International Journal of Medicine and Pharmaceutical Sciences (IJMPS)
ISSN(P): 2250-0049; ISSN(E): 2321-0095
Vol. 4, Issue 4, Aug 2014, 27-34

ANTIMICROBIAL ACTIVITIES OF IRAQI PROPOLIS; AN *INVITRO* STUDY

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ABSTRACT

Background: 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.

Aims: This paper aimed in part to evaluate the antibacterial activity of propolis against ten bacterial pathogens. The active components of propolis were also investigated.

Methods: Propolis samples were collected during spring and summer seasons, 2011. The antibacterial effects of propolis and active components of propolis against some Gram-positive isolates, Gram-negative isolates and yeast isolates. These organisms included local isolates represented by *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Listeria monocytogenes, Helicobacter pylori, Enterobacter aerugenes, Klebsiella pneumoniae,* and *Candida albicans* in addition to the standard strains represented by *Escherichia coli* ATCC 25922 and *Salmonella typhi TY21*. The local isolates were isolated from clinical cases and fully identified in our laboratory.

Results: Antimicrobial activities of crude extract of Al-Museiab propolis (CEMP) and active components of propolis at 10, 20 and 30% concentration against bacterial isolates were studied. The results of agar diffusion showed that most bacterial isolates were sensitive to CEMP. *Staphylococus aureus* was highly sensitive to CEMP than other Gram positive and Gram negative bacteria with an inhibition zones of 30mm. diameter. All bacterial isolates were highly sensitive to the component of propolis showing maximum an inhibition zone of 30 mm at the 25% concentration.

Conclusions: Propolis possesses considerable antimicrobial activity against gram-positive and gram-negative bacteria together with fungi presented by *Candida albicans*, accordingly propolis can be used for treatment of microbial infections as it appears to satisfy all of the criteria as antibacterial agents. is natural and safe for human use.

KEYWORDS: Propolis, Antibacterial Activity, Pathogenic Microorganisms

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. Its biological properties, e.g. antibacterial, antiviral, antifungal and others activities have attracted the researchers interest[1].

The biological properties and components of Propolis 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[2].

Worldwide studies have shown broad spectrum antimicrobial activity of various propolis extracts. 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[3]. Some other studies have reported that the antibacterial activity of Propolis can be attributed to a number of phenolic compounds, mainly flavonoids, phenolic acids and their esters[4,2,5,6]. Furthermore, the volatile compounds, diterpenes, cinnamic acid derivatives and flavonoids have been reported to be responsible for ununcoupling the energy transduction of cytoplasmic membrane inhibiting bacterial motility which might contribute to the antibacterial action of propolis[7]. Although numerous researchers have been reported However, the biological activities of propolis have investigated worldwide but information about Iraqi propolis has not been studied yet, accordingly, this study has suggested and designed to evaluate the antibacterial activity of propolis against ten microbial pathogens.

MATERIAL AND METHODS

Preparation of Ethanolic Extract of Propolis

Propolis samples were collected from hives of honey bees of Al-Museiab (Iraq) during all seasons of 2010. Propolis samples were cleaned, free of wax, paint, wood, cut it into small pieces and placed in clean container.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 with gently shaking 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 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[8].

Bacterial Strains

Standard bacterial strains and local isolates used in this study are listed in Table 1. The sources of these organisms are indicated opposite each one. The local isolates being obtained were fully reidentified in our laboratory[9].

Table 1: The Organisms Included in this Study

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

Antibacterial Susceptibility Test

This test was carried out according to NCCLS 2002[10] A Loop full growth from each isolate was inoculated into nutrient broth and incubated at 37 °C for 18 hours. The bacterial suspensions were diluted with normal saline. The turbidity broth culture was adjusted with standard tube (McFarland number 0.5) to yield a uniform suspension with cell density of 1.5×10^8 CFU / ml. A cotton swab was dipped and streaked on Mueller-Hinton agar plates and the plates were left for 5 -15 minutes at room temperature to dry. Four wells were prepared in each plates using the cork borer with a diameter of 5 mm. and a volume of 20µl of the propolis extracts was dropped in each well (the plates were prepared in triplicates). Then, the plates were incubated at 7 37°C for 24 hrs. After incubation period, the diameter of the inhibition zone was measured by measuring scale in millimeter (mm).

Statistical Analysis: Bonferroni test recommended by Danial, and colleagues[11] was used for statistical analysis ($P \leq 0.05$) to show if there is any significant differences between results of agar diffusion methods of propolis ethanolic extract.

RESULTS AND DISCUSSIONS

In this study, the antimicrobial activities of crude extract of EEMP at different concentration (10%, 20%, and 30%) against both bacteria and fungi were investigated. It has been stated that propolis is considered as an active reagent against microorganisms (bacteria and fungi) when the inhibition zone is greater than 6 mm[12]. Figure 1 shows the antimicrobial activities of crude ethanolic extract against bacteria and yeast. The results of agar diffusion test at 10% concentration showed that most bacterial isolates were sensitive toward EEMP. *S. aureus* revealed higher sensitive than other Gram-positive and Gram-negative bacteria as well, followed by *L. monocytogenes* with an inhibition zones of 25 mm. and 18 mm. respectively. Standard strain *E. coli* was quite sensitive compared with other Gram negative bacteria with an inhibition zones of 15 mm. The inhibition zone resulted by *S. pyogenes* was 14 mm., while the inhibition zones for each of *S. typhi* and *K. pneumoniae* were 12 mm. The susceptibility of *P. aeruginosa*, *H. pylori* and *E. aerugenes* seems to be moderate since the inhibition zone was 10 mm. in diameter for each. However, no considerable effect for EEMP be observed against *C. albicans*.

