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The Effect of Propolis on The Some Biological Parameters of Pathogenic Bacteria

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ABSTRACT

The ability of propolis to inhibit growth of different types of pathogenic bacteria was investigated. Different concentration were prepared from ethanolic extract of propolis using 5%, 10%, 15%, 20% and 25%. The diameter of inhibition zone increase with increase of concentration of propolis. The propolis was more effective against gram-positive bacteria (*S. aureus* and *Streptococcus mutans*) than the gram-negative bacteria (*E. coli, P. aeruginosa* and *Proteus mirabilis*).The redox status of such bacteria broth has been check and results shows that oxido index value reduced which reflect the tendency of bacteria to overcome such effect.

Keywords: *Streptococcus aureus, Streptococcus mutans, propolis , SOD, XO, Oxido index*

Introduction

Propolis, the natural resinous substance collected by honeybees from various plant sources, is considered as a good source of natural antioxidants and antibacterial [1]. Propolis composition is directly related to that of buds andexudates collected by bees from various trees. Propolis has a variety of chemical compounds such as polyphenols (flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols and ketones), sesquiterpenequinines, coumarins, steroids, amino acids and inorganic compounds. [2]. The most important active constituents of propolis are aromatic acids, phenolic compounds, especially flavonoids (flavones, flavonols and flavonones) and phenolic acids. Flavonoids, aromatic acids, diterpenic acids and phenolic compounds seem to be the principal components responsible for the biological activities of propolis samples [3]. Propolis has been reported to have various biological activities, such as antibacterial, antiviral, antitumor, anti-inflammatory, anticancer, antifungal and antitumoral properties , consequently, propolis has been used in food and pharmaceutical products to improve health and prevent diseases such as cancer, inflammation, diabetes, heart disease and erythrocyte membrane disorder [4]. It was demonstrated that propolis have antibacterial activity against *Enterococcus* spp, *Escherichia coli*, and *Staphylococcus aureus*. Reports have pointed out propolis efficient activity against Gram-positive bacteria and limited action against Gram-negative bacteria [5]. The antibacterial properties has been studied from other side by study such effect on the other biological parameters including broth total protein, super oxide dismutase (SOD), Xanthine oxidase (XO) and SOD / XO index as previous study shows important information from

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Materials and Methods

Ethanolic Extract of Propolis

Ten gm of propolis were mixed with 100 ml of absolute ethanol in dark brown bottle and left for 7 to 14 days in room temperature and in dark place with 2 or 3 times per day shaking the container and return to warm dark place,

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continue this for 14 days. The liquid was filtered through Whatman No.1 and ethanol evaporate by using oven at 45 °C, then weighed the extract and stored in dark clean container for using. Ethanolic extract was dissolved by Dimethyl Sulfoxide (DMSO), and sterilized by filtration (using Millipore 0.45 filter paper) and then requisite dilutions prepared according to Darwish *et al* [7].

Bacterial isolates:
Both gram negative and gram-positive microorganisms were used for the test. The gram-negative bacteria include *Escherichia coli, Pseudomonas aeruginosa and Proteus mirabilis* and gram-positive organisms include *Staphylococcus aureus* and *Streptococcus mutans*. All bacterial strains were maintained on freshly prepared nutrient agar. The identification of the local bacterial isolates was confirmed using conventional biochemical tests [8]. The bacterial strains were isolated from different site of infection procured from department of microbiology-College of Medicine-Babylon University

Agar well diffusion test:
The Mueller-Hinton agar was employed for in vitro evaluation of antibacterial activities. The propolis extracts was used as control in separated plates. The concentrations of the propolis extracts used in the study were ranged from: 5%, 10%, 15% and 25 mg/ml. The inoculums size was adjusted so as to deliver final inoculums of approximately 10^8 CFU/ml, comparison with the turbidity of sample to the 0.5 McFarland standards. After the plates were solidified at room temperature, cork borer in 5mm dimension made wells in the agar. All of the suspensions of the gram-positive bacteria and gram negative were spread on to different plates. Then, the same 40 µl of extracted propolis was added into the wells of plates. The plates were incubated for 24 h at 37°C for tested the bacteria Finally, diameters of inhibition zones around the wells were measured.

Detection of bacterial growth by optical density:
- Nutrient broth was prepared and distributed in tubes and extracted propolis was added to each tube at various concentrations to gain the final concentration (5, 10, 15, 20, 25 µg/ml).
- The tubes is inoculated with 0.5 ml of bacterial suspension, then the tubes were incubated at 37°C for intervals (1, 2, 4, 6, 24hr).
- After incubation, the absorbance is read at wavelength 560nm by using spectrophotometer to show the effect of extracted propolis on the growth of bacteria.

