Effect of Iraqi Propolis (Bees gum) Extract on Gram Negative and Positive Bacteria

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Abstract

The antibacterial effect of Iraqi propolis extract was evaluated by an in vitro study testing the growth of various Gram-negative and Gram-positive bacteria. The bactericidal activity of this extract was analyzed by serial dilution in tubes. This study, found that Gram positive bacteria are susceptible to very low propolis concentrations. On the other hand, Gram negative bacteria were more resistant, with the minimal bactericidal concentration of this extract ranging between 0.5 and 1 mg ml^{-1}, for the species of Gram positive bacteria and were ranging between 2 and 4 mg ml^{-1} for the species of Gram-negative bacteria. The results of this study suggest that propolis is effective in controlling of multiple pathologies as a natural resource to prevent many bacterial infections.

Introduction

Propolis (bees gum) is a resinous yellow-brown to dark brown substance collected by worker honey bees from the growing parts of trees and shrubs (e.g., leaf buds, trunk wounds). Propolis protects the hive in two ways: First, it reinforces the hive itself; second, it protects the hive from bacterial and viral infection. Propolis is collected from beehives through the use of traps or through scraping methods. However, it is the use of traps from which the highest quality propolis is obtained, due to a lower degree of contamination (1). And it is these latter properties which man has found so helpful through the centuries. Propolis has been used by man since early times, for various purposes, and especially as a medicine because of its antimicrobial properties. However, more recently there has been resurgence in its use, and currently, research is being carried out on its activity, effects and possible uses in biology and medicine. The most prominent are its application as a dietary supplement and its use in the pharmaceutical industry (2, 3, 4). The antibacterial and antifungal activities are the most popular and among the most extensively investigated biological actions of propolis (5, 6). There are many data about antiviral action, too (5, 7).For the purpose of communicable diseases with bacterial infection and resistant bacteria to manufactured antibiotics, the objectives of this work were: to study the activity of Iraqi propolis extract against several Gram-positive and Gram-negative bacteria.

Materials and Methods

Extraction of Propolis

Iraqi propolis samples were collected from an apiary located in Al-Tarmyia (a region 60 km north-east Baghdad) in different seasons and stored at 4 ^{0}C. For the purpose of extraction, one gram of propolis was cut into small pieces, and extracted at room temperature with 50 ml of 70% ethanol using ultrasonic bath (Decon FS 300, England) for 90 minutes. Then, the alcoholic extract was evaporated at 50°C until dryness (7)

Antibacterial assay

Six bacterial strains were used: ***Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia, Diplococcus pneumonia, Streptococcus faecalis*** and ***Bacillus subtillis***.These bacteria were kindly supplied by the Biotechnology department; college of science, university of Baghdad, Baghdad, Iraq .The bacterial suspension was prepared and adjusted by comparison against 0.5 Mc-Farland turbidity standard ($5x\ 10^{7}$ cell ml^{-1}) tubes. It was further diluted to obtain a final of 5×10^{6} cell ml^{-1}. All bacteria strains were sub- culture on nutrient broth (8). The broth was inoculated by the 0.2 ml/10ml broth either with all bacteria strains, then added 1 ml of (0.5, 1, 2, 4 and 6 mg) propolis. The tubes were incubated at 37 ^{0}C for 24 h. The growth of control bacterial growth due to propolis was measured by turbidity at 600 nm wavelength. The mean values of inhibition were calculated from triple reading in each test. The minimum bactericidal concentration (MBC) of propolis was determined by the ten-fold dilution method against bacterial strains in ***in vitro***.

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Statistical data analysis: Data were statistically analyzed using SPSS statistical software (version 11.5). The values are given as mean ± standard error.

