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Antimicrobial and Antioxidant Activity of *Typha* L. Pollen Extracts Against Selected Bacteria and Fungi

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ABSTRACT

Recently, humanity has turned to medicinal plants as a remedy and analgesia for various diseases. In this work, two procedures, oily and alcoholic, were employed to extract *Typha* pollen. Additionally, the extracts were subjected to qualitative analysis of the active components. Then, the inhibitory activity of the extracts was tested on three fungal and bacterial isolates. The antioxidant activity of both extracts was evaluated. The results showed that the inhibitory activity of the extracts varied depending on the extraction solvent and the microorganisms under study. The alcoholic extract gave a higher superiority than oily extract in inhibiting the test microorganisms isolating at all its concentrations. The percentage of growth inhibition reached 90%, 96% and 94% for fungi *Aspergillus niger*, *Fusarium* spp. and *Penicillium* spp., respectively, at the highest concentration of 100 mg/ml. The growth inhibition diameters were 36 mm, 43 mm, 37 mm for *Staphylococcus aureus*, *Bacillus sp*., and *Escherichia coli*, respectively, at the highest 100 mg/ml concentration. The alcoholic extract showed a higher DPPH free radical scavenging capacity than the oil extract, reaching 89.74% at the concentration of 10 mg/ml compared to the oil extract, which reached 76.64% at same concentration. According to the above results, it is believed that *Typha* pollen has promising potential in medical and pharmaceutical applications, especially due to its biologically active compounds that exhibit significant anti-inflammatory, antioxidant and antimicrobial properties.

Keywords: Antibacterial, Antifungal, Antioxidant, *Typha* pollen extract

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INTRODUCTION

The use of antibiotics has become widespread in the medical, agricultural and veterinary fields. This has enhanced the development of antibiotic resistance among infectious microbial strains, leading to a serious problem in treating pathogenic microbes [1]. Therefore, recent studies have turned to using plant extracts as natural antibiotics against pathogenic microorganisms, as they do not cause any side effects when used in specific doses [2]. Especially since, they have an antioxidant biological activity with therapeutic properties [3]. Plant extracts are used to combat diseases. However, plant extracts and medicinal herbs have received great attention recently. They are a source to produce medical drugs or active substances that enter into the composition of the drug, in addition to their use in cosmetics. Many studies have addressed the effect of these extracts on the growth of microorganisms, which has led to their use in treating various microbial diseases that affect plants and humans alike [4, 5].

Abbas and Al-Subaihawi [6] studied the effect of aqueous and alcoholic extracts of *Asparagus officinalis* roots on the growth of *Aspergillus niger* and noted that both extracts inhibited fungal growth with high efficiency. Siqueira [7] also showed that the alcoholic extract of propolis was characterized by inhibitory activity against three types of yeasts: *Candida albicans*, *C. glabrata*, and *C. tropicalis*. Richwagen [8] indicated the efficiency of the alcoholic extract of lavender wood stems in inhibiting both *Staphylococcus aureus* and *Acinetobacter baumannii*. Nayim [9] studied the inhibitory activity of the alcoholic extract of sweet potato leaves on *Klebsiella pneumonia* and confirmed its strong effectiveness. The efficiency of the extracts varies depending on the extraction method, the type of solvent used, and the experimental microorganism [10].

Based on the above, *Typha* pollen was chosen, which is a yellow substance with a pleasant smell and sweet taste, which specialists a complementary food that benefits pregnant women and patients with irritable bowel syndrome. It contributes to lowering blood pressure, reducing cholesterol, strengthening teeth in children, treating diarrhoea, intestinal colic, and healing wounds [11]. These pollens are a good source of many active ingredients, such as tannins, alkaloid glycosides, saponins, flavonoids, and polyphenols [12]. The pollens are collected from cattails, an aquatic flowering plant belonging to the Typhaceae family. This family includes more than 500 species worldwide and about 104 genera [13]. *Typha* L. is a perennial herbaceous plant with long leaves and is called the herbaceous pond [14]. It grows to a height of 2-3 m, is dioecious and is considered a columnar plant. This means that the plants have their roots in the water, while most of the plant body is outside the water and its origin is in Africa. It grows on the banks of rivers, swamps and lakes in large clusters, as it is a tropical plant rather than cold regions [15]. This explains its growth in the marsh and swamps of southern Iraq and on the banks of the Shatt al-Arab. Its

spread extends to the central and northern regions because its plants grow in shallow areas in clay soil, forests, fields, and semi-humid wetland environments [16]. For a long time, *Typha* has attracted the interest of scientists and researchers due to its ability to purify water by absorbing nutrients and dissolved impurities, hence fostering a clean environment[17]. Therefore, this study aimed to extract *Typha* Pollen powder using oil and alcoholic extraction and to detect the active compounds in those extracts qualitatively. Then, the effectiveness of these extracts against certain types of fungi and bacteria was tested, in addition to testing the ability of these extracts to scavenge DPPH free radicals.