Determination of Xanthine Oxidase (XO): Measured by using the method described by [9].

Determination of Superoxide dismutase (SOD): SOD activity measured using the method as described previously [10].

Determination of total protein: Using kit supplied by Biolabosa company (France)

Results and Discussion

Antimicrobial activity
The antimicrobial property of propolis was tested against Gram-positive (*S. aureus* and *Streptococcus mutans*) and Gram-negative (*E. coli, P. aeruginosa* and *Proteus mirabilis*. The results in figure 1 showed that propolis extracts using 5%, 10%, 15%, 20% and 25% ethanolic extract of propolis showed inhibitory zone against bacteria. The diameter of inhibition zone increase with increase of concentration of propolis. The antimicrobial effects of propolis against Gram-positive (*S. aureus*) and Gram-negative (*E. coli and Pseudomonas*) bacteria were also reported on korean propolis [11] and Portugalean propolis [12]. The results in Figure 2, Figure 3 and Figure 4 also suggested that propolis was more effective against Gram-positive bacteria (*S. aureus* and *Streptococcus mutans*) than the Gram-negative bacteria (*E. coli, P. aeruginosa* and *Proteus mirabilis*). This may be explained by the structural differences of the bacterial cell wall of Gram-positive and Gram-negative bacteria. Gram-negative bacteria, apart from the cell membrane, possess an additional outer layer membrane, which consists of phospholipids, proteins and lipopolysaccharides, and this membrane is impermeable to most molecules [13]. It was also reported that Gram-negative bacteria were less susceptible to propolis than Gram-positive strains [12].

Inhibition of bacterial and fungal growth were extrusive proportioning with increase of concentration of propolis due to increase of concentration of active component of propolis. This result was in agreement with [14-16], which they found that the efficiency of propolis extract was high when the concentration of propolis increased.

These results show that the antimicrobial activity of ethanol extract of propolis was due to propolis constituents [17]. In our study, there were not any inhibition zones in control and the results revealed that propolis has a strong antimicrobial activity against *S. aureus* and *Streptococcus mutans* as in Figure 2, Figure 3 and Figure 4 at different concentration..

Kilic *et al* [18] demonstrated in their research that dependence of antibacterial activity effect of ethanol extract of propolis is changeable and dependent on propolis fraction and bacteria species. However, Ophori *et al* [19] reported that the antimicrobial activity of propolis is a result of the high content of flavonoids. However, this activity varies according to geographic regions and pH of the culture medium [20]. The presence of flavonoids and derivatives of caffeic acid is associated with the bactericidal activity [21]. The mechanism of antibacterial action of propolis has been the subject of only a few publications [22], which showed through electron microscopy and micro-calorimetric assays that ethanolic extracts propolis (EEP) interferes with the division of *Streptococcus* through the formation of pseudo-multicellular forms, cytoplasm disorganization, inhibition of protein synthesis leading to lysis of the bacteria.

