sodium carbonate solution (75 g/l) was added to the different mixtures and incubated for another 2 hours at room temperature in the dark. The absorbance of the mixtures was measured at 765 nm using a UV-Vis spectrometer, and distilled water was used as blank. Gallic acid in the range of 0–1000 mg/l was used as a standard to make the calibration curve, and the total phenolic content was expressed in milligramsof gallic acid equivalents (GAE)/ 100 g of honey, propolis, or royal jelly.

Furthermore, the total flavonoid contents (TFC) of honey, propolis, and royal jelly samples were determined based on the methods of **Hegazi *et al.*** [11], with some modifications. In brief, 2.5 ml of (10%) ethanolic extract of propolis, honey, and royal jelly solutions were mixed with 2.5 ml of 2% aluminium chloride ($AlCl_3$) in methanol and incubated for 10 min at room temperature. The absorbance of the mixtures was measured at 415 nm using a UV-Vis spectrometer. The blank solution was prepared by mixing 2.5 ml of honey, propolis, and royal jelly solutions with 2.5 ml of methanol without the addition of $AlCl_3$. Quercetin in the range of 0–100 mg/l was established as a standard to make the calibration curve, and the total flavonoid content was expressed as Quercetin equivalent (QUE)/ 100 g of honey, propolis, or royal jelly.

Antimicrobial susceptibility testing:

During the winter of 2021, sixteen microbial isolates were collected from the Clinical Microbiology Department of some hospitals in Cairo, Egypt. These isolates were collected by hospital clinicians from the sputum and chest swabs of hospitalized COVID-19 patients who were present in intensive care units. The specimens were cultured immediately after collection on Blood and MacConkey, and aerobically incubated at 37°C for 24 hours. After the incubation period, the pure microbial isolates were Gram-stained, examined microscopically, and identified using the VITEK 2 system.

Antibiotic susceptibility testing of the bacterial isolates was performed on Muller-Hinton agar according to the Kirby-Bauer method, and the results were interpreted according to CLSI guidelines [12]. Thirteen antibiotics were tested against Gram-negative bacterial isolates and seventeen antibiotics were tested against Gram-positive bacterial isolates, including penicillins, cephalosporins, DNA synthesis inhibitors, protein synthesis inhibitors, and carbapenems. The following antibiotics were used: amoxicillin-clavulanic acid (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg)**,** ciprofloxacin (5 µg), norfloxacin (10 µg), gentamicin (10 µg), tigecycline (15 µg), ertapenem (10 µg), meropenem (10 µg), colistin (10 µg),

sulfamethoxazole/trimethoprim (25 μg), and piperacillin/tazobactam (110 μg), Gram-positive antibiotics: clindamycin (2 μg), erythromycin (15 μg), linezolid (30 μg), and oxacillin (1 μg).

Susceptibility testing of *Candida* spp. was performed on 2% glucose-supplemented Mueller-Hinton agar according to the CLSI guidelines. The following antifungal agents were used: clotrimazole (10 μg), itraconazole (10 μg), fluconazole (25 μg), nystatin (100 U), and terbinafine (1 μg).

The microbial isolates were cultured on nutrient agar, incubated aerobically at 35–37°C, and stored in the laboratory at 4°C on nutrient agar slants. All media used in this study were provided by Oxoid (UK).

Antimicrobial activity of different honeybee products by the agar well diffusion method:

The antimicrobial activity of different honeybee products was determined against MDR microbial isolates by the agar well diffusion method on Mueller-Hinton agar (Oxoid, UK). Each microbial isolate was freshly prepared and subcultured on nutrient agar at 35–37 °C overnight. A microbial suspension of 1.5×10^8 CFU/ml, equivalent to 0.5 McFarland turbidity standards was prepared in sterile saline (0.85%) and swabbed over the surface of a sterilized Mueller-Hinton agar plate by using a sterile cotton swab. Wells were made in each inoculated plate using a sterile cork borer with a diameter of 8 mm. A volume of 100 μl of EEP (100 mg/ml), honey (50% v/v), royal jelly (25% v/v), and 70% ethanol (control) was added to each plate well, and plates were kept in the refrigerator for 1–2 hours prior to incubation to allow the diffusion of different samples. The plates were incubated at 35–37 °C for 18–24 hours, the diameter of the inhibition zone around the wells was measured in mm, and the mean ± standard error (SE) was calculated.

