International Journal of Biotechnology and Microbiology
www.biotechnologyjournals.com
Online ISSN: 2664-7680, Print ISSN: 2664-7672
Received: 09-08-2023, Accepted: 26-08-2023, Published: 11-09-2023
Volume 5, Issue 3, 2023, Page No. 41-47

Evaluation of antifungal activity of aqueous extract of *Pulicaria undulata* against certain phytopathogenic fungi

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Abstract
Many medicinal plant extracts have been shown to have antifungal efficacy against a wide variety of phytopathogenic fungi. The aim of this study was to estimate the antifungal activity of the aqueous extract of *Pulicaria undulata* against nine pathogenic fungal species (*Fusarium solani, Fusarium oxysporum, Fusarium proliferatum, Fusarium verticilliodes, Macrophomina phaseolina, Curvularia austriaca, Drechslera halodes, Bipolaris* sp., *Alternaria alternata*). The study was performed using three concentrations of the *P. undulata* aqueous extracts, *i.e.*, 5%, 10% and 20%. Scanning Electron Microscopy (SEM) was used to examine ultrastructural and morphological alterations of treated and untreated mycelia of *Drechslera halodes.* Fourier Transform Infrared (FT-IR) spectrometry was employed to detect the present functional groups in *P. undulata* aqueous extracts. The results showed that the aqueous extracts of *P. undulata* had an effective activity against all tested phytopathogenic fungi, according of concentration dependents. SEM micrographs of untreated fungal cells (control) did not exhibit structural changes, while the treated fungal cells illustrated deformation of the cellular structure, distortion and folding of the external surfaces, and compacted spores. FTIR spectrum analysis of *P. undulata* aqueous extract exhibited different phytochemical including glycosides, fatty acids and aromatic compounds. The study concluded that the aqueous extract of *P. undulata* might be used as a natural fungicide, which control plant diseases caused by phytopathogenic fungi in the agricultural fields.

Keywords: *Pulicaria undulata*, antifungal activity, aqueous extracts, phytopathogenic fungi, FTIR

Introduction

In spite of the widespread use of antifungals, experts estimate that fungal pests destroy 10 to 23% of the world's crops every year, with an additional 10 to 20% lost post-harvest. Fungal diseases are steadily expanding into the Polar Regions as a result of global warming, increasing the number of nations where these infections are an issue since they impair both agricultural productivity and harvests. Because stem rust fungal infections are normally found in the tropics, it was previously exclusively detected in European nations. Furthermore, the researchers warn that fungi's tolerance to greater temperatures may increase the possibility that opportunistic diseases could develop the capacity to infect plants (crops) and animals (including humans).

Crop farmers mostly apply chemical fungicides as inhibitive and therapeutic methods to controlling of these diseases. The random and immoderate use of a wide range of synthetic fungicides has led to the pollution of the environment and the production of resistant pathogenic strains. For this reason, the demand for natural products planted without using any synthetic pesticides or chemicals is increasing. These matters have caused a lot of scientists to perform much research about integrated control of microbial plant diseases, including the natural products derived from plants [1].

Amongst various medicinal and aromatic plants, *Pulicaria undulata, (*also known as Desert Golden Daisy or AL-Githgath) Figure 1**.** *Pulicaria undulata* belongs to the Asteraceae family which is composed of over 100 herbaceous plant species, their homeland is in the Canary Islands, Southern Europe, the Mediterranean and North Africa. They are also widely distributed in the Arabian Peninsula together within the dry, and salty-land areas of Qassim- Saudi Arabia [2, 3]. Pulicaria undulata also called "Githgath" in Saudi Arabia, is an aromatic flowering plant. Its volatile constituents are one of the reasons for the plant's common use in herbal tea, perfumery, and as an insect-repellant **.**

Fig 1: *Pulicaria undulata*

P. undulata can tolerate high salt- soil and dry climate, so-called halophytes. In KSA, the plants of the genus *Pulicaria* are well-known as medicinal plants. Arabian nomads and herbalists used it as a remedy for inflammation, diabetes, and GIT disorders. tonic, food-preservative, in perfumes,

and as part of a salad. Pulicaria plant contains 12 constituents; flavonoids in nature, furthermore quercetin and caffeic acid were also reported in the *P. undulata* species planted in Egypt [3].

