Artículo de investigación

Evaluation of the antibacterial activity of iranian propolis on the strains of pseudomonas aeruginosa and staphylococcus aureus

Evaluación de la actividad antibacteriana del propóleo iraní en las cepas de pseudomonas aeruginosa y staphylococcus aureus

Avaliação da atividade antibacteriana da própolis irani nas pseudomonas aeruginosa e staphylococcus aureus scp

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Abstract

Propolis has been found to possess antibacterial activity and this has been attributed to specific chemicals in its composition, which depends on the region where it was collected. Our study evaluated the antimicrobial activity of propolis collected from Ardabil province located at northwest of Iran against *S.aureus* and *P.aeroginosa*.

Twenty propolis (*Apis mellifera*) samples were obtained from the beehives situated in different regions of Ardabil province, located at the northwest of Iran. The disc diffusion method was employed to test the antibacterial activity of extracts of propolis (EEP, CEP and AEP). *S.aureus* (PTCC 1431) and *P.aeruginosa* (PTCC 1707) were used in this investigation to test antimicrobial activity of propolis.
The extraction of propolis, regardless of how it is extracted, had the significantly higher inhibitory effect on the Gram-positive bacteria *S.aureus* compared to *P.aeruginosa* ($p<0.001$). Both MIC and MBC of EEP, AEP, and CEP on *S.aureus* determined 0.164 mg/ml and there was no statistically significant difference. On the other hand, for *P.aeruginosa*, the amount of MBC and MIC for the EEP, AEP, and CEP determined as 0.022 mg/ml, 0.082 mg/ml, and 0.041 mg/ml, respectively.

Resumen

Se ha encontrado que el propóleo posee actividad antibacteriana y esto se ha atribuido a productos químicos específicos en su composición, que depende de la región en la que se haya recolectado. Nuestro estudio evaluó la actividad antimicrobiana del propóleos recolectados en la provincia de Ardabil, ubicada al noroeste de Irán, contra S. aureus y P. aeroginosa.

Se obtuvieron 20 muestras de propóleos (Apis mellifera) de las colmenas situadas en diferentes regiones de la provincia de Ardabil, ubicadas al noroeste de Irán. El método de difusión de disco se empleó para probar la actividad antibacteriana de extractos de propóleo (EEP, CEP y AEP). Se usaron S. aureus (PTCC 1431) y P.aeruginosa (PTCC 1707) fueron usados en esta investigación para evaluar la actividad antimicrobiana del propóleos.

La extracción de propóleos, independientemente de cómo se extraiga, tuvo un efecto inhibidor significativamente mayor en las bacterias Gram positivas S. aureus en comparación con P. aeruginosa ($p <0,001$). Tanto la CIM como la MBC de EEP, AEP y CEP sobre S. aureus determinaron 0,164 mg / ml y no hubo diferencias estadísticamente significativas. Por otro parte, para P.aeruginosa, la cantidad de

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In conclude, in accordance with literature data the appropriate concentration of propolis might be effective on Gram-positive infectious bacteria but it was inactive against the Gram-negative bacteria. In vivo evaluations are required to find out concise antimicrobial mechanism of propolis and its appropriate dose. In addition, there is need for recognition of the antimicrobial active components in the propolis extracts.

Keywords: Propolis- Pseudomonas aeruginosa-Staphylococcus aureus

MBC y MIC para EEP, AEP y CEP se determinó como 0.022 mg / ml, 0.082 mg / ml y 0.041 mg / ml, respectivamente.

En conclusión, de acuerdo con los datos de la literatura, la concentración apropiada de propóleos podría ser efectiva en las bacterias infecciosas Gram-positivas, pero fue inactiva contra las bacterias Gram-negativas. Se requieren evaluaciones in vivo para descubrir el mecanismo antimicrobiano conciso del propóleos y su dosis apropiada. Adicionalmente, existe la necesidad de reconocimiento de los componentes activos antimicrobianos en los extractos de propóleos.

Palabras claves: Propóleo- Pseudomonas aeruginosa-Staphylococcus aureus.

Resumo

Constatou-se que a própolis possui atividade antibacteriana e isso tem sido atribuído a produtos químicos específicos em sua composição, que depende da região em que foi coletada. Nosso estudo avaliou a atividade antimicrobiana da própolis coletada na província de Ardabil, localizada no noroeste do Irã, contra S. aureus e P. aeroginosa.

