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Alcohol Extract Effect of Raw Propolis in Acinetobacter Baumannii Isolated from Diabetic Foot Ulcers

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Abstract

Background: Diabetic foot ulcers (DFU) are severe complications of diabetes, often leading to limb amputation due to bacterial infection, such as Acinetobacter baumannii. This study aims to isolate A. baumannii in DFU, assess its sensitivity to alcoholic propolis extract compared to antibiotics, and evaluate its potential as a natural antimicrobial. Methods: Fifty DFU samples from patients at Al-Kafeel Specialty Hospital in Karbala Governorate (September 2022 - February 2023) were cultured and diagnosed with 16 A. baumannii isolates, primarily in males. Results: Crude alcoholic propolis extract inhibited A. baumannii growth in a concentration-dependent manner. The largest inhibition diameter (23.67 mm) was at 150 mg/ml, while the smallest (8.33 mm) was at 12.5 mg/ml. Propolis outperformed Trimethoprim Sulfamethoxazole. Dermacyn extract showed a synergistic effect. MIC was 25 mg/mL, and MBC was 100 mg/ml. Conclusion: Alcoholic propolis extract demonstrates potential as a natural antimicrobial against A. baumannii in DFU infections.

Keywords:

Diabetes Foot, Ethanoic Propolis Extraction, Diabetes Foot Ulcers, Acinetobacter Baumannii, Dermacyn, Antibiotic.

Introduction

One global statistic suggests that about 537 million people currently have Diabetes Mellitus (DM), and that number is expected to double to more than 643 million by 2030. In another statistic, more than 15% of people with DM were found to have diabetic foot ulcers. Diabetes Foot Ulcer(DFU) [1] .Diabetes Foot (DF): It is one of the serious complications for diabetics[2]. which is located in the area below the heel of the foot and is a major source of many ailmentss involving inflammation of tissues, joints, tendons, bone marrow and muscles [3]. And it may develop and become more dangerous by invading deep tissue, causing it. Immune disorder, peripheral neuropathy, the leading cause of ulcer formation (neuropathic ulcer), once the ulcer state emerges bacteria can colonize the lesion (over 50% of cases), with inflammatory response and systemic infection [4].

Many organisms can cause diabetic foot infections, but Gram-positive pigments, especially Staphylococcus aureus, are the most common pathogens [5]. Gram-negative non-fermented species (GN), especially Pseudomonas aeruginosa and Acinetobacter baumannii have long been documented as common and recurring pathogens that cause DFUS Where between Pan [6].

Acinetobacter baumannii is a rare colonizer in human skin and a widespread opportunistic disease that causes hospital infections and causes many illnesses such as sepsis, urinary tract infection, meningitis, pneumonia, burn injuries and wounds. It is found in intensive care units, and its long-term environmental stability has contributed to the ability to acquire determinants to become resistant to MDR and the spread of disease[7].

It also exists in the soil and affects humans and animals, affects people with weak immunity and can cause death. And one of the most common causes of diabetes foot infection Diabetes Foot Infection (DFIs)[8].