MATERIALS AND METHODS

Materials

Typha Pollen was purchased from local markets in the form of stone blocks. The fungal isolates *Penicillium* spp., *Aspergillus niger*, *Fusarium* spp., and the bacterial isolates *Bacillus* sp., *Escherichia coli*, and *Staphylococcus aureus* were used. These isolations were diagnosed in the Food Research Center\Ministry of Science and Technology. Diphenyl-1-picrylhydrazyl (DPPH)-22, and Muller Hinton Agar were purchased from Sigma Aldrich Company, and Potato Dextrose Agar (PDA), Methanol, Hexane, and Dimethyl sulfoxide (DMSO) were purchased from Sinopharm Chemical Reagent.

Preparation of Pollen Extracts

The extraction was carried out according to the method of [18] by placing 100 g of pollen powder in the extraction thimble. Then, it was placed inside the steam extraction device (Soxhlet extractor). The powder was covered with a layer of glass wool that prevented the powder from rising to the top. 500 ml of methyl alcohol for alcoholic extraction and hexane for oil extraction were added slowly and gradually to the thimble containing the pollen powder. Then, the device was operated at 75 °C, and the extraction process continued for several hours and six cycles. The solvent was evaporated using a rotary evaporator under vacuum pressure at 40 °C. Then, the remaining sample in the flask was taken and placed in an oven at a temperature of 40 °C until the solvent evaporated. The extract remained and was stored in the refrigerator until use.

Preparation of Extract Concentrations

The stock solution was prepared from the plant extract by dissolving 10 g of dried plant powder in 20 ml of Dimethyl Sulfoxide (DMSO) solution. Thus, we obtained a concentration of 0.5 g/ml. DMSO was chosen as a solvent for the extract because the alcoholic extract does not dissolve in distilled water. Also, this solution does not affect microorganisms negatively or positively, nor does it affect the nature of the culture medimedium,the properties of the extract used. Then, the stock solution was filtered for sterilization using special filters (Whatman membrane filter 0.45µm). After that, specific concentrations were prepared as follows (100, 50, 25) mg/ml in the fungal inhibitory activity test. In comparison, another additional concentration of 12.5 mg/ml was made in the bacterial inhibitory activity test.

Qualitative Detection of active Compounds in the Extracts

Chemical tests were conducted on the methanolic and oil extracts using the method [19] to detect active compounds in pollen.

Assays the Effect of Extracts on Fungal Growth

After completing the extraction of pollen powder, fungal isolates were cultured from the plates with the plant extract at different concentrations that were prepared. A fixed amount of the prepared concentrations was used with a fixed amount of the PDA culture medium is as follows: 0.5 ml (extract) + (6 ml PDA). The stabbing method was used using a sterile needle. A small amount of the fungal suspension was cultured on the PDA medium mixed with the plant extract at different concentrations. The plates were incubated at 25 °C and the results were read after 3 to 5 days. It is worth noting that a control was made with each test for comparison purposes. That is, the fungi were cultured on plates containing only PDA without adding the extract to determine the effectiveness of the extract in inhibiting the growth of the fungi under study. After the incubation period was over, the results were read. The effectiveness of each concentration of the extract was determined by measuring the colony diameter of each type of fungi in the plates containing the plant extract, comparing it with the corresponding control treatment plates, and observing the difference in growth. Thus, the inhibitory effectiveness of the extract was determined. Note that three replicates were made for each concentration[20]. The percentage of inhibition was calculated according equation [21].

$$Inhibition\ \% = \frac{A-B}{A} \times 100 \qquad (1)$$

Where A is the radial growth of the control and B is the radial growth of the treated sample

Antibacterial Activity of Extracts

The agar well diffusion method was followed by [22] in testing the sensitivity of bacteria to extracts. Five holes were made with equal distances in the solid nutrient agar medium with a diameter of 5 mm using a cork bore. Extract concentrations of 0.1 ml were added to each hole after spreading 0.1 ml of bacterial suspension for each type of bacteria on the medium at concentrations of (12.5, 25, 50, 100) mg/ml of plant extract. Negative control treatment was also done by adding DMSO in one of the holes. The plates were incubated at 37 °C for 24 hours. The diameter of the inhibition zone formed around the holes was measured in millimeters. All tests were performed under sterile conditions and in three repetitions.