Minimum inhibitory concentration (MIC) for the most active honeybee products:

MIC values were calculated for the most active honeybee products (Egyptian honey, Turkish propolis, and Egyptian royal jelly 1) using the microdilution method, in which the microbial isolates were incubated in 96–well microtiter plates in broth containing different concentrations of Turkish propolis (0.1–125 mg/ml), Egyptian honey (1.0–75 %), and Egyptian royal jelly1 (0.5–50 %) for 24 hours at 37°C. MIC values were estimated by visual and spectroscopic methods using absorbance measurements at 620 nm [(13)]. Control tubes without EEP, honey, or royal jelly were used as negative controls.

Ethical approval and consent to participate:

This study was approved by the National Research Committee under Ethics Committee number 20072, as all patients were more than 18 years old. Informed consent was obtained from each patient before enrollment in the study. All patients were subjected to full history taking and clinical examination. All methods were performed in accordance with relevant guidelines and regulations.

RESULTS

Sixteen microbial isolates were collected from the chest and sputum swabs of COVID-19 patients and identified using the VITEK 2 system based on their phenotypic and biochemical features. These isolates included *Acinetobacter lwoffii* (n=1), *Acinetobacter baumannii* (n=3), *Klebsiella pneumonia* (n=2), *Klebsiella ozaenae* (n=1), *Pseudomonas aeruginosa* (n=1), *Serratia liquefaciens* (n=1), *Serratia rubidaea* (n=1), and *Staphylococcus aureus* (n=1) as well as three *Candida albicans* and two non-albicans strains (*Candida glabrata* and *Candida tropicalis*).

The secondary bacterial infection isolates from COVID-19 patients were resistant to most antibiotic classes, including cephalosporins, DNA synthesis inhibitors, protein synthesis inhibitors, and carbapenems, except for colistin and tigecycline in Gram-negative bacteria as well as tigecycline and linezolid in *S. aureus*, as illustrated in table (1), whereas *Candida* spp. were fluconazole-resistant strains, as shown in table (2).

Table (1): Sensitivity of Gram-negative and Gram-positive secondary bacterial infection isolates in COVID-19 patients to different antibiotics.

Antibiotic	***A. lwoffii* (n=1)**	***A. baumannii* (n=3)**	***K. ozaenae* (n=1)**	***K. pneumonia* (n=2)**	***P. aeruginosa* (n=1)**	***S. liquefaciens* (n=1)**	***S. rubidaea* (n=1)**	***S. aureus* (n=1)**
AMC 30	R	R	R	R	R	R	R	R
CAZ 30	R	R	R	R	R	R	R	R
CRO 30	R	R	R	R	R	R	R	R
CTX 30	R	R	R	R	R	R	R	R
CIP 5	R	R	R	R	R	R	R	R
NOR 10	R	R	R	R	R	R	R	R
CN 10	R	R	R	R	R	R	R	R
TGC 150	**I**	**I**	**I**	**I**	R	**I**	**I**	**S**
ETP 10	R	R	R	R	R	R	R	R
MEM 10	R	R	R	R	R	R	R	R
SXT 25	R	R	R	R	R	R	R	R
TZP 110	R	R	R	R	R	R	R	R
CT 10	**I**	**I**	**I**	**I**	R	**I**	**I**	R
DA 2	NA	NA	NA	NA	NA	NA	NA	R
E 15	NA	NA	NA	NA	NA	NA	NA	R
LZD 30	NA	NA	NA	NA	NA	NA	NA	**S**
OX 1	NA	NA	NA	NA	NA	NA	NA	R

AMC 30: Amoxicillin-clavulanic acid, **CAZ 30:** Ceftazidime, **CRO 30**: Ceftriaxone, **CTX 30**: Cefotaxime**, CIP 5:** Ciprofloxacin, **NOR 10:** Norfloxacin, **CN 10:** Gentamicin, **TGC 15:** Glycylcycline, **ETP 10**: Ertapenem, **MEM 10**: Meropenem, **CT 10**: Colistin, **SXT 25**: Sulphamethoxazole/trimethoprim, **TZP 110:** piperacillin/tazobactam, **DA 2**: Clindamycin, **E 15**: Erythromycin, **LZD 30**: linezolid, **OX 1**: Oxacillin, **S:** sensitive, **I:** Intermediate, **R:** Resistance, **NA:** not applicable.

