

Full Length Research Paper

***In vitro* antibacterial and antifungal activity of Iraqi propolis**

Nada K. K. Hendi¹, Habeeb S. Naher² and Alaa H. Al-Charrakh^{2*}

¹College of Nursing, Babylon University, Babylon province, Iraq.

²Department of Microbiology, College of Medicine, Babylon University, Babylon province, Iraq.

Accepted 23 May, 2011

The study was aimed at determining the antimicrobial activities of crude ethanolic extract of Al-Museiab propolis (EEMP) against some bacterial and fungal isolates by the method of disc diffusion and agar-well diffusion, respectively. MICs of propolis extracts using the two-fold agar dilution susceptibility method were also determined. Results revealed that *Staphylococcus aureus* was highly sensitive to EEMP than other Gram positive and Gram negative bacteria, while standard *Escherichia coli* strain was highly sensitive to EEMP than other Gram negative bacteria. The effect of EEMP was elevated when the concentration increased to 20 and 30%. EEMP was not effective against *Candida albicans*. Results of disc diffusion methods of crude EEMP at 10% concentration showed that *S. aureus* was highly sensitive to EEMP inhibition while *C. albicans* was resistant. Statistical analysis showed significant differences ($P \leq 0.05$) between results of disc and agar diffusion methods of EEP at concentration of 10%, while there was no significant difference ($P \leq 0.05$) at concentrations of 20 and 30% of extract, respectively. This study concluded that EEP was the most active of all propolis extracts, *S. aureus* was more sensitive to EEP and AEP than other bacteria, and agar diffusion method was better than disc diffusion method for detection of antimicrobial activity of propolis.

Key words: Propolis, antibacterial, antifungal, Iraq.

INTRODUCTION

Propolis is a resinous substance collected by worker bees (*Apis mellifera*) from the bark of trees and leaves of plants. This salivary and enzymatic secretions-enriched material is used by bees to cover hive walls to ensure a hospital-clean environment. As a natural honeybee hive product, propolis extracts have been used both internally and externally for thousands of years as a healing agent in traditional medicine. Propolis shows a complex chemical composition. Its biological properties- such as antibacterial, antiviral, antifungal, among other activities, have attracted the researchers' interest (Simone-Finstrom and Spivak, 2010). Its biological properties may vary according to different plant sources. In Brazil, there are many plants that could be visited by bees as sources of propolis, whose chemical composition may differ depending on the geographic location. Brazil produces

the best propolis in the world due to its tropic and sub-tropic climates and through its largest primitive forest (Trusheva et al., 2006).

In laboratory tests, studies have shown broad spectrum antimicrobial activity of various propolis extracts. Synergism with certain antibiotics has been demonstrated. Depending upon its composition, propolis may show powerful local antibiotic and antifungal properties. <http://en.wikipedia.org/wiki/Propolis> Many authors have demonstrated propolis antibacterial activity against *Enterococcus* spp, *Escherichia coli*, and *Staphylococcus aureus*. Reports have pointed out propolis efficient activity against Gram-positive bacteria and limited action against Gram-negative bacteria (Park et al., 2005). Different researchers (Sforzin et al., 2000; Trusheva et al., 2006; Katircio and Nazime, 2006; Yaghoubi et al., 2007) have reported that propolis antibacterial activity is attributed to a number of phenolic compounds, mainly flavonoids, phenolic acids and their esters. Some prenylated coumaric acids were isolated from propolis in several countries (Kosalec et al., 2004).

*Corresponding author. E-mail: aalcharrakh@yahoo.com. Tel: 009647707247994, 009647813216822.

Table 1. Standard and local bacterial strains.

Bacterial strain	Source
<i>E. coli</i> 25922	ATCC
<i>Salmonella typhi</i> TY21	Central health lab, Baghdad
<i>Listeria monocytogenes</i>	Kufa University/ College of science
<i>Helicobacter pylori</i>	Qadisiya University/ College of science
<i>Streptococcus pyogenes</i>	
<i>Pseudomonas aeruginosa</i>	
<i>Staphylococcus aureus</i>	Babylon University/ College of Medicine
<i>Klebsiella pneumoniae</i>	
<i>Enterobacter aerogenes</i>	

The antibacterial activity of volatile compounds and diterpenes from Brazilian propolis was identified by Bankova et al. (2000). Propolis and some of its cinnamic acid derivatives and flavonoids were responsible for uncoupling the energy transducing cytoplasmic membrane inhibiting bacterial motility, which might contribute to the antibacterial action (Bankova et al., 2000). Although, numerous researchers have reported the biological activities of propolis collected worldwide, information about Iraqi propolis are still absent. The aim of this study is to investigate antibacterial and antifungal activity of propolis samples from Museiab in Iraq.

MATERIALS AND METHODS

Propolis samples

Propolis samples were collected from hives of honey bees of Al-Museiab, Iraqi during spring and summer seasons of 2010. Propolis samples were cleaned, free of wax, paint, wood, cut into small pieces, and placed in clean container.

Aquatic extract of propolis

Ten gram of propolis were mixed with 100 ml of double D.W. in dark brown container and left for 7 to 14 days at room temperature in dark place. For 2 weeks, the container was shaken 2 or 3 times per day and returned to warm dark place. The liquid was filtered through Whatman No.1 filter paper and the water was evaporated by oven at 45°C, then the extract was weighed and stored in dark clean container for further using. Water or aqueous extract was dissolved by distilled water, sterilized by filtration (using Millipore 0.45 filter paper), and the requisite dilutions were prepared.

