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Inhibitory Effectiveness of *E. prostrata* and *E. hypericifolia* Extracts in Inhibiting the Growth of Two Pathogenic Fungi, *A. alternata* and *F. solani*

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ABSTRACT

The current study included the preparation of an aqueous-alcoholic extract of species *E. prostrate* and *E. hypericifolia* leaves (herbaceous plants belonging to the genus *Euphorbia* (Euphorbiaceae) spread in Iraq) and showing the effect of the extract on some pathogenic fungi *Alternaria alternata* and *Fusarium solani* as a comparative study between the two species. The disk-diffusion method (MIC) and the Well diffusion method were used to determine the minimum inhibitory concentration at 5, 10, 15 and 20% to evaluate the effectiveness of the plant extract of both the species against both the fungal isolates by calculating the percentage of inhibition. The methanolic extract of *E. prostrata* leaves had higher inhibitory activity than *E. hypericifolia* leaves. The highest inhibition rate for *A. alternata* was 100 and 100%, and for *F. solani* (91 and 100%) at concentrations of 15 and 20. While the inhibitory percentages of *E. hypericifolia* leaves extract for *A. alternata* (85, and 89%) and for *F. solani* (84 and 88%) at the same concentrations. Also, the fungus *A. alternata* was more sensitive to the plant extracts than *F. solani*, specifically using the Well method. The results of the current study confirmed the importance of the alcoholic extract in inhibiting or reducing the growth of fungi, especially *E. prostrata* leaves extract.

Key words: *Euphorbia prostrata*, *Euphorbia hypericifolia*, *Fusarium*, *Alternaria*, plant extract

INTRODUCTION

Recently, there has been a wide and significant interest in using plants that contain medicinal metabolites as an alternative method for chemical compounds for the possibility of controlling microorganisms that are causing many diseases (Barba-Ostria *et al.*, 2022).

Euphorbia prostrata and *Euphorbia hypericifolia* are annual herbaceous plants that belong to the Euphorbiceae family, which have been used in many digestive disorders (Meghana *et al.*, 2022). The whole plant as well as the aerial parts of these species has been used as antifungal and antibacterial (Hasan *et al.*, 2014).

Alternaria alternata and *Fusarium solani* are mostly saprotrophic fungi that derive their energy from cellular activity found in decomposing plant tissues or soil (Mašková *et al.*, 2019). These are found in different regions of the world, especially in field soils or plant residues (DeMers, 2022).

F. solani is characterized by its wide family range, as it causes many diseases to various field crops, including stem and root rot, as well as leaf wilt disease. It also causes vascular wilt and seedling death (Villarino *et al.*, 2019). The aim of study was to conduct a comparative study of two species of the genus *Euphorbia* to find out the inhibitory effect of the extract of the two species against the pathogenic fungi under study.

MATERIALS AND METHODS

Leaves of *E. prostrata* and *E. hypeircifolia* were collected in June and July 2022 from flowering adult plants from different regions of the country (Babylon, Karbala and Najaf) which were diagnosed by the botanist Dr. Shaemaa Muhi Hasson, Department of Biology, College of Sciences, University of Babylon.

After collecting and washing the fresh leaves, they were dried for 3-5 days in the shade and at room temperature, with continuous stirring to prevent any rotting. Then it was ground with

a blender to obtain a dry powder. To prepare the plant extract, the polar solvent 50% (methanol:distilled water) was used. Fifty g of dry powder of *E. prostrata* and *E. hypericifolia* leaves was mixed with 500 ml of the solvent in 1000 ml flask. Then, the flasks were placed in the rocking water bath at 37 °C for half an hour at high speed. The mixture was filtered using layers of medical gauze. Then, it was filtered by milipore filter (0.22 mm diameter). The filtered liquid was concentrated to dryness in oven at 45°C for 24 h. The dried concentrated material was milled by using electric mill and final powder was sterilized by UV equipment for 20 min.