Table (2): Sensitivity of *Candida* spp. secondary microbial infection isolates in COVID-19 patients to antifungals used

***Candida* spp.**	**Clotrimazole 10 µg**	**Fluconazole 25 µg**	**Itraconazole 10 µg**	**Nystatin 100 U**	**Terbinafine 1 µg**
***C. albicans* (n=3)**	R (2/3)	**R**	R	S	R (2/3)
***C. glabrata* (n=1)**	R	**R**	R	S	R
***C. tropicalis* (n=1)**	S	**R**	S	S	S

Polyphenol contents

From the results displayed in table (3), it can be concluded that Turkish propolis contained higher polyphenol content (TPC= 322.33 mg GAE/100 g and TFC= 88.97 mg QUE/100 g) compared to Egyptian types. Egyptian propolis contained TPC in the range of 201.88–289.39 mg GAE/100 g as well as TFC in the range of 83.80–94.19 mg QUE/100 g.

Table (3): Total phenolic and flavonoid contents of propolis from different origins

Polyphenol content	**Egyptian Propolis 1**	**Egyptian Propolis 2**	**Turkish Propolis**
TPC (mg GAE/100 g)	289.39 ± 1.85	201.88 ± 1.15	**322.33 ± 3.05**
TFC (mg QUE/100 g)	94.19 ± 1.95	83.80 ± 2.22	**88.97 ± 1.23**

Data were expressed as the means ± standard error of triplicate samples.

Also, table (4) indicated that Egyptian and Saudi honey contained higher polyphenol content compared to Turkish honey. The TPC in Egyptian, Saudi, and Turkish honey were 98.571, 97.925, and 27.744 mg GAE/100 g, respectively, while the TFC in Egyptian, Saudi, and Turkish honey were 48.077, 44.487, and 3.389 mg QUE/100 g, respectively.

Table (4): Total phenolic and flavonoid contents of honey from different origins

Polyphenol content	**Mean (mm) ± SE**		
	Egyptian honey	**Saudi honey**	**Turkish honey**
TPC (mg GAE/100 g)	**98.571 ± 0.865**	97.925 ± 1.121	27.744 ± 0.915
TFC (mg QUE/100 g)	**48.077 ± 2.001**	44.487 ± 1.521	3.389 ± 0.772

Furthermore, table (5) revealed the polyphenol contents of two Egyptian royal jelly samples, in which they contained the TPC in the range of 118.87–134.74 mg GAE/100 g as well as the TFC in the range of 73.67–78.16 mg QUE/100 g.

Table (5): Total phenolic and flavonoid contents of Egyptian royal jelly samples

Polyphenol content	**Mean (mm) ± SE**	
	Royal jelly 1	**Royal jelly 2**
TPC (mg GAE/100 g)	**134.74 ± 1.22**	118.87 ± 0.90
TFC (mg QUE/100 g)	**78.16 ± 3.11**	73.67 ± 1.21

Antimicrobial activity by the agar well diffusion method:

The agar-well diffusion method was used to determine the antimicrobial activity of eight honeybee products, by recording the inhibition zone diameters (IZD), on 10 MDR Gram-negative, 1 Gram-positive bacterium, as well as 5 fluconazole-resistant *Candida* spp. From the results displayed in table (6), Turkish propolis showed excellent antimicrobial activity compared to Egyptian propolis samples and expressed its activity IZD ranging from 15.33 mm in *S. rubidaea* to 28.33 mm in *C. albicans*.

On the other hand, Egyptian propolis1 showed moderate activity on all microbial isolates with IZD in the range of 9.00–21.66 mm, while Egyptian propolis 2 didn't give antibacterial action on most of the microbial isolates under study. In comparison, Ethanol 70% was considered an inert solvent and didn't report any inhibitory effect on any of the microbial isolates under study.

