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Study of the Effect of Inhibitory Activity on Palm Pollen Phoenix Sylvestris in Some Bacterial Isolates from Male Reproductive Infections

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Abstract

The aim of this study was to investigate the inhibitory activity of the hot alcoholic and water extracts (cold and hot)and acetonic extracts of Phoenix Sylvestris to the bacteria isolated from the *Staphylococcus aureus* and *Streptococcus pyogenes*, Which is a common cause of genital tract infection, *Klebsiella spp* and *Enterobacter spp* Cause inflammation and urinary tract infections. from the Sadr City Medical Hospital in Najaf, for its clinical importance as the cause of genital infection, where 320 samples of patients with symptoms and signs of genital tract infection were collected. The result was 46 isolates from The highest bacterial infection in the infected patients was *Staphylococcus aureus* (17) isolates and 40%, followed by *Streptococcus pyogenes* with 15 isolates and 32%, respectively. Followed by *Klebsiella spp bacteria* with a number of isolates (9) and a percentage of 19%. The lowest infection rate among bacterial patients was *Enterobacter spp* with (5) isolates and (9%) of the total number of bacterial infection patients (46).The results of the study showed that these extracts have an effective effect in inhibiting the growth of these bacteria outside the body of the organism The plant extracts varied against the bacterial species using the method of wells diffusion method in the agar at a concentration of 200 mg / ml. The acetonic extracts showed the highest inhibitory effect against the bacteria studied, followed by hot and cold alcohol extracts, while the hot water extract showed little effect by measuring Inhbition Zone diameter.

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The minimum inhibitory concentration MIC was determined for Acetonic and Alcoholic extracts against bacteria and was between 12.5-50 mg / ml. The hot water extract was (50 µg / ml) for the *Staphylococcus aureus* bacteria and the *Streptococcus pyogenes*, The concentration 50 mg / ml did not show effect on the bacteria *Enterobacter spp* and *Enterobacter spp*., MBC was determined with 25-50 mg / ml of acetone extract. MBC for hot alcohol extract was 25 mg / ml and cold alcohol extract was 50 mg / ml for bacteria, *Streptococcus pyogenes, Enterobacter spp, Staphylococcus aureus, Enterobacter spp*. The hot water extract was negative for all bacteria tested for 50 mg / ml concentration. The results of the first preliminary detection of cold and hot alcohol extract showed that it contains effective substances (Flavonids, Steriod, alkaloids, Aminoacid, saponin, glycosideTanins and Terpenoid), the hot water extract is to contain it (Tanins, Flavonids and saponin) only. The acetone extract contained Tanins, Flavonid, saponin, Terpenoid.

Keywords: Phoenix Sylvestri; Biochemical tests; Inhibition area; Genital infections; Bacteria.

1. Introduction

Infection of bacterial urinary tracts is one of the most common diseases in men, ranking second in prevalence after infection of respiratory tracts [1]. Genital tract infection usually results from attacking microorganisms of the reproductive system, which are often Gram-negative pathogens. As most of the infections of the reproductive system are caused by *Staphylococcus aureus* and *Streptococcus pyogenes*, which is a common cause of genital tract infection [2]. As well as other pathogens include *Klebsiella*. *Spp*, which causes inflammation of the urinary tract and genus *Enterobacter spp*, which represents another genus of intestinal bacteria of medical importance, causing genital infections. The virulence of the attacking bacteria and its susceptibility to the host is of fundamental importance in the occurrence and development of infection, which depends on a series of interactions between the pathogen and the host [3]. Where medicinal plants and herbs in developed countries used 80% to treat various diseases, including infertility caused by genital infections [4]. Plants manufacture secondary metabolites, which contribute to many biological events and are used as a defense and as an antibiotics to many diseases [5]. In the past, most of the infections of these germs could be treated with antibiotics. However, at present, the natural treatment of herbs of all kinds has spread without resorting to the drugs of the world of chemical laboratories and synthetic materials. The new sources were the use of medicinal plants as antimicrobial agents, such as palm pollen used in this study because it contains elements It contains essential amino acids and other essentials, fatty acids, proteins, carbohydrates, vitamins and minerals [6]. Palm pollen has the nutritional qualities that have enabled it to resist inflammation and increase immunity [7]. In addition, it contains estradio l, which is similar to estrogen, [8] and palm pollen has the ability to stimulate the ovaries. [9] The plant possesses important active groups It has been used in many important therapeutic applications used in reducing and regulating the proportion of microbes in the body. The plant has been known to have multiple uses, such as the production of highly effective antimicrobial agents such as bacteria, fungus and viruses [10]. Dietary and pharmacological analyzes have shown that palm pollen contains hormones such as hormones, esters, vitamins A, H, D, H, K (group of vitamins) 34% carbohydrate nutrients, 35% proteins, 5% calcium, Phosphorus, silicon salts, boron, aids of Siteo chromium, isomers, carotene pigments, Xanthophyll. , Other Materials Mineral acids, phenolic acids, monocrystalline, binary, and triple glycerides. There are many health benefits, including improved reproductive capacity, very useful for infertility