Ethanolic extract of propolis

Ten gram of propolis were mixed with 100 ml of ethanol in dark brown bottle and left for 7 to 14 days at room temperature and in dark place. For 2 weeks, the container was shaken 2 or 3 times per day and returned to warm dark place. The liquid was filtered through Whatman No. 1 filter paper and the water was evaporated by oven at 45°C, then the extract was weighed and stored in dark clean container for further usage. Ethanolic extract was dissolved

by Dimethyl Sulfoxide (DMSO), sterilized by filtration (using Millipore 0.45 filter paper), and the requisite dilutions were prepared.

Bacterial strains

Standard bacterial strains and local isolates used in this study are listed in Table 1. The standard bacterial strains were activated and cloned three successive times in nutrient agar and stored on nutrient agar slants at 4°C. The identification of the local bacterial isolates was confirmed using conventional biochemical tests (Forbes et al., 2007).

Isolation and identification of *Candida albicans*

C. albicans isolates were recovered from women with vaginitis attended to Marjan hospital, Hilla, Iraq. Swabs were taken from patient by using sterile cotton swabs with transport media. The samples were cultured on Sabouraud dextrose agar supplemented with chloramphenicol to prevent bacterial contamination and incubated at 37°C. The fungal culture was examined according to colonies, cellular morphology and germ tube formation (Forbes et al., 2007).

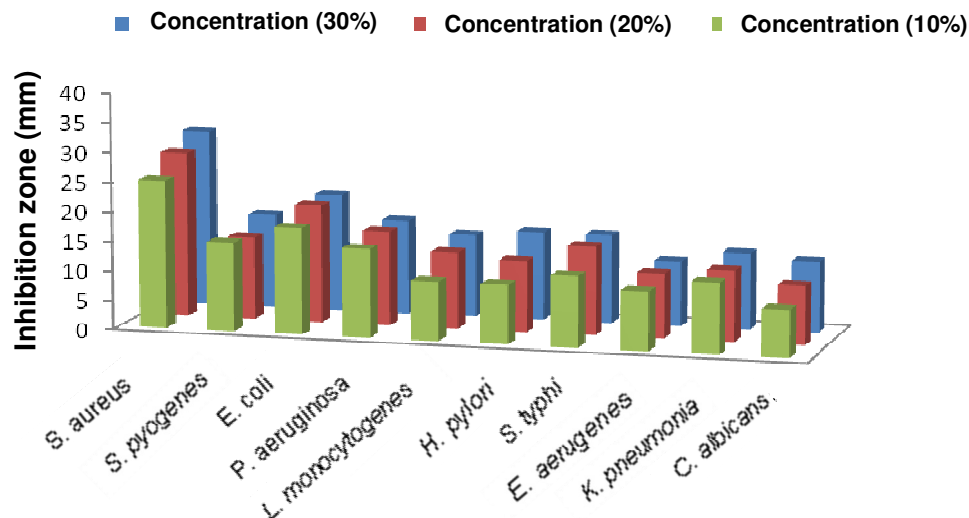
In vitro antibacterial and antifungal activities of crude propolis extract

Determination of activity by disk method

Antimicrobial susceptibility was tested using paper disc agar diffusion method (Bauer et al., 1966). Paper discs (5 mm) were sterilized by autoclave and soaked in a propolis extracts (ethanolic and aquatic extract) solution with different concentrations (10, 20 and 30%). Solutions containing different propolis extracts solution at varying concentrations were placed separately in the plate under aseptic conditions. Triple plates were used for each concentration. The agar plates maintained at room temperature for 2 h allowing for diffusion of the solution. All plates were then incubated at 37°C for 24 h, and the zones inhibition were subsequently measured in millimeters (Mukherjee et al., 1995).

Determination of activity by agar diffusion method (NCCLS, 2002)

Petri plates containing 25 ml of Mueller-Hinton agar for bacteria and



Bacterial and yeast isolates

Figure 1. Effect of ethanolic extracts of Al-Museiab crude propolis on the bacterial and fungal isolates at different concentrations by well diffusion test.

Sabouraud dextrose agar for *C. albicans* were used. Agar media were seeded with a 24 h old culture of the microorganism strains (by sterile cotton swab dipped into the broth of these microorganisms). Four wells (5 mm diameter) were cut into the agar by cork borer and 0.1 ml of the crude propolis extracts was applied in each well. The inoculum size was adjusted so as to deliver final inoculums of approximately 10^8 colony forming unit (CFU)/ml, comparison with the turbidity of sample to the 0.5 McFarland standards. Incubation was performed at 37°C for 24 h. The assessment of antibacterial and antifungal activity was based on measurement of the diameter of the inhibition zone formed around the well. Streptomycin was used as a reference antibacterial agent and Nystatin as a reference antifungal agent.

Determination of the minimum inhibitory concentration (MIC)

The two-fold agar dilution susceptibility method was used for determination of MICs of propolis extracts. The prepared dilutions of propolis extracts solutions were added to the molten Muller- Hinton agar media that have been allowed to equilibrate in a water bath to 45-50°C. The agar and propolis extracts solution were mixed thoroughly and the mixture was poured into Petri dishes. The agar was allowed to solidify at room temperature. A standardized inoculum for agar dilution method was prepared by growing bacteria to the turbidity of 0.5 McFarland standards.