Antioxidant Activity of Alcoholic and Oil Extracts

A 0.135 mM DPPH solution DPPH was prepared in methanol. Then, 10 µl of different concentrations of alcoholic and oil extracts were added to 1.0 ml of the DPPH solution. These concentrations ranged from (10 - 0.625) mg/ml. Ascorbic acid was used as a standard antioxidant as a positive control at the same concentrations. Then, the reaction mixture was vortexed well and left in the dark at room temperature for 30 minutes. Afterwards, the absorbance of the test samples was measured spectrophotometrically at 517 nm [23]. These samples were read in duplicate, and the ability to scavenge DPPH radicals was calculated using the following equation:

$$Free\ radical\ scavenging\ activity\ (\%) = \frac{A\ control - A\ sample}{A\ control} \times 100 \qquad (2)$$

$A_{control}$ represents the absorbance of the free radical solution DPPH + methanol, and A_{sample} is the absorbance of the free radical solution with the sample or positive control (ascorbic acid).

RESULTS AND DISCUSSION

Qualitative Chemical Detection of Active Compounds

The results of the chemical detection of active compounds in the methanolic and oil extract showed that it contains phenols,

flavonoids, alkaloids, glycosides, saponins and tannins [24], as shown in Table (1)

Table 1 Qualitative chemical detection of active compounds in the methanolic and oil extract

Effective groups	Alcoholic extract	Oil extract
Phenols	+++	+
Flavonoids	++	++
Alkaloids	+	+
Glycosides	+	-
Saponins	+	+
Tannins	+++	-

+ Weak positive result, ++ Moderate positive result, +++ Strong positive result, – Negative result

Effect of Oily and Alcoholic Extract on Fungal Growth

The procedure for extracting *Typha* pollen, the inhibitory action of the extracts on microbes, and antioxidant activity were investigated, as seen in the figure 1. As showed in figure 2 the inhibition percentages of the oily and alcoholic extract were (88%, 70%, 66%) and (90%, 85%, 78%) compared to the control for concentrations (100, 50, 25) mg/ml respectively for *Aspergillus niger*. In contrast, the inhibition percentages were (90%, 60%, 51%) and (96%, 78%, 76%) for the same previous concentrations, respectively, for *Fusarium* sp. As for the fungus *Penicillium* spp, the inhibition percentages were (78%, 72%, 50%) and (94%, 91%, 88%) for the same concentrations, as in Table (2).

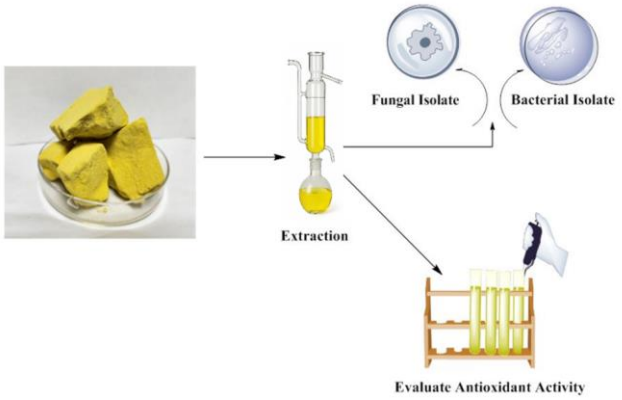

Fig. 1 Schematic represents extraction, inhibitory effect and antioxidant activity for *Typha* pollen