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A. baumannii are characterized by being gram-negative bacillus, spherical to bacillus-shaped, aerobic, immobile, non-fermented for lactose sugar, and are characterized by their ability to withstand dehydration and can and A. baumannii can survive exposure to common disinfectants - gluconate phenols, chlorhexidine on fingertips or dry surfaces, and long nutrients because of their ability to develop biofilms, these bacteria have been associated with long-term hospital admissions, morbidity, and deaths[9].Therefore, WHO listed Carbapenem Resistant Acinetobacter baumannii (CRAb) as the number one pathogen for urgent development of new treatment options. A global review of data analysis from 2019 listed A. baumannii among the six main pathogens of antimicrobial resistance-related death (AMR), with more than 10,000 deaths worldwide[10]. More recently, natural products have been used as important sources of antibiotics because they have bioactivity for different types of microorganisms due to these plants having effective secondary compounds. (Flavonoids, Tannins, Terpenoids, alkaloids, etc.) And its antioxidant properties, microbes and inflammation [11]. which has high anti-microbial efficacy[12]. Some studies have highlighted the propolis bee product for possessing these properties. Propolis is a resin made by bees, produced by mixing beeswax and salivary gland secretions with secretions collected from plants. Propolis is sometimes referred to as "bee penicillin[13]. A beehive is a very sterile environment found in nature[14]. Propolis not only acts as glue, but is also used to clean and disinfect honey tablets[15]. One of the honeybee products known to affect biofilm production[16]. Ancient Egyptians used it to preserve bodies during mummification [17]. More recently, propolis has become an interesting topic and has received increasing attention from chemists and biologists over the centuries, has had an important role in traditional medicine and is still used. Today as an alternative to antibiotics because of The growing problem of antimicrobial resistance coupled with the problem of antibiotic resistance, natural products are more valuable than synthetic products due to reduced cell toxicity. Propolis ("bee glue") is a powerful adhesive for blocking holes and cell Strengthening[18, 19], and smoothing the inner walls. Because propolis is collected by bees from different plants, its properties can be associated with seasonal time and geographical location. It was found that the chemical composition of the arachnid could be affected by the plant origin and assembly area. More than 150 ingredients such as polyphenols, phenolic aldehydes, quinones, comarine, amino acids, stimulants and inorganic ingredients were identified in the propolis samples. Among the active and bioactive compounds in these samples are phenolic acids and flavonoids [20]. As a result of the therapeutic importance of propolis, it was used in this study and focused on evaluating the efficacy of extract Ethanolic of propolis (EEP) on *Acinetobacter baumannii* bacteria Isolated from diabetic foot ulcers in Planted Dishes and Microtiter Plate

Materials and Methods

Collection of Samples

50 collected a sample of diabetic foot ulcers for people with diabetes (males and females) at the Kafil Hospital, peace be upon him specialist, diabetic foot department for the period from 1 September 2022 to 1 February 2023. By using sterile cotton swabs patients were diagnosed with diabetes by the department's specialist doctor, they were grown on container petri dishes at the centre of MacConkey agar and Blood Agar and incubated an antenna of 37C for 24 hours for the purpose of isolating and diagnosing the microorganisms that cause diabetic foot ulcers to be contaminated.

The container at the Media of MacConkey agar and Blood Agar and incubated an aerobic at 37C for 24 hours for the purpose of isolating and diagnosing the microorganisms causing diabetic foot ulcers. Diagnosis of isolated bacteria A. baumannii was diagnosed via: A-Microscopic Diagnosis B-Morphological Examinations, Blood Agar, MacConkey Agar and by Vitek-2 Compact System Diagnosis: Phytec-2 is an automatic device whose function is to identify the type of organism that causes disease in the sample, depending on the type of bacteria Gram-Negative Bacteria or Gram-Positive Bacteria or Yeast fungi. Also doing allergy tests (AST) s Antibiotic Sensitivity Test)

Collecting propolis

A 100g raw propolis sample was purchased from the local market during the months of April and May. The coarse impurities were removed. The models were kept in clean, dark and sealed packaging in the refrigerator pending use

Preparation of ethanol extract for propolis

It was attended by dissolving 25 g of propolis in 100 ml of ethanol by 70% in a dark and sterile glass bottle and was extracted at room temperature after three days with shaking for a short period of time in a dark and warm place (reshaking at least twice a day) to obtain a more effective extract then by filtering with filter paper. Whatman No.1 and kept at refrigerator temperature until different concentrations were prepared [21] Preparation of bacterial stuck, Test the Extract Effect in Bacteria My Way Well diffution method Disc diffusion method [22, 23].