Results

This study confirms the antibacterial effect of propolis extract on various Gram-negative and Gram-positive bacteria. In particular, crude of propolis is a very potent inhibitor of growth of bacteria such as clinical isolates of ***Pseudomonas aeruginosa***, ***Proteus vulgaris***, ***Klebsiella pneumonia***, ***Diplococcus pneumonia***, ***Streptococcus faecalis*** and ***Bacillus subtillis***. All bacterial pathogens failed to grow in higher concentrations of propolis extract (Table 1 and Figure 1, 2)

Table 1. Antibacterial properties of Iraqi propolis

Strains	Optical Density 600 nm					
	Concentration of propolis mg ml^{-1}					
	0.5	1	2	4	6	Control
Pseudomonas aeruginosa	1.59±0.34	1.41±0.11	1.21±0.20	0.45±0.04	0.15±0.13	0.58±0.28
Proteus vulgaris	1.45±0.25	1.40±0.30	1.15±0.22	0.21±0.07	0.11±0.04	1.43±0.19
Klebsiella pneumonia	1.36±0.24	1.33±0.19	1.23±0.22	0.21±0.04	0.19±0.06	1.32±0.10
Diplococcus pneumonia	1.30±0.12	1.29±0.20	0.85±0.12	0.41±0.12	0.12±0.08	1.39±0.11
Streptococcus faecalis	1.40±0.14	1.39±0.08	1.34±0.14	0.13±0.02	0.12±0.16	1.49±0.36
Bacillus subtillis	0.45±0.06	0.30±0.07	0.31±0.17	0.20±0.13	0.20±0.02	0.38±0.08

Pseudomonas aeruginosa: The results indicated that propolis exhibited antibacterial activity at concentrations of 4 mg ml^{-1} (0.45 ± 0.04) and above as compared with control 0.58 ± 0.28 (Table 1). There was also an obvious decrease in the number of viable cells of ***Pseudomonas aeruginosa*** especially at the higher concentration (6 mg ml^{-1}) was 1.12 x10^{9} CFU ml^{-1} as compared with control 4.35 x 10^{9} CFU ml^{-1} (Figure 1). The MBC of propolis was 4 mg ml^{-1}.

Proteus vulgaris: The results indicated that propolis exhibited antibacterial activity at concentrations of 1mg ml^{-1} (1.40±0.30) and above as compared with control 1.43±0.19 (Table 1). There was also an obvious decrease in the number of viable cells of ***Proteus vulgaris*** especially at the higher concentration (4 and 6 mg ml^{-1}) was 1.57 x10^{9} and1.42 x10^{8} CFU ml^{-1}, respectively as compared with control 1.07x10^{9} CFU ml^{-1}. The MBC of propolis was 2 mg ml^{-1}.

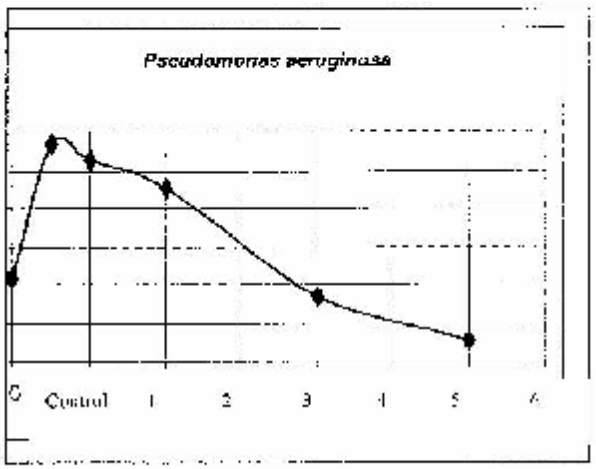
Klebsiella pneumonia: The results indicated that propolis exhibited antibacterial activity at concentrations of 1mg ml^{-1} (1.23±0.19) and above as compared with control 1.32±0.10 (Table 1). There was also an obvious decrease in the number of viable cells of ***Klebsiella pneumonia*** especially at the higher concentrations (4 and 6 mg ml^{-1}) was 1.57 x10^{8} and 1.42 x10^{8} CFU ml^{-1}, respectively as compared with control 9.80 x10^{9} CFU ml^{-1} (Figure 1). The MBC of propolis was 2 mg ml^{-1}.