Table (6): Antimicrobial activity of propolis from different origins on MDR secondary microbial infection from COVID-19 patients by agar well diffusion method

pathogen	**Mean (mm) ± SE**		
	Egyptian propolis 1	**Egyptian propolis 2**	**Turkish Propolis**
A. lwoffii	13.00± 0.57	10.33± 0.33	**20.66± 0.88**
***A. baumannii* I**	16.33± 0.88	9.00± 0.57	**21.33± 0.33**
***A. baumannii* II**	15.33± 0.33	0.00± 0.00	**23.00± 0.57**
***A. baumannii* III**	15.00± 0.57	0.00± 0.00	**20.66± 0.66**
***K. pneumonia* I**	10.66± 0.66	0.00± 0.00	**24.00± 0.57**
***K. pneumonia* II**	12.33± 0.33	0.00± 0.00	**25.66± 0.88**
K. ozaenae	13.66± 0.33	9.66± 0.33	**25.66± 0.88**
P. aeruginosa	10.66± 0.66	10.66± 0.33	**20.00± 0.57**
S. liquefaciens	9.00± 0.57	0.00± 0.00	**16.33± 0.33**
S. rubidaea	10.66± 0.66	0.00± 0.00	**15.33± 0.33**
S. aureus	19.33± 0.66	13.00± 0.57	**26.66± 0.88**
***C. albicans* I**	14.00± 0.57	9.66± 0.88	**26.66± 0.66**
***C. albicans* II**	15.66± 0.33	9.66± 0.33	**26.00± 0.57**
***C. albicans* III**	16.00± 0.57	10.33± 0.33	**28.33± 0.66**
C. glabrata	15.33± 0.33	9.33± 0.33	**24.66± 0.66**
C. tropicalis	21.66± 0.88	10.66± 0.66	**25.33± 0.88**

Data were expressed as the means ± standard error of triplicate samples.

Also, according to the results presented in table (7), Egyptian honey exhibited a significant antimicrobial activity among other honey types against Gram-negative and Gram-positive bacterial isolates, while both Saudi and Turkish honey showed nearby activities. Indeed, *S. aureus* and *Acinetobacter* spp. (*A. lwoffii* and *A. baumannii*) were the most sensitive Gram-negative bacterial pathogens to honey, with IZD ranging 17.66–22.66 mm, 13.66–21.33 mm, and 12.66–20.00 mm in Egyptian, Saudi, and Turkish honey, respectively. While, other bacterial isolates were inhibited in varying degrees.

In comparison, the different isolates of *Candida* spp. were not affected by honey from different origins.

Table (7): Antimicrobial activity of honey from different origins on MDR secondary microbial infection from COVID-19 patients by agar well diffusion method

pathogen	Mean (mm) ± SE		
	Saudi honey	Egyptian honey	Turkish honey
A. lwoffii	13.66± 0.88	**18.66± 0.33**	12.66± 0.66
***A. baumannii* I**	14.33± 0.33	**17.66± 0.88**	12.00± 0.57
***A. baumannii* II**	21.33± 0.66	**22.00± 0.57**	20.00± 0.57
***A. baumannii* III**	15.00± 0.57	**18.33± 0.88**	15.33± 0.66
***K. pneumonia* I**	10.33± 1.20	**12.33± 0.33**	10.66± 0.88
***K. pneumonia* II**	0.00± 0.00	**11.33± 0.66**	11.00± 0.57
K. ozaenae	10.33± 0.33	**14.00± 0.57**	13.33± 0.66
P. aeruginosa	11.00± 0.57	**13.66± 1.20**	11.33± 0.33
S. liquefaciens	10.66± 0.33	**14.66± 0.33**	11.33± 0.33
S. rubidaea	13.00± 0.57	**16.00± 0.57**	13.33± 0.88
S. aureus	17.66± 0.66	**20.33± 0.66**	17.66± 0.33
***C. albicans* I**	0.00± 0.00	0.00± 0.00	0.00± 0.00
***C. albicans* II**	0.00± 0.00	0.00± 0.00	0.00± 0.00
***C. albicans* III**	0.00± 0.00	0.00± 0.00	0.00± 0.00
C. glabrata	0.00± 0.00	0.00± 0.00	0.00± 0.00
C. tropicalis	0.00± 0.00	0.00± 0.00	0.00± 0.00

Further, table (8) indicated that Egyptian royal jelly sample 1 possessed antibacterial activity against all bacterial isolates, with IZD ranging from 13.00 mm in *K. ozaenae* I_{83} Ko_3 to 21.66 mm in *S. aureus*, while Egyptian royal jelly sample 2 had no action on most of the bacterial strains. In comparison, the different isolates of *C. albicans* showed complete resistance to both royal jelly samples.