Minimum inhibitory Concentration MIC

The MIC test was conducted in a method Microdilution method approved by (2022) CLSI, as follows: Set the lowest MIC inhibitor concentration and the lowest MBC killer concentration of ethanol extract of propolis by preparing a series of half concentrations using Mueller Hinton liquid medium and as follows (3.12-6.25-12.5-25-50-100-150) mg/ml used the microtitere plate has 96

holes (Wells). Put in each (150) micro-litre hole of all the concentrations I attended and then add 50 micro-liters of pre-prepared bacterial stuck per hole, and use Dermacyn solution. Synergistically with the raw alcohol extract of propolis where 75 micro-litres of both solutions were added to the hole containing 50 microliters of stuck, the results were compared with the drilling of the container control of the liquid-only. Mueller Hinton center and alcohol Only Athelian. The plate was then covered and incubated at 37 ° C for 24 hours and the results were recorded based on the presence or absence of turbidity in all fossils Alcoholic for raw propolis, in which no visible bacterial growth was observed. The lower killer concentration was then identified as the lowest concentration of alcoholic extract of the propolis inhibiting bacteria growth by 99.9.

Results

50 samples of diabetic foot ulcers were collected for patients after their diagnosis by a specialist doctor at Al-Kafil Hospital, peace be upon him, specialist hospital in the holy province of Kerbala, for the period from September2022 to February 2023. The samples taken were transplanted, microscopic tests were conducted and diagnosed with a VITEC-2 compact system-v.

The findings for the current study show that of the 76 isolations, 16 were isolated by 32% of the most common pathogen Acinetobacter baumanni in this study were isolated from males 38% by 76% and the bacteria were not isolated from females. The results indicate that the alcohol extract of the local raw material Propolis has an anti-growth and reproduction effect of A. baumannii bacteria in cultivated dishes with this effect varying depending on the concentration used. Between statistical analysis using contrast analysis and LSD, there is a moral difference between the Transactions and the probability level. The alcoholic extract of propolis also has an effect on bacteria

Table 1: The Effect of Raw Propolis Ethanol Extract in Inhibiting Growth of Acinetobacter baumannii in Culture Media

Concentration mg/ml	Bacteria inhibition ZoneInhibition diameter measured in mm Mean+S D
12.5	8.33 ±0.94
25	10 ±0.81
50	14 ±0.47
100	19.33. ±0.47
150	23.67 ±0.5
Trimetheprim -sulfamethoxazole)1.25/23.75µg) (Control Positive)	14 ± 0.47
Alcohol Ethanol control Negtive	
P value	0.0000*
LSD	1.7778

* means significant differences at P ≤ 0.05, (-) There is no disincentive diameter.

Table 2: Impact of raw propolis ethanol extract against A. baumannii bacteria using Microtiter plate.

Concentrations mg/ml	The result
3.12	–
6.25	–
12.5	–
25	+
50	+
100	++
150	++
ML ext75 + 75 Dermacyn ML	+

(-) means an ineffective act

(+) means an act of inhibition of bacteria

(+ +) means a fatal act of bacteria

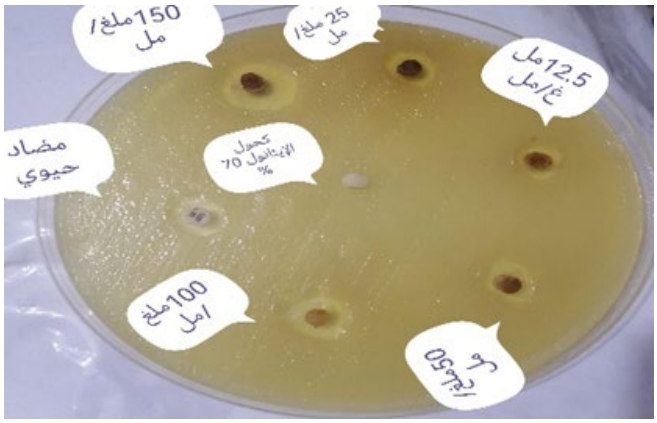

Figure 1: The different concentrations of raw propolis ethanol extract against *Acinetobacter baumannii* stagnant bacteria in cultivated dishes.

