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Evaluating the Inhibitory Efficacy for Southern Cattail (Typha Domingensis) Pollen Extracts against the Growth of Two Types of Human Pathogenic Bacterial and Fungal Isolates

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Abstract

The effect of inhibitory efficacy of water and alcoholic *Typha domingensis* pollen extracts and powder solution, as well as the effect of minimal inhibitory concentrations MIC on two types of bacterial isolates: Escherichia coli and *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger* pathogenic to humans. The results showed that the aqueous and alcoholic extracts and the powder solution of all concentrations (0.05, 0.1, 0.2, 0.3, 0.4, 0.5) mg. L^{-1} had an inhibitory effect against bacterial isolates and the greatest inhibition was at 0.5 mg. L^{-1} for the three extracts and for both types of bacteria, The powder solution recorded the highest inhibition diameter of *Staphylococcus aureus*, reaching (32.76) mm, and *Escherichia coli* (28.65) mm. The extracts of *Typha domingensis* pollen showed an inhibitory effect in all concentrations in inhibiting the growth of fungal isolates, where the powder solution recorded the largest inhibitory diameter against the growth of Candida albican and Aspergillus niger fungi at a concentration of 0.5 ml.L^{-1}, where the diameter of theinhibition zone reached (27.97, 33.71) mm, respectively. The minimum inhibitory concentration MIC ranged between (100-400) mg.ml^{-1}.

Keywords: Southern cattail, *Typha domingensis*, pollen extracts

INTRODUCTION

Typha domingensis is a flowering aquatic plant belonging to the Typhaceae family, which includes more than 500 species worldwide with about 104 genera (Keddy, 2010). It is a powerful perennial herbaceous plant with long leaves and is called a pond herbivore (Jane and Heidi, 2014).It grows to a length of 3-4 m, and it is a dioecious plant and is considered an vertical plant, meaning that the plants have their roots in the water, while most of the plant's body is outside the water. (Alwan, 2006), It has its country origin in Africa. It grows on the banks of rivers, swamps and lakes in large agglomerations, where it is considered a plant in tropical regions sensitive to frost. This explains its growth in the marshes and swamp areas in southern Iraq and on the banks of the Shatt al-Arab. Its spread extends to the central and northern regions. As its plants grow in shallow areas, in clay soils, forests, fields and semi-saline wetland environments (Shipley,1989), a long time ago, the *Typha domingensis* has attracted the attention of scientists and researchers because it is one of the plants capable of purifying water by taking nutrients and pollutants dissolved in the water and providing a clean environment. Many plants have been found to have Inhibiting efficacy against pathogens, where they contain active elements and compounds after they have been extracted and purified, in addition to their few side effects. The inability of germs to create resistance to

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them and there are many studies on the use of their plant extracts in inhibiting and killing microorganisms when scientific progress with all its capabilities fails to cure some diseases as well as being an economical, safe and highly efficient treatment (Al-Sayed, 2004)

The efficiency of these extracts varies according to the method of their extraction, the type of solvent used for the extraction and the experimental microorganism (Al-Zubaidi, 2005). *Typha domingensis* pollen is extracted from the *Typha domingensis*, which specialists consider a complementary food that benefits pregnant women and irritable bowel patients, and contributes to lowering blood, reducing cholesterol, strengthening teeth in children, treating diarrhea, intestinal colic, and treating wounds. In view of the absence of applied scientific studies at all on the biological effectiveness of *Typha domingensis* pollen against bacterial and fungal isolates that cause human pathogenicity, this study was conducted.

MATERIALS AND METHODS

1- Study plant:

Typha domingensis pollen grain was obtained from papyrus plants located in Al-Chibayish area Marshes in Nasiriyah province, They were taken and dried under the heat of the sun, and then the pollen grains were separated from the other materials with a piece of light cloth.

