Synergism activity between propolis and various antibacterial drugs against multidrug resistance gram positive and gram-negative bacteria isolated from upper respiratory tract (URT)

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Abstract

The majority of upper respiratory tract infections are of viral origin. A part from bacteria pathogens have been reported to cause (URTI) and these include Haemophilus influenzae, Streptococcus pneumoniae, and Klebsiella pneumonia. Because the increasing rate of antibiotic resistances by most bacteria of respiratory infections, recent research has been directed towards the use of natural products for treatment and control of infections. Propolis is one of such products that is being tested on pathogens. Two bacterial isolates were used throughout the current work, one gram negative Klebsiella pneumonia and the other is a gram positive Staphylococcus aureus. Synergism between three types of propolis and different antibacterial drugs against multidrug resistance K. pneumonia and S. aureus isolated from (URT) was estimated. Both S. aureus and K. pneumonia display high resistant Gram-positive and Gram-negative bacteria to a number of antibiotics that used in the current study, (gentamicin, ampicillin, cefotaxim, erythromycin, and tetracyclin). Our results demonstrated synergistic activity between ethanol extract of different types of propolis with antibiotics (gentamicin, ampicillin, erythromycin, and tetracyclin) against both S. aureus and K. pneumonia. Synergistic activity between water extract of two types of propolis (clover and eucalyptus) with antibiotics (gentamicin, ampicillin, erythromycin) was recorded against both S. aureus and K. pneumonia., but synergistic activity between water extract of sidr propolis with antibiotics dose not recorded.

Keywords: propolis, upper respiratory tract, bacterial resistance, antibiotics

1. Introduction

An upper respiratory tract infection (URTI) is a term used to describe acute infections involving the nose, paranasal sinuses, pharynx, larynx, trachea and bronchi 1. URTIs such as sore throat, earache, laryngitis, common cold, otitis media and sinusitis are the most frequently occurred infections of all human diseases and among the leading cause of health services worldwide and have been frequently documented 2. The prescription of an antibiotic for URTI especially broad-spectrum antimicrobial and second-generation macrolides is a common practice in the medical profession and with the ever increasing tendency to buy antibiotic, the emergence of resistant strains posses a great problem in the treatment and management of such pathogens 3. Propolis is a natural resin and nontoxic material produced by bees (Apis mellifera), with significant therapeutic and biological properties 4. Propolis contains an average of 50% resin, 30% wax, 10% essential and aromatic oil, 5% of pollen and 5% of other substances such as amino acids, minerals, and bioflavonoids. 5 The composition of propolis varies from hive to hive, from district to district, and depends on the time of collection, seasonality, illumination, collector type, and food availability and activity developed during propolis exploitation. The color of propolis is often dark brown, but it can be found in green, red, black, and white hues, depending on the sources of resin found in the particular hive area.

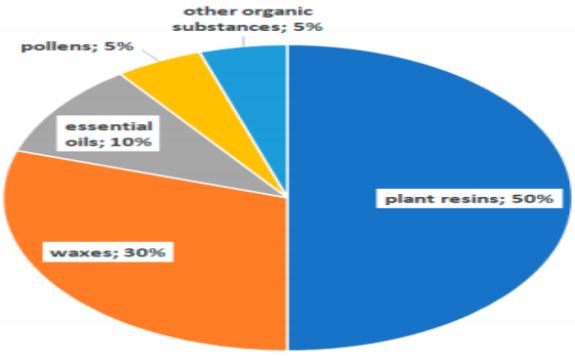

Figure (1) Composition of propolis

The antibacterial properties of propolis have been examined and confirmed in several studies, it is showed inhibitory effects on the growth of 15 microorganisms, including Streptococcus mutans, Staphylococcus aureus, and Enterococcus faecalis 7. The effect of propolis on bacteria has been demonstrated as it inhibits the growth and adhesion

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of bacteria to surfaces, inhibits glycosyltransferase enzyme activity in vitro and in vivo, inhibition of cell division, collapsing microbial cytoplasm cell membranes and cell walls, bacteriolysis, and protein synthesis inhibition 8, 9. Propolis has also been successful in inhibiting drug-resistant microorganisms 10 . There is a synergist effect between the propolis and antibiotics acting on the ribosome. In fact, propolis inhibits RNA polymerase which can explain partially the synergism of propolis with drugs that act on inhibition of protein synthesis. It has been reported that propolis cannot interact with the antibiotics acting on the DNA (ciprofloxacin and norfloxacin) and folic acid (cotrimoxazole) and only exerts bactericidal activity 11, 12.

2. Materials and methods

Microorganism

Two different bacterial isolates were used throughout the current work, one-gram negative Klebsiella pneumonia and the other is a gram positive Staphylococcus aureus.