Discussion

The results of the current study show that the ethanol extract of the raw substance propolis has a significant effect on Stagnant Pomanate Growth of bacteria in all of the concentrations used[24]. The study was conducted with variations in this effect by the concentration used. These results are consistent with studies by researchers on propolis collected from solvents located in several countries against the growth of all positive or negative germs of a gram pigment other than the stagnant Pomanium bacteria not addressed in previous studies. These studies indicated its effectiveness of antibacterial propolis, especially those that are Especially Gram-positive bacteria Moroccan propolis and its synthesis to be effective Inhibitory against a wide range of Gram-positive bacteria and limited potency against the growth of Gram-negative bacteria and in a study [25, 26]. On two samples of Turkish-origin propolis, a sample of Bulgarian-origin propulus, and a study of its effectiveness against my germ. Staphylococcus. aureus and coli Escherichia.

The method of drilling has shown all the samples to be High efficacy against bacteria *S. aureus* and limited effectiveness against *E. coli*. In this study, the alcoholic extract was shown to have a distinct effect in inhibiting the growth of Gram-negative *Acinetobacter baumannii*

bacteria compared to the antibiotic under study, which is consistent with. In general, propolis has been shown to exhibit stronger antimicrobial activity against Gram-negative bacteria due to the geographic location from which propolis was collected, where the chemical composition of the propolis varies greatly from region to region depending on the bee's diet [27].As explain, on the effectiveness of propolis against positive and Gram-negative microorganisms that are resistant to multiple drugs[17].

The influential act shown by the alcoholic extract of the raw material propolis against the Pomanium stagnant is due to the latter containing a number of active compounds, especially flavonoids, phenols and terpes, which previous studies have shown to contain raw propolis [28]. When he pointed out Components of propolis such as flavonoids and fenolic acid esters have an antibacterial effect. In general, the mechanics of alcohol extraction of propolis against microscopic biology is not known precisely are said [29], Propolis has an effect on the plasma membrane of microorganisms, and it also inhibits their movement and the enzyme effect, while both [30].

In their study of propolis using electron microscopy, the process of cell division of microbes stops with the alcoholic extract of propolis, and they suggested that this ability of this extract to stop. The process of cell division is the result of inhibiting the process of DNA division of bacteria. Because propolis contains a large number of antibacterial compounds, it can be highly efficient at inhibiting the growth of germs from more than the coming mechanics. It has also been observed from the results of this study that the diameter of inhibition of the growth of *A. baumannii* in Planted Dishes increases the concentration used[31]. That is, there is a direct correlation between the inhibition diameter and the concentration of the alcoholic extract used and that This explains the increase in bacterial growth inhibitors as well as the concentration used. It was also noted that effectiveness The extract decreases at one week of preparation of the concentrations of the extract. This explains the uncertainty of the material. Daily preparation is desirable when the experiment is performed or repeated in the laboratory for better results. So are most of the bacteria isolated from the Diabetes foot is highly resistant to many antibiotics that, after being treated with the antibiotic, can become resistant to it. After getting to know him. While they are sensitive to simple antibiotics, this explains why bacteria can mutate their genes.

Against more efficient antibiotics, that is, they have the potential to change course if effective antibiotics affect them or They use close bacterial species that increase their resistance at the same time the simple antibiotic inhibits their growth. Caution must therefore be taken when dealing with such a type of bacteria and an allergy test is performed before prescribing appropriate antibiotics so that their resistance is not increased by inaccurate antibiotic prescribing.

Conclusion

Concession The predominance of Gram-negative bacteria isolated from the diabetic foot in the holy province of Kerbala compared with cram-positive bacteria. We conclude from the current study that A. baumannii bacteria is the most pathological cause of diabetic foot ulcers.

We conclude that the proportion of diabetic foot ulcers in males is higher than in females. The alcoholic extract of the raw large is highly effective and has a high inhibitory effect against the bacteria A. baumannii. The synergistic action of the alcoholic extract of propolis with dermacin solution gave inhibition to bacteria.

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