Sci-Afric Journal of Scientific Issues, Research and Essays Vol. 2 (12), Pp. 551-553, December, 2014. ***(ISSN 2311-6188)***
www.sci-africpublishers.org

Research Paper

The Antibacterial Effect of Propolis against Clinical Isolates.

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Accepted December 22nd, 2014

ABSTRACT

Propolis, an old and a new natural product collected from beehives by honeybees (*Apis mellifera)* has been used from ancient times. The aim of this study is to determine the antibacterial activity of Propolis against gram positive and negative clinical isolates besides standard bacterial strains. Two different crude Propolis samples obtained from Konya-Turkey and Moscov-Russian Federation (2012 October) were used in this study. Methicillin sensitive Staphylococcus aureus (MSSA), methicillin resistant Staphylococcus aureus (MRSA), extended spectrum beta lactamases producing (ESBL) Escherichia coli and multi-drug resistant (MDR) Acinetobacter baumannii clinical isolates were tested. Thirty isolates from each bacterium enrolled. *S.aureus* ATCC 29213, MRSA ATCC 43300, *E.coli* ATCC 25922, *P.aeruginosa* ATCC 27853 strains were also tested as standard strains. The MICs of the strains were determined by broth dilution method. MSSA and MRSA clinical isolates' MIC ranges of Turkish Propolis extract were between 512-2048 µg/mL, while 256-1024 µg/mL for Russian Propolis extract. For ESBL+ *E.coli* and *A.baumannii* the MIC ranges of both Turkish Propolis extract and Russian Propolis extract were greater than 16,384 µg/mL. The MIC values of standard strains were also compatible with clinical isolates. The bactericidal concentration of both Propolis samples were greater than 16,384 µg/mL for all strains containing gram positive and gram negative microorganisms used in this study. There was no statistically difference between methicillin resistance and MIC values (p< 0,091). There was statistically significant difference between Gram positive MIC values and Gram negative MIC values for both Turkish and Russian Propolis samples (p<0,001). According to our results crude Propolis is more effective against gram positive clinical isolates than gram negatives. Clinical strains may show different susceptibility patterns from standard strains. Thus to evaluate clinical strains besides standard strains in vitro should demonstrate more accurate results. Before marketing of Propolis containing products standardization, further laboratory assays, laboratory animal researches and clinical trials are to be needed.

Key words: Propolis, Antimicrobiyal activity, Minimal inhibitory concantration.

INTRODUCTION

Spreading and increasing antimicrobial resistance rates make physicins to search new approaches. Natural products are being used from ancient times. Propolis is a natural resinous bee product collected from beehives by honeybees Apis mellifera. Propolis, an old and a new product has been used for a long time as the need arised. Propolis has various effects such as; antibacterial, antifungal, antiviral, local anaesthetic, antiinflammatory, antioxidant, and hepatoprotective.[1][2]. It is used for the treatment of eczema and infections of eye, throat and urinary tract. Although its composition differs due to the region, it is usually composed of resin, balsam, wax, aromatic oils, polen and other organic material. [2][3]

The aim of this study is to determine the antibacterial activitiy of Propolis on gram positive and gram negative clinical isolates besides standard bacterial strains.

MATERIAL AND METHODS

Propolis samples

Two different crude Propolis samples obtained from Konya-Turkey and Moscov-Russian Federation (2012 October) were used in this study. 25% ethanolic extract was used in this study.

Propolis samples were prepared as described before [3]. Samples were extracted in 95% (v/v) ethanol with shaking at room temperature for seven days. After centrifugation the supernatants were obtained and filtered. The ethanol was evaporated by lyofilization (2508C, 0.50 hPa LyoPro 3000; Thermo Scientific Heto, Allerod, Denmark, USA) Dimethyl sulfoxide (DMSO) was used as solvent. Until study the extracts were kept -20^{o}C in dark conditions.