The 0.5 McFarland suspensions were diluted 1:10 in sterile normal saline. 1 μ l aliquot of each inoculum was applied to the agar surface with standardized loop. Propolis extracts free media were used as negative controls. The inoculated plates were allowed to stand at room temperature (for no more than 30 min) until the moisture in the inoculum spots was absorbed by the agar. The plates were inverted and incubated at 35°C for 18 to 24 h. To determine agar dilution break points, the plates were placed on a dark surface, and "the MIC was recorded as the lowest concentration of the antimicrobial agent that completely inhibits growth" or that concentration (μ g/ml) at which no more than two colonies were detected (CLSI, 2010).

Statistical analysis

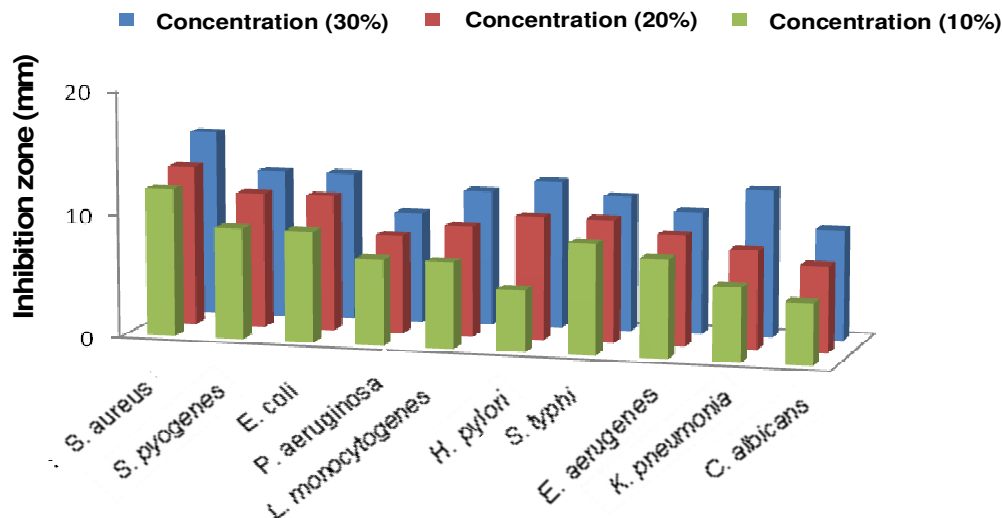
Bonferroni test was used for statistical analysis (Danial, 1988), to show if there is any significant difference ($P \leq 0.05$) between results of disc and agar diffusion methods of propolis ethanolic extract.

RESULTS AND DISCUSSION

In vitro antibacterial and antifungal activities of crude extract of propolis

As a general rule, an extract is considered active against both bacteria and fungi, if the zone of inhibition was greater than 6 mm (Muhammad and Muhammad, 2005). Antimicrobial activities of crude extract of Al-Museiab propolis (EEMP) at different concentration (10, 20, and 30%) against both bacterial and fungi isolates were studied. Antibacterial and antifungal activities of crude ethanolic extract against bacteria and fungi are shown in Figure 1. The results of agar diffusion at 10% concentration showed that most bacterial isolates were sensitive to EEMP.

S. aureus was highly sensitive to EEMP than other Gram positive and Gram negative bacteria followed by *Listeria monocytogenes* with inhibition zones of 25 and 18 mm respectively while standard strain *E. coli* was highly sensitive to EEMP than other Gram negative bacteria with inhibition zones of 15 mm. The zone of inhibition for *Streptococcus pyogenes* was 14 mm while the zones of inhibition for each of *Salmonella typhi*, and *Klebsiella pneumoniae* were 12 mm. The zone of inhibition was 10 mm for each of *Pseudomonas*



Bacterial and yeast isolates

Figure 2. Effect of ethanolic extracts of Al-Museiab crude propolis on the bacterial and yeast isolates at different concentration by disc diffusion method.

aeruginosa, *Helicobacter pylori*, and *Enterobacter aerogenes*. EEMP was not effective against *C. albicans*. On the other hand, the effect of EEMP was elevated when the concentration increased to 20 and 30%. The zones of inhibition of *S. aureus* were 28 and 30 mm respectively, whereas the zones of inhibition of *C. albicans* were 10 and 12 mm respectively. EEMP possessed a good antibacterial and antifungal activity against bacteria and fungi at different concentrations (10, 20, and 30%). Inhibition zones were extrusive proportioning with increasing of concentration. Statistical analysis showed no significant differences after treating the microorganisms with propolis ethanolic extract at different concentrations of agar diffusion ($P \leq 0.05$).

This result indicated that the active components of propolis were concentrated in the sample. This was in agreement with reports of several papers which indicated that each propolis sample contained 80 to 100 chemical compounds with different concentrations (Bankova et al., 2000; Kosalec et al., 2004; Trusheva et al., 2006; Park et al., 2005; Yaghoubi et al., 2007; Darwish et al., 2010). The present results on *S. aureus* were in agreement with those obtained by several authors who found that the inhibition zones obtained by propolis from Mongolia, Albania, Egypt and Brazil were 24, 21.8, 24.3, and 21.8 mm respectively (Kujumgiev et al., 1999). These results are comparable with results obtained by Prytyk et al. (2003) who found that the inhibition zone for Bulgarian propolis was 20 mm, also with results obtained by Stepanovi et al. (2003) who found out that the inhibition zone of propolis form different geographical areas of Serbia ranged from 18 to 23 mm. These differences in

antibacterial activity of propolis from the different regions in the world supported the commonly reported statements in literature which indicated that sensitivity of microbes and differences in chemical composition of propolis are greatly affected by variations in geographical origins (Bankova et al., 2000; Abd El Hady and Hegazi, 2002; Kartal et al., 2003; Trusheva et al., 2006). Furthermore, the result of disc diffusion methods of crude EEMP at 10% concentration was studied (Figure 2). *S. aureus* was highly sensitive to EEMP with 13 mm as zone of inhibition while *C. albicans* was resistant. The zones inhibition for each of standard *E. coli* strain and *S. pyogenes* were 12 mm. The zones of inhibitions were 11, 10, and 7 mm for each of *S. typhi*, *K. pneumoniae* and *H. pylori* respectively while the zones of inhibition for each of *L. monocytogenes*, *P. aeruginosa*, and *E. aerogenes* were 10 mm. The effect of EEMP was elevated when concentration of crude propolis increased to 20 and 30%.

