Effect of Water & Methanol Extracts of *Turkish Propolis* Against Some Species of Pathogenic Bacteria

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Abstract

Background: Propolis (bee glue) is a mixture of wax and resin. This products have some properties such as bactericidal and fungicidal and also it was could be used as an alternative treatment against infections.

Objective: The study was shed light to evaluate the effect of alcoholic and water (hot& cold) propolis extract against some pathogenic bacteria and also to investigate the antimicrobial activity "in vitro".

Material & Methods: Both alcoholic and water (hot & cold) extract from Propolis which used to obtained the inhibited growth and the effectiveness against some bacterial strain (*Escherichia coli, Pseudomonas aeruginosa, Proteus Spp., Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pyogenes* and *Bacillus subtilus*) and this effected will be measured by the minimum inhibitory concentration (MIC) on the bacterial species and this occurs by using agar distribution methods with Propolis extract in serial concentration by treat each of bacteria with different concentration (6.2,12.5,25,50,75,100,125,150and 200mg/ml).

Results: The result showed that the alcoholic extract which have more effective as an inhibitor on the growth compared with water extract. When used alcoholic Propolis extract the results obtained that the diameter of inhibition zone was(6 ,18 .and 19 mm) respectively when treat each of bacteria (*Escherichia coli,., Staphylococcus aureus, Streptococcus pyogenes*,) with different concentration (100,125, 150, 200)µg/ml .While in the type of bacteria (*Proteus Spp*) the inhibition zone was 6mm in diameter when used the concentration 125 µg/ml from extract Also the results obtained there was a positive effect against *Pseudomonas aeruginosa*, and *Bacillus subtilus* when treat it with propolis extract in the concentration (6.2, 12.5, 25,50)µg/ml and the inhibition zone showed (8and5mm) in diameter respectively, While the inhibition zone for *Klebsiella pneumonia* was (6mm) in diameter when treat it with the concentration (6.2) µg/ml

Conclusion: The results in this study refer to the ability of propolis extract to inhibitory effect on the growth and effectiveness of the bacteria and the effect depend used...

Key words: Turkish propolis, methanol extract, antimicrobial activity.

Introduction

Propolis is a mixture of wax and resin which are collected by honey bees (*A. mellifera L.*). Propolis has very important functions in the bee hive. It is used for closing the holes and cracks in the hive, for making the entrance hole smaller, and for covering the insects which die in the hive that cannot be taken out due to their size.

Propolis contains 45-55% resin, 25-35% wax and fatty acids, 5% pollen, 5% other organic substances and minerals. Methanolic extracts of propolis contain flavanoids, flavanons, aliphatic acids and their esters, alcohols, aldehydes, calcons, dihydrocalcons, ketones, terpenoids, and many other biologically active substances [(1)].

The term propolis originates from the Greek words *Pro-* mening before/ in front and *Polis-* meaning town[(2)] and denotes the fact that bees use propolis to construct the entrance to the beehive "town". It is also known as "bee glue".

Propolis has bactericidal and fungicidal properties and it is used as an alternative treatment for infections. The wide range of action of propolis on various microorganisms is the result of the combined activities of flavonoids and aromatic compounds [(3)]

These compounds are polyphenols and phenolic acids are active antimicrobial agents. Most propolis components are of phenolic nature, mainly flavonoids are synthesized by plants as a response to microbial infections and recognized to have effective antimicrobial effects against a wide range of microorganism [(4)].

However the chemical properties of propolis vary according to the climate and vegetation of the region. Thus, the reported medicinal properties of propolis may vary as well [(5)].

There is a long history of use of propolis, at least to 300BC [(6)]. Propolis has remarkable therapeutic qualities, and much sought after in some countries for the treatment of a range of human aliments and for cosmetic purposes.

General medicinal uses of propolis include treatment of the cardiovascular and blood system disorder (anemia), respiratory apparatus (for various infection), dental care[(7)], dermatology (tissue regeneration, ulcers , eczema, wound healing – particularly burn wounds, mycosis, mucous membrane infection and lesions), cancer treatment [(8)].