On the other hand, the effectiveness of EEMP was elevated at an increased concentrations up to 20% and 30%. The inhibition zones of *S. aureus* were 28 mm. and 30 mm, respectively, whereas the inhibition zones of *C. albicans* were 10 mm. and 12 mm. respectively. Accordingly, one can conclude that EEMP possesses an influential antibacterial and antifungal activity against bacteria and fungi. This activity is extrusive proportioning with an increase concentration. Statistical analysis showed no significant differences after treating the microorganisms with propolis ethanolic extract at different concentration by agar diffusion method ($P\leq 0.05$). These results were in agreement with Goodwin[13], who stated that the inhibition zones were extrusive proportioning with an increas of concentration which can be sttributed to the active components of propolis which estimated as high as 80 – 100 types of chemical compounds being extensively studied worldwide[14, 15, 16, 17, 18,19].

The results being recorded for *S. aureus* are 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[20].

These results are also comparable with results obtained by Prytzyk and colleagues[21] who found that the inhibition zone with Bulgarian propolis was 20 mm. and 18 - 23 mm. with propolis from different geographical areas of Serbia[22].

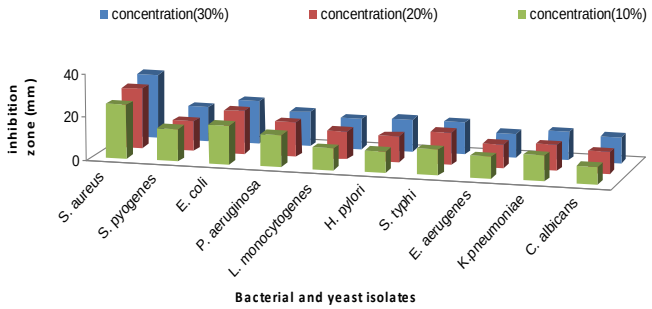

Figure 1: Effect of Ethanol Crude Extracts of Al-Museiab Propolis on the Bacteria and Yeast Isolates at Different Concentrations in Agar Diffusion Test

The susceotiblity of microorganisms towards propolis extract was also carried out by determination of minimum inhibitory concentration (MIC) at 10%, 20% and 30% concentrations as shown in table 1 below. MIC values at 10% concentration of *S. aureus* and *S. pyogenes* were ≥1280 µg /ml, while it was ≥2560 µg /ml against each of *E. coli*, *K. pneumoniae*, *S. typhi*, *L. monocytogenes* and *P. aeruginosa*. MIC value was increased (≥5120 µg /ml) against each of *C. albicans E. aerugenes* and *H. pylori* at the same concentration.

However the results can be varied according to type and source of propolis Sforcin *et al*. (2000). The variation might reflect the difference in the composition of the propolis. The lower sensitivity (or resistance) of *E. coli* is in agreement with the findings by many researchers where this bacterium showed either very low sensitivity or total lack of sensitivity against propolis[20,23,24]. This emphasizes the fact that, Gram-negative bacteria are less sensitive than Gram-positive strains, which is in agreement with several other reports[25,26,27,28,24].

The most possible explanation for the low sensitivity of gram-negative bacteria is due to the fact that their outer membrane inhibits and/or retards the penetration of propolis molecules or the organisms is already a multi-drugs resistant by possession some mechanisms, e.g. for drugs resistance[29].

Regarding anti- *L. monocytogenes*, the results in this study was in accordance with Bayoub *et al*.[30] who mentioned that the diameter of inhibition zone of ethanolic extract against *L. monocytogenes* accounted for 14mm.-26mm. and the MIC ranged 0.25-11.75 mg\ml.

Activity of 30 % EEP against of *H. pylori* was evaluated by using the agar diffusion method and the diameter of inhibition zone was 21.4mm.[31]. It was noted that disk diffusion assay and agar well diffusion method exhibited coordinated results, but in another study, the disc diffusion revealed a low activity of ethanolic extracts[32].

However, it is agreed that, the variability of propolis activity can be attributed in part to geographic regions, cliamatis factors, e.g. temperature, moisture, type of plantation and experimental conditions[33,34,35].

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
S. 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. aerugenes	5120 ≥	5120 ≥	2560≥
K. pneumoniae	2560 ≥	2560 ≥	2560 ≥
C. albicans	5120 ≥	5120 ≥	2560 ≥

The mechanism of antibacterial action of propolis has been the subject of only a few publications. It has been shown that by using electron microscopy and micro-calorimetric assays that propolis interferes with the division of bacterial cell through the formation of pseudo-multicellular forms, cytoplasm disorganization or bacterial cytoplasm, cell membrane and cell wall collapse and inhibition of protein synthesis leading to lysis of the bacteria[36]. Furthermore, found that EEP and some of phenolic components in propolis 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 bacteria[37]. A synergistic effect with conventional anti-mycotic drugs was also observed[38,39].

Analysis of propolis compostions revealed various active components, e.g. mucilage, alkaloids, terpenoids, saponin, flavonoids, phenolic compounds, tannins and others[40].

CONCLUSIONS

In light of the results documented in this study, one can conclude that propolis possesses considerable antimicrobial activity against gram-positve and gram-negative bacteria together with fungi pepresented by *Candida albicans*, accordiningly propolis can be used for treatment of microbial infections as it is natural and safe for human use.

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