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Biological Activity of Different Botanical Origin of Propolis

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ABSTRACT

The present study was designed to investigate the effect of antimicrobial ethanolic extract of propolis (EEP) on gram negative and gram positive bacterial. The tested bacterial strains were *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* and *Proteus* as Gram negative, while *Staphylococcus aureus* and *Streptococcus pyogenes* consider as Gram positive. Four different types of propolis (Saudi, Turkish, Chinese and Egyptian) were used in this study. There was highly significant effect of Saudi, Egyptian and Turkish propolis on tested bacteria. The highest inhibition zone in Egyptian propolis was 12 mm with *Proteus mirabilis* at 10% concentration while the lowest inhibition zone was 1.6 mm with *E.coli* at 1.25% concentration at the same propolis. *Proteus mirabilis* showed the highest inhibition diameter which record 14 mm at 10% concentration in Saudi propolis while the lowest diameter was recorded as 2.6 mm at 1.25% concentration with *P.aeruginosa*. In Chinese propolis the *Proteus* showed the highest inhibition zone at 1.25 %, 2.50%, 5%, 7.50% and 10% concentration that recorded (5mm), (7.3mm), (9.6mm), (11mm) and (13 mm) respectively. In Turkish propolis the 10.5 mm was recorded as the highest inhibition zone at 7.5% and 10% concentration in *Klebsiella pneumoniae*, on the other hand, the lowest inhibition zone in *S.aureus* was 1.6 mm at 1.25% concentration. the antibacterial activity of propolis was concentration depends and depends upon its botanical origin

INTRODUCTION

Bees have been in existence for 125 million years and their evolutionary success has allowed them to become perennial species that can exploit virtually all habitats on Earth. This success is largely because of the application of the specific products that bees manufacture: honey, beeswax, venom, propolis (bee gum), pollen and royal jelly. Propolis regarded as the most important 'chemical weapon' of bees against pathogenic microorganisms (Wollenweber *et al*., 1990). Propolis (bee glue) is a natural products collected from beehives by worker bees from the bark of trees and leaves of plants. The color of propolis depend on the bees food, the common color is dark brown. This salivary and enzymatic secretions-enriched material is used by bees to cover hive walls to ensure a hospital-clean environment. propolis has a powerful effect on human health especially in fighting upper respiratory infections, such as common cold and influenza viruses (Focht *et al.,* 1993*)*.

The chemical composition of propolis is quite complicated. More than 300 compounds such as polyphenols, phenolic aldehydes, sequiterpene quinines, coumarins, amino acids, steroids and inorganic compounds have been identified in propolis samples. The contents depend on the collecting location, time and plant source. Consequently, biological activities of propolis gathered from different phytogeographical areas and time periods vary greatly (Lotfy, 2006).

The anterior nares are the major site of *Staphylococcus aureus* colonization in humans (Wertheim *et al.,* 2005). *S.aureus* often cause β-hemolysis (Ryan and Ray, 2004). Common disease of *S.aureus* are pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome. Other Gram –positive that we used in this study is *streptococcus pyogenues. S. pyogenus* consider as the most actually life threating (Lamagni *et al*., 2009). *S .pyogenes* (beta-hemolytic; Lancefield group A) is a human pathogen that causes pharyngitis (strep throat), skin disease and many other infections.

Proteus usually infects urinary tract, blood, abdominal wound, groin, neck and ankle (Kishore, 2010). *Klepsiella* live in human intestine flora (Tsai *et al*., 2010). It's able to cause urinary and biliary tract infections, osteomyelitis (Varaldo *et al*., 1990).

The *Pseudomonas aeruginosa* can cause pneumonias, urinary tract infections as well as causing high morbidity and mortality in patients with cystic fibrosis due to chronic infections that eventually cause pulmonary damage and respiratory insufficiency (Ochoa *et al*., 2013).

