Original Article

EVALUATION OF INHIBITORY EFFECTS OF IRANIAN PROPOLIS AGAINST FILAMENTOUS BACTERIA

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ABSTRACT

Objective: To investigate the antibacterial activities of propolis in samples collected from Zanjan province IRAN, against 25 pathogenic strains of bacteria.

Methodology: In order to evaluate the biological properties of methanol extract of propolis using agar distribution methods (disk and drop plate). Seven concentrations of methanolic extract of propolis were prepared and added drop wise to the bacterial seed layer cultured agar media individually. The diameter of the clear zone formed in each concentration was measured and correlated to the ability of the extracts to inhibit the growth of bacteria.

Results: *Nocardia asteroides* and *N. brasiliensis* has nearly shown the same susceptibility to various concentrations of propolis extract, and the complete clear zones revealed that this effect was quite remarkable. For other bacteria, different degrees of susceptibility to propolis were observed.

Conclusions: We came to this conclusion that zones formed by 50mg/ml Amikacin in agar was similar to that of 5% concentration of propolis, and that the potency of propolis is 80% of Amikacin potency, which is the most effective antibiotic against *Nocardia*.

KEYWORDS: Propolis, *Nocardia*, Antibacterial activity, Flavonoids, Caffeic acid.

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INTRODUCTION

One of the most useful products of the beehive is propolis (bee glue), a brownish and resinous substance that is collected by worker bees from the leaf buds and cracks in the bark of numerous tree species. Bees mix the original propolis with beeswax and ß-glucosidase they secrete during the propolis collection.[1] Since ancient time, propolis was employed as an antiseptic[2,3] and cicatrizant in wound treatment,[4,5] and as mouth disinfectant,[5] an antipyretic agent.[6,7] Flavonoids content and caffeic acid phenethyl esters (CAPE), active component of propolis,[2,4,8] may be responsible for most of this biological activities.[9-11] At a concentration of 10 µM, CAPE completely blocks the production of reactive oxygen species in human neutrophils and in the xanthine /xanthine oxidase (X/XO) system.[12] These observations attracted the attentions to the antibacterial properties of this substance. The main purpose of the present study was to evaluate the antibacterial activity of methanolic extract of propolis.

Propolis Compounds: Propolis is composed of 50% resin (composed of flavonoids and related phenolic acids), 30% wax, 10% essential oils, 5% pollen and 5% various organic compounds.[6] Polyphenols due to their proven abil-

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ity to inhibit specific enzymes are considered as the main pharmacologically active molecules in propolis.[13] Clinical pharmacological studies show the potential beneficial effects of propolis product as an adjuvant to treat asthmatic[14] and gastro duodenal ulcers.[11] Propolis contains a high concentration of flavonoids, which are used in a wide range of cosmetics and health food preparations for their antimicrobial properties.[15,16] At present, propolis is one of the most popular health food products and is available commercially through all over the world.[14-17]

Microbiology: Nocardia, a gram positive variably acid-fast aerobic bacterium is an opportunistic pathogen in immunocompromised hosts. The species have pathogenic potential to human, including *Nocardia asteroides, N. farcinica* and *N. brasiliensis.*[18-19] Because of influence of propolis in the treatment of both cutaneous and respiratory disorders, the efficacious and antibacterial properties of propolis on the above bacteria have been studied.

MATERIALS AND METHODS

Microorganisms: In this study a total number of 25 different bacterial strains were provided from School of Public Health, Teheran University of Medical Sciences, Iran. The bacteria used were included of six strains *Nocardia asteroides*, seven strains *Nocardia brasiliensis* as original bacteria and two strains of each *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterobacter cloaceae, Shigella flexneri* and *Staphylococcus aureus* as elective bacteria. The cultures were stored at –70ºC in brain heart infusion (BHI) broth containing 20% glycerol.

Media and Sensi-Disk: The following microbiological media and antimicrobial susceptibility test discs were supplied from Becton Dickinson Microbiology Systems Cockeysville Maryland (BBL products):

Glycerol Yeast Extract agar (GYEA), Brain Heart Infusion Broth (BHI) containing 20% glycerol, Sabouraud's Dextrose Agar (SDA), Mueller Hinton Agar (MHA), Blood Agar base (BA) and test disks including; Amikacin (AN-30) (31597), Amoxicillin(AmC-30)(31629), Ceftizoxime (ZOX-30)(31623), Cephalothin (CF-30) (31271), Chloramphenicol (C-30) (31274), Gentamicin (GM-10)(31299), Kanamycin (K-30) (31301), Neomycin (N-30) (31313), Ceftazidime (CAZ-30) (31633), Streptomycin (S-10) (31328), (Co-trimoxazole) Sulfamethoxazole with Trimethoprim (SXT-23.75) (31539), Tetracycline (Te-30) (31344). Antimicrobial susceptibility test against *Nocardia* strains was performed using the above antimicrobial susceptibility test discs.