2- Used isolates

Two types of bacterial isolates were selected: Escherichia coli and *Staphylococcus aureus*. Candida albicans and *Aspergillus niger*, obtained from the microbiology laboratory at the College of Science - Basra University, were also used to study the inhibitory effect of *Typha domingensis* pollen grain.

3- Preparation of extracts

3-1: Aqueous Extract:

Mix 20 g of pollen powder with 200 ml of sterile distilled water and leave the solution with constant stirring by shaker for 24 hours at room temperature after which the solution is filtered through several layers of gauze. Then the extract was placed in a dish petri dish and left exposed at room temperature to dry. Then it was kept in sterile glass bottles until the inhibition activity of the tested bacteria and fungi was tested (Harb, 2011).

3-2: Alcoholic Extract:

The alcohol extract was prepared in the same way as before, except for replacing the water with 70% ethanol.

3-3: Preparation solution powder

Mix 10 g of powdered powder with 25 ml of distilled water to make a concentration of 40 $mg.ml^{-1}$was used directly in testing the inhibition activity against bacteria and fungi (Reuter, 1996).

3-4: determination of minimum Inhibiting concentrations (MIC)

The minimum Inhibiting concentrations of *Typha domingensis* pollen extracts against bacterial and fungal isolates were determined by preparing a stock solution by dissolving 2 g of the extract in 5 ml of solvent (DMSO) Dimethyl sulfoxaide. A series of dilution was prepared as 10, 30, 50, 150, 250, 300, 350, 400 mg. L^{-1} (Al-Jubouri, 1990).

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4- Sterilization of prepared plant extracts:

4-1: Sterilization of aqueous extracts

For the purpose of sterilization, 2 g of the extract was taken and dissolved in 5 ml of distilled water, thus obtaining an extract with a concentration of 40 $mg.ml^{-1}$ where a standard concentration and then was sterilized through membrane filters with a diameter of 0.22 m. This was the standard concentration for the dilution preparation used in the study (Al-Nu'man, 1998).

4-2: Sterilization of alcoholic extracts

The 2 g of the alcoholic extract were dissolved in 5 ml of ethylene glycol to a concentration of 40 ml. Ml as standard concentration for dilution preparation and then sterilization by pasteurization at 62 ° C for 10 minutes. This was the standard concentration for dilution preparation used in the study (Al-Numan, 1998).

5: Chemical detection:

Samples from crude extracts were used to detect some of the basic chemical components that may be present in the papyrus pollen under study. Table (1) shows the results of these revelations. The approved color detection methods were used for the chemical compounds mentioned in the table as follows:

5-1: Detection of Cuomarin: a little of the crude extract was added in a test tube and covered with a filter paper moistened with a solution of dilute NaOH, then placed in a boiling water bath, and the filter paper was exposed to ultraviolet rays and the appearance of a greenish-yellow color was evidence of the presence of comrin (Geismann, 1992) .

5-2: Detection of Saponin: The aqueous extract was shaken in a test tube and a thick foam appeared index of the presence of saponins (Shihata, 1951).

5-3: Detection of Alkaloid: Use of the Drageendorf reagent, which gives an orange color when interaction with the alkaloids present in the extract (Fahmy, 1933).

5-4: Detection of glycosid: Kadde droplets are added to 5 cm^3 of aqueous extract of the sample and the appearance of a purple color is evidence of the presence of glycosides (Alshahat, 1986).

5-5: Detection of Tannin: When 1% lead acetate is added to the fragrance from the aqueous extract, it leads to the appearance of a gelatinous precipitate, evidence of the presence of tannin (Dalali et al., 1987).

5-6: Detection of Volatile Oil: When adding a little extract to a filter paper to the limit of saturation and exposure to ultraviolet rays, it causes a gray color to appear, indicating the presence of volatile oils (Geismann, 1992).