in women, elimination of stress and elimination of all symptoms during menstruation. Strengthen the liver and prevent toxins and prevent the deposition of fat in the liver and protect the liver from fibrosis. Preventing nervous tension and prostate diseases. Strengthens the bodies of children, youth and adults and supplies them with vitamins, enzymes and important mineral elements. Treatment of hemorrhoids, weakness of veins and capillaries. Rebuilding and strengthening the immune system. Helps to tighten the body's skin and soften it. Treatment of indigestion, wasting and thinness and helps to open the appetite and regulate the process of food conversion in the body. Treatment of diseases of aging. Increase body resistance to popular flu and flu. Increase red blood cells and raise hemoglobin. Useful for the health of pregnant women and fetuses. It is useful for patients treated with radioactive drugs and for patients exposed to radiological examination. Treatment of wounds and regeneration of burning skin. Treatment of fine intestinal inflammation. Treatment of bleeding conjunctivitis. , Where it was found that the smell of pollen (dry) for the treatment of colic and gastric disorders of the clutch and anti-diarrhea [11].

2. Materials and Methods

2.1 Plant sampling

The palm pollen available in the local market was collected. The pollen was then peeled and cleaned from the soil in the tap water and placed in the shade to dry at room temperature of 25 ° C for two weeks, then grinded by an electric grinder to obtain powdered cereal. Palm. Place the powder in clean, sterile, dry nylon bags in the refrigerator until used in the microbial study.

2.2 Preparation of a hot water extract and cold and hot alcohol for palm pollen seeds

A method [12] was adopted in the preparation of the hot water extract of the palm pollen and the The acetone was prepared according to [13].It involves taking 40 g of dry palm pollen and placing it in a glass flask with 350 ml of hot distilled water and, the flask and its contents placed on the hot plat stirrer for mixing for an hour and a half and leave the solution for 24 hours to settle the parts of the plant after its covered, after which the solution was filtered through a clean cloth and then using the filter type Watman (No.1) The centrifugal filter was placed at 3000 cycles / min for 10 minutes for the purpose of separating the precipitation and extracting the extract. Then put the extract in the rotary evaporator device to concentrate the extract at a temperature of(45 – 40) () m, and then kept the powders in sterilized bottles in the refrigerator until use.

2.3 Preparation of the original solution (storage) for the extraction of palm pollen seeds

A reservoir solution was prepared with a concentration of 200 mg / ml. The solution was then sterilized using 0.45 µm diaphragm filters. And was stored in the refrigerator at a temperature of 4 m. After that, the different concentrations of each extract were present from the original solution, using the dilution law: N1 × V1 = N2 × V2.

2.4 Preparation of concentrations of plant extracts

In order to prepare the stock solution for the hot water extract, take 2 g of plant extract and solvent in 10 ml of sterilized distilled water. We have a storage solution with a concentration of (0.2) g / ml sterile solution by filtration using the filter and filtration papers What man No.10) to get rid of the microbial contaminants present in it and obtain a sterile storage solution. This solution was used to make the concentration of 100, 200 mg / ml for the preparation of the alcohol extract, 2 gm. and the solvent in 3 ml of ethyl alcohol and complete the volume to 10 ml with the distilled water. The solution concentration was 200 mg / The remaining concentrations are 200,100 mg / ml.

2.5 Chemical detection of some active ingredients in palm pollen extracts

- Detection of Alkaloides reagent alkaloids followed the method [14] and [15].
- Detection of phenols reagent followed the method [16] and [17].
- Detection of terpenoid reagent turbines followed method [18] and [19].
- Detection of amino acids followed the method [20].

2.6 Method of collecting, isolating, diagnosing and preserving clinical bacterial samples

A total of 320 isolated and isolated bacterial samples were collected at the Sadr City Medical Hospital in Najaf Governorate exclusively for male sex. The samples were collected in sterile tubes. The samples were then examined before incubation at 37 ° C. And then examined the eye and microscopy and then cultured on the various types of the culture media to know the type of bacteria causing bacterial infection. The diagnosis of bacterial isolates based on [21] To diagnose some of the characteristics of the culture and the appearance of the characteristics of the chemical biochemical on the basis of the following

• Microscopy

• Formal and culture characteristics

After completion of incubation period and conditions for each medium, the dishes were removed from the incubator. The culture characteristics were observed on the medium of blood agar and the other culture medium: Nutrient agar, MacConkeysager, Mannitol salt agar, and the shapes, colors and sizes of the colonies were recorded in these media.