Preparation of Propolis extracts: Crude propolis samples collected by *Apis mellifera* were obtained from hive bee's located in the province of Zanjan (Northwestern IRAN). About 2 g of propolis was dissolved in an appropriate amount of Merck ethanol. The extract was evaporated and filtrated aseptically under flow cabinet. The sticky extract yielded (specific gravity of 1.3), was used to prepare appropriate concentrations for tests. Seven concentrations of methanolic extract of propolis were prepared using 80% methanol.

Determination of the inhibitory activity: Two distribution methods were used to evaluate the inhibitory effects of propolis against *Nocardia* strains. The appropriate amounts of each concentration (0.1, 0.2, 1, 2, 5, 8 and 10%) of propolis were added to the surface of GYEA and MHA plates in the middle of every marked area using Gilson sampler (drop plate as a quantitative method). The similar concentrations of propolis were used for disk plate procedure as a qualitative method. The media used for the distribution methods mentioned above were prepared by inoculating the agar media with a pure culture of *Nocardia asteroides* and *Nocardia brasiliensis* respectively. The duplicate cultured plates were incubated in two different temperatures (37°C and 42°C). The same extract concentrations were applied to the surface of appropriate media inoculated with a pure culture of each following bacteria; *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterobacter cloaceae, Shigella flexneri* and *Staphylococcus aureus.* The cultured plates were incubated at 37°C. Following incubation,

Table-I: The inhibitory effects of propolis against selected pathogenic bacteria using drop and disk plate methods respectively

Concentrations of Propolis Bacteria	*0.1(%)*	*0.5(%)*	*1(%)*	*2(%)*	*5(%)*
	Inhibitory zone diameter (mm) Drop Plate/Disk Plate				
Nocardia asteroides	0/0	14/13	16/20	24/23	30/24
Nocardia brasiliensis	12/12	17/16	23/20	27/22	35/23
Escherichia coli	14/24	15/24	16/23	17/17	19/19
Pseudomonas aeruginosa	12/22	14/20	16/19	18/18	18/18
Klebsiella pneumonia	10/20	11/17	14/15	16/16	18/18
Enterobacter cloaceae	9/18	12/16	14/15	14/18	17/17
Shigella flexneri	10/21	12/21	17/19	18/18	18/18
Staphylococcus aureus	11/27	14/25	17/19	20/20	21/21

the plates were examined and the clear zones of inhibitory surrounding of the bacterial strains were investigated using standard antibiotic clear zones.

RESULTS

According to the results, *Nocardia* strains have nearly shown the same susceptibility to various concentrations of propolis extracts. The comparison of inhibitory effects of propolis on glycerol yeast extract agar plate, against *Nocardia asteroides* using two different distribution methods are demonstrated in Fig-1. The comparison of antibacterial activity, revealed the clearly inhibitory effects of the propolis extracts against all the bacterial strains using drop and disk plate procedures (Table-I). The antibacterial susceptibility test against *Nocardia* strains using different media, showed clear susceptibility to most of standard antimicrobial discs (Table-I). The effects of antibacterial activity of propolis against *Nocardia* strains using disk plate method on different media are demonstrated in figures 2 and 3 respectively.

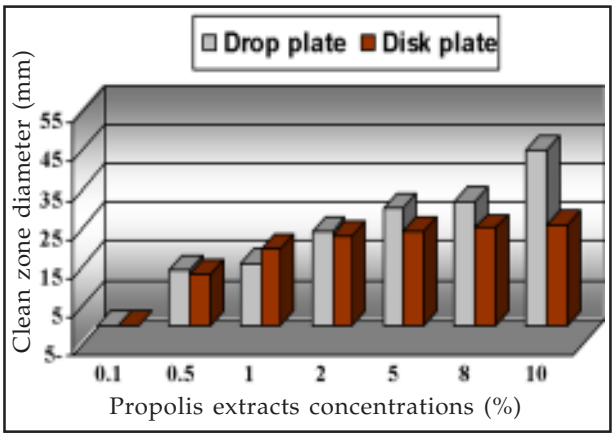

Fig-1: The bacterial activities of propolis against *Nocardia asteroides* on GYEA, using two distribution methods.

DISCUSSION

The studies of propolis against a wide variety of microorganisms all over the world show strong antimicrobial activities.[20-25] However fewer investigations were related to *Nocardia* strains and thus the present study could be unique.