Table (8): Antimicrobial activity of Egyptian royal jelly samples on MDR secondary microbial infection from COVID-19 patients by agar well diffusion method

pathogen	Mean (mm) ± SE	
	Egyptian Royal jelly 1	Egyptian Royal jelly 2
A. lwoffii	**15.33± 0.33**	10.66± 0.88
***A. baumannii* I**	**15.00± 1.15**	12.00± 0.577
***A. baumannii* II**	**15.33± 0.66**	0.00± 0.00
***A. baumannii* III**	**16.33± 0.33**	0.00± 0.00
***K. pneumonia* I**	**14.33± 0.88**	0.00± 0.00
***K. pneumonia* II**	**13.66± 0.66**	0.00± 0.00
K. ozaenae	**13.00± 0.57**	0.00± 0.00
P. aeruginosa	**17.00± 0.57**	10.33± 0.33
S. liquefaciens	**15.00± 0.57**	0.00± 0.00
S. rubidaea	**14.00± 0.00**	0.00± 0.00
S. aureus	**21.66± 0.33**	15.00± 0.57
***C. albicans* I**	0.00± 0.00	0.00± 0.00
***C. albicans* II**	0.00± 0.00	0.00± 0.00
***C. albicans* III**	0.00± 0.00	0.00± 0.00
C. glabrata	0.00± 0.00	0.00± 0.00
C. tropicalis	0.00± 0.00	0.00± 0.00

Antimicrobial activity by MIC determination:

Table (9) showed MIC values of Turkish propolis, Egyptian honey, and Egyptian royal jelly 1 on sixteen MDR secondary microbial infections from COVID-19 patients, in which the mean of MIC values were calculated for the repeated microbial isolates. The MIC value for Turkish propolis ranged between 0.105 mg/ml and 7.5 mg/ml, while its value ranged 6.25–37.50 % for Egyptian honey and 1.15–4.68 % for Egyptian royal jelly 1. *Candida* isolates were inhibited by Turkish propolis in the range of 0.50–0.625 mg/ml, while not affected by Egyptian honey or Egyptian royal jelly 1 samples. In general, propolis exhibited lower MIC values compared to honey and royal jelly.

Indeed, *A. baumannii* and *Klebsiella* spp. **(***K. pneumonia* and *K. ozaenae*) were the most sensitive pathogens to Turkish propolis and were inhibited by 0.105 mg/ml, while *A. lwoffii* and *Serratia* spp. (*S. liquefaciens* and *S. rubidaea*) were inhibited by higher propolis concentrations of 7.5 mg/ml. In comparison, *S. aureus* and *A. baumannii* were the most susceptible microbial pathogen to Egyptian honey, with MIC of 6.25 and 9.37%, respectively, while *K. pneumonia, K. ozaenae*, and *P. aeruginosa* were suppressed at higher honey concentrations of 37.5, 18.75, and 18.75% respectively. On the other hand, *S. aureus* was the most sensitive microbial isolate to Egyptian royal jelly1 (MIC= 1.150 %), followed by *A. baumannii* and *P. aeruginosa* (MIC= 2.34%), *A. lwoffii*, *Klebsiella* spp. **(***K. pneumonia* and *K. ozaenae*), and *Serratia* spp. (*S. liquefaciens* and *S. rubidaea*) (MIC= 4.688%).