Inhibition of bacterial and fungal growth were extrusive proportioning with increase of concentration of propolis due to increase of concentration of active component of propolis. This result was in agreement with Taylor et al. (1996) and Hernandez et al. (1994) who found that the efficiency of propolis extract was high when the concentration of propolis increased. Statistical analysis showed significant differences after treating the microorganisms with 10% concentration of ethanolic extract at using disc and agar diffusion methods at level ($P \leq 0.05$), while there was no significant difference ($P \leq 0.05$) at concentrations of 20 and 30% of propolis ethanolic extract, respectively.

Biological and pharmaceutical activity of propolis may

Table 2. Effect of ethanol extracts of Al-Museiab crude propolis 30% on the bacterial and yeast isolates by determination of MIC of the extract.

Microorganism	Concentration		
	10%	20%	30%
	MIC ($\mu\text{g/ml}$)	MIC ($\mu\text{g/ml}$)	MIC ($\mu\text{g/ml}$)
<i>S. aureus</i>	1280 \geq	1280 \geq	\geq 640
<i>S. pyogenes</i>	1280 \geq	1280 \geq	\geq 640
<i>E. coli</i>	2560 \geq	2560 \geq	1280 \geq
<i>P. aeruginosa</i>	2560 \geq	2560 \geq	2560 \geq
<i>L. monocytogenes</i>	2560 \geq	2560 \geq	1280 \geq
<i>H. pylori</i>	5120 \geq	5120 \geq	2560 \geq
<i>S. typhi</i>	2560 \geq	2560 \geq	2560 \geq
<i>E. aerogenes</i>	5120 \geq	5120 \geq	2560 \geq
<i>K. pneumoniae</i>	2560 \geq	2560 \geq	2560 \geq
<i>Candida albicans</i>	5120 \geq	5120 \geq	2560 \geq

contribute to the fact that propolis contains active compounds such as phenols, flavonoids and alkaloids that possess antibacterial and antifungal activities against bacteria and fungi. These results were comparable with results obtained by several authors (Scheller et al., 1999; Abd-El-Salam et al., 1989). Moreover, determination of minimum inhibitory concentration of EEMP at different concentrations (10, 20, and 30%) against bacterial and fungal isolates was determined (Table 2). MIC of EEMP at 10% concentration against *S. aureus* and *S. pyogenes* were \leq 1280 $\mu\text{g/ml}$ while it was \leq 2560 $\mu\text{g/ml}$ against each of Standard *E. coli* strain, *S. typhi*, *L. monocytogenes* and *P. aeruginosa*. The MIC value was increased (5120 $\mu\text{g/ml}$) against each of *E. aerogenes*, *K. pneumoniae*, *H. pylori* and *C. albicans* at the same concentration.

MIC values of EEMP in 20% concentration of bacterial and fungal isolates were similar to that of 10% concentration. The MIC value in 30% concentration of propolis against *S. aureus* and *S. pyogenes* was $640 \leq \mu\text{g/ml}$. The MIC was increased (\leq 1280 $\mu\text{g/ml}$) against standard *E. coli* strain and *L. monocytogenes* and the value was dramatically increased (\leq 2560 $\mu\text{g/ml}$) against each of *E. aerogenes*, *S. typhi*, *K. pneumoniae*, *H. pylori* and *C. albicans*. The MIC values of EEM propolis in this study, was similar to that reported by Sforcin et al. (2000) on propolis collected from Brazil, and Darwish et al. (2010) on propolis collected from Jordan but they were higher than those reported in Egypt by Hegazi and Abd El Hady (2002) in which the MIC value of their propolis was 2.2 mg/ml. However, Moreno et al. (2000) reported that propolis collected from Argentina had lower MIC value of 0.04 mg/ml against the same strain. This difference in MIC values of propolis was related to the different constituents of propolis collected from different geographical regions (Bankova et al., 2000; Abd El Hady and Hegazi, 2002). Several researchers (Kujumgiev et al., 1999; Moreno et al., 2000; Sforcin et al., 2000; Stepanovi

et al., 2003; Gonzalez et al., 2005) reported that there was no effect of propolis from different geographical regions on standard *E. coli*.

Our results however, show that there is some antibacterial effect of propolis on Gram negative bacteria, but it is rather limited with a zone of inhibition of 15 mm for crude propolis. This again might reflect the fact that chemical composition of propolis differs greatly from one region to another (Burdoc, 1998; Bankova et al., 2000; Prytyk et al., 2003; Stepanovi et al., 2003). The MIC value against standard *E. coli* was 2560 $\mu\text{g/ml}$. This MIC value is higher than that reported by Sforcin et al. (2000) of 8 mg/ml on the same strain. However, the variation might reflect the difference in the composition of the propolis, since the bacterial strain used was the same. The lower sensitivity (or resistance) of *E. coli* to propolis, was in agreement with the findings obtained by many researchers who revealed that this bacterium showed either very low sensitivity or total lack of sensitivity against propolis (Marcucci, 1995; Kujumgiev et al., 1999; Gonzalez et al., 2005). This emphasizes the fact that, Gram negative bacteria are less sensitive than Gram positive strains, which is in agreement with several previous reports (Burdoc, 1998; Moreno et al., 2000; Sforcin et al., 2000; Abd El Hady and Hegazi, 2002; Gonzalez et al., 2005).

