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Efficacy of propolis extract against *S. aureus*, *P. aeruginosa*, and *K. pneumoniae* strains isolated from patients with wound infection

Abstract

The wound is a suitable site for the incidence of resistance infection. Thus, the research for the finding of effective drugs against this infection is necessary. The study was planned to determine the minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) of propolis ethanolic extract against three pathogens isolated from patients with wound infection. For the aim of the present study, 26 bacteria (10 *S. aureus*, 10 *P. aeruginosa*, 6 *K. pneumoniae*) isolated from wound infection were assessed for their sensitivity to 12.5, 25, 50,100, and 200 mg/ml concentrations of propolis extract using broth dilution method. The majority (70%) of *S. aureus* isolates were showed a MIC and MBC at 100 and 200 mg/ml concentrations of propolis extract, respectively. In contrast, 50% of *P. aeruginosa* isolates reported MBC at 200 mg/ml. Notably, 66.7% of *K. Pneumoniae* isolates were resistance to the used concentrations of propolis extract. Accordingly, this study underlined the antimicrobial activity of Propolis ethanol extract against *S. aureus, P. aeruginosa,* and *K. pneumoniae* isolates. Further deep and confirmatory studies are important.

Keywords: *S. aureus, P. aeruginosa, K. pneumoniae*, propolis extract, wound infection

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Introduction

Propolis is a natural non-toxic beehive product, which uses for building and restoration of the honeycomb.[1] The term propolis comes from the Greek 'pro' (in front) and 'polis' that means town or city. Bees use propolis to seal their hives against the attack of the other insects.[2] The Greek world propolis means also to glue and describes the role of propolis to cement openings of the beehive. Another name of propolis is bee glue. Propolis was mentioned by the Greek philosopher Aristoteles in his *Historia animalium,* it was referred to a substance that the bees smeared at the hive entrance and used as a cure for bruises and sores. In the hive, propolis act as a biocide, being active against the invasive bacteria, fungi and even invading larvae.[3] Other biological activities have also been depicted for propolis, including antibacterial,[4] antifungal,[5] antiviral,[6] antitumor,[7] immunomodulation,[8] and anti-inflammatory[9] activities. Bee uses the propolis to keep their homes dry, free of drafts and hygienic. The inside wall of bee trees is remarkably smooth with varnish of propolis sealing cracks where volatile component of propolis are thought to serve as an antiseptic air-freshener. The thin layer of propolis varnish inside the brood cells strengthens the comb and establishes a more hygienic space in which eggs, larva and pupae complete their metamorphic processes. The space within the brood nest is dark, humid from honey processing and filled with the microbes associated with pollen conversion to bread. The thin propolis layer on much of the wood surface as well as on the wax comb apparently helps the bees maintain colony on healthy homeostasis.[10] Propolis is a resinous substance collected by worker bees (*Apis mellifera*) from the bark of trees and leaves of plants. This salivary and enzymatic secretions-enriched material is used by bees to cover hive walls to ensure a hospital-clean environment. Bees use propolis as a "chemical weapon" against pathogenic microorganisms. Propolis shows a complex chemical composition. It contains a variety of chemical compounds such as polyphenols (flavonoid aglycones, phenolic acids, esters, phenolic aldehydes and alcohols), terpenoids, steroids, amino acids, and inorganic compounds.[11] Propolis has also traditionally been used in curing infections and healing wounds and burns. The Greeks used propolis as the primary ingredient of *polyanthus*, a perfume which combined propolis, olibanum, styrax, and aromatic herbs.[12] More than 15 Greek and Roman research's were reported on the preparation and application of propolis. It was also already known in ancient Egypt, where it was probably used as an adhesive. Arabs have known propolis as well. For instance, Avicenna wrote about two different kinds of wax, which are clean and black wax; the latter being probably propolis. He said that by its strong smell it makes you sneeze and it has the characteristics to eliminating the spikes of the bolts and the stakes.[13] In the Persian research, propolis was described as a drug against eczemas, myalgia, and rheumatism. Recently, there is increasing evidence about the emergence of antimicrobial resistance particularly among the wound isolates that suggest the need for safe and most effect alternatives.[14,15] Identifying the antimicrobial activity of Propolis may help in treatment and prevention of infections. Therefore, the current study was planned to determine the minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) of propolis extract against three pathogens isolated from patients with wound infection.

