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Full Length Research Paper

In vitro antibacterial and antifungal activity of Iraqi propolis

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Abstract:

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 higher sensitive to EEMP than other Gram positive and Gram negative bacteria, while standard *E. 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 *C. 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 differences ($P \leq 0.05$) 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. 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 (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. 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).

Although numerous researchers have been 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 gm 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 shaked 2 or 3 times per day and returned to warm dark place. The liquid was filtered through Whatman No.1 and the water was evaporated by oven at

45 ºC, then the extract was weighed and stored in dark clean container for further using. Water or aqueous extract was dissolved by distillated water, sterilized by filtration (using Millipore 0.45 filter paper), and the requisite dilutions were prepared.

Ethanolic extract of propolis:

Ten gm 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 shaked 2 or 3 times per day and returned to warm dark place. The liquid was filtered through Whatman No.1 and the water was evaporated by oven at 45 ºC, then the extract was weighed and stored in dark clean container for further using. 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 ºC. The identification of the local bacterial isolates was confirmed using conventional biochemical tests (Forbes *et al*.,2007).

Isolation and identification of *Candida albicans*

Candida 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 37ºC. The fungal culture was examined according to colonies, cellular morphology and germ tube formation (Forbes *et al*.,2007).

Table (1): Standard and local bacterial strains

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

***In vitro* antibacterial and antifungal activities of crude propolis extract**

1-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%, 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 hours allowing for diffusion of the solution. All plates were then incubated at 37 ºC for 24 hr, and the zones inhibition were subsequently measured in millimeters (Mukherjee *et al*., 1995).

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

Petri plates containing 25 ml of Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for *Candida albicans* were used. Agar media were seeded with a 24 hr- old culture of the microorganism strains (by sterile cotton swab dipped into the broth of these microorganism). Four wells (5 mm diameter) were cut into the agar by cork borer and 0.1ml of the crude propolis extracts was applied in each well. The inoculums 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 ºC for 24hr. 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-µL 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 hours.

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 (µg/ml) at which no more than two colonies were detected (CLSI, 2010).

Statistical analysis

Bonferroni test was used for statistical analysis ($P \leq 0.05$) to show if there is any significant differences 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%, 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 higher sensitive to EEMP than other Gram positive and Gram negative bacteria followed by *L. monocytogenes* with inhibition zones of 25 mm 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 *S. pyogene*s was 14 mm while the zones of inhibition for each of *S. typhi, and K. pneumoniae* were12 mm. The zone of inhibition was10 mm for each of *P. aeruginosa*, *H. pylori*, and *E. aerugenes.* 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 mm and 30 mm respectively, whereas the zones of inhibition of *C.albicans* were 10 mm and 12 mm respectively. EEMP possessed a good antibacterial and antifungal activity against bacteria and fungi at different concentrations 10%, 20%, 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 80–100 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 and 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 Prytzyk *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 - 23 mm.

These differences in antibacterial activity of propolis from the different regions in 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

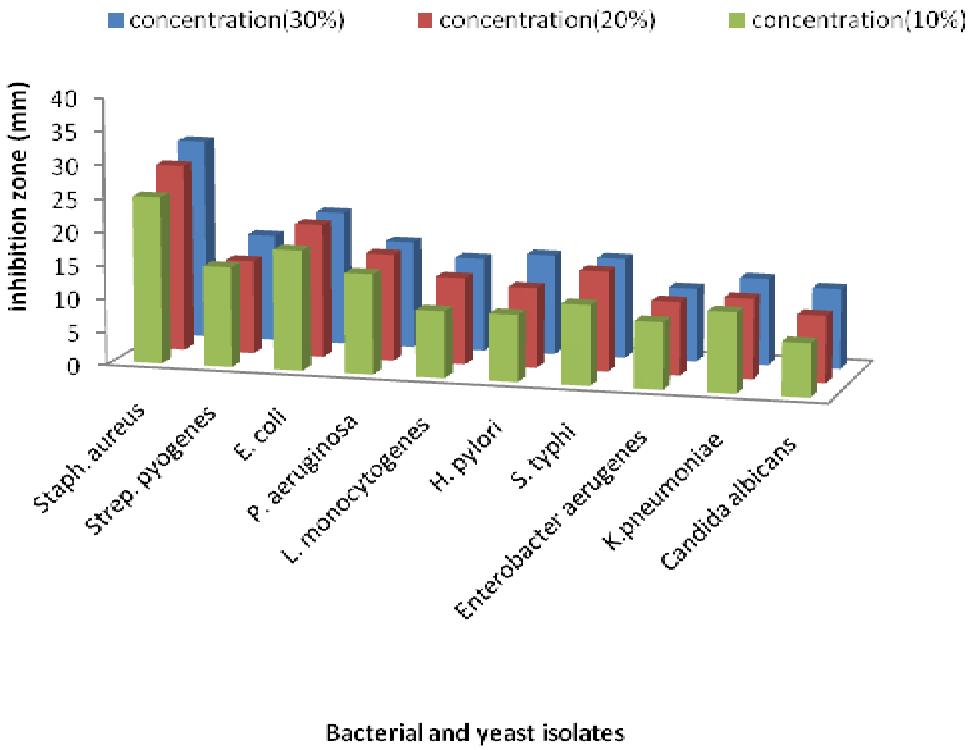

Figure (1) Effect of ethanolic extracts of Al-Museiab crude propolis on the bacterial and fungal isolates at different concentrations by well diffusion test.