The most possible explanation for the low sensitivity of Gram negative bacteria to propolis extract is that, their outer membrane inhibits and/or retards the penetration of propolis (Tegos et al., 2002). Another possible reason is their possession of multi drug resistance (MDR) pumps, which extrude amphipathic toxins across the outer membrane (Tegos et al., 2002). Several authors (Sforcin et al., 2000; Trusheva et al., 2006; Katircio and Nazime, 2006; Yaghoubi et al., 2007) have reported that propolis antibacterial activity is attributed to a number of phenolic compounds, mainly flavonoids, phenolic acids and their esters and some prenylated-coumaric acids were isolated

from propolis in several countries (Kosalec et al., 2004). The antibacterial activity of volatile compounds and diterpenes from Brazilian propolis was identified by Bankova et al. (2000).

Propolis and some of its cinnamic acid derivatives and flavonoids were responsible for uncoupling the energy transducing cytoplasmic membrane inhibiting bacterial motility, which might contribute to the antibacterial action (Bankova et al., 2000). Regarding anti- *L. monocytogenes*, the results of this study was in agreement with Bayoub et al. (2010) who mentioned that the diameter of inhibition zone of ethanolic extract against *L. monocytogenes* was 26 to 14 mm and MIC value was 0.25 to 11.75 mg/ml.

The activity of 30% of ethanolic extract of propolis (EEP) against of *H. pylori* was evaluated by using agar well diffusion method and the diameter of inhibition zone was 21.4 mm (Kimoto et al., 1998). It was found that a concentration of 15 to 30 mg/ml of propolis was needed to inhibit the growth of *C. albicans* (Pepeljnjak et al., 1982). It was noted that disk diffusion assay and agar well diffusion method exhibited similar results, but the agar well diffusion revealed a low activity of ethanolic extracts (Olila et al., 2001).

Most of the antimicrobial constituents such as monoterpenes contributed to the antimicrobial effect particularly against *L. monocytogenes* (Mourey and Canillac, 2002). Prindle and Wright (1997) reported that the antimicrobial activity of phenolic compounds was concentration dependent, affecting enzymatic activity related to energy production at low concentrations and causing protein precipitation at high concentrations. Many plants contain non toxic glycosides which can get hydrolyzed to release phenolics which are toxic to microbial pathogens (Aboaba and Efuwape, 2001). An important characteristic of essential oils and their components is their hydrophobicity, which enabled them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable (Sikkema et al., 1994).

Ophori et al. (2010) reported that the antimicrobial activity of propolis is as a result of the high content of flavonoids. However, this activity varies according to geographic regions and pH of the culture medium (Meresta and Meresta, 1980; Glinski and Meresta, 1993). The presence of flavonoids and derivatives of caffeic acid is associated with the bactericidal activity (Bosio et al., 2000).

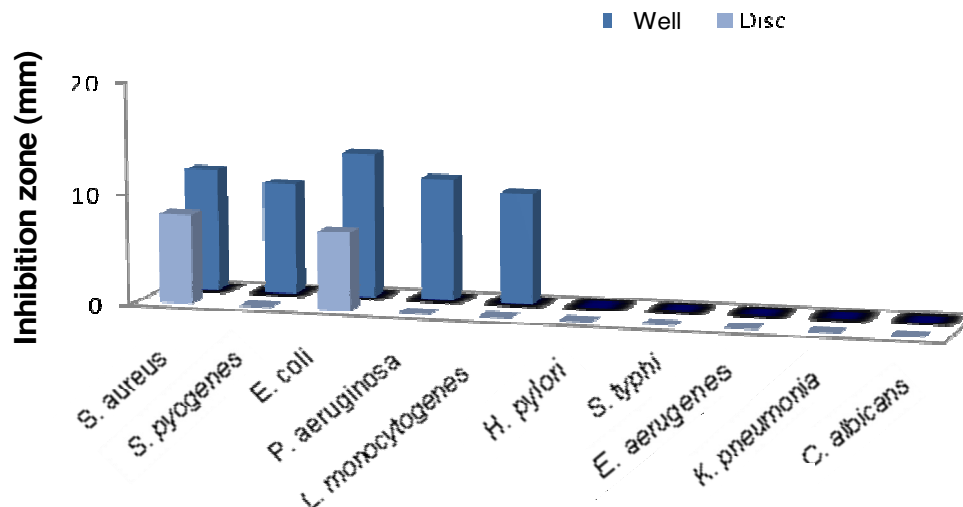
The mechanism of antibacterial action of propolis has been the subject of only a few publications. Takalsi-Kilkuni and Schilcher (1994) showed through electron microscopy and micro-calorimetric assays that ethanolic extracts propolis (EEP) interferes with the division of *Streptococcus* through the formation of pseudo-multicellular forms, cytoplasm disorganization, inhibition of protein synthesis leading to lysis of the bacteria. Mirzoeva et al. (1997) found that EEP and some phenolic

components affect the bioenergetical status of the membrane by inhibition of the membrane potential leading to increased permeability of the membrane to ions and to immobility of *Bacillus subtilis*. A synergistic effect with conventional anti-mycotic drugs was also observed (Holderma and Kedzia, 1987; Scheller et al., 1998). Takaisi-Kilkuni and Schilcher (1994) stated that the propolis inhibits bacterial growth by preventing cell division, thus resulting in the formation of pseudo-multicellular *Streptococci*.

