

Study of antibacterial activity in the local Iraqi propolis

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Objective This study includes the investigation of antibacterial activity of the local propolis against four types of bacteria isolated from patients.

Methods Bacteria were tested including *Pseudomonas* sp, *Streptococcus* sp, *Escherichia coli* and *Staphylococcus aureus*. Six concentrations (0, 5, 10, 15, 20 and 25) mg/ml of propolis extracts were tested against bacteria.

Results Results revealed the presence of significant difference ($P < 0.05$) in the effect of propolis extract against the four types of bacteria in this study. *Pseudomonas* sp. was the most sensitive among the others toward the propolis extract followed by *Streptococcus* sp., *E. coli* and *Staphylococcus aureus* at a rate of inhibition zones (14.09, 10.39, 8.78 and 8.39) mm, respectively. Results of this study also showed increasing rate of inhibition zone if the concentration of propolis extract was increased.

Conclusion This study provided that local propolis has antibacterial activity against gram positive and gram negative bacteria.

Keywords antibacterial activity, propolis, inhibition zone, extract

Introduction

Propolis is a natural product derived from plant resins collected by honeybees; it is used by bees as glue, general purpose sealer and as draught-extruder for beehives. Propolis has been used in folk medicine for cosmetic purpose. The chemical composition of propolis is quite complicated, more than 300 compounds such as polyphenol, phenolic, aldehydes, sesquiterpene quinones, coumarin, amino acids, steroids and inorganic components have been identified in propolis sample. The content depends on collection location, time and plant source.¹ Among the types of chemical substances found in propolis are waxes, resins, balsams, aromatic and ethereal oil, pollen and other organic matters.² Propolis contain some enzymes like succinic dehydrogenase, glucose-6-phosphatase, adenosine triphosphatase and acid phosphatase and small quantities of terpenes, tannins, traces of secretion from salivary gland of bees and possible contaminants. It also contains some minerals such as manganese and iron in addition to vitamins like B1, B2, B6, C and E and number of fatty acids.³

Propolis shows a complex chemical composition responsible for its biological properties such as antibacterial, antifungal and antiviral, among other activities which have attracted the researcher's interest. The laboratory tests studies have shown broad spectrum antimicrobial activity of various propolis extracts, depending upon its composition. Propolis may show powerful local antibiotics and antifungal properties. Many authors have demonstrated antibacterial activity of propolis against *Enterococcus* spp., *E. coli* and *Staphylococcus aureus*. Reports have pointed out the efficient activity of propolis against Gram-positive bacteria and limited action against Gram-negative bacteria.⁴

Many researches had investigated the antibacterial activity of propolis and its extract against Gram-positive and Gram-negative strain and they found that propolis had antibacterial activity against a wide range of Gram-positive bacteria. Antimicrobial activity of all propolis increases with increasing dosage without reaching a plateau at the highest dosage tested.¹ The aim of the present study is to investigate

the antibacterial activity for local propolis against G⁺ and G⁻ bacteria.

Materials and Methods

Microorganisms: Four types of bacteria were obtained from clinical laboratory of Al-Hindiyya Hospital: *E. coli*, *Pseudomonas* sp., *Staphylococcus* sp. and *Streptococcus* sp.

Propolis extraction: Propolis was grinded several times to get a very fine powder. The samples of propolis have been obtained from the apiaries of holy Karbala province. The method described by Ahmed et al. (1998)⁵ to obtain extracts is as follows:

1. 15 gm of propolis powder was added to 150 ml of 70% ethanol in a beaker and the beaker was covered with an aluminum foil.
2. The beaker was incubated in a shaker incubator at 35°C with 100 rotary/min for 48 h.
3. After the completion of the incubation process, the extracted liquid was filtered with gauze and then the filtrate was poured into a petri dish and was allowed to dry.
4. The dry extraction is collected and stored in a container for later use.

Preparation of propolis extract concentrations: The first step is to prepare stock solution by weighing 0.375 gm from the dry extract and dissolving in 15 ml of 70% ethanol to obtain the final concentration, 25 mg/ml in stock solution. Then make the following concentration as given:

Stock solution ml	Ethanol 70% ml	Final concentrate mg/ml
2	–	25
1.6	0.4	20
1.2	0.8	15
0.8	1.2	10
0.4	1.6	5
–	2	0

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Table 1. Inhibition zone (mm) of propolis extract against four types of bacteria

Concentration of propolis extract (mg/ml)	Types of bacteria				Mean of concentration	LSD _{0.05} conc.
	<i>Pseudomonas</i> sp.	<i>Streptococcus</i> sp.	<i>E. coli</i>	<i>Staphylococcus aureus</i>		
0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 G	
5	4 ± 0.8	2.7 ± 0.3	3.2 ± 0.12	2.9 ± 0.75	3.20 F	
10	8 ± 1.2	5 ± 0.95	4.1 ± 0.83	5.3 ± 0.91	5.60 E	
15	16.6 ± 1.7	10.7 ± 0.99	7.9 ± 0.65	8.2 ± 0.77	10.85 D	1.451
20	17.3 ± 1.85	16.6 ± 1.1	12.6 ± 1.04	11.4 ± 0.9	14.48 C	
25	24.7 ± 1.52	16.7 ± 0.86	15.7 ± 1.32	13.9 ± 1.14	17.75 B	
Chloramphenicol 50 µg/ml	28 ± 1.07	21 ± 1.42	18 ± 0.89	17 ± 0.92	21.00 A	
Mean of bacteria	14.09 a	10.39 b	8.78 c	8.39 c	LSD _{0.05} Interference	2.903
LSD _{0.05} bacteria		1.185				

LSD: less significant difference. The numbers refer to mean ± standard error. The capital letters indicate significant differences ($P < 0.05$) between the concentrations. Small letters indicate significant differences ($P < 0.05$) between bacteria.

