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Effect of Honey Bee Propolis on Certain Human Bacterial Species (*Bacillus subtilis, Escherichia coli and Klebsiella pneumoniae*)

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Abstract

Using propolis ethanol 70% extract on the growth of three bacterial species showed that by increasing extract concentration, the zone of growth inhibition increased in the three tested bacterial species where, at 8 ppm the growth inhibition zones were 14.3, 21.3 and 30.0 mm for *Bacillus subtilis, Escherichia coli and Klebsiella pneumoniae*, respectively. On the other hand the lowest concentration (2ppm) showed the lowest growth inhibition for all bacterial species (9.3, 15.3 and 22.6 mm, respectively). The medium concentration (4ppm) was intermediate. However, propolis-water resulted no growth inhibition except for the *B. subtilis* which was affected by 4 and 8 ppm; (4.3 and 8.0 mm extract growth inhibition respectively).

Introduction

The honey bee (*Apis mellifera* L.) is considered as one of the most important beneficial insects. Honey bee colonies give nutritional, medical and industrial products (Honey, bee-wax, royal jelly, pollen grains, propolis and bee venom).

Propolis (bee glue) is a hard resinous and aromatic material derived by bees from plant juices and used to seal openings in the hives and to varnish and disinfect the cells after emergence of the workers and drones before the queen relays eggs in them. It contains pollen, resins, waxes and large amount of flavonoids which are found in photosynthesizing cells. Flavonoids have many biological effects in animal systems and have received some attention from pharmacologists (Havsteen 1983, Zumla and Lulat 1989 and Dandiya 1991).

B. subtilis is a Gram-positive, rod-shaped bacterium. It has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions and is a normal gut commensal in humans.

E.coli is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some can cause food poisoning in humans. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K and by preventing the establishment of pathogenic bacteria within the intestine. The increasing resistance of *E. coli*, bacteria most frequently isolated during urinary tract infection to diverse antibiotics is an international public health problem making new solutions essential (Philippe et al. (2011)

K. pneumoniae is a Gram-negative, rod shaped bacterium. Although found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human lungs if aspirated. In recent years, *K. pneumoniae* have become important pathogens in nosocomial infections.

Material and methods

In this experiment the used propolis samples were collected from the apiary at El-Quanater El-Khairia, El-Qualiobia Governorate during 2009, 2010 and 2011. The aim of study was to test different propolis extracts on the growth of some bacteria species (*B. subtilis, E. coli and K. pneumoniae*). Two propolis extracts were used (propolis-ethanol extract and propolis-water extract) in three concentrations (2, 4 and 8 ppm):

Propolis Preparations:

Propolis-Ethanol Extracts (PEE):

To get ethanol extract; 10g of row propolis was dissolved in 100 ml ethanol 70% for 30 min and left in the laboratory for 24 hours. The procedure was repeated five times. After five days the extract was filtered by filter paper. The obtained solution was evaporated to a thick mass on a water path under vacuum and hardened after cooling to give a gummy matter of propolis (Boeru and Derevici, 1978).

Propolis-Water Extracts (PWE):

Ten grams of cleaned crude propolis was taken and dissolved in 100 ml distilled water. The mixture was gradually heated and allowed to boil for three min. The extract was then cooled in the refrigerator. This was repeated for three times. After three days the extract was filtered by filter paper. The obtained liquid was condensed to thick mass under a water bath and vacuum, which hardened after cooling. From the prepared extract 10% propolis solution was made in distilled water.

Antibacterial Effect of the Propolis:

The agar-well diffusion method (Crespo *et al.* 1990) was performed to study the in-vitro effects of the two propolis extracts (PEE and PWE) as inhibitors of the growth of three bacterial species (*B. subtilis, E. coli* and *K. pneumoniae*). Three concentrations were tested (2, 4 and 8 ppm), and 4 replicates for each treatment were used. The inhibition rates of growth were observed and evaluated. The data were statistically analyzed by ANOVA and tabulated.