A previous study in Egypt revealed that *Pulicaria undulata* contains significant phytochemicals; phenols, flavonoids terpenoids, saponins, alkaloids, and others highlighting which can developed and use as an alternative way to the serious synthetic fungicides. In addition, the methanolic *P. undulata* extract showed antifungal activity against the tested six phytopathogenic fungal species. The cytotoxicity assessment of methanolic extract revealed a good antifungal activity against the four fungal strains which examined in this study [4]. In another study conducted to evaluate the potential activity of *Pulicaria incisa* extract as an eco-friendly method for controlling *Fusarium oxysporum* which causes rot and wilt diseases for many economic crops. The results showed that *Pulicaria incisa* rich in phenolic and flavonoid contents as well as antioxidant capacity. Additionally, the extract exhibited high antifungal activity of the tested extract against *F. oxysporum. P. incisa* extract not just affects the growth and enzymes of *F. oxysporum* but also affects the ultrastructure alterations in the fungal mycelium, as confirmed by transmission electron microscopy examination (TEM) [5].

Although numerous studies have evaluated the antifungal activities of some plant extracts against a variety of pathogens [6, 7], the antifungal activity of *P. undulata* against plant phytopathogenic fungi remains elusive. Therefore, the current study aims to evaluate *in vitro* antifungal activity of *P. undulata* extract against nine selected phytopathogenic fungi; *Fusarium solani, Fusarium oxysporum, Fusarium proliferatum, Fusarium verticilliodes, Macrophomina phaseolina, Curvularia austriaca, Drechslera halodes, Bipolaris* sp., and *Alternaria alternata*. In addition, to determine the chemical composition of aqueous extract by Fourier Transform infrared (FTIR) technique and to characterize the ultra-structural alteration of the treated fungi with *P. undulata* aqueous extract with employ of Scanning Electron Microscope (SEM).

Materials and Methods

1. Collection of plant samples

P. undulata samples was harvested from Rawdat Al-Khafs region, north of Riyadh, Saudi Arabia, during the period of March 2022. The fresh aerial parts and leaves were separated from the stems. Then plant parts were washed with running water from the tap followed by distilled water and dried in shade for two weeks. Dried samples were ground into a fine powder with the help of a milling machine. After polverization, to avoid contamination, the samples were labeled and stored in air-sealed plastic containers at 4ºC for extraction and further analysis [8].

2. Preparation of Aqueous Extract of *Pulicaria undulata*

First, 40 gm of plant samples materials was soaked in a glass beaker containing 400 ml of sterilized distilled water. The glass beakers were covered with foil well then subjected to vortexing on the rotary shaker (250 rpm at 45 °C) at 24 hours. The next day, the crude aqueous plant extracts were filtered across from Whatman′s filter paper (No. 1). After that, all studied wild plant extracts were centrifuged at 10,000 rpm for 30 minutes to remove any suspended deposits, then the supernatants were dried in a rotary evaporator. Finally, the plant extract was collected and kept in sterile glass bottles at 4°C until practical use [6].

3. Fungal species

The phytopathogens used in the study were obtained from the Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, KSA. The nine following fungal species were examined in the current study: (*Fusarium solani, Fusarium oxysporum, Fusarium verticilliodes, Fusarium proliferatum, Drechslera halodes, Bipolaris sp., Curvularia austriaca, Macrophomina phaseolina* and *Alternaria alternata*). *In vitro*, all the fungal strains were cultured and preserved at 18º C on Potato dextrose agar media (PDA).