Historical documents in medicine and pharmacy consist of valuable information about propolis. This herbal substance has been used in ancient time for the treatment of malignant tumors and injuries especially in world war.[26-29] Recent pharmacological studies reveal propolis has a vide range of sophisticated composition including anti-inflammatory, anti-oxidative anti-microbial,[30] immunomodulatory properties[31] and polyphenols including flavonoids, phenolic acids.[16] Generally the major antibacterial properties of propolis (especially gram positive bacteria) is attributed to its high percentage of flavonoids content and also the presence of caffeic acid esters.[10,11,16,20-21,27] Since these substances and other polyphenols contents of propolis are portions of the strong antibacterial activities. Analysis of these active ingredients in propolis demonstrated that the composition and contents of these active com-

Table-II: The comparison of clean zone diameter of standard antibiotic disks against *Nocardia* strains

No	*Antibiotic disk*	*Media*	*Clean zone diameter (mm)*	
			N. brasiliensis	*N. asteroides*
1	Ceftazidime	GYEA, BHIA, BA	25	23
2	Ceftizoxime	GYEA, BHIA, SDA	21	20
3	Cephalothin	GYEA, BA	27	22
4	Cotrimoxazole	MHA, SDA	23	25
5	Amikacin	GYEA, BHIA	28	29
6	Amoxicillin	GYEA, BHIA	26	22
7	Tetracycline	GYEA, BHIA, MHA	18	17
8	Neomycin	GYEA, BA, BHIA	19	17
9	Streptomycin	GYEA	17	19
10	Chloramphenicol	GYEA, BHIA	15	14
11	Gentamicin	GYEA, BA, BHIA	11	12
12	Kanamycin	BHIA	13	11

BA = Blood Agar, BHIA = Brain Heart Infusion Agar, MHA = Mueller Hinton,
SDA = Sabouraud's Dextrose Agar, GYEA = Glucose Yeast Extract Agar

ponents depend on various factors such as season,[32] and vegetation of the area, geographical origin[33] and the state of propolis (fresh or aged).[34] There were about 180 kinds of polyphenols isolated from propolis.[7] Among these compounds, rutin, ferulic acid, apigenin, luteolin, quercetin, caffeic acid, flavonoids and phenolic acids are the important active ingredients in propolis.[16] The chemical composition of propolis is variable depending on the geographical origin of this natural substance.[1,16,20,32-34] In this descriptive study, we report a new variety of Iranian propolis which is collected from the Northwestern of the country (Zanjan). As the tables indicate that the inhibitory effect of propolis was complete against *E. coli* and *S. aureus*, while for the other four bacteria the effect was the same and also less than the two mentioned. Theses results indicate that the propolis undoubtedly has antibacterial effects as reported in the previous studies.[20-25]

The results demonstrated that the propolis, especially Iranian sample, could be a promising anti-bacterial agent; however, further *in-vitro* and *in- vivo* studies need to be performed. Although the flavonoids content and caffeic acid phenethyl esters (CAPE), active component of propolis, may be responsible for most of these biological activities,[8-11] but the biological effects of propolis cannot be attributed solely to these contents.

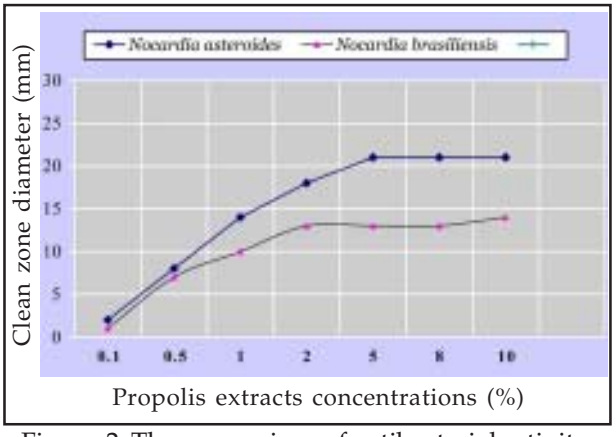

Figure-2: The comparison of antibacterial activity of propolis against *Nocardia* species using Muller Hinton agar

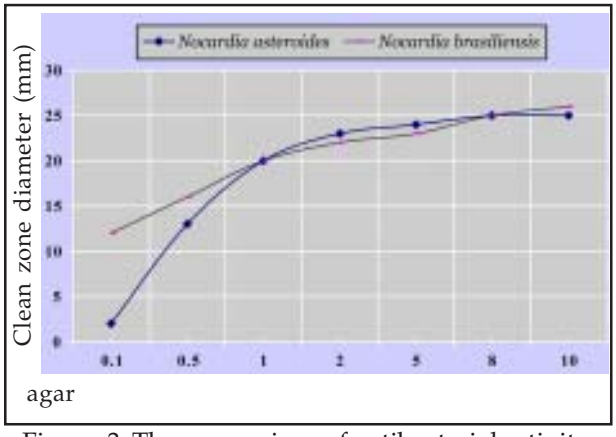

Figure-3: The comparison of antibacterial activity of propolis against *Nocardia* species using glucose yeast extract agar.

CONCLUSIONS

From the present study it is concluded that:

1. Zones formed by 50mg/ml Amikacin in agar was similar to that of 5% concentration of propolis.
2. The potency of propolis is 80% of Amikacin potency, which is the most effective antibiotic against *Nocardia*.

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