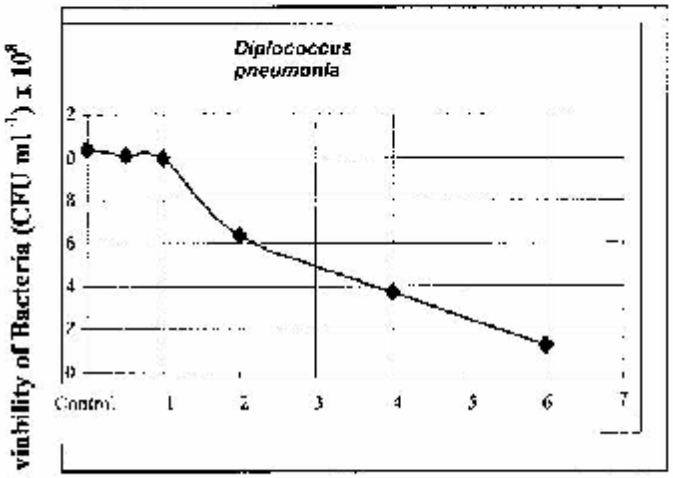
Diplococcus pneumonia: The results indicated that propolis exhibited antibacterial activity at concentrations of 2 mg ml^{-1} (0.85±0.12) and above as compared with control 1.39±0.11 (Table 1). There was also an obvious decrease in the number of viable cells of ***Diplococcus pneumonia*** especially at the higher concentration (4 and 6 mg ml^{-1}) was 3.67 x10^{8} and 1.27 x10^{8} CFU ml^{-1}, respectively as compared with control 1.04x10^{9} CFU ml^{-1}(Figure 2). The MBC of propolis was 0.5 mg ml^{-1}.

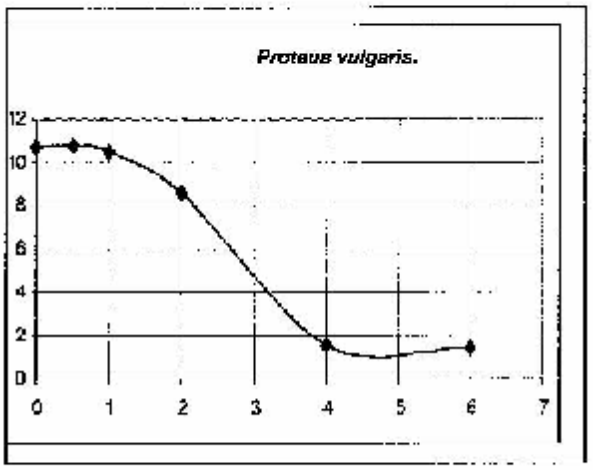
Streptococcus faecalis: The results indicated that propolis exhibited antibacterial activity at concentrations of 4 mg (0.13±0.02) and above as compared with control 1.49±0.36 (Table 1). There was also an obvious decrease in the number of viable cells of ***Streptococcus faecalis*** especially at

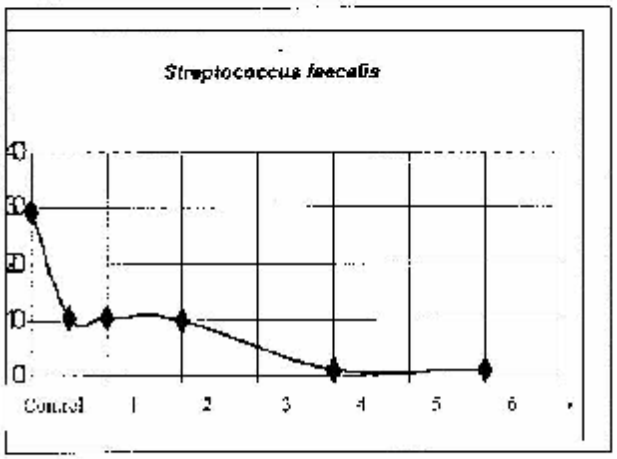
the higher concentration (4 and 6 mg ml^{-1}) was 3.67×10^{8} and 1.27×10^{8} CFU ml^{-1}, respectively as compared with control 1.04×10^{9} CFU ml^{-1} (Figure 2). The MBC of propolis was 0.5 mg ml^{-1}.