4. Growth Inhibition Assay *In Vitro*

The agar dilution method was used for identification of the mycelial fungal growth inhibition of the selected fungi by *Pulicaria* extract. The aqueous extract under study was diluted in sterilized distilled water to obtained on the required concentration, then sterilized by passing through 0.45 µm sterile filters. To avoid any contamination, the performing was conducted in a laminar flow cabinet. to preparation the different concentration, known amount of the aqueous extract was mixed aseptically with equal volume of molted cooled (40-45°C) PDA to give the required concentrations (5, 10 and 20 mg/ml), according of the weight of dried samples of the plant materials. A Control was done from PDA medium only. After pouring the medium into sterilized petri dishes, the plates were left a while for solidification. Subsequently, for the bioassay, 6 mm disc was taken from the edge of the activity growing colonies of the tested fungal strains and placed in the center of PDA plates that contains specific concentration of plant extract (as treated). The cultures of tested fungi were incubated for 7 days at 25 ± 2°C. All assays were replicated three times. The mycelial fungal growth was evaluated by calculating the mean of two perpendicular colony diameters for each replicate. The values were expressed in millimeters diameter and calculated as percentage of mycelial growth inhibition (according to the formula mentioned below).

$$\text{Growth Inhibition (\%)} = \frac{DC - DT}{DC} \times 100$$

Where: DC= colony diameter of control (untreated), and DT = colony diameter of treated cells [7].

5. Scanning Electron Microscopy (SEM) examination

SEM was employed to examine the cellular structure of selected mycelial fungus; *Drechslera halodes* which was treated with the aqueous extract of *P. undulata*, and compared with the untreated control mycelia. Primary fixation by buffered Glutaraldehyde (2.5%) overnight was performed in a refrigerator, then washed by phosphate buffer (pH 7.2) and later fixed using buffered Osmium Tetroxide 1%. The specimens were then dehydrated by a series of different ethanol concentrations. They were then freeze dried in a critical point dryer and mounted on gold

plated stubs. Finally, the samples were observed through scanning under a JEOL scanning electron microscope (Model JSM-6060LV, Japan).

6. Characterization of the aqueous plant extracts Infrared Spectrometry Analysis

FT-IR is a method of measuring infrared absorption and emission spectra of the biomolecules found in the prepared plant extract. The aqueous plant extract of *P. undulata* was analysed using FTIR spectroscopy with a Perkin Elmer spectrum BX FTIR (PerkinElmer, Waltham, USA) to determine any significant information about the existent functional groups of chemical components in the plant extract, which were separated based on their peak ratio. The percent transmittance (%T) was recorded in the spectral range 400 – 4000 cm-1.

Statistical Analysis

In order to clarify the effect of plant extract concentrations and types of fungi as well as their interaction on the studied parameters, all values given in this work were means of 3 replicates. Analysis of variance (ANOVA) was used. The data were analyzed by aid of Statistical Package for the Social Sciences (SPSS) VERSION 18.

Results

1. Evaluation of Antifungal Activity

The antifungal activity of the aqueous extract of *P. undulata* at concentrations of 5%, 10% and 20% were summarized in Table 1. The finding showed that *P. undulata* aqueous extract showed a strong antifungal activity against most of the tested nine phytopathogenic fungi, where completely inhibited the growth (100%) of four pathogenic fungus (*F. oxysporum*, *F. proliferatum*, *F. verticilliodes*, and *M. phaseolina*) at a 20% concentration. *D. halodes* and *Bipolaris sp* showed 98.96% and 88.53% inhibition of growth respectively, at 20% concentration. *P. undulata* extract appears to have a different effect on *C. austriaca*, appearing to inhibit growth more at 5% conc (76%) than at concentration of 20% (65.60%). When compared to the control, the two other tested fungi that were also exhibited good inhibition of growth. In general, the effect of aqueous extract of *Pulicaria undulata* on morphology of the tested fungi was clearly visible *in vitro*. All fungal strains showed weak hypha and pale growth especially at 20% concentration of the plant extract. In addition, some fungi occurred color change other than their natural for instance, *Drechslera, halodes Alternaria alternata, Curvularia austriaca* and *Bipolaris sp* (Table 1, Figures 2, 3 and 4).

Table 1: Effect of different concentrations of aqueous extracts of *Pulicaria undulata* on growth diameter (mm) and percentage of inhibition of growth against selected phytopathogenic fungi on PDA media after 7 days of incubation at 25º C.