Escherichia coli typically colonizes the gastrointestinal tract of human infants within a few hours after birth. *E.coli* that cause diarrhea, nonspecific gastroenteritis especially in children and the hemolytic uremic syndrome (Nguyen *et al*., 2006).

The objective of this work was to investigate antimicrobial properties of four ethanolic extract propolis samples obtained from different regions of the world against six microorganisms.

MATERIALS AND METHODS

The present investigation was carried out at the research laboratory, Biology section, faculty of Science, Taibah University, Al-medina to study the antibacterial activity of four samples of propolis on six types of bacteria.

Propolis samples:

The four samples of propolis were Saudi, Egyptian, Chinese and Turkish propolis. All these samples were obtained from commercial bee products market in Saudi and stored in the refrigerator until using.

Preparation of propolis extract solution:

Propolis samples were extracted by maceration at room temperature, with occasional shaking, in the proportion of 10 g of (Saudi, Egyptian, Chinese and Turkish) propolis to 100 ml of solvent (ethanol 80%v/v), extracts were obtained after 7 days of maceration and the ethanolic extracts were then filtered by Whatman (No.1) filter paper and incubated at room temperature until ethanol evaporated and the product obtained a honey-like consistence are referred to as ethanolic extract propolis, this method was reported by Ildenize *et al*. (2004).

Preparation of different concentrations of Saudi, Egyptian, Chinese and Turkish ethanolic extract propolis:

According to (Bauer *et al*., 1959), 1.0 g of each ethanolic extract propolis was dissolved in 10 ml ethanol (80%) by shaking, for 30 min protected from light. The resulting aqueous-ethanol extract was filtered through a Whatman (No.1) paper (Menezes *et al*., 1997). This final solution was (100mg/ml) employed for the antibacterial assay and Five serial concentrations were10.00, 7.50, 5.00, 2.50 and 1.25 mg/ml of the ethanolic extract propolis to the assay.

Microorganisms:

Six bacterial strains were studied. *Staphylococcus aurous* and *Streptococcus pyogenus, which are gram-positive bacteria, Klebsiella pneumoniae, Pseudomonas, Escherichia coli* and *Proteus mirabilis,* are gram-negative bacteria. All types of tested bacteria obtained from the hospital (Selection of sample One thousand patients attending gastroenteritis in childern of two general hospitals (hood and the Maternity hospitals Al-Madina Al- Munawarah)

Antimicrobial activity:

Antimicrobial activity of propolis extracts was investigated using agar diffusion method. Test plates, were prepared of Muller-Hinton agar (MHA) according to (Muller *and Hinton,* 1941), and three wells were punched in the agar plates by using sterile glass-made pipettes attached to a vacuum pump. Sterile swabs were dipped into the bacterial suspension containing., and inculcated on to plate surfaces. Each well was filled with 100 µm of extracts (80 % ethanol and distilled water) the plates were kept for 2 hours at room temperature to allow the diffusion of the agents through the agar. Afterwards, the plates were incubated for 24 hours at 37° C. The control for all experiments was 80% ethanol; determination of the antimicrobial activity was done by measuring zones of inhibition using a ruler based on (Johnson and Case, 1995).

Statistical Analysis

Results were analysed using prism program and the probability p=0.05 was considered the critical value for all tests.

RESULTS

Antimicrobial activities of Saudi, Egyptian, Chinese and Turkish Ethanolic Extract Propolis against positive and negative bacteria test organisms are summarized in table 1 and illustrated in figure 1 in generally, all ethanol extracts of propolis samples showed inhibitory action on all tested bacteria, in addition antimicrobial activities of these extracts showed different inhibitory effect against all the tested bacteria, Gram-positive and Gram-negative bacteria.