In addition, propolis disorganized 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 components (Ravn et al., 1989). Results of antibacterial and antifungal activities of crude aquatic extract of Al-Museiab propolis (AEMP) against bacteria and fungi were also determined (Figure 3). The results of agar diffusion and disc diffusion at 10% concentration showed that standard *E. coli* strain was the highest sensitive bacteria to AEMP with zone of inhibition reached to 13 mm followed by *S. aureus* and *P. aeruginosa* (11 mm), while isolates of *S. typhi*, *K. pneumoniae*, *H. pylori*, *E. aerogenes*, and *C. albicans* were not to be affected by AEMP.

Moreover, the results of disc diffusion of AEMP were effective only against standard *E. coli* strain and *S. aureus* which they were sensitive to AEMP with zones of inhibition 7 and 9 mm, respectively. Statistical analysis showed significant differences after treating the microorganisms with inhibition zones of propolis aquatic extract at 10% concentration of agar diffusion and disc diffusion at level ($P \leq 0.05$). These results were in agreement with Al-Ammar (2001) who pointed out that zones of inhibition of *S. aureus* and *E. coli* were 8 and 7 mm respectively. Furthermore, determination of minimum inhibitory concentration of AEMP at 10% concentration against bacterial and fungal isolates was studied (Table 3). MIC values of *S. aureus*, *S. pyogenes* and standard strain *E. coli* were ≤ 2560 $\mu\text{g/ml}$, whereas *S. typhi*, *H. pylori*, *L. monocytogenes*, *P. aeruginosa*, *E. aerogenes*, *K. pneumoniae*, and *C. albicans* were ≤ 5120 $\mu\text{g/ml}$.

The activities variation depend on types extract (ethanolic or aquatic), types of microbes, and propolis concentration in the media. AEMP had lower antimicrobial activity than EEMP. This may be due to different techniques in extraction methods and solvent nature (Hernandez et al., 1994; Musa and Muhamed, 1992; Twaij et al., 1988) in addition to different active components of propolis extracted. EEMP possessing a number of active components that had inhibition effect on microbial growth more than AEMP but the antimicrobial activity depends on type of extracts with increase of concentration. AEMP were not effective against *S. typhi*, *H. pylori*, *L. monocytogenes*, *P. aeruginosa*, *E. aerogenes*, *K. pneumoniae*, and *C. albicans* due to



Bacterial and yeast isolates

Figure 3. Effect of aquatic extracts of Al-Museiab crude propolis on the bacterial and yeast isolates at 10% concentration disc and well.

Table 3. Effect of aquatic extracts of Al-Museiab crude propolis on the bacterial and yeast isolates by determination of MIC of the extract.

Microorganism	Concentration 10%
	MIC ($\mu\text{g/ml}$)
<i>S. aureus</i>	2560 \geq
<i>S. pyogenes</i>	2560 \geq
<i>E. coli</i>	2560 \geq
<i>P. aeruginosa</i>	5120 \geq
<i>L. monocytogenes</i>	5120 \geq
<i>H. pylori</i>	5120 \geq
<i>S. typhi</i>	5120 \geq
<i>E. aerogenes</i>	5120 \geq
<i>K. pneumoniae</i>	5120 \geq
<i>Candida albicans</i>	5120 \geq

decreased number of active components of propolis extracts and bacterial resistance to these extracts, these results were in agreement with Nieva-Moreno et al. (2000).

Al-Zubady (2009) reported that the zones of inhibition of *S. aureus* was 16 mm at 150% concentration of propolis collected from Al-Kufa, Iraq. Al-Salamy (2000) pointed out that the phenolic compound was causing protein denaturation of microbes through the pause of the enzymes action of metabolic reactions and dead the microorganism. Flavonoids were regarded as the largest component of the phenolic compound and it had pharmaceutical and antimicrobial activities. The concentration of flavonoids differ from sample to other sample of propolis, attributed to geographical area and

concentration of propolis extracts (Bonhevi and Jorda, 1999; Kumer et al., 2008). Tannins are toxic for bacteria, fungi and yeast due to their ability to combine with the microbial cell wall and cause growth inhibition (Jones et al., 1984). Antimicrobial activity of propolis contributed to the presence of alkaloids (metabolite products of proteins), nitrogen alkaline which has pharmaceutical properties, it helps in the treatment of wounds and burn infection (Harbone, 1984).

Conclusion

Thus, it was concluded that the EEP was the most active of all the extracts, showing the maximum zone of

inhibition of 30 mm at the 30% concentration and the MIC value was 640 µg/ml. *S. aureus* was more sensitive to propolis extract than other bacteria and agar diffusion method was better than disc diffusion method for detection of antimicrobial activity of propolis. Further studies can be done for the identification of the chemical compounds responsible for the antimicrobial activity and its isolation along with its characterization.