Plant pathogenies	Growth diameter (mm)				Inhibition of growth %		
	Control	5%	10%	20%	5%	10%	20%
Fusarium solani	80.0± 0.00	57.5± 1.44*	39.17± 0.83*	29.17±0.83*	28.13±1.8*	51.03±1.00*	63.53±1.03*
Fusarium oxysporum	80.00± 0.00	39.16±0.833*	24.17±0.83*	0.00±0.00*	51.03±1.03*	69.83±1.0*	100.0±0.00*
Fusarium proliferatum	80.00± 0.00	48.33±0.83*	35.83±0.83*	0.00±0.00*	39.57±1.03*	55.23±1.0*	100.00±0.0*
Fusarium verticilliodes	80.00± 0.00	55.0±0.0*	32.50±1.44*	0.00±0.00*	31.30±0.00*	59.40±1.70*	100.00±0.0*
Macrophomina phaseolina	80.00±0.00	48.33±0.83*	11.67±0.83*	0.00±0.00*	39.57±1.03*	85.43±1.00*	100.00±0.00*
Curvularia austriaca	80.00±0.00	19.16±0.83*	40.83±0.83*	27.50±0.00*	76.03±1.03*	48.96±1.0*	65.60±0.00*
Drechslera halodes	80.00±0.00	50.0±0.00*	30.83±0.83*	9.17±0.83*	37.50±0.0*	61.46±1.0*	88.53±1.03*
Bipolaris sp	80.00±0.00	49.17±0.83*	18.33±0.83*	0.83±0.833*	38.53±1.0*	77.06±1.0*	98.96±1.03*
Alternaria alternata	80.00±0.00	63.3±1.66*	33.33±0.83*	17.50±0.00*	20.87±2.0*	58.36±1.0*	78.10±0.00*

Data represented as mean ± standard error of triplicates. **P-values* < 0.05 were considered significant.

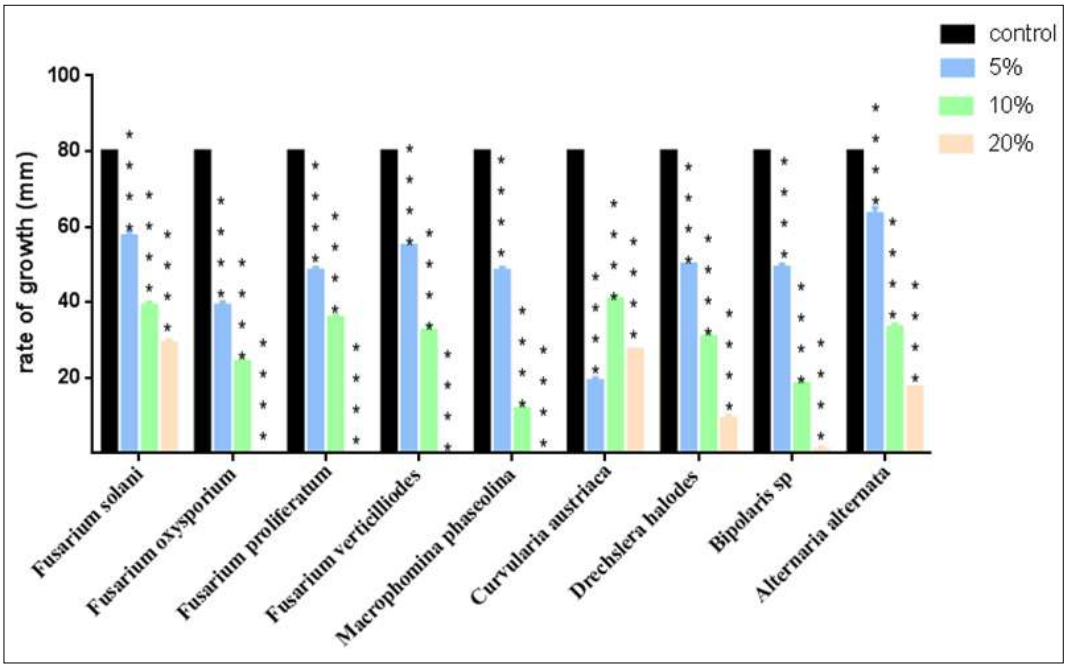


Fig 2: Influence of Pulicaria undulata plant extracts at various concentrations on growth rate (mm) on selected phytopathogenic fungi. (Data represented as mean ± standard error of triplicates. * P-values < 0.05 were considered significant)