The aim of study was to define which concentration of propolis had most antimicrobial activity so that bactericidal effect of propolis extracted by different species of bacteria and compared with antimicrobial drug.

Material and methods

Standardized pure cultures of bacterial strains procured from the Laboratory of the Faculty of college of Science for women / University of Baghdad, which had been isolated from pathological specimens to hold a series of biochemical tests, were used in this research. The bacterial species were chosen according to the frequency that they were used in various researches and also according to the frequency of infections in humans. The bacterial

strains chosen to assay antimicrobial activity were *Staphylococcus aureus*

Methanol extract

Propolis was collected from beehives located in Turkey (Izmir).

In the first step propolis was extracted by methanol, propolis (80) g was added in to 300ml of 96% methanol and mixed. The mixture was centrifuged at 3500 rpm for 30 min at 20 C. the supernatant was collected and the insoluble fraction was separated by filtration. Which used in the experiment.

Water extracts of propolis

a- About 30g of propolis was added to 300 ml cold phosphate buffer mixed for 20 min at 37c°, left it over night and centrifuged at 3000 rpm for 15min, then filtered by (Whatman No.1) the supernatant was used in this work.

b- Hot extraction was prepared by mixing hot distilled water (300 ml) with 30g of propolis, mixed for 20 min at 37c°, left it over night and centrifuged at 3000 rpm for 15min, filtered by (Whatman No.1) then used.

Susceptibility test

Antimicrobial activity of propolis samples was investigated by the agar dilution method, following the National committee of clinical Laboratory standard guidelines [(9)].

Bacterial strains were grown in Mueller Hinton Agar; the turbidity of the suspensions was adjusted to the Mac Farland 0.5 turbidity standards.

Each antimicrobial test was also reproduced with plates containing the culture medium plus propolis extracts .The concentration of propolis in the media was expressed in micrograms per milliliter.

After the inoculation procedures, plates were incubated at 37c° /24h and MIC endpoints were read as the lowest concentration of propolis that resulted in no visible growth on the surface of the culture medium.

Detection of active compounds in the propolis extract

Detection of **alkaloids** have been using the detector Drajndov which consists orange color when treated extract detector indication of the presence of alkaloids.

Detecting **Flavonoids** by taking the amount of the extract and placed on filter paper and then added drops of Hcl and acid-ray examination UV, and the appearance of brown color, a sign of the presence of flavonoids [(10).]

Determination of the minimum inhibitory concentration (MIC)

The MICs of the propolis and the fractions were conventionally determined in triplicate for each strain by the macro dilution broth method as described by the NCCLS [(11)].

Serial dilutions of each propolis and fractions were prepared in macro dilution tubes. Bacterial suspensions were adjusted to the 0.5 Mc Farland standards (approximately 1 to 2×10^8 cfu /ml). Final inoculate were adjusted to the 10^4 cfu/ml. A constant amount of bacteria were added to all tubes and they were incubated at 37°C for 18 – 24 h. Each tube was examined for growth, comparing each tube to the control.

The MIC was defined as the lowest concentration of propolis at which there was no visible growth of the organism. MICs of the antibiotics were determined in the same way. A positive growth control was included where bacterial suspension was added to a tube filled with nutrient broth without crude propolis. An uninoculated tube of nutrient broth was also added to serve as negative growth control.

Antimicrobial drugs

Test the sensitivity of antibiotics Gentamicin, Rifampin, Kanamycn, and Vancomycin against isolates of bacteria studied:

Used antibiotics, processed from a company Bio-analyze, as it tested the sensitivity of bacteria to antibiotics and using the diffusion agar method compared the result with MIC of bacteria, by measure inhibition zones of antibiotic.

Statistical Analysis

Treatments of gram positive and negative bacteria were carried out under laboratory conditions using a split plot design with complete randomized design (CRD) arrangement and analyzed according to **Duncan Test (12**). Three replicates of Petri dishes were used for each treatment, mean zone of inhibition and standard deviations were calculated and presented in Table 4, 5.