Samples of propolis

Three types of propolis was obtained from a honey bee market located in Karbala City. The samples were kept in sterile plastic containers in the dark before its use. 13

Water extract preparation of propolis

Aqueous propolis extract was prepared as designated by Miguel et al. with slight modification. 10 g of the dried powder of propolis was crushed into a very fine powder in a blender, dissolved in 20 mL of sterile water and kept at 60 ◦C for 7 h. The suspensions were separated by centrifuge at 28,000×g for 30 min and then filtrated by filter paper. The extract was kept under 4 ◦C in the dark until testing. 14

Ethanol extract preparation of propolis

Ethanolic extracts of propolis were prepared and applied as described by ISLA et al. 15 with slight changes. The sample was frizzed and chopped into a small blender and dissolved in 70% ethyl alcohol with a ratio of 3:10 (30 g of propolis in 100 mL of 70% ethyl alcohol). Then, the propolis samples were kept for 1 week at room temperature in a laboratory shaker in a dark place. Then, centrifuged at 26,000×g for 30 min and filtrated by filter paper. The pure propolis was kept at 4◦C in the dark until use.

Isolation and identification of bacteria from upper respiratory tract infections

Bacterial identification depending on the reference cited in, 16 and by using the methods used by 17, 18.

Evaluation of antibacterial activity of aqueous propolis extract and ethanolic propolis extracts using well diffusion method.

The inhibition zones were reported in millimeter (mm). Briefly, Muller Hinton Agar (MHA) plates were inoculated with bacterial strain under aseptic conditions and wells (diameter=6mm) were filled with 50 µl of the test samples and incubated at 37°C for 24 hours. After the incubation period, the diameter of the growth inhibition zones was measured. 19

Determination minimum inhibitory concentration (MIC) of different types of propolis by agar dilution method

Determination of the minimal inhibitory concentrations (MIC) by the agar dilution method was performed, following the National Committee of Clinical Laboratory Standards Guidelines (NCCLS 2002). Serial concentrations were achieved in plates containing Mueller Hinton Agar, ranging from (2.5, 3, 5, 10, 20, 30 and 40 % v/v), plates were incubated at 37°C / 24 h and MIC endpoints were read as the lowest concentration of propolis that resulted in no visible growth or haze on the surface of the culture medium 20.

The antibiotic susceptibility testing

The antibiotic sensitivity testing was determined using the disc diffusion method, 20 with minor modifications. Mueller Hinton Agar was employed as the standard test medium for bacteria. The agar plate was spread with the inoculum having 108 CFU/mL pathogenic bacteria as MacFerland standards 0.5% 21, was prepared for disc diffusion sensitivity testing using discs: ampicillin, tetracyclin (10 µg), ciprofloxacine (10µg), gentamycine (10µg), erythromycine (15µg), and cefotaxime (30µg) Bioanalyse, Turkey. Resistance was judged by the inhibition zone diameter 22, 23. When the antibiotic agent was 16 mm or higher, it was recorded as sensitive and resistant when less than 16 mm. 24

Determination minimum inhibitory concentration (MIC) of different antibacterial agents by agar dilution method

In order to measure MIC, agar dilution method was used. Stock solution was prepared by dissolving 0.1 gm of the antibacterial agents in a few amounts of distile water, then completed the volum to 100 ml. The concentration of stock solution became 1000 µg / ml. Other concentrations were prepared from the stock solution. The MIC of these antibiotics was determined by serial dilutions in Mueller-Hinton agar, ranging from 0.008 µg/mL to 1024.0 µg/mL. Aliquots (20 µL) of bacterial suspensions (1.0 x 106 CFU/ mL) of bacteria were added to petri dishes containing Mueller-Hinton agar (2.5 mL) plus the antibiotics. Petri dishes were incubated for 24 h at 37°C and MIC endpoints were read as the lowest concentration of antibiotics that resulted in no visible growth. 25

Synergism between propolis and various antimicrobial drugs against multidrug resistance gram positive and gram-negative bacteria isolated from URT

After determination of MIC of various antibiotics and propolis, various concentrations of different

antibiotics and propolis below their MIC were prepared. Mixtures of various antibiotics and propolis were prepared by mixing ½ and ¼ of the propolis samples and the antibiotics respectively. These mixtures were tested against the Klebsiella pneumonia and Staphylococcus aureus to identify whether there was synergism between various antibiotics and propolis. Synergism was identified when the MIC of antibiotics and propolis in combination was lower than the MIC of antibiotics or propolis alone. 25 ,26

3. Results and Discussion

Two different bacterial isolates were used throughout the current work, one-gram positive Staphylococcus aureus and the other is a gram negative Klebsiella pneumonia. Both S. aureus and K. pneumonia display high resistant Gram-positive and Gram-negative bacteria to a number of antibiotics that used in the current study, as indicated in table (1).

