



Research Article

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Study the Antimicrobial Activity of Alcoholic Extract of Propolis and Balsam against a Imipenem-Resistant *Pseudomonas Aeruginosa*

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ABSTRACT

Propolis and balsam these two substances known to be curative. In ancient times they have been used for wound healing skin. In this study the antimicrobial activity of alcoholic extract of propolis and balsam against a imipenem-resistant *Pseudomonas aeruginosa* have been examined 50 *Pseudomonas aeruginosa* in a period of 4 months from February to May 2014, were isolated. The strains from different clinical samples such as blood, urine, pus, etc., were isolated using microscopic and biochemical tests such as catalase, oxidase, the growth of the TSI, the response of the OF, grown on agar cetrimide ability to grow at 42degrees C were identified. The antibiotic susceptibility testing against 10 different antibiotics using the disk diffusion method was used. The 50 isolates of *Pseudomonas aeruginosa* resistance patterns of the isolates tested by disk diffusion antibiogram were as follows: Imipenem (30%), ampicillin (75%), tetracycline (70%), cephalothin (42.5%), cefotaxime (47.5%), amikacin (52.5%), nalidixic acid (45%), gentamicin (50%) ciprofloxacin (40%), cotrimoxazole (65%). Antimicrobial activity of the extracts was carried out by the agar dilution of the test minimum inhibitory concentration of propolis extracts against imipenem-resistant *Pseudomonas aeruginosa* 125 to 500 micrograms per milliliter variable. The results of this study, the use of propolis and balsam in the treatment of skin infections and scars suggested course of these substances can be used in the prevention and treatment of skin diseases.

Keywords: alcoholic extract of propolis and balsam, Antimicrobial activity, *Pseudomonas aeruginosa*

INTRODUCTION

Increasing and promoting indiscriminate use of antibiotics has led bacterial resistance to any antibiotics day after day, where microbes are becoming increasingly resistant to that kind of antibiotics. One of the big problems of global scientific community is to find alternative antibiotics or substances against resistant bacteria's. *Pseudomonas Aeruginosa* is a hot-negative and opportunistic bacteria which is separated from land, water and the environment. *Pseudomonas Aeruginosa* as the second most common pathogenic bacteria is common at surgeries and the third prevalent and popular factor in hospitals' infection after *Staphylococcus Aureus*, which constitute about ten percent of hospital infections. Elderly patients with lymphoma, AIDS, undergoing to chemotherapy and burned are those who are prone to severe infections caused by *Pseudomonas aeruginosa* factor such as endocarditis, meningitis and septicemia which are toxic (Brooks, 2010). One of the most serious complications in burn patients is the infections caused by *Pseudomonas aeruginosa*. Carbapenems like imipenem and meropenem are of the most important antimicrobial antibiotics is used to treat infections caused by *Pseudomonas Aeruginosa* multi-drug resistant strains. Metallo beta lactamases is capable to hydrolyzing a wide range of beta-lactams such as penicillins, cephalosporin and carbapenem, but it's not able to aztreonam hydrolysis (aztreonam) (Hassett, 2004). Imipenem by the brand of Primaxin is used to treatment of pulmonary infections, urinary tract - bone infections, skin lesions, Gynecological Infections, bacterial infection of the kidneys or heart valves. This drug works by containment of Mokoptaed synthesis at the bacterial cell wall then applies beta-lactam antibiotics mechanism. Researchers are studying how nature works and those methods used by insects against germs and contaminations in order to find out resistant barriers for onslaught of resistant microbes against antibiotics (Brooks, 2010). Bee makes propolis by combination

of gum herbs, beeswax, flowers' nectar and pollen. Propolis resins are collected from trees and shrubs. It seems that each region and colony has its own preferred resins resources that lead to lots of differences in color, smell and composition (Khodadadi, 1391). Propolis is a substance that is made by honey bees to protect the hive which has a good effect against fungi, bacteria and viruses. Many studies have been done on the effect of propolis. Also now it is being reviewed by many researchers. The present study evaluates the efficacy of propolis over imipenem-resistant *Pseudomonas aeruginosa* bacteria (Hassett, 2004).

This review has been done by collecting suspicious isolates of *Pseudomonas aeruginosa* from early December 2014 until February 2015. By referring to the hospital laboratory at the first stage, those plates containing *Pseudomonas* isolated from clinical samples were collected and taken immediately to lab. In the laboratory, the samples inoculated with the Mac Cancan agar environment, then the plates were incubated for 44 hours at 37 °C. In order to recognize these separation from other hot-negative bacteria and to ensure genus and species of bacteria standard biochemical tests including oxidase test, catalase test, reaction at TSI environment, OF test, cultivation in SIM environment to check the mobility and indole and gas production; growth at 44 °C and Pigment Plocianin production have been performed at the Mueller-Hinton Agar environment. Determining sensitivity towards antibiotics has been assessed by disk diffusion method.