20 amostras de própolis (Apis mellifera) foram obtidas de colmeias localizadas em diferentes regiões da província de Ardabil, localizada no noroeste do Irã. O método de difusão em disco foi utilizado para testar a atividade antibacteriana dos extratos de própolis (EEP, CEP e AEP). S. aureus (PTCC 1431) e P. aeruginosa (PTCC 1707) foram utilizados nesta investigação para avaliar a atividade antimicrobiana da própolis.

A extração de própolis, independentemente da forma como foi extraída, teve um efeito inibitório significativamente maior nas bactérias Gram positivas S. aureus em comparação com P. aeruginosa ($p < 0,001$). Tanto o CIM como o MBC do EEP, AEP e CEP em S. aureus determinaram 0,164 mg / ml e não houve diferenças estatisticamente significativas. Além disso, P. aeruginosa, a quantidade de CBM e CIM para EEP, AEP e CEP foi determinada como 0,022 mg / ml, 0,082 mg / ml e 0,041 mg / ml, respectivamente. Em conclusão, de acordo com dados da literatura, a concentração adequada de própolis pode ser eficaz em bactérias infecciosas Gram-positivas, mas foi inativa contra bactérias Gram-negativas. Avaliações in vivo são necessárias para descobrir o mecanismo antimicrobiano conciso da própolis e sua dose adequada. Além disso, há uma necessidade de reconhecimento dos componentes ativos antimicrobianos nos extratos de própolis.

Palavras-chave: Própolis-Pseudomonas aeruginosa-Staphylococcus aureus

Introduction

Recently released World Health Organization (WHO) reports stated that antibiotic resistance is now a major threat to public health. In 2050 estimated that there will be around 10 million deaths attributable to antimicrobial resistance every year. In last decades, efforts have been made to recognize naturally occurring mediators that could prevent antibiotic resistant infections development without (or with minimal) side effects (Ventola, 2015; Berendonk et al, 2015; O'Neill 2016).

Propolis or bee glue is a sticky dark colored material resinous mixture of saliva and beeswax, that has been used to treat many diseases since ancient times, and is a significant source of drug derivatives and bioactive natural compounds (Pasupuleti et al, 2017).

A vast number of recently published papers indicated that propolis presents several pharmacological and biological properties, such as antitumor, antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, anti-parasite activities and immunomodulatory effects. The biological properties and chemical composition of propolis vary depending on seasonal, geographical, vegetational and changes in plant sources from which it is collected by the bees (Aminimoghadamfarouj & Nematollahi, 2017; Bankova et al, 2016).

More than 300 different compounds have been known so far in propolis, including inorganic substances, amino acids, aromatic acids, esters, vitamins, aliphatic acids, terpenoids, carbohydrates, aldehydes, ketones, chalcones, dihydrochalcones, and fatty acids (Saleh et al, 2015).

The major components of propolis in Brazilian propolis are terpenoids and prenylated derivatives of coumaric acids, whereas major components in Europe and China are flavonoids and phenolic acid esters and major components of Iranian and Indian propolis are aromatic acids and fatty acids derivatives, respectively (Bittencourt et al, 2015; AL-Ani et al, 2018; Xuan et al, 2016; Afrouzan et al, 2018). The many investigators, who have demonstrated these properties of propolis, have done their survey using propolis from different geographic locations around the world (Oryan et al, 2018; Savka et al, 2015).

The application of propolis against a broad spectrum of bacteria may be beneficial for improving antibiotic resistant infections (Savka et al, 2015). Additionally, the current view is that the use of standardized preparations of propolis is safe and less toxic than many other antibiotics (Afrouzan et al, 2018).

In this study, we wish to report the results of our survey on the antibacterial activity of propolis samples (ethanol, chloroform and acetone extracts), obtained from different regions of Ardabil province of Iran, against Staphylococcus aureus and Pseudomonas aeruginosa strains.