Table 1 shows the results of some biological parameters under

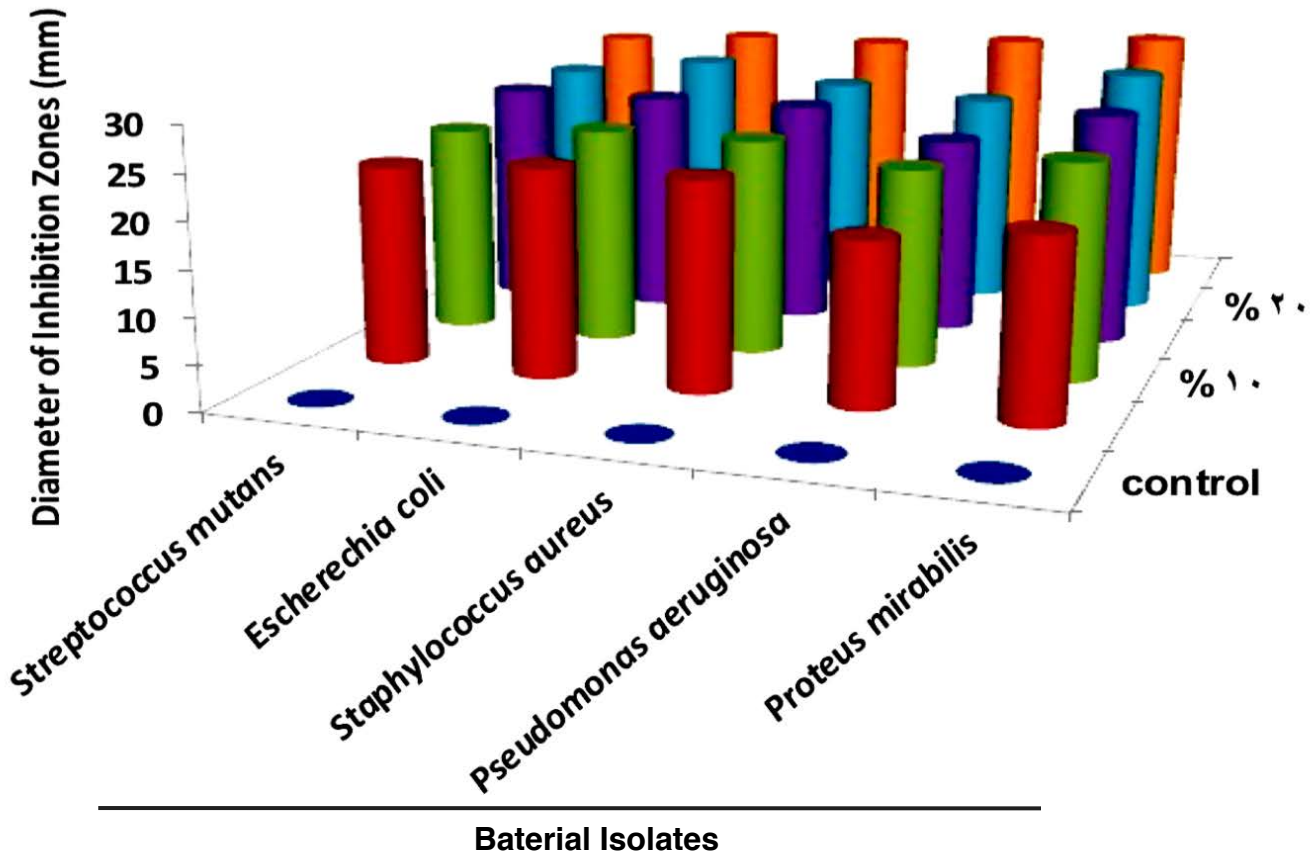

Figure 1: Inhibition Zones of different Propolis concentration against different bacterial isolates