Activation of Bacteria

Nutrient broth was prepared according to the information of company (HiMedia, India). Thirteen grams of media powder was taken and dissolved in 1 litre of distil water. The media was poured into tubes and sterilized in an autoclave. After this process, the media was left to cool. This can be used as activation media for bacteria by taking specimen from origin cultures of bacteria by swab and transport to nutrient broth tube, and then incubated at 37°C for 24 h. Also we can use the sterilizing nutrient broth tubes to obtain an appropriate dilution for each bacteria by making serial dilutions and comparing with tube containing McFarland solution.

Determination of Antibacterial Activity of Propolis

The antibacterial activity was determined by using the agar diffusion technique described by Egorove (1985).⁶ Mueller Hintonagar was used for this purpose. This media was prepared according to the information of the company (Himedia, India). Thirty-eight grams of powder was weighed and dissolved in 1 litre of distil water. Then it was sterilized in an autoclave, left to reach 45–55°C and poured into petri dishes. Each petri dish (Mueller Hinton agar media) was drilled with three wells and 50 µl of propolis extract concentrations was added to each well and waited for 1 h. These dishes were inoculated with an appropriate dilution for each bacteria separately by spreading 50 µl from the L-shaped spreader, and incubating at 37°C for 24 h. Inhibition zone around wells was measured in

millimetres. Chloramphenicol 50 µg/ml was used as control for comparing the results.

Statistical Analysis

Statistical analysis included factorial experiences analysis 7×4 with 3 replicates. The factors analyzed are the concentration of propolis extract and types of bacteria. A P value of 0.05 is the level of probability that was used to identify a significant difference. The significant differences between the averages were also tested by using less significant difference (LSD) test at the level of probability of 0.05.⁷

Results

As shown in Table 1, the results of statistical analysis showed there were significant differences ($P < 0.05$) between the types of bacteria and between the concentrations of propolis extract compared with control (chloramphenicol). *Pseudomonas* sp. is more sensitive towards propolis extract followed by *Streptococcus* sp. Moreover *E. coli* and *Staphylococcus aureus* are less sensitive and without significant differences ($P > 0.05$) between them. The rate of inhibition zones were 14.09, 10.39, 8.78 and 8.39 mm of *Pseudomonas* sp., *Streptococcus* sp., *E. coli* and *Staphylococcus aureus*, respectively.

On the other hand, results showed significant differences ($P < 0.05$) between the concentrations that were used in the present study. The results also showed that the propolis extract concentration increased with increased inhibition zone compared with the control on the one

hand and with concentrations on the other hand. The inhibition zones were 0, 3.20, 5.60, 10.85, 14.48 and 17.75 mm of propolis extract concentrations 0, 5, 10, 15, 20, 25 mg/ml, respectively.

Discussion

One study showed that the Iranian propolis was mainly active against gram-positive; however, it has been reported the ethanol extract propolis (EEP) was effective on Gram-negative bacteria at the higher concentration.⁴

In another study, propolis showed good antimicrobial activity against most of the isolates that include *Pseudomonas aeruginosa*, *E. coli*, *Streptococcus pneumonia* and *Staphylococcus aureus*.⁸ One of the studies also showed that the Brazilian propolis was effective against *Staphylococcus aureus* more than *E. coli*.⁹

The composition of propolis can vary depending on the location of the bees and what trees and flowers they have access to. The composition of the plant source determines the chemical composition of propolis, for example in Europe, China and North America, propolis was generally considered to be of the poplar-type, and other types can also be found.¹⁰ Significant amounts of phenolic glycerides such as dicoumaroyl acetyl glycerol, diferuloyl acetyl glycerol, feruloyl coumaroyl acetyl glycerol and caffeoyl coumaroyl acetyl glycerol have been isolated from the propolis obtained in northern Russia.¹¹

The Brazilian propolis represents 10–15% of the worldwide production, Brazil being the third world producer, behind Russia and China.¹⁴ Among the types produced in Brazil, green propolis

(from greenish-yellow to deep green) prevails, gaining preference in the world propolis market.¹² Prenylated derivatives of p-coumaric, artemillin C (4-hydroxy-3,5-diprenyl cinnamic acid), dupranin (4-hydroxy-3-prenyl cinnamic acid),

(E)-3-prenyl-4-(dihydroxycinnamoyloxy)-cinnamic acid and diterpenic acids were detected in green propolis as well as in the buds of *Baccharis dracunculifolia*, an *Asteraceae* family from southeast and western-central Brazil.¹³⁻¹⁶

Conclusion

The local Iraqi propolis that was collected from the apiaries of holy Karbala province has antibacterial activity against Gram-positive negative bacteria. ■

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