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 *Strep. pyogene*s were 12 mm. The zones inhibitions were 11, 10, and 7 mm for each of *S. typhi, K. pneumoniae* and *H. pylori* respectively while the zones 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 increased of concentration of propolis due to increased 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 differences ($P \leq 0.05$) at concentrations of 20% and 30% of propolis ethanolic extract, respectively.

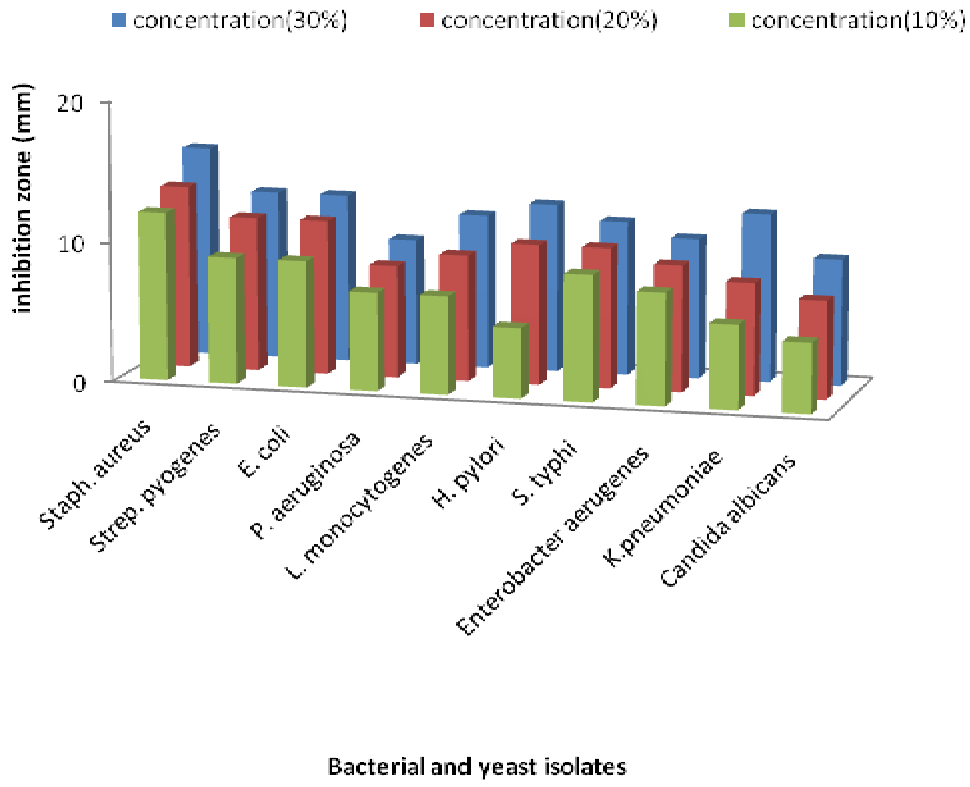

Figure (2) Effect of ethanolic extracts of Al-Museiab crude propoli on the bacterial and yeast isolates at different concentration by disc diffusion method.

Biological and pharmaceutical activity of propolis may contributed to the fact that propolis contains active compound such as phenols, flavonoids and alkaloids that possessing antibacterial and antifungal activities against bacteria and fungi. This results were comparable with results obtain by several authors (Scheller *et al.,*1999 ; Abd-El- Salam, 1989).

Moreover, determination of minimum inhibitory concentration of EEMP at different concentrations (10%, 20%, 30%) against bacterial and fungal isolates was determined (Table 2). MIC of EEMP at 10% concentration against *S. aureus* and *St. pyogene*s were ≥1280 µg /ml while it was ≥ 2560 µg /ml against each of Standard *E. coli* strain*, S. typhi*, *L. monocytogenes* and *P. aeruginosa.* The MIC value was increased (5120 µg /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 *St. pyogene*s was ≥640 µg/ml. The MIC was increased (≥1280 µg/ml) against standard *E. coli* strain and *L. monocytogenes* and the value was dramatically increased (≥2560 µg /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*. (1999) reported that propolis collected from Argentine 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.,* 1999; 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.*