5- Preparing the media

Mueller Hinton agar was used to test the effectiveness against bacteria, which was prepared according to the instructions of the supplier. Agar diffusion (Cruickshank et al., 1975) was used ,In which the diameters of the growth

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inhibition zones are measured (the areas free from bacterial growth due to the effect of the extract used).The medium was inoculated with 0.1 ml of microbial suspension, a concentration of 10^8 bacterial cells at an age of 18 hours, which were measured by a spectrophotometer at a wavelength of 450 nm. Spread the bacterial suspension by sterilized cotton swap droppers. The dishes are left for 15 minutes to absorb the suspension in the culture medium. Three replicates were made for each isolate, then drilled with a diameter of 5 mm was made to inject the extracts into the dishes grown with bacterial isolates, then the plates were incubated at a temperature of 37 C° for 24 hours, then the results were recorded by measuring the diameter of the inhibition zone in milliliters and the method was repeated for all isolates under study. For testing the effectiveness against fungi, the Sabouraud Dextros Agar culture medium was prepared, which was prepared by dissolving 28 g of it in a liter of distilled water and dissolving the materials by heating with stirring with the help of a magnetic stirrer, and sterilized with an Autoclave device under a temperature of 121 m and a pressure of 1.5 atmosphere for a period of 30 minutes. Cool then pour into sterilized Petri dishes and allow to harden. The Agar diffusion technique was used, as 0.1 ml of the fungal culture suspension at the age of 18-24 hours grown in sterile distilled water was used so that the number of fungal cells at this stage was about 10^8 cells. The dishes were inoculated with *Aspergillus niger* or *Candida albicans*, and the diffusion technique was used. ,Then pits with a diameter of 5 mm were made on the dishes grown with fungal isolates, then the dishes were incubated at a temperature of 37 ° C for 24 hours and the result was recorded by measuring the damping diameter in mm with a ruler.

6- Statistical analysis

The experiment was conducted using a Completely randomized design with three iterations for each treatment, then the averages were compared using the Least Significant Difference Test (L.S.D) (Alrawi and Khalaf Allah, 1980).

RESULTS AND DISCUSSION

The results in tables (2,3) showed that the powdered solution of *Typha domingensis* pollen grain had a significant effect in inhibiting the growth of bacteria, both Escherichia coli and *Staphylococcus aureus*, and the highest inhibition was the concentration of 0.5 ml. L^{-1}.The diameter of the inhibition zone was (28.65, 32.76) mm, respectively, compared to the lowest diameter of inhibition recorded by the aqueous extract with a concentration of 0.05 ml.L^{-1}, if the diameter of the inhibition zone reached (10.50, 12.72) mm, respectively, which is thus excelled to the effect of the two antibiotics Cefodizine and Erythromycin (Table, 7).

Table (2) The effect of concentrations and extract type on inhibiting Escherichia coli bacterial diameters

Extract concentrations (ml / L) and inhibition zone diameters (mm)						
0.5	**0.4**	**0.3**	**0.2**	**0.1**	**0.05**	**extract**
22.00	**16.98**	**17.11**	**14.98**	**12.00**	**10.50**	**aqueous**
20.17	**18.65**	**18.89**	**18.07**	**15.09**	**12.00**	**alcoholic**

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28.65	**23.00**	**25.87**	**19.98**	**15.00**	**13.00**	**Powder**

Table (3) the effect of concentrations and type of extract on inhibiting *staphylococcus aureus* bacteria

Extract concentrations (ml / L) and inhibition zone diameters (mm)						
0.5	**0.4**	**0.3**	**0.2**	**0.1**	**0.05**	**extract**
25.30	**20.71**	**19.16**	**15.53**	**16.81**	**12.72**	**aqueous**
20.54	**20.09**	**18.85**	**17.82**	**14.13**	**15.87**	**alcoholic**
32.76	**25.90**	**26.98**	**19.65**	**16.90**	**16.86**	**Powder**

the results in Table (4)showed that the excelled of the alcoholic extract with a concentration of 0.5 ml. L^{-1} was significant in inhibiting the growth of Candida albican fungi ,The diameter of the inhibition zone was (27.97) mm compared to the lowest diameter of inhibition recorded by the aqueous extract with a concentration of 0.05 ml. L^{-1}, as it reached 10.76 mm.