Table (9): Antimicrobial activity of honeybee products on MDR secondary microbial infection from COVID-19 patients by MIC determination

pathogen	MIC		
	Egyptian Royal jelly1 (%)	Egyptian Honey (%)	Turkish Propolis (mg/ml)
A. lwoffii	4.68	12.50	7.50
A. baumannii	2.34	9.375	0.105
K. pneumonia	4.68	37.50	0.105
K. ozaenae	4.68	18.75	0.105
P. aeruginosa	2.34	18.75	3.75
S. liquefaciens	4.68	12.50	7.50
S. rubidaea	4.68	12.50	7.50
S. aureus	1.15	6.25	0.185
C. albicans	--	--	0.50
C. glabrata	--	--	0.625
C. tropicalis	--	--	0.625

DISCUSSION

During the COVID-19 pandemic, the changes in nosocomial infection prevention and control, and excessive use of antibiotics or antifungals had implications for infection rates and increased drug resistance [(14)]. Our findings showed that *Acinetobacter* spp., *P. aeruginosa*, *Klebsiella* spp., *Serratia* spp., *S. aureus*, and *Candida* spp. were the predominant secondary microbial infection isolates from COVID-19 patients and exhibited complete resistance to most antibiotic classes, as previously reported in **Sharifipour *et al.*** [(2)] and **Helmy *et al.*** [(3)] studies. **Sharifipour *et al.*** [(2)] study focused on the co-infection in COVID-19 respiratory patients and reported that *A. baumannii* was the most predominant pathogen, followed by *S. aureus*. On the other hand, **Timsit *et al.*** [(15)] found that *Candida* spp. was the most prevalent fungal isolate in respiratory specimens and colonized the lower respiratory tract of patients who were receiving mechanical ventilation. According to early reports from Wuhan in China, about half of the patients who died from COVID-19 developed secondary microbial infections due to the widespread use of antibiotics during this pandemic [(16)].

Polyphenols have attracted researchers' interest even though most other compounds found in honeybee products have demonstrated biomedical potential. This is primarily because of their widespread distribution across all honeybee products (particularly propolis, honey and royal jelly) in varying relative amounts, as well as their complex composition and biological properties, including antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant, and antineoplastic effects [(4)]. Our results are consistent with those reported by **Mouhoubi-Tafinine *et al.*** [(17)] on which propolis extract contains more polyphenol contents compared to honey and royal jelly. Also, **Mouhoubi-Tafinine *et al.*** [(17)] reported that propolis is the source of more than 25% of honey phenolic acids and flavonoids. Consequently, the findings of the current investigation highlighted Turkish propolis extract as a superior antimicrobial compared to other honeybee products. This activity may differ due to the presence of flavonoids, phenolic compounds, esters, and aromatic acids in different concentrations [(18)].

The polyphenol contents in Turkish propolis in the current study were higher than the results reported by **Kurek-Górecka *et al.*** [(19)], in which the TPC and TFC in Turkish propolis were recorded to be 135.982 mg GAE/100g and 60.427 mg QUE/100g, respectively. Moreover, a previous study on Egyptian and Chinese propolis found that the TPC content ranged between 269 and 313.67 mg GAE/100 g, which is similar to our result. Also, another study by **Nadir *et al.*** [(20)] supported our findings and reported that Turkish propolis contained higher polyphenol contents than the Egyptian type.

Furthermore, the polyphenol contents in all honey types in our study were higher than the results indicated by **Abdulaziz *et al.*** [(21)], who found that the TPC for some locally produced honey types in Egypt and Saudi Arabia ranged from 44.0 to 84.0 mg/kg. In addition, our findings were higher than a previous study by **Roby *et al.*** [(22)], who found that the TPC of Egyptian honey ranged from 33.85 to 53.64 mg/100 g. On the other hand, another study reported the TPC in Turkish honey ranged from 24.20 to 124.05 mg/100 g, which support the present results [(23)].

Also, the polyphenol contents in the Egyptian royal jelly samples in our study were higher than the findings indicated by **Darwish *et al.*** [(24)], who found the TPC and TFC in Egyptian royal jelly were 66.35 µg/g and 15.29 µg/g, respectively. Moreover, The value of TPC in the Egyptian royal jelly samples in the present study were higher than those reported by **Özkök and Silici** [(25)] for Turkish royal jelly (59.2 mg GAE/100 g) and lower than **El-Guendouz *et al.*** [(26)] study, who found that the TPC ranged between 3.1 and 9.0 mg GAE/g in Mediterranean royal jelly. Also, the TFC in our study was higher than those reported by **El-Guendouz *et al.*** [(26)] for Mediterranean royal jelly (10–50 mg QE/100g).