EXPERIMENTAL SECTION

Preparing Extract of Propolis

25 grams of propolis is quite chunked, accurately weighed then poured in a 250 ml flask, after that the sample size reached to 100 ml by ethanol 96% and the mixed material has been homogenized well. This procedure was repeated twice a day for three days, then it was kept in a warm and dark place for 1-2 weeks, after that time the mixture is smoothen. The smooth material was kept at a temperature of 1-4 °C for one day in the refrigerator. Then, the solution was filtered and the obtained extract was kept in a dark and twisted glass. The remaining alcohol was obtained in suspension completely isolated by Soxhlet and the pure alcohol extract has been acquired, respectively. To determine MIC and MBC, Broth Microdilution MIC testing method were used. This method is used Polystyrene sterilized panel containing 96 pits. Serial dilutions half times was prepared from the alcoholic extract of propolis in microplates [at density of 800 µg/ml in the first cell, 15/62 µg/ml in tenth cell, and for 12 cell one microplate] with nutrient broth (Merck, Germany). A micro plate has been allocated as control environment and solvent (80 degree alcohol) and stated dilutions was prepared in it.

Statistical Analysis

Statistical analysis was performed using SPSS version 20. Statistical differences between experimental groups have been defeminised using Pearson and two-way analysis of variance.

RESULTS

After identification testing's, by preparing McFarland suspension of 0.5, antimicrobial sensitivity of *Pseudomonas aeruginosa* isolates against 10 antibiotics were tested. The results of Antibigram which is obtained by Kirby - Bauer method, according to guidelines and CLSI table on 40 isolates of *Pseudomonas aeruginosa* isolated from clinical samples were performed. of 40 isolated strains from clinical samples, the results showed 14 strains were resistant and 6 ones have intermediate resistance against antibiotic imipenem. Finally, 20 strains were resistant to imipenem.

Table 1: Model of Antimicrobial Resistance of *Pseudomonas Aeruginosa* Isolated Strains

%	Sensitivity	%	Intermediate	%	Resistance	Antibiotics
16	8	8	4	76	38	Ampicillin
38	19	8	4	54	27	Amikacin
50	25	10	5	40	20	Imipenem
40	20	8	4	52	26	Tetracycline
40	20	12	6	48	24	Gentamicin
48	24	8	4	44	22	Cefalotin
38	19	10	5	52	26	Cefotaxime
46	23	16	8	38	19	Ciprofloxacin
42	21	6	3	52	26	Nalidixic Acid
34	17	6	3	65	30	Cotrimoxazole

MIC and MBC Test Results

Minimum inhibitory compactness (MIC) and Minimal bactericidal concentration (MBC), propolis and henna extract on resistant *Pseudomonas aeruginosa* bacteria to imipenem were obtained as follows.

Table 2: MIC and MBC Determining Results

Continous Dilution of Propolis Extracts *										Pseudomonas Aeruginosa Resistant to Imipenem
Continous Dilution of Propolis *										
1	2	3	4	5	6	7	8	9	10	
32	28	22	17	12						Containment Zone Diameter (mm)

µg/ml 62/15:75/31 ,5/62 ,125 ,250 ,500 ,1000 ,2000 ,4000 ,8000

Table 3: Assessing the effect of propolis extract against resistant Pseudomonas aeruginosa to imipenem

compactness / Dilution µg/ml Propolis Extract									
8000	4000	2000	1000	500	125	62/5	31/75	15/62	Repeat
			MIC	MBC					1
			MIC	MBC					2

In order to compare the effect of alcoholic extract of propolis against imipenem-resistant Pseudomonas, agar diffusion method was used. The obtained results for each plate have been repeated three times and the results of these three steps were together in accordance, respectively.

15/62, 31/75, 62/5, 125, 250, 500, 1000, 2000, 4000, 8000 µg / ml *

The obtained results show the effectiveness of antibacterial properties in these two materials of propolis extract. To investigate the proper effect of these two materials, we combine them in different dilutions and by resistant strains to imipenem of isolated Pseudomonas. The combination results of propolis extracts have shown better effect. Univariate analysis of variance results showed that by increasing different compactness of propolis extract against resistant Pseudomonas aeruginosa to imipenem in comparison to the control group was significantly affective ($p < 0/01$). Pearson correlation test results showed that among Pseudomonas aeruginosa and different compactness of propolis extract and Hana has been observed negative correlations ($p < 0/01$). This means that by increasing compactness of propolis extracts the number of Pseudomonas aeruginosa are significantly reduced.

The results of antimicrobial activities of Propolis extracts have been shown over different species. Alcoholic extract of propolis showed compactness of 500 micrograms. But investigated Pseudomonas aeruginosa bacteria were resistant to imipenem; however, it could eradicate the bacteria at compactness of 250 micrograms. In a study conducted by Moradi in Karaj, Iran, as "the bacterial effect of bee propolis on Paenibacillus Larae bacteria which was the disease symptom for American Luke bee" spoke about the anti-bacterial effect of propolis and reported about its capability to spread in solid culture environment. The results of his study are in consistence with the results of ours (Yuan, 2004).

DISCUSSION AND CONCLUSION

Due to the effect of alcoholic extract of propolis and Hana, it is natural, non-toxic and has not destructive effects and with respect to other advantages such as low price and availability; it appears that the use of these materials as an effective solution for treating and preventing skin infections have been suggested in developing countries. It also may be a useful solution to reduce the rising consumption of antibiotics that bacteria are increasingly going to resistance to them.

Suggestions

The prepared propolis has been bought. Bee propolis is extracted from unknown species of trees and flowers. In order to get better and more accurate results, we suggest that only those honey bees examine which only make propolis from lemon and sumac trees. The effect of Propolis and henna extracts on each other has been shown better impacts against pseudomonas aeruginosa resistance to imipenem. Therefore, it is suggested that these extracts examined by GC-MS method and analyzed may be used in order to find effective materials to replace these substances to annihilate bacteria.

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