Material and Methods

Propolis extract preparation

Twenty propolis (*Apis mellifera*) samples were obtained from the beehives situated in different regions of Ardabil province, located at the northwest of Iran. Samples collected into sterile tubes to avoid contamination, kept in a dry place, and stored at -20 °C until its processing. Propolis samples were cut into 2 mm pieces, divided into 3 parts and separately extracted with ethanol, chloroform and acetone to compare these extraction methods. 30g of unrefined propolis was accurately weighed, dissolved in 300 ml of chloroform/acetone or 96% ethanol and left at room temperature for 20 days. The suspension was shaken (150 rpm) daily during this time. Then the opaque liquid was then filtered through Wattman filter paper No. 41, placed inside a rotary device, and concentrated at 45 °C. Finally, 7.5 gram of dry propolis were obtained. After extraction, 10 mg of dry propolis subjected to 10 ml 2% DMSO, heated and diluted to 1:2 proportion. Then, propolis disks were made in pharmacological department of Shahid Beheshti Medical University. Sterile paper discs (Wattman no.4 paper, 6mm diameter) were loaded with 2 μl of propolis extracts dilution and dried for 5 hours at 37 C in a sterile incubator.

Microorganisms and Antimicrobial activity

Staphylococcus aureus PTCC 1431 and *pseudomonas aeruginosa* PTCC 1707 were used in this investigation to test antimicrobial activity of propolis. All microorganisms were provided by Iran Pasteur institute and Iranian Research Organization for Sciences and Technology of Iran, Tehran. The disc diffusion method was employed to test the antibacterial activity of extracts of propolis (EEP, CEP and AEP), as described elsewhere (Ertürk et al, 2016). Ceftazidime and Imipenem were used as positive control and the turbidity of the suspension was adjusted to the McFarland 0.5 turbidity standard. The commercial antibiotic and laboratory made propolis extracts discs were placed on the surface of Muller Hinton (MH) agar culture plates previously inoculated by the test microorganism. The inhibition zone was measured for each disc in millimeters and compared with Imipenem and Ceftazidime inhibition zone, as showed in figures 1 and 2. Tests were performed in triplicate.

MIC and MBC determination

The MIC and MBC values were determined according to the guidelines by Clinical Laboratory

Standards Institute (CLSI) and the microdilution broth method was used to evaluate the inhibitory effects of propolis extracts. Serial dilutions of each EEP,AEP and CEP extracts were prepared under aseptic conditions and the microdilution broth method were performed as described by Acka et al (Akca et al, 2016).

Statistical analysis

Results were analyzed using Analysis of Variance (ANOVA) with the probability p= 0.05 as the critical value for all test (SPSS 19.0 Version).

Results

The extraction of propolis, regardless of how it is extracted, had the significantly higher inhibitory effect on the Gram-positive bacteria *Staphylococcus aureus* compared to *Pseudomonas aeruginosa* ($p<0.001$)(Fig.1and 2). Both MIC and MBC of EEP, AEP, and CEP on *S.aureus* determined 0.164 mg/ml and there was no statistically significant difference. On the other hand, for Pseudomonas aeruginosa, the amount of MBC and MIC for the EEP, AEP, and CEP determined as 0.022 mg/ml, 0.082 mg/ml, and 0.041 mg/ml, respectively (Tables 1 and 2). Disc diffusion method results, showed similar data. Propolis in dried discs retained antibacterial activity, resulting in a strong growth inhibition in *S.aureus* strains (>15 mm) and no inhibition in *P.aeruginosa* strains.

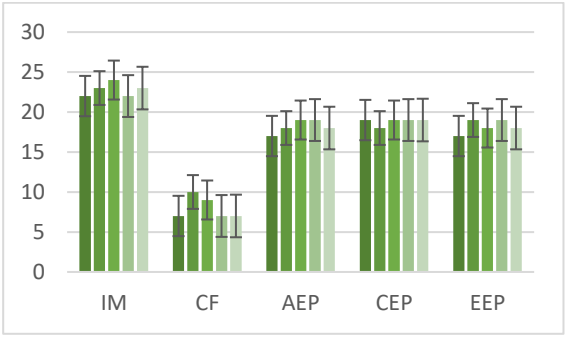

Figure 1: Summary of the antimicrobial activity of extracts of Propolis against S.aureus

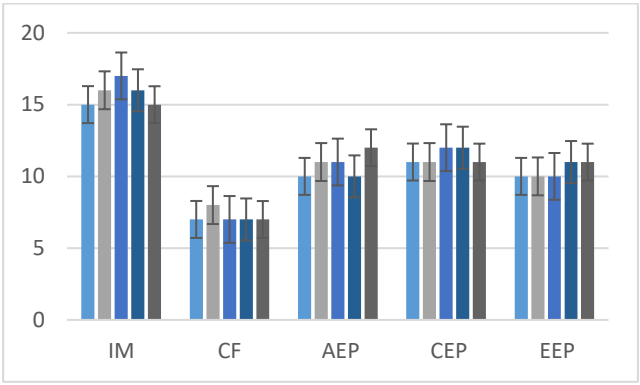

Figure 2: Summary of the antimicrobial activity of extracts of Propolis against P.aeruginosa

Table1: Minimum inhibitory concentrations and minimum bactericidal concentration for EEP in different tests against P.aeroginosa.