• Biochemical tests

The following biochemical tests were carried out:

• Catalase Reagent test.Oxidase test Reagent.

• Voxesproskaur indicator.

• Hemolysis Test:

• Indole test: Indol test medium.

• Methyl- red reagent test.

• Test citrate consumption: citrate agareSimmon

• IronKligler test: Kligler Iron agar.

• Growth on the mediumMannitol salt agar.

• Growth on the medium EMB.

• Motility medium:.

And the bacterial isolates were saved in two ways: the short-term conservation method, where The tubes containing the solid nutritious medium slant with bacteria were to be vaccinated and incubated at 37 ° C for 24 h and then incubated at a refrigerator temperature of 4 ° C until used .The second method is the long-term preservation, where the tubes containing the liquid nutrient-containing medium of glycerol were cleared with a 15% concentration cultured with bacteria and stored for 24 hours after being kept at -20 ° C [22]. Where 3 bacterial isolates were taken and calibrated with The McFarland tube was sprayed on the center of Muller Hinton for the purpose of studying the effect of the extract.

2.7 Test the sensitivity of bacterial isolates to the plant extract recorded in the growth of bacteria

The concentration of 200 mg / ml for all extracts were obtained using distilled water of the water extract and 10% DMSO solution for the alcoholic and acetone extracts. The sensitivity of all bacterial isolates was tested and thewell diffusion method was used by [23] to test the bacterial sensitivity of the plant extract. (0.1) ml of bacterial suspension for each microbiology type of bacteria was published on thesolid mediumof Muller Hinton. Using the Cork borer and then work (3) wells with a diameter of 6 mm per well. Add (0.1) mm of plant extract concentration as mentioned in the above paragraph and add distilled water to one of the well as a negative control. The dishes were left for 15 minutes and incubated at 37 ° C for 24 hours. The diameter of the inhibition zone was then measured by the ruler [24].

2.8 Determination of the minimum inhibitory concentration MIC and the minimum bactericidal concentration MBC for all plant extracts

A series of diluted concentrations of all plant extracts (50,25,12.5) mg / ml using Nutrient broth were obtained. The tubes were cultured with 0.1 mL from the 24-hour bacterial farm and the container was 1.5 x 10 ^ 8 cell / Ml and then incubated the tubes at 37 ° C for 48 hours. The results were compared with the control of the culture medium with the plant extract only. The minimum inhibitory concentration (MIC) was determined to be the lowest concentration of the extract, which prevents a clear inversion of the naked eye in the culture medium. The minimum bactericidal concentration (MBC) was determined by transferring 0.1 milliliters from all the tubes

that did not have an acorn to container dishes on the nutrient agar and incubated dishes at 37 ° C and the value of MBC was determined to be less than the concentration of the extract, which inhibits the growth of bacteria [25].

3. Results and Discussion

The results of the present study aimed at investigating the presence of bacteria causing genital infection through 320 swabs of genital infection from patients in Al Sadr Medical City / Najaf Governorate. A total of 46 bacterial isolates were obtained from these swabs for different types of bacteria that are most harmful and dangerous to the reproductive system, i.e. 100%.*(Staphylococcus aureus)* was the most common form of bacterial genital infection, with 40% bacterial isolation . This is consistent with the findings of the researchers [26] in Nigeria where *Staphylococcus aureus* was isolated by 4758.8% of the total number of samples (229) this ratio is the highest isolation of bacterial species isolated ratio, and The percentage of isolating the bacteria *Streptococcus pyogenes*32% (bacterial isolation), These results differed with the study of the researcher [27] In Italy, which isolated the bacteria by (10%) of its total samples (417). The percentage of isolation of *Klebsiella* Spp. bacteria was(19%) bacterial isolates, Where the results of isolation of *Klebsiella spp bacteria* have converged with the study of the researcher [28]. The results of isolating the intestinal bacteria (*Enterobacter Spp*.) were (9%) also agreed with the results of the researcher [29]. This variation in the isolation rate of bacterial species is due to the varying length of time of isolation of bacteria and the techniques of isolation and diagnosis used in the study [30]. It was found that there were differences between the percentages and the number of different bacterial isolates in general as in Table (1).

Table 1: Shows the percentage of pathogenic and isolated bacterial species of infected patients with severe genital infections.