Strains Tested

Methicillin sensitive *Staphylococcus aureus* (MSSA), methicillin resistant *Staphylococcus aureus* (MRSA), extended spectrum beta lactamases producing (ESBL) *Escherichia coli* and multi-drug resistant (MDR) *Acinetobacter baumannii* clinical isolates were tested in this study. Thirty isolates from each bacterium enrolled. *S.aureus* ATCC 29213, MRSA ATCC 43300, *E.coli* ATCC 25922, *P.aeruginosa* ATCC 27853 strains were also tested as standard strains. The distributions of the samples are shown in Table I. The isolates were identified by using conventional bacteriologic procedures and the Phonenix system (Becton Dickinson; BD, Diagnostic Systems, Sparks, MD, USA).

Each isolate was stored –20^{0}C until use. Before test procedure, they were subcultured on blood agar and MacConkey agar for determination of contamination, and then Mueller Hinton agar for in vitro testing.

Table I: Distribution of Clinical Isolates

	MSSA	MRSA	ESBL+E.coli	A.baumannii
Sputum	2	2	0	2
Tracheal aspirate	1	7	0	5
Urine	2	4	0	4
Blood	7	7	30	6
Catheter	1	2	0	1
Pus	0	0	0	5
Wound	15	8	0	7
Peritoneal lavage	1	0	0	0
Vagen	1	0	0	0
Total	30	30	30	30

Determination of minimum inhibitory concentrations (MICs) for antimicrobial agents tested

The minimal inhibitory concantrations (MIC) of Propolis were determined by a 2-fold serial broth dilution method in Mueller Hinton medium (BD) according to the CLSI recommendations. Two fold serial dilutions of Propolis were 16,384 µg/mL to 32 µg/mL. After 24 hours of incubation at 37^{o}C, the MIC was determined as the lowest concentration of the agent that completely inhibits visible growth as evaluated by the naked eye. Each isolate was evaluated at least two times. MIC50, MIC90 and minimal bacterisidal concentration (MBC) of isolates were also determined. Although criteria for Propolis susceptibility for bacteria are not available, results were interpreted according to susceptibility ranges selected by the researchers.

Statistical analysis

Compliance with the normal distribution of the variables was checked with Shapiro-Wilk test. Homogeneity of groups' variances was checked by Levene's test. Parametric test assumptions were not available so groups' medians were compared by using Mann Whitney U test.

The results of statistical analysis were expressed as number of observations (n), mean ± standard deviation ($\overline{X} \mp S_{\overline{x}}$), median and minimum–maximum values [M (min–max)], interquartile ranges (IQR) and geometric means. Data analyses were performed using the Statistical Package for the Social Sciences, version 17.0 (SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered statistically significant.

RESULTS

MSSA and MRSA clinical isolates' MIC range of Turkish Propolis extract was between 512-2048 µg/mL, while 256-1024 µg/mL for Russian Propolis extract.

For ESBL+ *E.coli* and *A.baumannii* the MIC ranges of both Turkish Propolis extract and Russian Propolis extract were greater than 16,384 µg/mL. The MIC values of standard strains were also compatible with clinical isolates. The bactericidal

concentration of both Propolis samples were greater than 16,384 µg/mL for all strains containing gram positive and gram negative microorganisms used in this study. MIC50, MIC90 and MBC of isolates are shown in Table II. The MIC values of Turkish and Russian Propolis varied for only one dilution against gram positive strains. There was no statistically difference between methicillin resistance and MIC values (p< 0,091). There was statistically significant difference between Gram positive MIC values and Gram negative MIC values for both Turkish and Russian Propolis samples (p<0,001). (Table III).

Table 2: MIC50, MIC90 and MBC of Clinical Isolates.

	Russian F Moscov Region Propolis				Turkey Konya Region Propolis			
	MSSA	**MRSA**	**ESBL+E.coli**	**MDR A.baumannii**	**MSSA**	**MRSA**	**ESBL+E.coli**	**MDR A.baumannii**
MIC50(µg/mL)	256	256	>16.384	>16.384	512	512	>16.384	>16.384
MIC90(µg/mL)	1024	1024	>16.385	>16.385	2048	2048	>16.385	>16.385
MBC(µg/mL)	>16.384	>16.384	>16.386	>16.386	>16.384	>16.384	>16.386	>16.386

Table 3: Statistically evaluation of the strains.