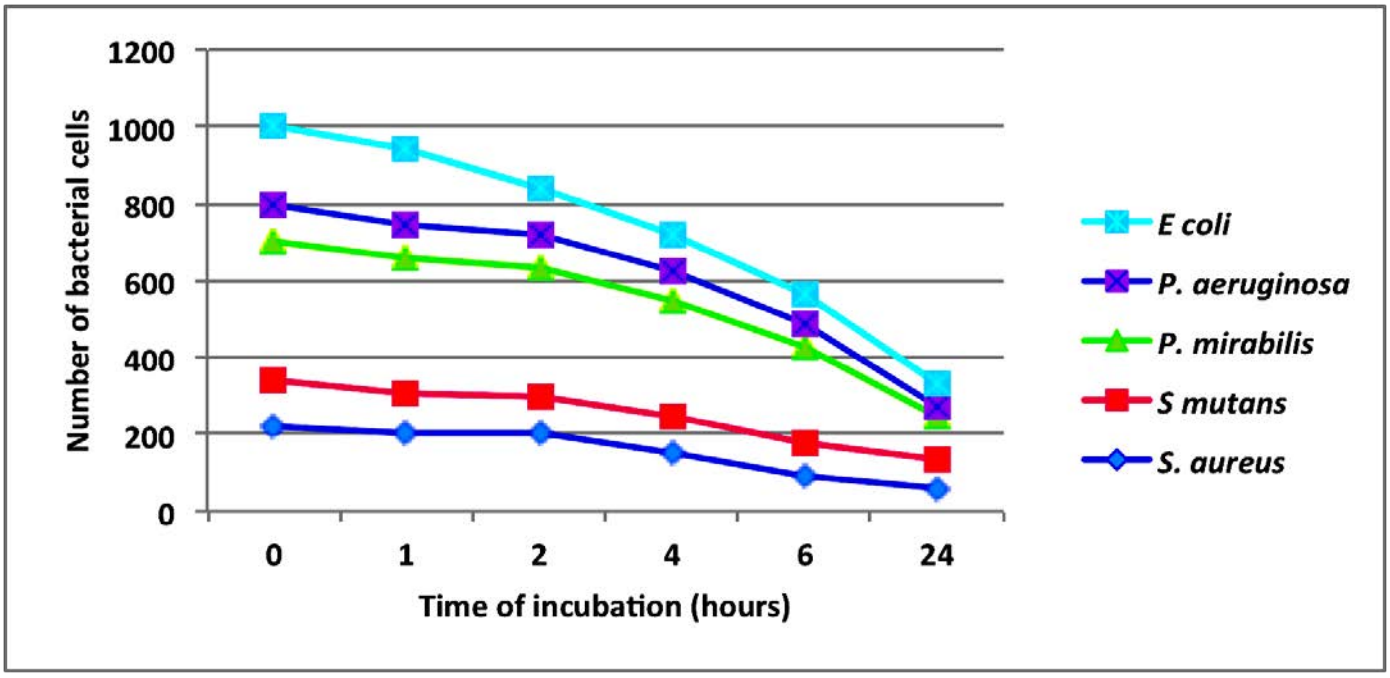

Figure 2: Optical density referring to growth inhibition after incubation at various time intervals of different bacterial isolates with Propolis (concentration 15%).

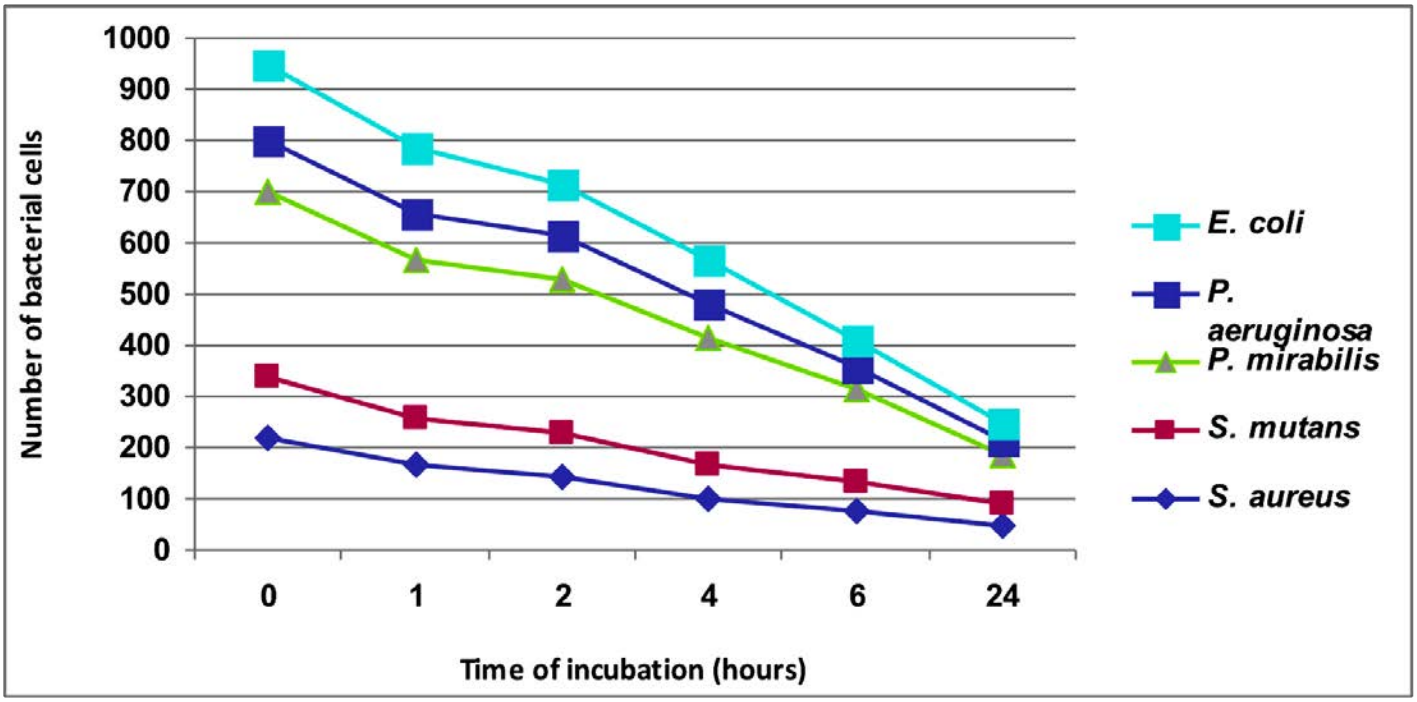

Figure 3: Optical density referring to growth inhibition after incubation at various time intervals of different bacterial isolates with Propolis (concentration 20%).