These variations in polyphenol contents might result from the different geographical region, the season of collection, and floral sources of different honeybee products [(18)].

Interestingly, various MDR bacteria and *Candida* isolates responded differently to various honeybee products. Propolis, honey, and royal jelly from different origins were able to inhibit both Gram-negative and Gram-positive bacteria, but only propolis was able to inhibit *Candida* species. This comes in agreement with

Maželienė *et al.* [27] study, which reported that the tested honey and royal jelly were not effective on *C. albicans*, although other authors demonstrated that the growth of *Candida* spp. was inhibited at high concentrations of honey [28]. Moreover, **Wahdan [29]** noted that *Candida* spp. were typically more tolerant to honey than bacteria, because of the strong osmotic effect produced by the honey. In this study, Candida spp. were suppressed by Turkish propolis with MIC value ranged from 0.50 to 0.625 mg/ml, and these findings are similar to **Lopez *et al.* [30]** study (MIC= 0.250–1.00 mg/ml) and lower than **Leite *et al.* [31]** study on red propolis (MIC= 1.0–7.0 mg/ml).

Generally, propolis in the present study showed the best antimicrobial activity compared to honey and royal jelly. These results are consistent with **Gaber *et al.* [32]** who studied the effect of various bee products on MDR *P. aeruginosa* and *A. baumannii* from Hospital-acquired pneumonic patients and found that propolis showed the best antimicrobial activity in comparison with honey and bee venom. However, MDR *A. baumannii* isolates in our study were inhibited by lower concentrations of Turkish propolis (MIC= 0.105 mg/ml) compared to **Hannan *et al.* [33]** (MIC= 1.5–2.0 mg/ml) and **Gaber *et al.* [32]** (MIC= 5.6–22.5%) studies. Additionally, a prior study on stingless honey supported our findings and reported its activity with IZD of 15.8 and 19.4 mm on *P. aeruginosa* and *S. aureus*, respectively [34]. In comparison, the Turkish propolis in **Segueni *et al.* [35]** study exhibited better activity than our findings against *P. aeruginosa*, with MIC values ranging from 0.20 to 0.60 mg/ml, while no activity was noted on the same pathogen in **Kahraman *et al.* [36]** study. In our study, *K. pneumonia* and *K. ozaenae* were inhibited by a higher concentration of Egyptian honey compared to other honeybee products in the range of 18.5–37.5%. This is supported by the results of **Wasihun and Kasa [37]** on Tigray honey, who reported that MDR *K. pneumonia* clinical isolates were inhibited by 25% honey concentration. Indeed, the Gram-positive *S. aureus* strain in our study was more sensitive to Egyptian royal jelly than Gram-negative pathogens. Similar results were reported by **Dundar *et al.* [38]**, who found that the range of royal jelly's MIC values for Gram-positive bacteria was 7.81–15.63 mg/ml (= 0.78–1.56%), and between 31.25 and 62.5 mg/ml (= 3.12–6.25%) for Gram-negative bacteria.

Thus, the variations observed in the antimicrobial activity of honeybee products from different origins could be related to the existence of active components in different quantities depending on flora, botanical regions, weather, and season as well as extraction method [5, 18].

CONCLUSION

Antimicrobial resistance is a serious problem, particularly in COVID-19 patients, that endangers public health and has created a growing demand for the development of alternative antimicrobials. In the present study, Egyptian honey, Egyptian royal Jelly1, and Turkish propolis are promising bee products that exhibited good antibacterial activity against MDR secondary bacterial infections from COVID-19 patients. Fluconazole-resistant *Candida* spp. isolates were inhibited only by propolis in the range of 0.50 mg/ml to 0.625 mg/ml and it's recommended for the treatment of *Candida* infections. Propolis showed excellent antimicrobial activity and contained more polyphenols compared to honey and royal jelly. The differences in the antimicrobial activity and MIC values may be related to the polyphenol content of honeybee products associated with their floral sources and geographical location.

- **Conflict of interest:** No conflict of interest to be declared.
- **Funding:** We didn't receive special grants from funding agencies.

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