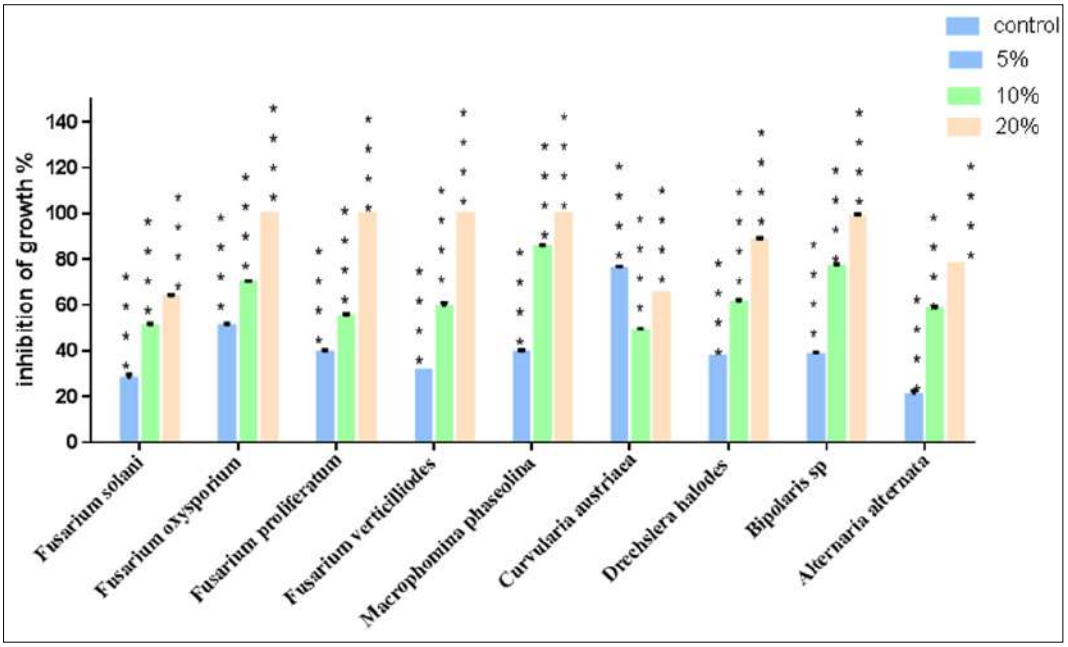


Fig 3: Influence of *Pulicaria undulata* plant extracts at various concentrations on inhibition of growth percentage of selected phytopathogenic fungi. (Data represented as mean ± standard error of triplicates. **P-values* < 0.05 were considered significant).

Fungi	Control	5%	10%	20%
F. solani				
F. oxysporum				
F. verticilliodes				
F. proliferatum				
M. phaseolina				
C. austriaca				