Materials and methods

Study design, area, duration, and subjects

This was a cross sectional carried out in Kosti teaching Hospital of Kosti city, White Nile state, Sudan**.** During the period of October 2016 to January 2017, the entire of patients attended to Kosti Teaching Hospital complained from wound infection were included. The causative pathogens were isolated by cultivation and subjected to assessment to their susceptibility to propolis extract.

Ethical clearance

The ethical approval was obtained from the university of El Imam El Mahdi and Kosti Teaching Hospital administration. Also, all samples were collected after he or she accepted and known that they are participating in the scientific study.

Isolation and identification of bacteria

Collection of samples: From every patient lesion, one wound swab was collected under aseptic condition. The dry sterile cotton swab was dipped in the wound to collected pus or any exudates. All specimens were collected before dressing the wound and processed immediately.

Macroscopic examination: First the color of pus was examined macroscopically, since the macroscopic examination can be of great help and give a clue to the causative agent.

Culture: Each swab was cultured on Blood agar plates and MacConkey agar plates. The culture plates were incubated aerobically at 37°C overnight.

Identification: At the end of incubation period, all plates were examined for growth. All mixed growth was purified by sub culturing on another suitable media according to the type of growth. All the subculture plates were incubated aerobically at 37°C overnight. The organism were identified by their cultural morphology, gram stain and biochemical characteristics. The biochemical tests carried out include Oxidase test, culture on Kligler iron agar, Urease test, Citrate utilization test, and Indole production test for gram negative bacteria; whereas Catalase test and Coagulase test were used for gram positive bacteria.[16]

Antimicrobial activity of propolis extract

The antimicrobial activity of propolis extract was assessed by broth macro dilution method. Briefly, serial concentration (12.5, 25, 50, 100, and 200mg/ml) of propolis extract was prepared in broth medium using sterile 5 test tubes. Afterward, the turbidity of test organisms were prepared and matched to turbidity of 0.5 McFarland standards. Two tube of broth medium that are free of propolis extract were used as positive and negative control.[17] Using the standard wire lop 0.5, each test organism was in inoculated into the medium and incubated at 37°C overnight. Positive and negative control tubes were inoculated with the test organism and sterile broth medium, respectively. The minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) of propolis extract were determined as described previously.[17] MIC is lowest concentration that can cause invisible growth. MBC is the lowest concentration that can prevent the growth of bacteria as confirmed by subculture.

Statistical analysis

The statistical package for social sciences (SPSS) software version 21was involved in data analysis. The results have expressed as number and percentage. Fisher's exact test assessed the difference between groups. A $P<0.05$ was considered significant.

Results

In this study a total of twenty six bacteria were isolated (10 *S. aureus*, 10 *P. aeruginosa*, 6 *K. pneumoniae*), Table 1. All isolates were tested for determination of the minimum inhibitory and minimum bactericidal concentration of propolis by using 12,5mg/ml, 25mg/ml, 50mg/ml, 100mg/ml, and 200mg/ml concentrations of propolis extract. Seven (70%) of *S. aureus* isolates were showed a MIC and MBC at 100mg/ml and of 200mg/ml concentrations of propolis extract, respectively. Whereas, 3 (30%) isolates were revealed a MIC at 200mg/ml. The reported MBC for *P. aeruginosa* isolates were 100mg/ml in 5 isolates (50%). Notably, 5 (50%) *P. aeruginosa* isolates were presented MIC at 100mg/ml. Moreover, the majority of *K. Pneumoniae* isolates 4 (66.7%) were resistance to the used concentrations of propolis extract. In contrast, 2 (33.3%) of *K. Pneumoniae* isolates were stated a MIC at 100mg/ml, Table 2. There was no significant variation in the MIC and MBC between the isolates, Table 2.