Table (4) the effect of concentrations and type of extract on inhibiting *Candida albicans* diameters

(Extract concentrations (ml / L) and inhibition zone diameters (mm						**extract**
0.5	**0.4**	**0.3**	**0.2**	**0.1**	**0.05**	
20.55	**20.41**	**20.78**	**10.65**	**15.80**	**10.76**	**aqueous**
27.97	**19.76**	**13.70**	**14.90**	**14.77**	**13.51**	**alcoholic**
24.18	**23.60**	**21.60**	**17.00**	**16.90**	**17.87**	**Powder**

Either from Table (5), it can be seen that the powder solution with a concentration of 0.5 ml.L^{-1} was significantly excelled in inhibiting *Aspergillus niger*, where the diameter of the inhibition zone was (33.71) ml. L^{-1} compared to the lowest inhibition diameter recorded by the aqueous extract of *Typha domingensis* pollen at a concentration of 0.05 ml. Liter-1, where the diameter of the damping area reached (10.98) mm. Thus, the alcohol extract and the powder solution are excelled to the two antibiotics Oxyteracyclin and Amoxicillin, which reached the highest inhibition diameter (16.00 and 20.00) mm, respectively, Table (7).

The minimum Inhibiting concentration MIC ranged between (100-400) mg. ml^{-1} as shown in Table (6), and the minimum Inhibiting concentration may vary depending on the preparation method and the method of extraction (Lawson, 1996).The inhibition activity is due to the presence of glycosides, alkaloids, saponins and tannins, which make the extracts more effective against the experimental organisms (Draughon, 2004).Table (7) shows the sensitivity of bacterial and fungal isolates to antibiotics. where it showed the effectiveness of similar to that shown by the extracts of *Typha domingensis* pollen and less than that of the powder solution, as shown in the tables (2,3,4,5).Therefore, extracts of Typha domingensis pollen and powder solution can be used to inhibit the effect of bacteria and fungi with minimal cost and less side effect, after conducting detailed clinical studies.

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Table (5) The effect of concentrations and type of extract on inhibiting the diameters of Aspergillus niger

Extract concentrations (ml / L) and inhibition zone diameters (mm)						**extract**
0.5	**0.4**	**0.3**	**0.2**	**0.1**	**0.05**	
27.54	**22.95**	**20.61**	**18.96**	**12.65**	**10.98**	**aqueous**
25.28	**22.73**	**21.70**	**20.57**	**15.05**	**16.84**	**alcoholic**
33.71	**28.60**	**25.43**	**23.87**	**17.84**	**16.50**	**Powder**

Table (6): Minimum inhibiting concentration of extracts (aqueous, alcoholic, powder)

MIC mg /ml	**extract**	**Isolates**
150	**aqueous**	
100	**alcoholic**	
300	**Powder**	***Escherichia coli***
150	**aqueous**	
150	**alcoholic**	***Staphylococcus aureus***
250	**Powder**	
350	**aqueous**	
200	**alcoholic**	***Candida albicans***
250	**Powder**	
300	**aqueous**	
300	**alcoholic**	***Aspergillus niger***
450	**Powder**	

Table (7) The sensitivity of bacterial and fungal isolates to antibiotics

The diameter of the inhibition area (n				**Concentratio**	**isolates**	**antibiotics**
19.00	21.00	22.00	12.00	30	*Escherichia Coli*	Cefodizine
20.00	23.00	18.00	18.00	30	*Pseudomonas aeruginosa*	Erythromycin
16.00	17.00	13.00	16.00	30	*Candida albicans*	Oxyteracyclin
20.00	14.00	10.00	11.00	30	*Aspergillus niger*	Amoxicillin

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