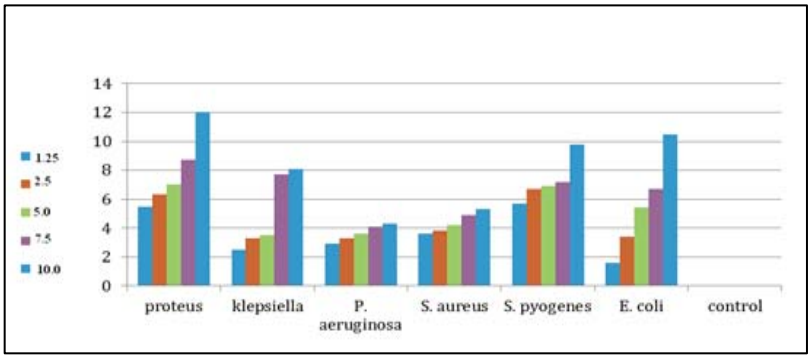
Data recorded in table 1, and figure 1 showed that, Egyptian propolis had highly significant effect on different types of bacterial compared to control group which record 0.0 mm. The highest diameter of inhibition zone at 1.25and 2.5% concentration was (5.7and 6.7 mm) with *Streptococcus pyogenes* respectively, in addition. At 5% concentration the highest diameter inhibition zone was (7.0 mm) with *Proteus mirabilis* and The highest diameter inhibition zone was (8.7and 12.0 mm) At 7.5 and 10.0 % concentration with *Proteus,* respectively. in addition Saudi propolis recorded a highly significant effect against different types of bacteria compared to control group, the highest diameter inhibition zone at all concentration of study was on *Proteus mirabilis* was (6.4,9.0, 9.1,9.4 and 14.0mm) at 1.25,2.5,5.0,7.5 and 10% concentration respectively . in the same line the Chinese propolis showed effect against different types of bacteria and *Proteus* showed the highest diameter inhibition zone at 1.25, 2.50, 5, 7.50 and 10% concentrations (5, 7.3, 9.6, 11 ,13 mm) respectively.

While, Data recorded showed the effect of Turkish propolis against different types of bacteria, and its obvious that Turkish propolis showed the highest diameter inhibition zone on *Klebsiella pneumoniae* was (5.1, 6.3, 7.5, 10.5 and 10.5 mm) at 1.25, 2.5, 5.0, 7.5 and 10% concentration respectively.

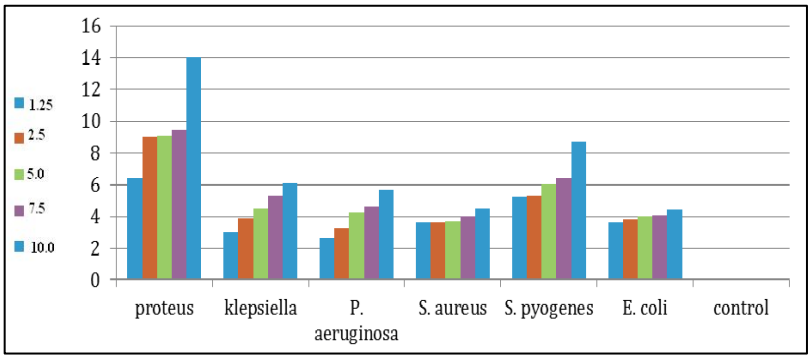
From the previous results we noticed that, the lowest diameter inhibition zone among the four types of propolis under study were recorded in *Proteus mirabilis* by Turkish propolis and *Streptococcus pyogenes* by Chinese propolis but on other hand the highest diameter inhibition zone were recorded in *Escherichia coli* by Egyptian propolis , *Pseudomonas aeruginosa* by Chinese propolis and *Klebsiella pneumoniae* by Turkish propolis.

Table 1: the antibacterial effect of Saudi, Egyptian, Chinese and Turkish propolis against different types of bacteria. (Zones of Inhibition / mm).