Table 1 Number of the study isolates and their susceptibility to 12.5, 25, 50mg/ml of propolis extract

Isolates		Concentration of propolis extract in mg/ml		
Name	**N**	**12.5**	**25**	**50**
S. aureus	10	NA	NA	NA
P. aeruginosa	10	NA	NA	NA
K. Pneumoniae	6	NA	NA	NA

Table 2 Effect of 100 and 200mg/ml of propolis extract on *S. aureus, P. aeruginosa,* and *K. Pneumoniae* isolates

Concentration of propolis extract in mg/ml		Isolates: N (%)			P value
		S. aureus	*P. aeruginosa*	*K. Pneumoniae*	
100	NA	-	-	-	-
	MIC	7 (70%)	5 (50%)	-	0.112
	MBC	-	-	-	-
200	NA	-	-	-	-
	MIC	3 (30%)	5 (50%)	2(33.3)	0.372
	MBC	7 (70%)	5 (50%)	-	0.112

N: number, NA: not active, MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration. Fisher's exact test evaluated the difference between groups

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Discussion

This study focused on antibacterial activity of ethanol extract of propolis against three pathogens isolated from wound infection (*S. aureus, P. aeruginosa, K. pneumoniae*). The result of this study showed that the ethanol extract of propolis has a better antibacterial activity on *S. aureus* isolates compared to *P. aeruginosa* and *K. pneumoniae*. This may attributed to the difference in genetic composition of isolates. Previously, it has reported that the antibiotic resistance rate was more in *P. aeruginosa* and *K. pneumoniae* compared to *S. aureus*.[18]

Notably, 70% of *S. aureus* isolates were displayed a MIC and MBC at 100mg/ml and of 200mg/ml concentrations of propolis extract, respectively. In contrast, 50% of *P. aeruginosa* isolates stated MIC and MBC at 100mg/ml and of 200mg/ml, correspondingly. Likewise, only 2 (33.3%) of *K. Pneumoniae* isolates were reported a MIC of 100mg/ml. Furthermore, 66.7% of *K. Pneumoniae* isolates were resistance to the used concentrations of propolis extract. This suggests that the susceptibility of these bacteria to ethanol extract of propolis were different, which need further deep confirmatory studies. Unlike our study, Anibijuwon et al.,[19] study, which was performed on *S. aureus*, *P. aeruginosa* and *K. pneumoniae* showed that ethanol extract of propolis at concentration 12,5 mg/ml inhibited the growth of bacteria and 50 mg/ml concentration completely killed bacteria. Moreover, another study by Sichani et al.,[20] which assessed the antimicrobial efficacy of ethanol propolis extract against beta-lactamase producing *S. aureus* and *P. aeruginosa* isolates, was presented that ethanol extract of propolis at 11.7 and 15.6mg/ml concentrations were inhibited the growth of bacteria of *S. areus* isolates and at 23.4 and 31.2mg/ml concentrations were completely killed bacteria. As well, ethanol extract of propolis was effective against *P. aeruginosa* with a MIC at 750mg/ml and MBC at 1500mg/ml.20 Previously, Grange et al.,[21] study found that propolis completely inhibited the growth of *S. aureus* (including the MRSA strains), and partially inhibited the growth of *P. aeruginosa,* whereas, no effect on *K. pneumoniae* isolates was observed. In Marco et al.,[22] study, propolis showed a MIC against *P. aeruginosa* at 125μg/ml and MBC at 2000μg/ml concentration of the extract. Formerly, Seidel et al.,[23] research was also further confirmed the effect of propolis on Staphylococcus and *P. aeruginosa*. Altogether, these findings were showed the antimicrobial activity of the extract against bacteria and suggested the need for further deep studies. The variation between the studies may be attributed to the difference in the sample size, and genetic constitution of isolated bacteria and their susceptibility to antibacterial agents.

In this research, the low sample size, and lack of many important bacteria such as *E. coli* may also affect the findings of the research and generalizability of the result. Furthermore, one of the main limitations is that the study did not perform disc diffusion method to assess the zone inhibition for the MIC of propolis extract. The study was also lack the uses of antibiotics susceptibly test.

Conclusion

Our study underlined the antimicrobial activity of Propolis ethanol extract that was showed different antimicrobial activity against the *S. aureus, P. aeruginosa,* and *K. pneumoniae* isolates. Other studies are important for further verification of the antimicrobial effect of Propolis ethanol extract and determining the suitable concentrations for antimicrobial use.

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Conflicts of interest

All authors declare that there was no conflict of interest.

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