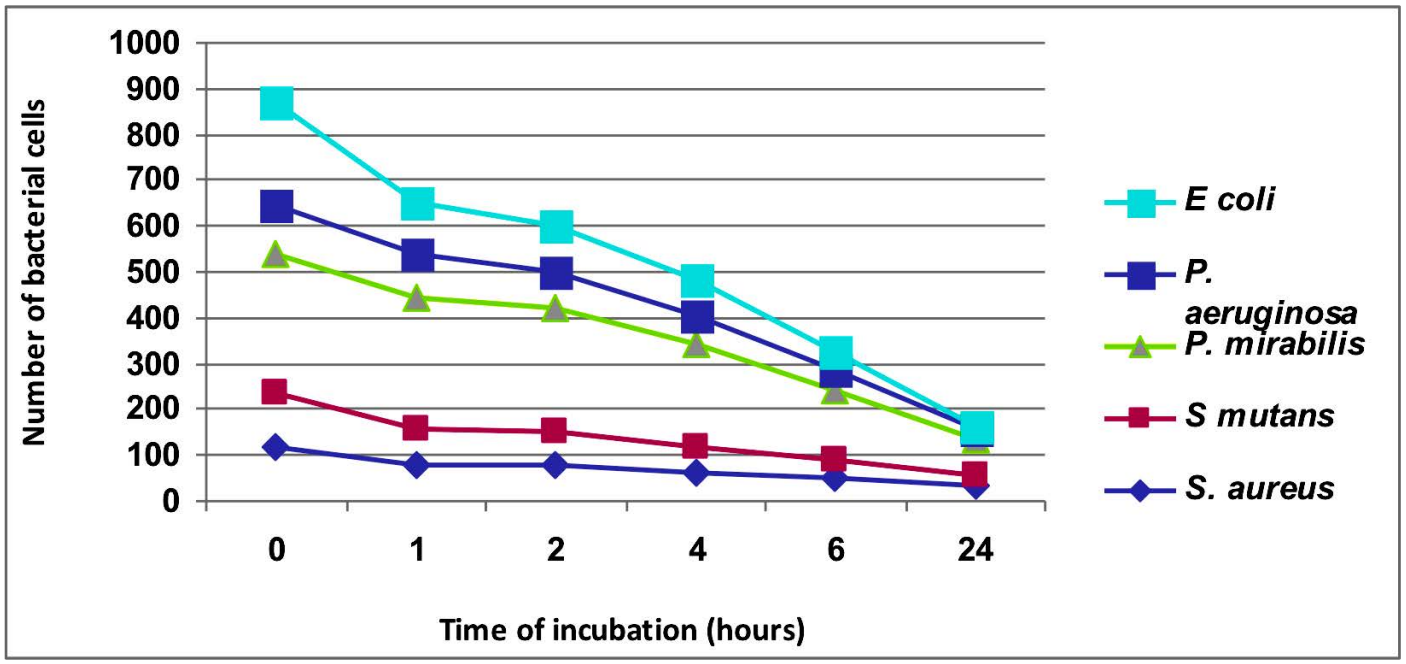

Figure 4: Optical density referring to growth inhibition after incubation at various time intervals of different bacterial isolates with Propolis (concentration 25%).

Table 1: The effect of higher concentration of propolies on biological parameters of two types of bacteria, *S. auras* **and Proteus before and after addition.**

Parameters	P1	P2	S1	S2
Total Protein (g/dl)	0.637	1.125	0.586	1.655
SOD (U/ml)	1.78	1.58	1.46	0.82
XO (U/ml)	0.0172	0.0434	0.010	0.094
(SOD/XO) index	103.4	36.40	146	9

P1 and P2 : Proteus before and after Propolies addition; S1 and S2 : St.auras before and after Propolies addition

the effect of propolis before and after addition on bacterial growth and as mentioned before that the situation of oxidant-antioxidant is not enough to evaluate precise situation of bacteria unless there is a measurement of oxido sensitive index (SOD / XO) [23]. The result shows that total protein has an increment in its value in both bacteria (*S. auras* and *Proteus*) and it can explained that the increment is not some biological changes of protein in bacteria but it reflects the amount of propels protein after addition to bacteria broth while the important changes has been appeared in SOD especially in the *S. auras* (11.2 % reduced in activity in Proteus while it shows decrement in its activity about (41 % in *S. auras*), however the activity of Xanthine oxidase increase in both bacteria after addition of propolis which shows the tendency of bacteria to over come the effect of free radicals appears after addition of such material such as uric acid (unpublished data) ,so the oxido index (SOD / XO) shows a very important decrement after propolis addition which evaluates the bacteria situation turnover the effect of propolis [6].

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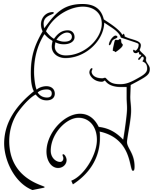

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