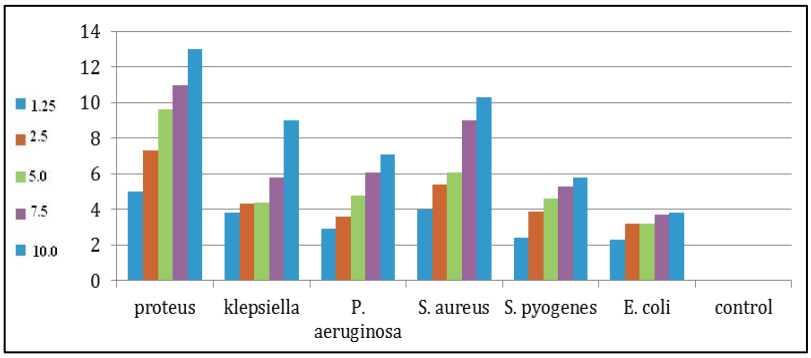
Bacteria		**Concentrations of ethanolic extract propolis**				
		1.25%	2.50%	5%	7.50%	10%
	Egyptian propolis					
Gram negative	*Proteus mirabilis*	5.5	6.3	7.0	8.7	12.0
	Klebsiella pneumoniae	2.5	3.3	3.5	7.7	8.1
	Pseudomonas aeruginosa	2.9	3.3	3.6	4.1	4.3
	Escherichia coli	1.6	3.4	5.4	6.7	10.5
Gram positive	*Staphyloccocus aureus*	3.6	3.8	4.2	4.9	5.3
	Streptococcus pyogenes	5.7	6.7	6.9	7.2	9.8
	Control	0.0	0.0	0.0	0.0	0.0
	P* value= 0.0003 **		**F=6.65**			
	Saudi propolis					
Gram negative	*Proteus mirabilis*	6.4	9.0	9.1	9.4	14.0
	Klebsiella pneumoniae	3.0	3.9	4.5	5.3	6.1
	Pseudomonas aeruginosa	2.6	3.3	4.3	4.6	5.7
	Escherichia coli	3.6	3.8	4.0	4.1	4.4
Gram positive	*Staphyloccocus aureus*	3.6	3.6	3.7	4.0	4.5
	Streptococcus pyogenes	5.2	5.3	6.0	6.4	8.7
	Control	0.0	0.0	0.0	0.0	0.0
	P* value= 0.0006 **		**F=5.933**			
	Chinese propolis					
Gram negative	*Proteus mirabilis*	5.0	7.3	9.6	11.0	13.0
	Klebsiella pneumoniae	3.8	4.3	4.4	5.8	9.0
	Pseudomonas aeruginosa	2.9	3.6	4.8	6.1	7.1
	Escherichia coli	2.3	3.2	3.2	3.7	3.8
Gram positive	*Staphyloccocus aureus*	4.0	5.4	6.1	9.0	10.3
	Streptococcus pyogenes	2.4	3.9	4.6	5.3	5.8
	Control	0.0	0.0	0.0	0.0	0.0
	P* value= 0.0015*		**F=5.170**			
	Turkish propolis					
Gram negative	*Proteus mirabilis*	4.2	4.6	5.9	6.3	6.3
	Klebsiella pneumoniae	5.1	6.3	7.5	10.5	10.5
	Pseudomonas aeruginosa	2.3	2.4	4.1	4.4	5.3
	Escherichia coli	4.3	4.4	4.7	4.8	5.7
Gram positive	*Staphyloccocus aureus*	1.6	3.1	3.6	3.6	4.8
	Streptococcus pyogenes	3.7	4.0	5.4	7.0	9.8
	Control	0.0	0.0	0.0	0.0	0.0
	P* value= 0.0006 **		**F=6.017**			



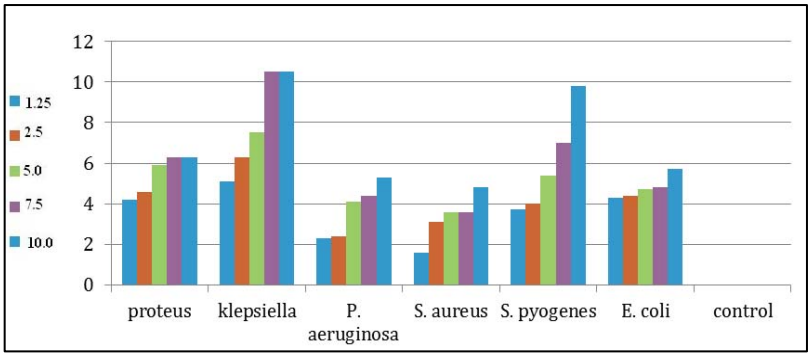
Egyptian propolis.