Dilution ration	Concentration mg/ml	Test number											
		1		2		3		4		5		6	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1/1	5/25	-	-	-	-	-	-	-	-	-	-	-	-
1/2	2/62	-	-	-	-	-	-	-	-	-	-	-	-
¼	1/31	-	-	-	-	-	-	-	-	-	-	-	-
1/8	0/656	-	-	-	-	-	-	-	-	-	-	-	-
1/16	0/328	-	-	-	-	-	-	-	-	-	-	-	-
1/32	0/164	-	-	-	-	-	-	-	-	-	-	-	-
1/64	0/082	-	-	-	-	-	-	-	-	-	-	-	-
1/128	0/041	+	-	-	-	-	-	-	-	+	-	+	-
1/256	0/02	+	+	+	+	+	+	+	+	+	+	+	+

Table 2: Minimum inhibitory concentrations and minimum bactericidal concentration for EEP in different tests against *Staphylococcus aureus*.

Dilution ration	Concentration mg/ml	Test number											
		1		2		3		4		5		6	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1/1	5/25	-	-	-	-	-	-	-	-	-	-	-	-
1/2	2/62	-	-	-	-	-	-	-	-	-	-	-	-
1/4	1/31	-	-	-	-	-	-	-	-	-	-	-	-
1/8	0/656	-	-	-	-	-	-	-	-	-	-	-	-
1/16	0/328	-	-	+	+	+	+	+	+	+	+	+	+
1/32	0/164	+	+	+	+	+	+	+	+	+	+	+	+
1/64	0/082	+	+	+	+	+	+	+	+	+	+	+	+
1/128	0/041	+	+	+	+	+	+	+	+	+	+	+	+
1/256	0/02	+	+	+	+	+	+	+	+	+	+	+	+
1/512	0.01	+	+	+	+	+	+	+	+	+	+	+	+

Discussion

Propolis has been found to possess antibacterial activity and this has been attributed to specific chemicals in its composition, which depends on the region where it was collected. There are conflicting results on the antimicrobial activity of Propolis (9). Current study evaluated the antimicrobial activity of propolis collected from Ardabil province located at northwest of Iran against *S.aureus* and *P.aeruginosa* and indicated a high inhibitory effect on the Gram-positive bacteria *Staphylococcus aureus*, whereas no activity was observed against *Pseudomonas aeruginosa*.

Similar results have been reported in previous investigations, which support our findings that propolis is mainly active against Gram-positive microorganisms. However, it has been reported that AEP, EEP or CEP are effective on Gram-negative bacteria at higher or lower concentration (Sforcin et al, 2000; Yaghoubi & Satari, 2007; Ugur & Arslan, 2004). The effect of Ardabil propolis on S.aureus growth, detected by the disc diffusion technique, was confirmed by the microdilution method. Both methods confirmed small variations in the antimicrobial activity. There was very slight difference between MIC/MBC values of EEP, AEP and CEP against both strains, which showed no statistically significant difference. Generally, the MIC/MBC values determined in our study were in line with other studies stating that Gram-positive bacteria were more susceptible to propolis than the Gram-negative bacteria.

Though the antimicrobial activities of Propolis have been the subject of many surveys, it is hard to compare the outcomes of different investigations, due to the different compositions of Propolis and different methods used for the evaluation of propolis antibacterial properties. However, it is well known that the inhibitory effect of Propolis is more effective on Gram-positive than Gram-negative bacteria, that is confirmed in current study on *S.aureus and P.aeroginosa*. The result of particular interest of this investigation is that resistance of tested bacteria to propolis was not related to the method of its extraction.

Conclusion

In conclude, in accordance with literature data the appropriate concentration of propolis might be effective on Gram-positive infectious bacteria but it was inactive against the Gram-negative bacteria. In vivo evaluations are required to find out concise antimicrobial mechanism of propolis and its appropriate dose. In addition, there is need for recognition of the antimicrobial active components in the propolis extracts.

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