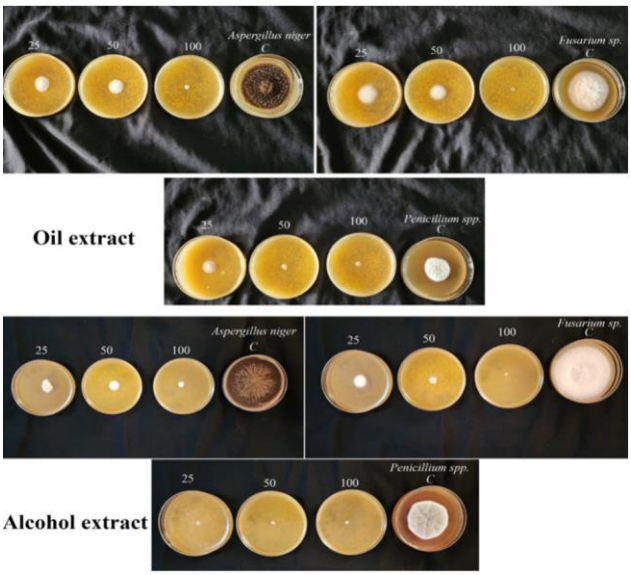

Fig. 2 Effect of Oily and Alcohol extract on growth of fungi

Effectiveness of *Typha* Pollen Extracts in Inhibiting Bacterial Growth

Three types of bacteria were used in this study. Two were Gram-positive: *Bacillus* sp. and *Staph. aureus*, while one was Gram-negative: *E. coli*, because these microorganisms are considered common causes of some diseases that humans and animals are exposed to, in addition to being considered one of the main contaminants of some foods [25]. The antibacterial activity of the extracts at their four concentrations against the test organisms ranged with different inhibition zones ranging from 0 to 43 mm. As shown in Figure 3 and Table 3, the diameters of the inhibition zones for the oily and alcoholic extracts were (19, 30, 29, 19) mm and (30, 32, 34, 36) mm for concentrations (12.5, 25, 50, 100) mg/ml, respectively, for *Staph. aureus* bacteria. The inhibition diameters were (0, 0, 13, 18) mm and (28, 35, 37, 43) mm for the same concentrations, respectively, for *Bacillus* sp. bacteria.

Table 2 The inhibition percentage of the oily and alcoholic extract in the three fungi

Penicillium spp.			*Aspergillus niger*		*Fusarium* sp.	
Concentration mg/ ml	oil	alcoholic	oil	alcoholic	oil	alcoholic
25	66%	78%	51%	76%	50%	88%
50	70%	85%	60%	78%	72%	91%
100	88%	90%	90%	96%	78%	94%

As for the inhibition diameters for *E. coli* bacteria for the oily and alcoholic extracts, they were (0, 0, 11, 20) mm and (27, 28, 32, 37) mm for the four concentrations, respectively. The results showed a variation in the effect of the extracts on fungal and bacterial isolates. Due to the variation in the extraction methods used and the difference in the polarity of the solvent used, it led to a difference in the content of active groups [26]. According to Marcas [27], the active chemicals in plant extracts may inhibit enzymes by oxidizing molecules, which is how they operate to suppress microbial activity. They are a source of stable free radicals. They often lead to protein disruption and loss of function. They can form complexes with dissolved extracellular fluids and proteins, form complexes with bacterial and microbial cell walls, and disrupt microbial membranes. Some can interfere with DNA formation and form ion channels in the microbial membrane.

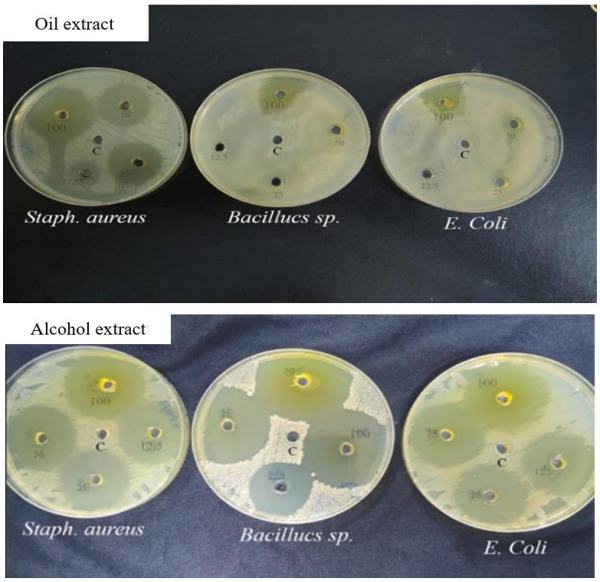

Fig. 3 Effect of Oily and Alcohol extract on growth of Bacteria.