Table (1) Antibacterial activity of different types of drugs against S. aureus and K. pneumonia

Inhibition zone of bacterial growth (mm)	Type of drug	Microorganism
9 (resist)	Gentamicin	S. aureus
8 (resist)	Ampicillin	
11 (resist)	Cefotaxim	
12 (resist)	Erythromycin	
8 (resist)	Tetracyclin	
24 (sensitive)	Ciprofloxacin	
7 (resist)	Gentamicin	K. pneumonia
6 (resist)	Ampicillin	
8 (resist)	Cefotaxim	
10 (resist)	Erythromycin	
7 (resist)	Tetracyclin	
18 (sensitive)	Ciprofloxacin	

Bacterial resistance to antibiotics is an increasing global concern. Therefore, new chemotherapeutic agents and new methods are urgently needed to combat such multiantibiotic-resistant bacteria. Combined antibiotic therapy has been shown to delay the emergency of bacteria resistance. Several groups of researchers documented that all types of propolis have antibacterial properties. The reference cited in 27 reported that propolis has antibacterial effect against both Gram-positive and Gram-negative microorganisms including multidrug-resistant bacteria. Our results show activity of both water and ethanolic extract of three types of propolis against S. aureus (MIC range 5-10 mg/m1 for water extract and 3-5 mg/m1 for ethanolic extract) and K. pneumonia (MIC range 10- 20 mg/ml for water extract and 5-10 mg/m1 for ethanolic extract), as indicated in table (2) and (3) below, this may be due to the non-abuse of propolis by patients. Propolis is costly to buy and therefore is not within reach by low-income earners, it has also been suggested that propolis, the antimicrobial agent of choice in the treatment of URTI, because of its antimicrobial activity which antibiotics lack against S. aureus and K. pneumonia, the most active was ethanol extract of propolis. 28

A number of studies have found that propolis are active against gram-positive bacteria, whereas they showed limited activity against the gram-negative bacteria 29. Such properties may be due to differences in the bacterial structure and cell wall and can be explained by presence of the negatively charged lipopolysaccharide which acts as a barrier in the gram-negative bacteria 30. Another author 31 reported that propolis has antibacterial activity against some bacterial species. In particular, 32 showed that the use of the ethanolic extract of propolis inhibited the growth of gram-positive bacteria.

Table (2) Minimum inhibitory concentrations (MIC) for water extract of different types of propolis

Concentration MIC (mg/ ml)							Type of propolis	Microorganism
40	30	20	10	5	3	2.5		
-	-	-	-	-	+	+	Clover	*S. aureus*
-	-	-	-	+	+	+	Sidr	
-	-	-	-	+	+	+	Eucalyptus	
-	-	-	-	+	+	+	Clover	*K. pneumonia*
-	-	-	+	+	+	+	Sidr	
-	-	-	+	+	+	+	Eucalyptus	
Represent inhibition* (-) : Represent growth* (+) :								

Table (3) Minimum inhibitory concentrations (MIC) for ethanolic extract of different types of propolis

Concentration MIC (mg/ ml)							Type of propolis	Microorganism
40	30	20	10	5	3	2.5		
-	-	-	-	-	-	+	Clover	*S. aureus*
-	-	-	-	-	+	+	Sidr	
-	-	-	-	-	-	+	Eucalyptus	
-	-	-	-	-	+	+	Clover	*K. pneumonia*
-	-	-	-	+	+	+	Sidr	
-	-	-	-	-	+	+	Eucalyptus	
Represent inhibition* (-) : Represent growth* (+) :								

Minimum inhibitory concentrations (MIC) for different antibacterial drugs also measured against S. aureus (MIC range 5-10 mg/m1) and K. pneumonia (MIC range 10- 20 mg/ml), as indicated in table (4) below.

Table (4) Minimum inhibitory concentrations (MIC) for different antibacterial drugs Represent inhibition : * (-)

Microorganism	antibacterial drug	Concentration						
		2.5	3	5	10	20	30	40
		MIC (mg/ ml)						
S. aureus	Gentamicin	+	+	+	-	-	-	-
	Ampicillin	+	+	-	-	-	-	-
	Cefotaxim	+	+	+	-	-	-	-
	Erythromycin	+	+	+	-	-	-	-
	Tetracyclin	+	+	-	-	-	-	-
K. pneumonia	Gentamicin	+	+	+	+	-	-	-
	Ampicillin	+	+	+	-	-	-	-
	Cefotaxim	+	+	+	+	-	-	-
	Erythromycin	+	+	+	+	-	-	-
	Tetracyclin	+	+	+	-	-	-	-
Represent growth* (+) :								