Results and Discussion:

The results showed that the propolis extract was highly effective against bacteria(positive and negative) Table (1,2.) , while the aqueous extract of hot and cold did not have any effectiveness as a result of discouraging both Gram positive and Gram negative bacteria . prpolis effect could be attributed to the synergistic activity between phenolic and other compounds [(13)] mainly flavonoids pinocembrin [(14)] also several mechanisms of the activity of propolis on bacterial growth have been reported (1) inhibition of cell division (2)bacterial cytoplasm, cell membranes and cell walls collapse[(3)] bacteriolysis and [(4)] protein synthesis inhibition [(15)] Takasi et al. [(16)] stated that propolis inhibits bacterial growth by preventing cell division, thus resulting in the formation of pseudo-multicellular streptococci. In addition, propolis disorganized the cytoplasm, the cytoplasmic membrane and the cell wall, caused a

partial bacteriolysis and inhibited protein synthesis. It was evidenced that the mechanism of action of propolis on bacterial cell is complex and a simple analogy cannot be made to the mode of action of any classic antibiotics. However in *Escherichia coli*

The inhibition of bacterial RNA-polymerase by the components of propolis was probably due to the loss of their ability to bind to DNA [17].

Table (1) Show the effect of the concentration of (µg) methanol extract and the Inhibition zone (mm) against G+ bacteria

Propolis Extraction. / Bacteria Name	*200 µg*	*150 µg*	*125 µg*	*100 µg*	*75 µg*	*50 µg*	*25 µg*	*12.5 µg*	*6.2 µg*
Staphylococcus aureus	23	20	19	18	0	0	0	0	0
Bacillus subtulus	0	0	0	0	0	20	13	11	8
Streptococcus pyogenes	25	21	20	19	16	0	0	0	0

Table (2) Show the effect of the concentration of (µg) methanol extract and the Inhibition zone (mm) against G- bacteria

Propolis Extraction. / Bacteria Name	*200 µg*	*150 µg*	*125 µg*	*100 µg*	*75 µg*	*50 µg*	*25 µg*	*12.5 µg*	*6.2 µg*
E .coli	20	16	10	6	0	0	0	0	0
Pseudomonas spp.	0	0	0	0	0	17	12	11	5
Klebsiella pneumoniae	22	21	20	20	19	18	15	11	6
Proteus spp.	8	0	6	0	0	0	0	0	0

Table (3) Show the Susceptibility test against *Staphylococcus aureus* and E. *coli*

Treatment	• Zone of inhibition (mm)	
	Standard	
	E. coli	*S. aureus*
Gentamicin	12	12
Rifampin	11	31
Kanamycn	11	24
Vancomycin	17	15

Table (4) Show the Mean of inhibition zone ±SD of G+ bacteria

Propolis Extraction / **Bacteria Name**	**Mean ±SD**								
	200 µg	*150 µg*	*125 µg*	*100 µg*	*75 µg*	*50 µg*	*25 µg*	*12.5 µg*	*6.2 µg*
Staphylococcus aureus	22.67±0.33 a	19.67±0.33 b	18.67±0.33 c	17.33±0.33 d	0.00±0.00 e	0.00±0.00 e	0.00±0.00 e	0.00±0.00 e	0.00±0.00 e
Bacillus subtulus	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	19.33±0.33 b	12.33±0.33 c	10.67±0.33 d	7.67±0.33 E
Streptococcus pyogenes	24.67±0.33 a	20.67±0.33 b	19.67±0.33 c	18.67±0.33 d	15.67±0.33 e	0.00±0.00 f	0.00±0.00 f	0.00±0.00 f	0.00±0.00 F

Table (5) Show the Mean ±SD of G- bacteria

Propolis Extraction. / **Bacteria Name**	**Mean ±SD**								
	200 µg	*150 µg*	*125 µg*	*100 µg*	*75 µg*	*50 µg*	*25 µg*	*12.5 µg*	*6.2 µg*
E. coli	19.67±0.33 a	15.67±0.33 b	9.67±0.33 c	5.33±0.33 d	0.00±0.00 e	0.00±0.00 e	0.00±0.00 e	0.00±0.00 e	0.00±0.00 e
Pseudomonas aeruginosa.	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	17.33±0.33 b	12.33±0.33 c	11.67±0.33 d	5.67±0.33 E
Proteus Spp	7.67±0.33 a	6.67±0.33 b	5.33±0.33 c	0.00±0.00 d	0.00±0.00 d	0.00±0.00 d	0.00±0.00 d	0.00±0.00 d	0.00±0.00 d
Klebsiella pneumoniae	21.67±0.33 a	20.67±0.33 b	19.67±0.33 c	19.67±0.33 d	18.67±0.33 e	17.67±0.33 f	14.33±0.33 g	10.67±0.33 h	5.33±0.33 I