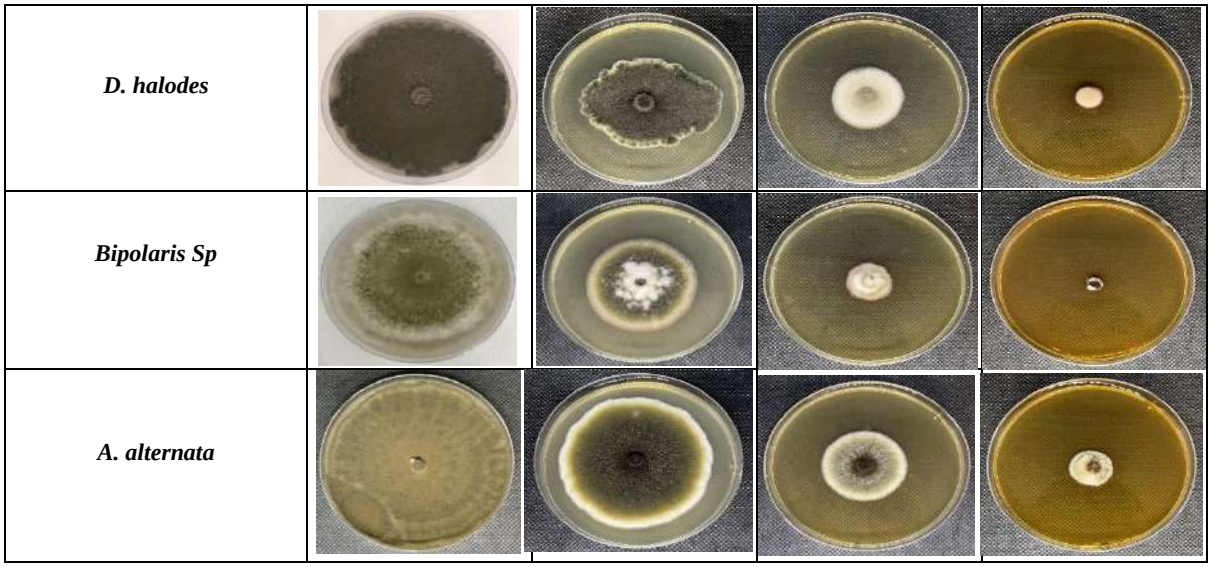
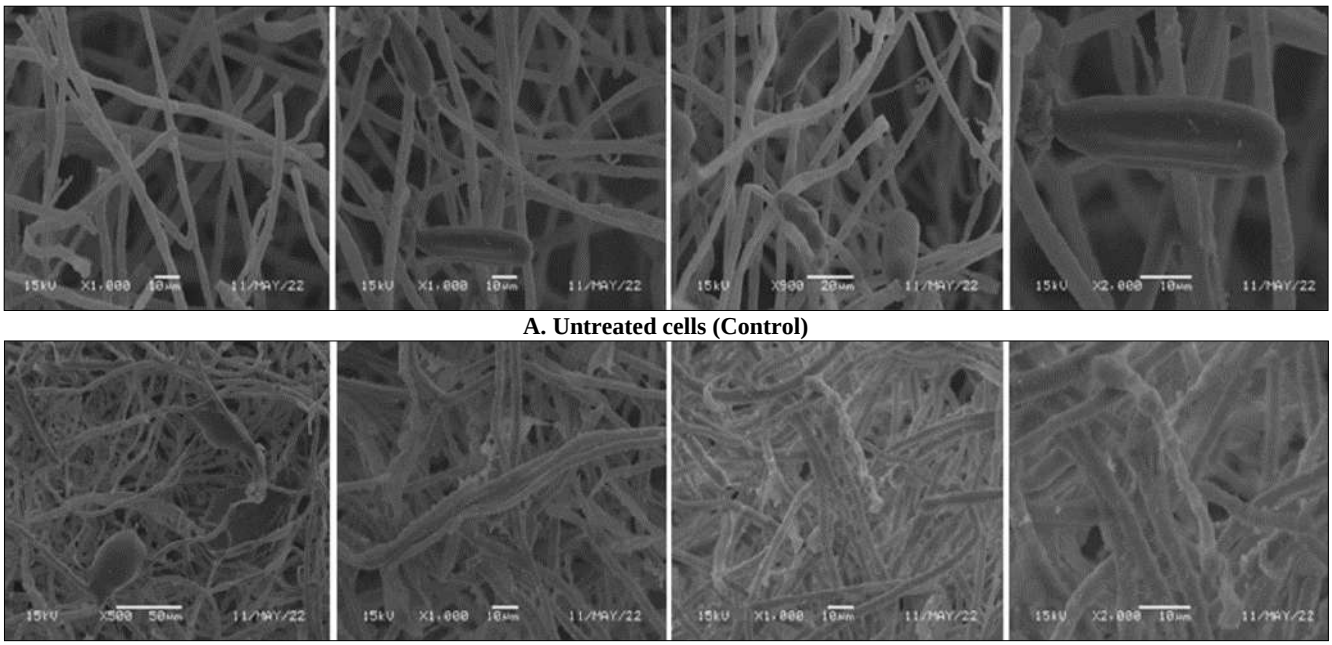


Fig 4: *In vitro* activity of aqueous extract of *Pulicaria undulata* on nine phytopathogenic fungi