As indicated in the table (5) below, the results demonstrated synergistic activity between ethanol extract of different types of propolis with antibiotics (gentamicin, ampicillin, erythromycin, and tetracyclin) against both S. aureus and K. pneumonia. Synergistic activity between water extract of tow types of propolis (clover and eucalyptus) with antibiotics (gentamicin, ampicillin, erythromycin) was recorded against both S. aureus and K. pneumonia., but synergistic activity between water extract of sidr propolis with antibiotics dose not recorded. The results that we obtained are in some ways correlated to the various patterns of initial susceptibility to the antibiotics or to the active agents and propolis. Synergistic properties between EEP and antibiotics have been described by 33; they described a synergism between EEP and antimicrobial substances targeting microbial ribosomes, but not with antimicrobial effective the biosynthesis of DNA (ciprofloxacin and norfloxacin) nor those inhibiting metabolic pathways. The reference cited in 34 mentioned synergistic interaction between EEP and antibiotics (chloramphenicol, gentamicin, netilmicin, tetracycline, tobramycin, and linezolid) interfering with bacterial protein biosynthesis against drug-resistant bacterial pathogens.

Table (5) Synrigistic effect between water and ethanolic extract of three different types of propolis and antibiotics against S. aureus and K. pneumonia

Concentration			Antibiotics and ethanolic extract of propolis		Concentration			Antibiotics and water extract of propolis	Microorganism
5	3	2.5		10	5	3	2.5		
MIC (mg/ ml)					MIC (mg/ ml)				
-	-	+	Gentamicin + clover	-	-	-	-	Gentamicin + clover	*S. aureus*
-	-	+	Ampicillin + clover	-	-	+	-	Ampicillin + clover	
-	-		Cefotaxim + clover	-	-	-	-	Cefotaxim + clover	
-	-	+	Erythromycin + clover	-	-	+	-	Erythromycin+ clover	
-	-	+	Tetracyclin + clover	-	-	-	-	Tetracyclin + clover	
-	+	+	Gentamicin + sidr	-	-	-	-	Gentamicin + sidr	
-	+	+	Ampicillin + sidr	-	-	-	-	Ampicillin + sidr	
-	-	-	Cefotaxim + sidr	-	-	-	-	Cefotaxim + sidr	
-	+	+	Erythromycin + sidr	-	-	-	-	Erythromycin + sidr	
-	+	-	Tetracyclin + sidr	-	-	-	-	Tetracyclin + sidr	
-	-	+	Gentamicin + eucalyptus	-	+	-	-	Gentamicin + eucalyptus	
-	-	+	Ampicillin + eucalyptus	-	-	+	-	Ampicillin + eucalyptus	
-	-		Cefotaxim + eucalyptus	-	-		-	Cefotaxim + eucalyptus	
-	-	+	Erythromycin + eucalyptus	-	+	-	-	Erythromycin + eucalyptus	
-	-	+	Tetracyclin + eucalyptus	-	-	-	-	Tetracyclin + eucalyptus	
-	+	-	Gentamicin + clover	-	-	-	-	Gentamicin + clover	*K. pneumonia*
-	+	+	Ampicillin + clover	-	+	-	-	Ampicillin + clover	
-		-	Cefotaxim + clover	-	-	-	-	Cefotaxim + clover	
-	+	+	Erythromycin + clover	-	+	-	-	Erythromycin + clover	
-	+	-	Tetracyclin + clover	-	-	-	-	Tetracyclin + clover	
+	+	-	Gentamicin + sidr	-	-	-	-	Gentamicin + sidr	
+	+	+	Ampicillin + sidr	-	-	-	-	Ampicillin + sidr	
-	-	-	Cefotaxim + sidr	-	-	-	-	Cefotaxim + sidr	
+	+	-	Erythromycin + sidr	-	-	-	-	Erythromycin + sidr	
+	+	-	Tetracyclin + sidr	-	-	-	-	Tetracyclin + sidr	
-	+	-	Gentamicin + eucalyptus	+	-	-	-	Gentamicin + eucalyptus	
-	+	+	Ampicillin + eucalyptus	-	-	-	-	Ampicillin + eucalyptus	
-			Cefotaxim + eucalyptus		-	-	-	Cefotaxim + eucalyptus	
-	+	+	Erythromycin + eucalyptus	+	-	-	-	Erythromycin + eucalyptus	
-	+	-	Tetracyclin + eucalyptus	-	-	-	-	Tetracyclin + eucalyptus	
Represent no synrigistic effect* (-) : Represent synrigistic effect:* (+)									

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