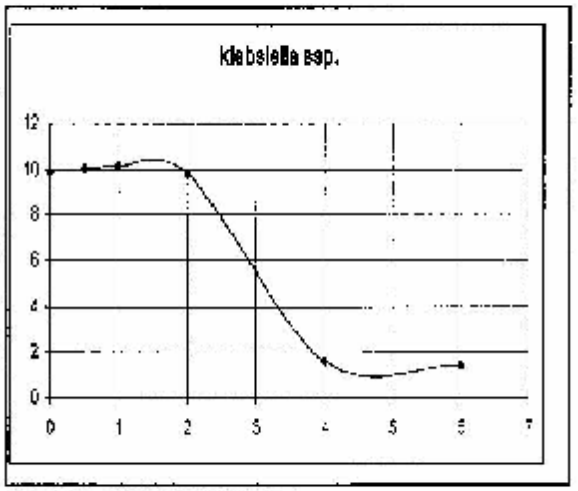
Bacillus subtillis: The results indicated that propolis exhibited antibacterial activity at concentrations of 4mg ml^{-1} (0.20±0.13) and above as compared with control 0.38±0.08 (Table 1). There was also an obvious decrease in the number of viable cells of ***Bacillus subtillis*** especially at the higher concentration (6 mg ml^{-1}) was 1.50×10^{8} CFU ml^{-1} as compared with control 2.62×10^{8} CFU ml^{-1} (Figure 2). The MBC of propolis was 1 mg ml^{-1}