2. Scanning Electron Microscopy Examination

The morphological changes observation by SEM of *Drechslera halodes* treated with the aqueous extract of *P. undulata* were selected and compared with untreated cells (control). The biomass of the untreated *D. halodes control* group was characterized normal tubular hyphae, smooth mycelium surface with normal conidial growth, and no structural modifications were noticed (Figure 5 A)**.** On the other hands, The SEM micrographs of treated samples by *P. undulata* extract (20mg/ml) showed sever hyphal adhesions and deformed of external surfaces, besides, shrinking in the conidial size and stopped of growth. In addition, the SEM results demonstrated some meanders to the external surfaces of hyphae and lightly slender conidia forms. Furthermore, revealed structural changes included; adherent and shriveling of hyphae and the presence of abnormal structures with small embedded and overlapping conidia (Figure 5B)**.**



A. Untreated cells (Control)

B. Cells treated with the aqueous extract of *P. undulata*

Fig 5: SEM micrographs of *Drechslera halodes* (A) control; (B) treated by *P. undulata* 20% concentration

3. FTIR analysis

Figure 6 demonstrates the FTIR spectra of the aqueous. extract of *P. undulata*. The extract's band at 3404 cm-1 was ascribed to the hydroxyl group's OH stretching vibration, which suggests the presence of alcoholic, phenolic and acidic compounds or even water. However, the bands are wide because hydrogen bonds are being formed. The bands at 2934 cm-1 and 2878 cm-1 correspond to the asymmetric and symmetric C−H stretches. While the peaks at 2366 cm−1 and 2341 cm−1 related to substituted benzene rings. The peaks at 1702 cm-1, 1621 cm-1 and 1517 cm-1 clearly demonstrate C=O stretching in ester, or C-N bending in the amide group as well as C=C. The methyl, methylene and methoxy groups' aliphatic and aromatic C-H plane deformation vibrations were observable in the vibration band at 1402 cm-1 for plant extract. Moreover, the peaks at 1267 and 1047 cm−1 represent C-O-C and C−O stretches groups of phenol, fatty acids and ether group.