Results and discussion

This experiment was performed to evaluate the effect of propolis extracts on the human bacterial growth (Gram-positive and Gram-negative species). The tested bacterial species were *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae*. Two propolis extracts were used in this experiment (propolis-ethanol extract and propolis-water extract). The obtained data are explained and discussed as follows:

Effects of propolis ethanol 70% extract on the bacterial growth:

Data in table (1) show the in-vitro effect of propolis-ethanol extract (70%) with three different concentrations (2, 4 and 8 ppm) on the growth of the three tested bacterial species. Increasing extract concentration resulted in increased zone of growth inhibition of the three tested bacteria species where, at 8 ppm the growth inhibition zones were 14.3, 21.3 and 30.0 mm for *B. subtilis*, *E. coli* and *K. pneumoniae*, respectively. On the other hand, the lowest concentrations showed the lowest growth inhibition for all bacterial species (9.3, 15.3 and 22.6 mm, respectively). The medium concentration (4 ppm) was intermediate (Table 1). The results are matching with what found by Pepeljnjak *et al.* (1985) who mentioned that the amounts of 3,5,7-trihydroxyflavone and of 5,7-dihydroxyflavonone were determined chromatographically in individual propolis samples and affected the growth of *B. subtilis*.

These findings confirm the study of Hellinger (2000) who stated that propolis has antibacterial activity against a range of commonly encountered cocci and Gram-positive rods, including the human tubercle bacillus, but only limited activity against Gram-negative bacilli and possibly attributable to its high flavonoid content. Also the results are close to what was reached by Sforcin, and Bankova (2011).

K. pneumoniae was the most affected species by propolis-ethanol 70% extraction with an average inhibition zone of 25.2 mm followed by *E. coli* (18.2 mm) and then *B. subtilis* (11.8 mm). The findings are close to what was gained by Gebara et al. (2002) and Ophori and Wemabu (2010).

Table 1. Effect of propolis-ethanol extraction on three bacterial species

Concentration	Growth inhibition zone (mm)		
	B. subtilis	*E. coli*	*K. pneumoniae*
2 ppm	9.3	15.3	22.6
4 ppm	11.7	18.0	23.0
8 ppm	14.3	21.3	30.0
Total	35.3	54.6	75.6
Mean	11.8	18.2	25.2
Control	0.0	0.0	0.0

F-values: For *B. subtilis*= 35.176 p= 0.106. For *E. coli*= 17.694 p= 0.149. For *K. pneumoniae*= 12.545 p= 0. 012.

1. Effect of propolis-water extract on the bacterial growth:

Table (2) represents the inhibition zone caused by propolis-water extract on the three tested bacteria species. No inhibition could be found except for the *B. subtilis* which was affected by propolis Concentrations 4and 8 ppm.(growth inhibition zone 4.3 and 8 mm, respectively).

Table 2. Effect of propolis-water extraction on three bacteria species

Concentrations	Growth inhibition zone (mm)		
	B. subtilis	*E. coli*	*K. pneumoniae*
2 ppm	0.0	0.0	0.0
4 ppm	4.3	0.0	0.0
8 ppm	8.0	0.0	0.0
Total	12.3	0.0	0.0
Mean	4.1	0.0	0.0
Control	0.0	0.0	0.0

F-values: For *B. subtilis*= 56.333 p= 0.084. For *E. coli*= 0. For *K. pneumoniae*= 0.

More studies are needed and further proof that propolis has countless health benefits.

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الملخص العربي

تأثير صمغ النحل (البروبوليس) على بعض أنواع البكتيريا التي تصيب الإنسان

(***Bacillus subtilis, Escherichia coli and Klebsiella pneumoniae***)

أظهر استعمال مستخلص صمغ النحل الكحولي (إيثانول 70%) على نمو بعض أنواع البكتيريا أنه بزيادة تركيز المستخلص زادت منطقة تثبيط النمو في الأنواع البكتيرية الثلاث حيث كانت مناطق التثبيط عند 8 جزء في المليون 14.3 ، 21.3 ، 30.0 مم لكل من *Bacillus subtilis,* *Escherichia coli and Klebsiella pneumoniae* على التوالي. ومن جهة أخرى أظهرت التركيزات الأقل مناطق تثبيط أقل في أنواع البكتيريا الثلاثة المذكورة حيث كانت 9.3 ، 15.3 ، 22.6 على التوالي عند تركيز 2 جزء في المليون. أما التركيز المتوسط (4 جزء في المليون) فقد نتجت عن استخدامه مناطق تثبيط نمو متوسطة لأنواع البكتيريا الثلاثة. أما المستخلص المائي لصمغ النحل فلم يثبط نموانواع البكتيريا المختبرة عند استخدام التركيزات المختلفة باستثناء اختبار البكتيريا *Bacillus subtilis* حيث وجدت منطقة تثبيط للنمو البكتيري عند تركيزي 4 ، 8 جزء في المليون ، وبلغت هذه المناطق 4.3 ، 8.0 مم على الترتيب.