MIC	N (number of strains)	Mean ±SD	Median (min-max)	IQR*	Geometric mean
Gram positive microorganisms	30	1147,73±519,105	1152,00 (512-2048)	768	1033,1275
Gram negative microorganisms	30	16,384±0	16,384 (16,384)	0	16,384

Note: *IQR: interquartile ranges

DISCUSSION

Propolis also called as bee glue is one of the few resinous substance collected from plant sources by honeybees.[1][2]. It is a chemical compound and the compound varies depending on the geographical areas, collection time and plant source. [4][5]

In this study the Propolis collected regions were Turkey Konya region and Russian Federation Moscov region. The plant sources of Propolis samples included in this study were unknown. The collection time of Propolis samples were 2012 October period.

Propolis has various biological and pharmacological activities such as antioxidant, anti-inflammatory, immunomodulatory, antitumor, anticancer, antiulcer, hepatoprotective, cardioprotective, and neuroprotective.[5]. It has also been used as a health drink in various Asian, European and American countries.[6]. Despite its different effects it is usually used tradiationally as an antimicrobial agent. [1][2].

It is reported that the mode of action is Propolis attacs cytoplasm, cytoplasmic membrane, inhibit bacterial enzymes, cell division, and protein synthesis. The antibacterial mechanism also depends on the inhibition of bacterial RNA polymerase [7].

Studies evaluating in vitro activitiy of Propolis were performed by using standart bacterial strains as well as clinical strains. The most evaluated bacterial strains were S. aureus, E.coli, Streptococcus pyogenes, Enterococcus spp., and some anaerobes. The reported data reveals that the Propolis extract has a broad antibacterial activitiy aginst gram positive and negative strains.[3][8]-[13]

Although Propolis have either gram positive or gram negative activitiy as mentioned above, Stepanović S et al reported that antimicrobial activity of Propolis were frank against gram positive bacteria while gram negatives were less susceptible. [14]

Banskota AH et al also emphisized this relationship in their review article.[6]. Scazzocchio F et al reported that Propolis is an active agent against various Staphylococcal clinical strains.[15] Similar results were found by different authors [16][17]. Our data also supports these findings. Either Turkish or Russian Propolis were found to be active against both standard and clinical strains of MSSA and MRSA between 256-2048 µg/mL while gram negative effectiveness were not observed between these MIC ranges. Minimal bactericidal activitiy of two different Propolis extracts against gram positive and negative bacteria were greater than 16,384 µg/mL. And also according to our data resistance to methicillin is not an important factor against antistaphylococcal activitiy of crude Propolis extract.

The effective MIC range against gram positive bacteria (either MRSA and/or MSSA) differed according to the researchers. The Propolis samples from Africa and Asia showed antibacterial activity, with MICs ranging from 0.0156 to >0.5 mg/mL and 0.0078 to >0.5 mg/mL, respectively. Samples from North and South America and samples collected in Europe showed anti-staphylococcal activity, with the MIC values in the range of 0.125 to >0.5 mg/mL. Wojtyczka RD et al showed this antistaphylococcal activity between the MIC ranges of 0.39 to 0.78 mg/mL, while Pamplona-Zomenhan LC et al reported MIC ranges between 710 to 2.850 µg/mL. [18][19]

Berretta et al. demonstrated that the MBC of three samples of standardized Propolis extract tested against S. aureus ATCC 25923 and S. aureus ATCC 43300 were in the range from 6.96 to 7.02 mg/mL and 3.48 to 3.51 mg/mL respectively. [18]. The diverse antimicrobial activity of Propolis extract shown by many authors and in our report may be due to the differences in the origin of Propolis, collection time and its chemical composition.

CONCLUSION

According to our results crude Propolis is more effective against gram positive clinical isolates than gram negative clinical isolates. As antibiotic resistance is increasing and spreading worldwide, resistance between clinical strains is a concerning problem. Clinical strains may show different susceptibility patterns from standart strains. Thus to evaluate clinical strains besides standart strains in vitro should demonstrate more accurate results. Although standardization of this product is difficult to determine the antibacterial effect standardization is crucial.

Before marketing of Propolis containing products further laboratory assays, laboratory animal researches and clinical trials are to be needed.

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