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(µg /ml)	**MIC(µg /ml)**	**MIC(µg /ml)**
S. aureus	1280≥	1280≥	≥640
St. pyogenes	1280≥	1280≥	≥640
E. coli	2560≥	2560≥	1280≥
P. aeruginosa	2560≥	2560≥	2560≥
L. monocytogenes	2560≥	2560≥	1280≥
H. pylori	5120≥	5120≥	2560≥
S. typhi	2560≥	2560≥	2560≥
E. aerogenes	5120≥	5120≥	2560≥
K. pneumoniae	2560≥	2560≥	2560≥
Candida albicans	≥5120	≥5120	2560≥

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 15mm 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; Prytzyk *et al*., 2003; Stepanovi *et al.,* 2003). The MIC value against standard *E. coli* was 2560 µg/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.,* 1999; 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 transuding 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 26-14mm and MIC value was 0.25-11.75 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- 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 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 & 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. Takaisi-Klkuni and Schilcher (1994) showed through electron microscopy and micro-calorimetric assays that ethanolic extracs 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 of 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-Klkuni and Schilcher (1994) stated that the propolis inhibits bacterial growth by preventing cell division, thus resulting in the formation of pseudo-multiceullar 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. aerugenes*, and *C. albicans* were not to be affected by AEMP.

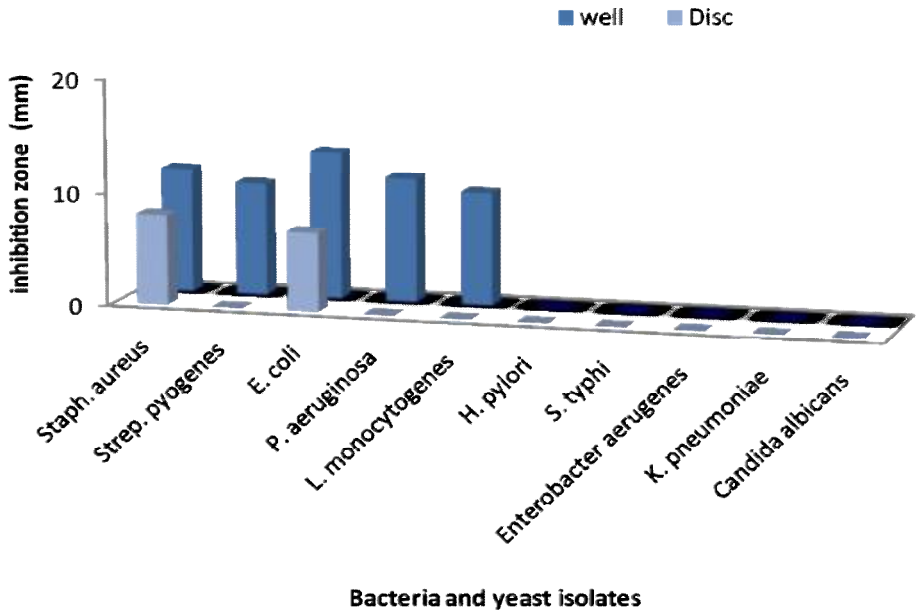

Figure (3) Effect of aquatic extracts of Al-Museiab crude propolis on the bacterial and yeast isolates at 10% concentration disc and well.

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 7mm 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 ($P \leq 0.05$).

This results were in agreement with Al-Ammar (2001) who pointed out that zones of inhibition of *S. aureus* and *E. coli* were 8 mm and 7mm 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*, *St. pyogene*s and standard strain *E. coli* were ≥2560 µg/ml, whereas *S. typhi*, *H. pylori L. monocytogenes*, *P. aeruginosa*, *E. aerugenes* , *K. pneumoniae,* and *C. albicans* were ≥5120 µg /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 the active components of propolis extracted. EEMP possessing number of active components that had inhibition effect on microbial growth more than AEMP but the antimicrobial activity depends on type of extracts with increased of concentration. AEMP were not effective against *S. typhi*, *H. pylori L. monocytogenes*, *P. aeruginosa*, *E. aerogenes*, *K. pneumoniae,* and *C.albicans* due to decreased number of active components of propolis extracts and bacterial resistance to these extracts, this results were in agreement with (Nieva-Moreno *et al.,*1999).

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 (µg /ml)
S. aureus	2560≥
S. pyogenes	2560≥
E. coli	2560≥
P. aeruginosa	5120≥
L. monocytogenes	5120≥
H. pylori	5120≥
S. typhi	5120≥
E. aerugenes	5120≥
K. pneumoniae	5120≥
Candida albicans	5120≥

Al-Zubiedy (2009) reported that the zones of inhibition of *S. aureus* was 16 mm at 150% concentration of propolis collected from Al-Kufa. 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 regarding

largest component of that 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 combine with the microbial cell wall and growth inhibition (Jones *et al*.,1984).

Antimicrobial activity of propolis contributed to the present of alkaloids (metabolite products of proteins) is Nitrogen alkaline which had pharma-ceutical properties it help in 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.

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