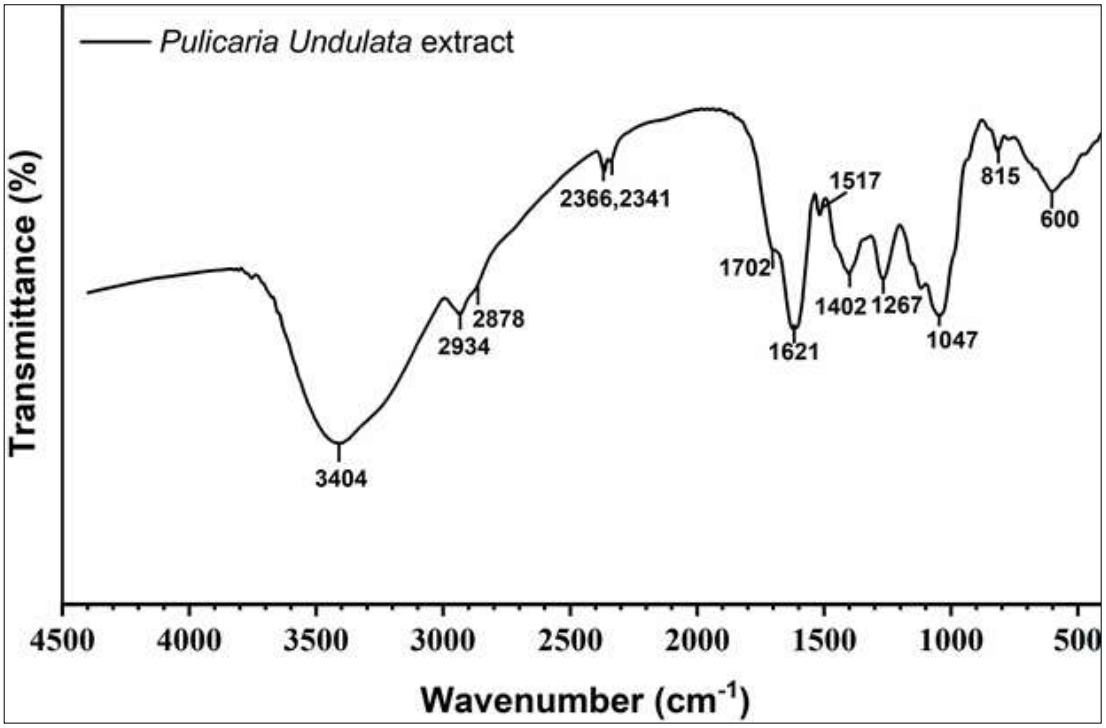


Fig 6: FTIR spectra of the *Pulicaria undulata* extract

Discussion

Plant pathogenic fungi cause different diseases including economically important crops around the world, they can cause severe damage to crops at pre- or post-harvest stages. Many fungal species cause necrotic fungal infections in plants, welt, rot and blights which have negative economic losses in agriculture production. Most of these species belonged to the genera; *Fusarium, Alternaria, Drechslera, Macrophomina* and *Bipolaris* [9]. Although synthetic fungicides are the first line of defense to control fungal plant diseases, where farmers generally apply them as preventive and therapeutic measures, these chemicals are toxic and led to environmental pollution and negative effects on the plant, soil and human and animal health, as well as it had resulted in of pathogen-resistant strains [10].

In this study, the antifungal activity of the aqueous extract of *Pulicaria undulata* was assessed against 9 pathogenic fungi species; *Fusarium solani*, *Fusarium oxysporum*, *Fusarium proliferatum*, *Fusarium verticilliodes*, *Macrophomina phaseolina*, *Curvularia austriaca*, *Drechslera halodes*, *Bipolaris* sp., and *Alternaria alternata*. The study results of *Pulicaria undulata* extract effects showed a high inhibitory rate against most of the tested fungal species, which completely inhibited the growth of four pathogenic fungi (*F. oxysporum, F. proliferatum, F. verticilliodes,* and *M. phaseolina*) at a 20% concentration. These scavenging abilities against fungal pathogens may return to the presence of phytochemicals in *Pulicaria* extract such as; Benzin rings, phenolic compounds, fatty acids and glycosides, which are characterized by their antifungal properties, as this composition of *P. undulata* is also confirmed in this study by the FTIR analysis results [11, 12]. FTIR is an effective method to detect specific chemical bonds or functional groups present in the plant extracts, The bioactive substances associated with the cytoplasmic membrane, of the fungal cell causing membrane damage, forms pits on the surface of the cell, and alters the cell wall's permeability [13, 14]. These findings are supported by several previous reports, which displayed the antimicrobial, antioxidant and anti-inflammatory properties of *Pulicaria* extract against some fungal strains such as; *Aspergillus flavus, Aspergillus fumigatus*, and dermatophytes, to aim the development of natural antimicrobial products with less toxicity [4]. One interesting finding in this study is the morphological changes on both of mycelia and conidia of the selected fungal cells before (untreated samples; control) and after treatment by the *Pulicaria* extract utilizing SEM photographs. The SEM images exhibited significant alterations in the treated *D. halodes* occurs by *Pulicaria* extract compared to untreated specimens, represented in; hyphal weaknesses or enlargement, thick cell walls, hyphae adhesion to each other, twisted conidia, hyphal shrinking and decrease in the conidia size. These findings reflect those of Elsherbiny who also found the morphological modification in *Fusarium sambucinum's* hyphae when treated with *Pulicaria undulata* extract, including curling, twisting and collapse [15].

These effects could be attributed to the bioactive phytochemical compounds present in the most of plant extracts. Phenols, tannins, glycosides and flavonoids cause disorders in the organization of the cell. Similarly, the phenols have antifungal activity, that is due to they are lipophilic and diffuse easily across the cell membrane and settle down by interfering with the ergosterol, mannans, and glucans synthesis process [8, 16]. In conclusion, the study results proven the antifungal activity of Pulicaria undulata in Saudi Arabia. That might suggest using them as an alternative to chemical fungicides, due their easy economical availability, high safety, unique chemical properties and eco-friendly characteristics. therefore, it can be used to aim to eliminate phytopathogens and to develop viable options for fungal disease control in cultivation [17, 18].

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