REFERENCES

- Abd EFK, Hegazi AG (2002). Egyptian Propolis: Chemical composition, antiviral and antimicrobial activities of East Nile Delta propolis. *Z Nat. Sci., Afr. J. Biotechnol.*, 57: 386-394.
- Abd-El-Salam KS, Mohamed MI, El-Nawawy MA (1989). Effect of propolis on some bacterial species.. *Egypt J. phytopathol. (Egypt)*, 21(1): 61-67.
- Aboaba OO, Efuwape BM (2001). Antibacterial properties of some Nigerian spices. *Bio. Res. Comm.*, 13: 183-188.
- Al-Ammar MHM (2001). Effects activity of propolis components on some species of pathogenic bacteria. M. Sc. thesis. Kufa University, Iraq.
- Al-Salamy NYA (2000). Study of effect the *Myrtus communis* and *Allium staviium* L. extract on *Pseudomonas*. M. Sc. thesis., Kufa University, Iraq.
- Al-Zubady BA (2009). Study of effect ethanolic extract of propolis on growth of *Staphylococcus aureus* in vitro. *Karbala Univ. J.*, 389: 25-30
- Bankova VS, Castro DSL, Marcucci MC (2000). Propolis: recent advances in chemistry and plant origin. *Apidologie*, 31: 3-15.
- Bauer AW, Kirby WAW, Sherris JS, Turk M (1966). Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.*, 45: 493-496.
- Bayoub K, Baibai T, Mountassif D, Retmane A, Soukri A (2010). Antibacterial activities of the crude ethanol extracts of medicinal plants against *Listeria monocytogenes* and some other pathogenic strains. *Afr. J. Biotechnol.*, 9(27): 4251-4258.
- Bonhevi JS, Jorda RE (1994). The composition active components and bacteriostatic activity of propolis dietetics. *J. Am. Oil Chem. Soc.*, 7: 532-538.
- Bosio K, Avanzini C, D'Avolio A, Ozino O, Savoia D (2000). *In vitro* activity of propolis against *Streptococcus pyogenes*. *Lett. Appl. Microbiol.*, 31: 174-177.
- Burdock GA (1998). Review of the biological properties and toxicity of bee propolis. *Food Chem. Toxicol.*, 36: 347-363.
- Clinical and Laboratory Standards Institute (CLSI) (2010). Performance standards for antimicrobial susceptibility testing. Approved standard M100-S20. Vol. 30, No. 1. National Committee for Clinical Laboratory Standards, Wayne, PA, USA.
- Daniel WW (1988). *Biostatistics: A foundation for analysis in health sciences*. 4th ed. John Wiley and sons Inc, USA.
- Darwish RM, Abu FRJ, Abu ZMH, Nazer IK (2010). Antibacterial effect of Jordanian propolis and isolated flavonoids against human pathogenic bacteria. *Afr. J. Biotechnol.*, 9(36): 5966-5974.
- Forbes BA, Sahm DF, Weissfeld AS (2007). *Bailey and Scotts' Diagnostic microbiology*. 12th ed. Elsevier. Germany.
- Glinski Z, Meresta T (1993). Trials to standardize an antibacterial activity of propolis. *Vet. Med.*, 49: 27-29.
- Gonzalez BG, Martinez-Aguilar G, Hulten KG (2005). Severe staphylococcal sepsis in adolescents in the era of community acquired methicillin-resistant *Staphylococcus aureus*. *Pediatrics*, 115: 642-648.
- Harbone JB (1984). *Phytochemical methods. A guide to modern techniques of plants analysis*. 2nd.ed. New York, Chapman and Hall.
- Hegazi AG, Abd EFK (2002). Egyptian propolis: 3. antioxidant, antimicrobial activities and chemical composition of propolis from reclaimed lands. *Z Nat. Sci.*, 57: 395-402.
- Hernandez M, Lopez R, Abanal RM, Daria V, Arias A (1994). Antimicrobial activity of *Visnea mocanera* leaf extracts. *J. Ethnopharmacol.*, 41: 115-119.
- Holderma E, Kedzia B (1987). Investigations upon combined action of propolis and antimycotic drugs on *Candida albicans*. *Herba Pol.*, 33: 145-151.
- Jones GA, Macalisher TA, Muri AD, Cheng KJ (1984). Effects of *Sainfion conobrychis* and proteolysis by four of luminal Bacteria. *Appl. J. Environ. Microbiol.*, 60: 1374-1378.
- Kartal M, Yildiz S, Kaya S, Kurucu S, Topçu G (2003). Antimicrobial activity of propolis samples from two different regions of Anatolia. *J. Ethnopharmacol.*, 86: 69-73.
- Katircio H, Nazime M (2006). Antimicrobial activity and chemical compositions of Turkish propolis from different regions. *Afr. J. Biotechnol.*, 5: 1151-1153.
- Kimoto T, Aral S, Kohguchi M, Aga M, Nomura Y, Micallef MJ, Kurimoto M, Mito K (1998). Apoptosis and suppression of tumor growth by artemillin C extracted from Brazilian propolis. *Cancer Detect Prev.*, 22: 506-515.
- Kosalec I, Bakmaz M, Pepeejnjak S, Kneevi SV (2004). Quantitative analysis of the flavonoids in raw propolis from northern Croatia. *Acta Pharm.*, 54: 65-72.
- Kujumgiev A, Tsvetkova I, Serkedjieva Y, Bankova V, Christov R, Popov S (1999). Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. *J. Ethnopharmacol.*, 64: 235-240.
- Kumer N, Mueen AKK, Raman D, Ahmed H (2008). Antioxidant and antimicrobial activity of propolis from Tamil Nadu zone. *J. Med. Plants Res.*, 2(12): 361-364.
- Marcucci MC (1995). Propolis: chemical composition, biological properties and therapeutical activity. *Apidologie*, 26: 83-99.
- Meresta T, Meresta T (1980). Effect of pH on bactericidal activity of propolis. *Bull. Vet. Inst. Pulawy*, 24: 1-4.
- Mirzoeva OK, Grishanin RN, Calder PC (1997). Antimicrobial action of propolis and some of its components: the effects on growth, membrane potential and motility of bacteria. *Microbiol. Res.*, 152: 239-246.
- Moreno MIN, Isia MI, Sampietro AR, Vattuone MA (2000). Comparison of the free radical- scavenging activity of propolis from several regions of Argentina. *J. Ethnopharmacol.*, 71: 109-114.
- Mourey A, Canillac N (2002). Anti-*Listeria monocytogenes* activity of essential oils components of conifers. *Food Control*, 13: 289-292.
- Muhammad H, Muhammad S (2005). The used of *Lawsonia inermis* Linn. (Henna) in the management of burn wound infections. *Afr. J. Biotechnol.*, 4(9): 934-937.
- Mukherjee PK, Giri SN, Saha K, Pal M, Saha BP (1995). Antifungal screening of *Nelumbo nucifera* (Nymphaeaceae) rhizome extract, *Indian J. Microbiol.*, 35: 320-327.
- Musa TN, Mohamed SH (1992). Inhibitory effect of *Peganum harmala* extract on some pathogenic bacteria and microbial content of minced beef. *Basrah Agric. Sci.*, 5(2): 189-194.
- NCCLS (2002). National Committee for Clinical Laboratory Standards, Methods for dilution antimicrobial susceptibility tests of bacteria that grow aerobically. Approved Standard M100-S12. Wayne, PA.
- Nieva-Moreno MI, Isla MI, Cudmani NG, Vattuone MA, Sampietro AR (1999). Screening of antibacterial activity of *Amaicha delvalle* (Tucuman, Argentine) propolis *Ethno pharmacol.*, 168: 97-102.
- Olila D, Olwa O, Opuda-Asibo J (2001). Antibacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis*. *Ugandan medicinal plants, Afr. Health Sci.*, 1: 66-72.
- Ophori EA, Eriagbonye BN, Ugboadaga P (2010). Antimicrobial activity of propolis against *Streptococcus mutans*. *Afr. J. Biotechnol.*, 9(31): 4966-4969.
- Park YK, Alencar SM, Aguiar CL (2005). Botanical Origin and Chemical Composition of Brazilian Propolis. *J. Agric. Food Chem.*, 50: 2502-2506.
- Pepejnjak S, Maysinger D, Jalsenjak I (1982). Effect of propolis extract on some fungi. *Sci. Pharm.*, 50: 165-167.
- Prindle RF, Wright ES (1997). Phenolic compounds. In S. S. Block (Ed.). *Disinfection, sterilization and preservation*. Philadelphia: Lea and Febiger.
- Prytyk E, Dantas AP, Salomão K, Pereira AS, Bankova VS, De CSL, Neto FRA (2003). Flavonoids and trypanocidal activity of Bulgarian propolis. *J. Ethnopharmacol.*, 88: 189-193.