Figure (1) Inhibition zone in G+bacteria

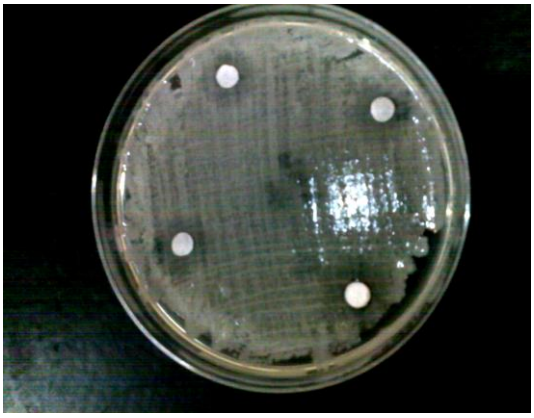

Figure (2) inhibition zone in G-bacteria

Figure (3) Antimicrobial activity against *S. aureus*

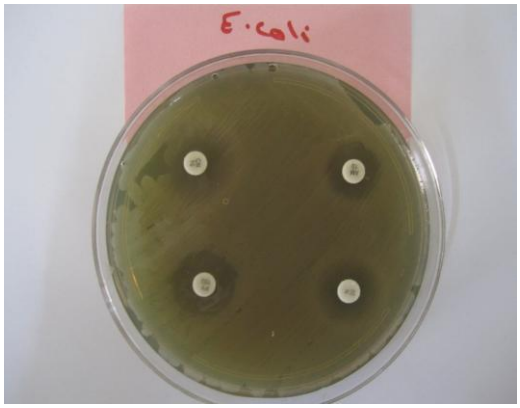

Figure (4) Antimicrobial activity against E. coli

represent the diameters of zone of inhibition measured propolis Either from a statistical standpoint has been treated bacterial species different concentrations mentioned previously extracted, and according to results of statistical analysis that the effect of propolis extract on the bacterial cells a significant difference ($p \leq 0.05$) starting from the focus 6.2 µg / ml in some species, while this is not focus a significant effect in other species, and as shown in the table above.

It has been observed that the concentrations (6.2, 12.5, 25,50) µg/ml did not have any effect, effective against bacteria *E. coli* and *Streptococcus* while the concentrations (75, 100 125 150, 200) µg/ml significant differences clear, as referred to in Table (4,5). But in *Pseudomonas* it was observed that concentrations (75,100,125) µg/ml did not show any significant differences compared to other different concentrations, which also showed variation in the degree of effectiveness against bacteria.

As well as can be seen bacteria *Bacillus subtilus* that did not show any effectiveness in high concentrations compared to different concentrations of low-lying, which was discouraging and effectively.

In the bacteria *Klebsiella pneumoniae* was the effectiveness of different concentrations are very high and significant effect with a clear focus, starting from the least to the top concentration.

As for *S. aureus* and Proteus bacteria it was observed that concentrations of low-lying did not show any significant effectiveness compared with the high concentrations that showed significant differences clear to the bacterial cells. there is only one research said that the fruit of this plant after extracted using methanol did not give any effective against bacteria, positive and negative.

The results show to those previously reported [4]. Being an effective against bacteria because it contains flovonoids and alkaloids these compounds have antimicrobial properties mainly against (*Staphylococci* and *Streptococci spp*.) and gram negative bacteria (*E. coli, K. pneumonia, P. vulgaris and P. aeruginosa*) (2) this result was agree with the present result; while, Meresta and Meresta, (17)

agree with all species of bacteria except *K. pneumonia* which has no effect

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