Table 3 represents the inhibitory effectiveness of the extracts in bacterial species

	Staph. aureus		*Bacillus* sp.		*E. coli*	
	Inhibition zone diameter (mm)					
Concentration mg/ ml	Oil	Alcoholic	Oil	Alcoholic	Oil	Alcoholic
12.5	19	30	0	28	0	27
25	29	32	0	35	0	28
50	30	34	13	37	11	32
100	33	36	18	43	20	37

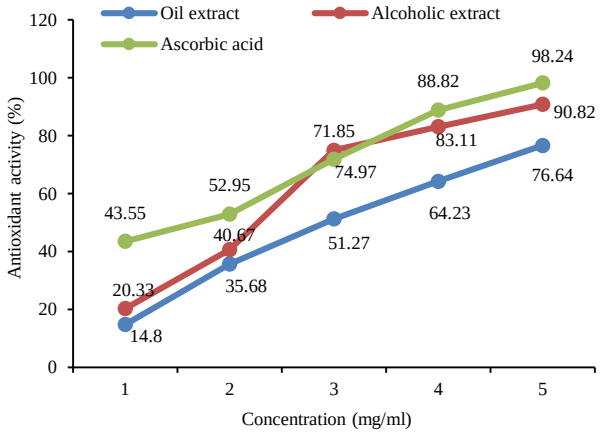

Fig. 4 shows the antioxidant activity of the extracts and ascorbic acid.

They compete with microbial proteins in adhesion to the host's polysaccharide receptors [28]. Bhattacharya [29] confirmed that the effectiveness of these extracts is due to their containing some types of phenols that have promising capabilities in treating common diseases that affect both humans and animals. Puljula [30] Also indicated that the mechanism of action of antimicrobial tannins may be due to their harmful effects on membranes, which may lead to blockage of the metabolic pathway and death of microorganisms.

Antioxidant Activity of Oily andA Extracts

The results in Figure (4) show that the antioxidant activity percentages of the extracts went up as the concentrations used in the test went up, compared to ascorbic acid concentrations, which had higher activity. The antioxidant activity of the oily extract was (14.88%, 35.68%, 51.27%, 64.23%, 76.64%) at concentrations of (0.625, 1.25, 2.5, 5, 10) mg/ml. The antioxidant activity of the alcoholic extract was 20.33%, 40.67%, 73.97%, 83.11, 90.82%) at the same concentrations. In contrast, the antioxidant activity of ascorbic acid reached (43.55%, 52.95%, 71.85%, 88.82, 98.24%) for concentrations (0.625, 1.25, 2.5, 5, 10) mg/ml, respectively. The IC50 value at a concentration of 2.4 mg/ml of the oil extract was enough to inhibit 50% of DPPH free radicals. While the alcoholic extract inhibited 50% of DPPH free radicals, the IC50 value was at a concentration of 1.7 mg/ml. As for the IC50 value of ascorbic acid, it required a concentration of 1.18 mg/ml to inhibit 50% of DPPH free radicals.

DPPH is a stable organic radical and has been widely used as a simple, rapid and sensitive method to evaluate the free radical scavenging capabilities of natural antioxidants. The alcoholic extract showed a higher free radical scavenging capacity, as evident from the lower IC50 value compared to the oil extract. This is due to the high polarity of methanol in the extraction process in addition to its ability to obtain a higher amount of phenols, which play a major role in resisting oxidation [31]. *Typha* pollen contains two specific components worth noting: Typhaneoside and Isorhamnetin-3-O-neohesperidoside (I3ON). The former promotes cell proliferation and reduces levels of malondialdehyde (MDA) and nitric oxide (NO). This suggests antioxidant and anti-inflammatory effects. The latter, similar to the former, also contributes (I3ON) to antioxidant and cell-protective activities [32]. As previously mentioned, the antioxidant and anti-inflammatory properties of *Typha* pollen components suggest that they could be a novel therapeutic strategy for conditions involving inflammation, such as those caused by lipopolysaccharide (LPS) [33].

CONCLUSION

In general, *Typha* pollen was found to have a high antimicrobial capacity and effective effects. In vitro, free radical scavenging demonstrated that both extracts exhibited distinct antioxidant activity. The results indicated that the methanolic extract had the highest total phenolic content and the strongest reducing power, as it could bind to the plant's total phenolic content. As a result, pharmaceutical applications are advised to utilize methanolic extracts of *Typha* pollen.

Conflict of Interest

The authors declare no conflict of interest

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