- Ravn H, Andary C, Kovacs G, Moelgaard P (1989) Caffeic acid esters as in vitro inhibitors of plant pathogenic bacteria and fungi. *Biochem. Syst. Ecol.*, 17: 175-184.
- Scheller S, Kawalski H, Okiek K, Dworniczak S, Matsuno T, Waldemar-Klimmek K, Rajca M, Shani J (1998). Correlation between virulence of various strains of mycobacteria and their susceptibility to ethanolic extract of propolis. *Z Nat. Sci.*, 53C: 1040-1044.
- Scheller S, Dworniczak S, Waldemar-Klimmek K, Rajca M, Tomczyk A, Shani J (1999). Synergism between ethanolic extract of propolis (EEP) and anti-tuberculosis drugs on growth of mycobacteria. *Z Nat. Sci.*, C54(7-8): 449-453.
- Sforcin JM, Fernandes JRA, Lopes, CAM, Bankova V, Funari SRC (2000). Seasonal effect on Brazilian propolis antibacterial activity. *J. Ethnopharmacol.*, 73: 243-249.
- Sikkema J, De BJAM, Poolman B (1994). Interactions of cyclic hydrocarbons with biological membranes. *J. Biol. Chem.*, 269: 8022-8028.
- Simone-Finstrom M, Spivak M (2010). Propolis and bee health: the natural history and significance of resin use by honey bees. *Apidologie*, 41: 295-311
- Stepanovi S, Anti N, Daki IV (2003). *In vitro* antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. *Microbiol. Res.*, 158: 353-357.
- Takalsi-Kikuni NB, Schilcher H (1994). Electron microscopic and Microcalorimetric investigations of the possible mechanism of the antibacterial action of a defined propolis provenance. *Planta Med.*, 60: 222-227.
- Taylor RSL, Manandhar NP, Hudson JB, Towers GHN (1996) . Antiviral activity of Nepales medicinal plants. *J. Ethno. pharmacol.*, 52: 156-163.
- Tegos G, Stermitz FR, Lomovskaya O, Lewis K (2002). Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. *Antimicrob. Agents Chemother.*, 46: 3133-3141.
- Trusheva B, Popova M, Bankova V, Simova S, Marcucci MC, Miorin PL, Pasin FR, Tsvetkova I (2006). Bioactive constituents of Brazilian red propolis. *Evid-Based Compl. Alt.*, 3: 294-254.
- Twajj HAA, Sayed AHM, AL-Zohayri AM (1988). Pharmacologic, phytochemical and antimicrobial studies on *Myrtus communis*. *J. Biol. Sci. Ros.*, 19(11): 441-452.
- Yaghoubi SMJ, Ghorbani GR, Soleimani Zad S, Satari R (2007). Antimicrobial activity of Iranian propolis and its chemical composition. *DARU*, 15: 45-48.