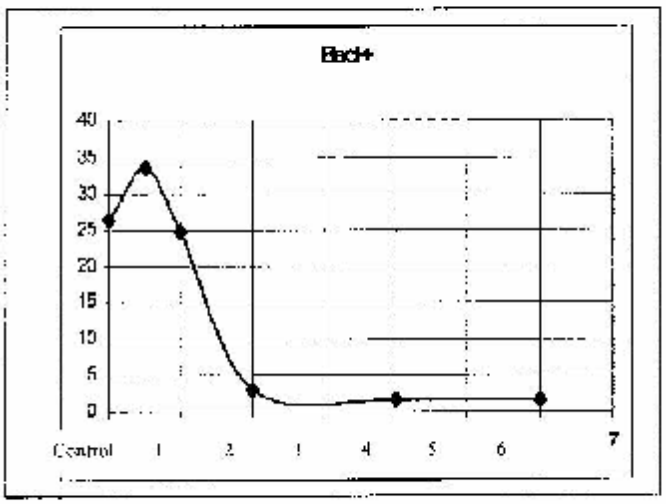












Concentration of propolis mg ml^{-1}

Figure 1. Propolis effect against cell viability of Gram negative bacteria

Concentration of propolis mg ml^{-1}

Figure 2. Propolis effect against cell viability of Gram positive bacteria

antibacterial activity of Iraqi propolis (9). In this Study, we could verify that Gram positive bacteria (***Diplococcus pneumonia, Streptococcus faecalis*** and ***Bacillus subtillis***) are susceptible to very low propolis concentrations. On the other hand, Gram negative bacteria (***Pseudomonas aeruginosa, Proteus vulgaris*** and ***Klebsiella pneumonia***) were more resistant. Previous studies also reported that Gram-negative bacteria were less susceptible to lower minimal inhibitory concentrations (MIC) than Gram-positive strains (10, 11, 12, 13, 14, 15). However, with respect to the magnitude of the MIC, Iraqi propolis showed lower activity against ***Pseudomonas aeruginosa*** than ***Proteus vulgaris*** and ***Klebsiella pneumonia*** and was more active against ***Diplococcus pneumonia*** and ***Streptococcus faecalis*** than ***Bacillus subtillis***. The quantitative and qualitative chemical composition could provide an explanation for the observed differences. Crude propolis contains a mixture of a large number of biologically active substances (16) that belong chemically to the terpenes, caffeic acid and its esters, flavonoids, free amino acids, aldehydes and ketones. Many studies have shown that fatty acid esters, phenolic compounds and cinnamic acid were the main propolis constituents and some of them were shown to possess antibacterial activity (17, 18). Crude propolis was shown more effective than single chemicals, a possible explanation of why propolis is more effective than its individual compounds (19). Of course, mixtures are more likely to contain toxic constituents, and they must be thoroughly investigated and standardized before approved for use on a large-scale basis in the West (20). Many researchers had investigated the antibacterial activity of propolis and its extracts against Gram-positive and Gram-negative strains and found that propolis had antibacterial activity against a wide range of Gram-positive rods but had a limited activity against Gram-negative bacilli (21, 22).An in vitro studies have demonstrated that propolis extracts are more effective against Gram-positive cocci (***Staphylococcus aureus, Streptococcus*** β-haemolyticus), but are only active against some Gram-negative bacteria, such as ***Escherichia coli*** or ***Pseudomonas aeruginosa*** (12). On the other hand, other studies (7,22), have indicated that the bacteriostatic or bactericidal effects of propolis depend on the dose and that Gram-negative aerobic bacteria may also be inhibited at concentrations higher than 2.8 mg ml $^{-1}$. Also, the minimum inhibitory concentration of propolis against 35 ***S. aureus*** strains and 92 other bacterial strains were determined by Gizmarik and Trupl (23). Scheller and co-workers (24) found 19 elements in propolis, three fractions were obtained and tested against ***Staphylococcus spp.***. In another study, Scheller et al. (25) found that the sensitivity of 90% of ***Staphylococci*** to ethanolic extract of propolis was lower than in a standard strain of ***S. aureus***. Shub et al. (26) prepared ethanolic extracts from samples of propolis collected in 18 regions of the former USSR. These extracts were serially diluted in agar, in Petri dishes. The dishes were then inoculated with the bacteria ***Bacillus cereus***, ***S. aureus***, ***Escherichia coli*** and ***Pseudomonas aeraginosa***, and the fungus ***Candida albicans***, and incubated at 37 °C or 20-25 °C for 48h. Propolis at 125-500 μg ml^{-1} inhibited the growth of ***B.Cereus*** and ***S. aureus***, but usually not that of the other two bacteria, or the fungus, even at concentrations higher than 1000 μg ml^{-1}. These findings confirm that, the antimicrobial properties of propolis possibly were attributed to its high flavanoids content. The anti-bacterial activity of European propolis is due to its flavonoid aglycones (galangin & pinocembrin) and phenolic compounds (pinobanksin, pinobanksin 3-O-acetate, benzyl-p-coumarate, caffeic acid esters, and ferulic and caffeic acids), and in propolis from the Canary Islands, lignan furofurans. The German variety, rich in phenylethyl-trans-caffeate, benzyl ferulate and galangin, is more effective against ***S. aureus*** and ***Escherichia coli***, than the French variety, rich in benzyl caffeate and pinocembrin (27). The inhibition of bacterial viability by propolis extract was probably due to the loss of their ability to bind to DNA (28). This fact suggested that propolis might act by inhibiting DNA replication and cell reproduction. In conclusion the Iraqi propolis extract exhibit significant antibacterial activity. This is an expected result, since propolis is thought to be the defense of bees against infections. These results confirm that antibacterial properties of propolis possibly could attributed to its high flavonoids content (9) and volatiles (29). Hence, propolis should be viewed more appropriately as a complex natural resource for the control of microorganisms rather than antimicrobial drugs.

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الخلاصة

تم تقييم الفعالية المضادة للبكتيريا لمستخلص العكبر العراقي في الزجاج على سلالات مختلفة من البكتيريا السالبة والموجبة لصبغة غرام .تم استخدام طريقة التخافيف المتسلسلة في الانابيب لقياس الفعالية المثبطة للنمو البكتيري .اظهرت الدراسة الحالية بان البكتيريا الموجبة لصبغة غرام كانت حساسة لتراكيز المكبر الواطئة . من ناحية اخرى اظهرت البكتريا السالبة لصبغة غرام مقاومة اكبرفقد تراوحت قيم التركيز الادنى لتثبيط البكتيري بين 0.5 و 1 ملليغرام/ملليلتر البكتيريا الموجبة لصبغة غرام بينما تراوحت بين 2 و4 ملليغرام/ملليلتر البكتريا السالبة لصبغة غرام . تقترح نتائج هذه الدراسة بان للعكبر فعالية تسيطرة على العديد من الامراض من خلال كونه مصدرا طبيعيا لمنع العديد من الاصابات الجرثومية .