Saudi propolis against.



Chinese propolis.



Turkish propolis.

Fig. 1: the antibacterial effect of Saudi, Egyptian, Chinese and Turkish propolis against different types of bacteria. (Zones of Inhibition / mm).

DISCUSSION

As indicated from the results of Antimicrobial activities of these extracts which showed different inhibitory effect against all the tested bacteria, In addition, all ethanol extracts of propolis samples showed inhibitory action on all tested bacteria, on Gram-positive and Gram-negative bacteria, and the results of the high concentration of the propolis exerted a high antimicrobial activity, while the low concentrations revealed a low response. This activity may be due to the high concentration of each extract, such as flavonoid and total phenolic compounds because this compounds play an important role as bactericidal agent which reported by many researchers, so the bactericidal action of propolis was concentration dependant, more over The present findings indicate that the antibacterial activity of propolis, could not be correlated with their propolis concentration only but mostly to their chemical composition which can be variable according to the collection site and vegetal source and depends upon its botanical origin.

The studies of propolis against a wide variety of microorganisms all over the world show strong antibacterial activities (Koo, *et al.,* 2000 and Steinberg, *et al*., 1996).

The present study showed a noticed effect of different types of propolis against the different types of tested bacteria, similar finding was reported by (Miyataka, *et al*., 1997 and Buono, *et al*., 2001). Propolis extracts also have antibacterial properties against some micro-organisms (Takaisi and Schilcher, 1994 and Nieva, *et al.,* 1999). Recent pharmacological studies stated that, propolis has a wide range of sophisticated composition including anti-inflammatory, anti-oxidative and anti-microbial (Burdock, 1998), immunomodulatory properties (Orsolic and Basic, 2003) also antitumor, anticancer, antiulcer, hepatoprotective, cardioprotective, and neuroprotective (Drago and Vecchi, 2007*).* Propolis also had polyphenols including flavonoids and phenolic acids (Cao, *et al.,* 2004). The antibacterial effects of propolis in this study are parallel to (Soley, *et al.,* 2011*),* whose found that, propolis had good *in vitro* antibacterial activity against *E. faecalis* in the root canals, suggesting that, it could be used as an alternative intra-canal medicament. Ferreira, *et al.,* (2007) also found that, propolis had an antibacterial effect on selected endodontic anaerobic bacteria (*Prevotella nigrescens, Fusobacterium nucleatum, Actinomyces israelii, Clostridium perfringens and E. faecalis)*. Bruschi, *et al.,* (2006) found that, propolis has an inhibitory effect on microorganism of oral importance (*E. faecalis, Streptococcus salivarius, Streptococcus sanguinis, Streptococcus mitis, Streptococcus mutans and Streptococcus sobrinus*). The preparation of propolis completely inhibited the growth of *Staphylococcus aureus, Staph- epidermidis, Enterococcus sp., Corynebacterium sp*. *and Bacillus cereus*. It partially inhibited growth of *Pseudomonas aeruginosa* and *Escherichia coli*. Tube dilution studies showed that, it was bactericidal for *B. cereus* at dilutions of 1: 160 to 1: 320 and that, growth of *Mycobacterium tuberculosis* was totally inhibited at 1: 320 and partially inhibited at 1: 640 (Grange and Davey, 1990). Propolis is mainly active against *S. aureus* and it is effective on *P. aeruginosa* at higher concentrations. That is sensitivity of *S. aureus* strains against antibacterial activity of ethanol extract of propolis with mean inhibitory diameter (17.66 ± 0.47-10 mm) followed by *P. aeruginosa* strains (7 mm) were recorded by (Zeighampour, *et al.,* 2013). The chemical composition of propolis is quite complicated and its compounds and biological activities depend on many different factors such as the geographical region, collected time and plant source (Haggag, *et al*., 2006; Sforcin, *et al.*, 2000 and Bankova, *et al.,* 2002*).* Among the numerous groups of substances identified in propolis samples from different localities, the most common are caffeic, p-Coumaric acid, aromatic acids, esters, chalcones,