Type of bacteria	Number of isolates	%
Staphylococcus aureus	17	40
streptococcus pyogenes	15	32
Klebsiella. Spp	9	19
Enterobacter spp.	5	9
	46	100%

The results of the qualitative detection of some of the active compounds in the plant extracts under study showed that the pollen palm was carried out using several methods to detect these compounds in Table 2 and showed the results of the chemical surveys of the pollen palm. The water extract contains several active substances, including saponin, flavonoid, Tannin only, and the cold and hot extract of alcohol contains all these compounds found in the water extract, as well as glycoside, alkaloids and AminoacidSteriod., The acetone extract contained saponin and flavonoid and tannin and Terpenoid [31]. And many active ingredients including oils and compounds responsible for anti-microbial activity and this is consistent with [32]. In addition to

containing oleic acid and fatty acid containers on the ester instance of Fattyacid methyl esters this is consistent with [33]. Phenolic compounds are a rich source of antioxidants [34].As for the nature of the extracts were characterized by viscous strength and smell aromatic characteristic that resemble the smell of semen for men, The aromatic aroma of the palm pollen is attributed to its containment of volatile oils, which contain essential oils in various parts. There are basic compounds found in this oil, Sitosterol, linolenicacid, linoleicacidoleic acid) [35].

Table 2: Shows the active compounds in palm pollen extracts.

Active compounds	Acetone extract	Cold ethanol extract	Hot ethanol extract	Hot water extract
Flavonoid	+	+	+	+
Saponin	+	+	+	+
Tannin	+	+	+	+
Glycoside	-	+	+	-
Aminoacid	-	+	+	-
Terpenoid	+	+	+	-
Steriod	-	+	+	-

The inhibitory effect of palm pollen extracts on bacteria isolated from genital infections in men was studied. The effect of different concentrations of water, alcohol, acetonitrile, and palm pollen was studied on bacterial isolates isolated from patients with genital infections and the most harmful resistance of bacterial isolates *Staphylococcus aureus, Enterobacter spp, Klebsiella.Spp, Streptococcus pyogenes* Sensitivity of bacteria to plant extracts, The results of the use of the concentration of 200 mg / ml for the extracts (hot water, coldand hot ethanol and acetone) were shown by measuring the diameter of the inhibition zone. Table (3) Concentrate 200 mg / ml for extracts against bacterial species isolated from genital infections.

Table 3: The effect of concentration (200) mg / ml for extracts on isolated bacteria

Type of bacteria	Diameter of inhibition zone (mg / ml) for extracts			
	Acetone	Ethanol Hot	Cold Ethanol	Hot Water
Staphylococcus aureus	16	11	8	6
Streptococcus pyogenes	15	13	11	7
Klebsiella. Spp	13	9	8	7
Enterobacter spp	11	10	8	10

The table shows the diameter of the inhibition zone with the highest effect of the acetone extract followed by the hot and cold ethanol extract and the lower effect of the hot water extract. Determination of MIC and MBC for extracts against bacterial species where concentrations (25,50,12.5 mg / ml) were used for extracts. The results indicated in Table (4) and (5) showed variations based on the type of extract and the type of bacteria. It was

found that the negative bacteria were more sensitive than the positive bacteria for all extracts.

Table 4: Shows MIC values for extracts against bacterial species.

Type of bacteria	Plant extracts (mg / ml)			
	Acetone	Ethanol Hot	Cold Ethanol	Hot Water
Staphylococcus aureus	50	50	50	50
Streptococcus pyogenes	25	25	50	50
Klebsiella. Spp	12.5	12.5	25	-
Enterobacter spp	25	12.5	25	-

(-): The negative sign symbol indicates that there is no increase in bacterial growth.

Table 5: Shows the MBC values of the extracts against bacterial species.

Type of bacteria	Plant extracts (mg / ml)			
	Acetone	Ethanol Hot	Cold Ethanol	Hot Water
Staphylococcus aureus	50	50	50	-
Streptococcus pyogenes	50	25	50	-
Klebsiella. Spp	25	25	50	-
Enterobacter spp	25	25	-	-

(-): The negative sign symbol indicates that there is no increase in bacterial growth. It is clear from Table (4) and (5) that hot acetone and alcohol extracts had a greater inhibitory effect on bacterial isolates followed by cold alcohol and hot water extracts. This may be due to the fact that these extracts contain the active substances listed in Table (2) and may contain additional active substances because they are raw extracts As well as the ability of alcohol extracts to dissolve in the cell membrane of bacteria due to the fitting of lipid membrane to the fat in the extract [36,37]. Ethyl alcohol also has a high ability to withdraw active compounds from the plant sample due to its high polarity, It was found that the negative bacteria were more affected by the Gram positive bacteria, because they had an external membrane made of fatty proteins and phospholipids compared to the Gram-positive bacteria containing a small percentage of fat and a high percentage peptidoglycan [38].

4. Conclusion

It was found that the raw extracts of palm pollen have Antibiotic effectiveness against certain bacterial species that cause genital infection in males because they contain some active substances that inhibit the growth of bacteria,and when separating the active compounds of the raw extracts and using them separately, a more effective result is in inhibiting the growth of the bacterial species under study.

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