flavonoids, terpenoids, waxy acids, cinnamic acid derivatives, amino acid, fatty acids, vitamins like (B1, B2, B6, C and E), minerals like (Mg, Ca, I, Na, Cu, Zn, Mn and Fe) and enzymes like (adenosine triphosphatase, succinic dehydrogenase and glucose 6-phosphatase) (Marcucci and Bankova, 1999; Velikova, *et al*., 2000; Marcucci, *et al*., 2001 and Zeighampour, *et al.,* 2013). Flavonoids are thought to account for much of the biologic activity in propolis. The antimicrobial properties of this mixture of natural substances are mainly attributed to the flavonone pinocembrin, to the flavonol galangin and to the caffeic acid phenethyl ester (Takaisi and Schilcher, 1994 and Bosio, *et al.,* 2000). Active component of propolis, may be responsible for most of these biological activities (Hepsen, *et al., 1977* and Koltuksuz*, et al.,* 1999).

The mode of action is Propolis inhibits bacterial growth by preventing cell division, thus resulting in the formation of pseudo-multicellular streptococci. In addition, propolis disorganized the cytoplasm, cytoplasmic membranes and cell wall, caused a partial bacteriolysis, inhibited protein synthesis also inhibit bacterial enzymes. The antibacterial mechanism also depends on the inhibition of bacterial RNA-polymerase (Takaisi and Schilcher 1994).

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ARABIC SUMMERY

النشاط البيولوجى للبروبوليس من مختلف المصادر النباتيه

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تم اجراء هذا البحث لدراسة تأثير المستخلص الكحولي لأربعة أنواع من البروبوليس و هي السعودي و التركي و المصري والصيني على ستة أنواع وهي : (*E.coli, Klebsiella pneumoniae, Proteus mirabilis, P. aeruginosa, S. pyogenes and S. aureus*)وقد أعد لكل نوع من انواع البروبوليس خمسة تركيزات (١.٢٥، ٢.٥، ٥.٠، ٧.٥ و ١٠)٪. وقد تم قياس قطر منطقة التثبيط بـ(المليميتر) في المنطقة التي لم يحدث بها نمو، بعد التحضين عند درجة ٣٧ مئوية لمدة ٢٤ ساعة. وقد أظهرت جميع أنواع البروبوليس فعالية معنوية ضد الأنواع المختلفة من البكتيريا. أظهر البروبوليس المصري النشاط المضاد للبكتيريا العالي على*Proteus*). وكانت أعلى منطقة قطر التثبيط في البروبوليس المصري هي ١٢ ملم عند تركيز ١٠٪، وأظهر البروبوليس السعودي النشاط المضاد للبكتيريا العالي على*Proteus*). وكانت أعلى منطقة قطر التثبيط في البروبوليس السعودي هي١٤ ملم عند تركيز ١٠٪، وأظهر أن البروبوليس التركي النشاط المضاد للبكتيريا العالي على*Klebsiella pneumoniae*). وكانت أعلى منطقة قطر التثبيط في البروبوليس التركي هي ١٠.٥ ملم عند تركيز ١٠٪، وأظهر البروبوليس الصيني النشاط المضاد للبكتيريا العالي على *Proteus*). حيث كانت أعلى منطقة قطر التثبيط في البروبوليس الصيني هي ١٣ ملم عند تركيز ١٠ %. و بشكل عام كل ما زاد تركيز البروبوليس زاد التاثير المثبط وكذلك باختلاف النوع تختلف قوة التاثير المضاد.