A stock solution with a concentration of 20 mg/ml was prepared by dissolving 4 g of the plant extract in 200 ml of methanolic alcohol. Through serial dilutions of the standard solution, other concentrations 5, 10 and 15 mg/ml were prepared to test the least inhibitory concentration. The concentrations were prepared according to the following equation:

$$C1 \times V1 = C2 \times V2$$

The culture medium (PDA) was prepared according to the manufacturer's instructions by dissolving 39 g of agar media in 1000 ml distilled water. It was sterilized in an autoclave at 121°C for 15 min under a pressure of 15 bar. After which the mixture was left to cool and 250 mg antibiotic (cephalexin) was added for bacterial growth. The medium was poured into sterile Petri dishes for the growth and maintenance of the isolates. The Petri dishes were kept at 4°C until use.

F. solani and *A. alternata* isolates were obtained from the Fungi and Mycotoxins unit of the Department of Biology, University of Babylon, in addition to the Crop Protection Laboratory of the College of Agriculture, University of Kufa. The two fungi were grown on PDA culture medium, and sterilized by autoclaving at 25°C for 5 days in an incubator. These were kept in the refrigerator for further experiments (Mohsen *et al.*, 2017).

The dishes were treated with different concentrations 5, 10, 15 and 20 mg/ml by adding 1 ml of each concentration to three dishes above the culture medium, leaving six (control) three for each fungus. All dishes were inoculated in 10 mm diameter discs, taken from the edge of the growing fungus colony on PDA at the age of five days, with three replications for each concentration. These were tightly closed and incubated at 25°C. After seven days, the (radial) growth was measured by taking two orthogonal diameters from the back of the colony passing through the center of the disc. The percentage of fungal growth inhibition was calculated according to Hamed and Hussein (2020) when the growth in the control treatment reached the edge of the dish as:

$$\text{Inhibition \%} = [(A - B)/A] \times 100$$

Where, A = the diagonal growth rate in the control treatment and B = the diagonal growth rate in the growth inhibition treatment

According to the Well method, a well was made on the surface of the culture medium (PDA) of 10 mm diameter, and it was filled with serial concentrations of 5, 10, 15 and 20 mg/ml of methanolic extract. After the incubation period, fungal growth inhibition zones around (well) were measured and the percentage inhibition equation was applied to calculate the inhibition rate of the two fungi extracts.

The data were analyzed using the statistical software (SPSS Inc. version 23, Chicago, Illinois, USA). The value of the LSD was adopted for significance at level $P \leq 0.05$.

RESULTS AND DISCUSSION

The growth rate of fungal colonies was inversely affected with increasing the concentration of the plant extracts as the percentage of inhibition of *F. solani* was 66.3, 83.7, 87.0 and 89.5 at concentrations of 5, 10, 15 and 20 mg/ml, respectively (Table 1; Fig. 1) when the methyl alcohol was 14%. Similarly, in *A. alternata* the percentages of inhibition at concentrations of 5, 10, 15 and 20 mg/ml were 68.7, 88, 91 and 100, respectively, when the percentage of inhibition using methyl alcohol was 39.

There were significant differences between most of the concentrations as well as between the fungus species and the concentration used where the percentage of inhibition of *Fusarium* 100 at a concentration 20 and at the same concentration, the percentage of inhibition of *Aternaria* was 89.5 (Figs. 2 and 3).

When compared the plant extract and methyl alcohol, the latter showed a weak inhibitory

Table 1. The effect of the methanolic extract of *E. prostrata* leaves on the inhibition of the radial growth of *F. solani* and *A. alternata* (MIC) method

Concentration (mg/ml)	Fungi species		Average (%)
	Alternaria alternate	*Fusarium solani*	
	(Average %±Standard deviation)		
Control (1)	0.0±0.0	0.0±0.0	0.0
Control (2) methanol	39.0±5.0	14.0±3.0	26.5
5	68.7±1.5	66.3±8.1	67.5
10	88.0±1.0	83.7±5.5	85.8
15	91.0±2.0	87.0±1.7	89.0
20	100.0±0.0	89.5±4.2	94.8
Average	64.4±3.2	56.7±5.3	

Fungi species = 2.078, Concentration = 3.599 and Concentration x Fungi species = 5.089.

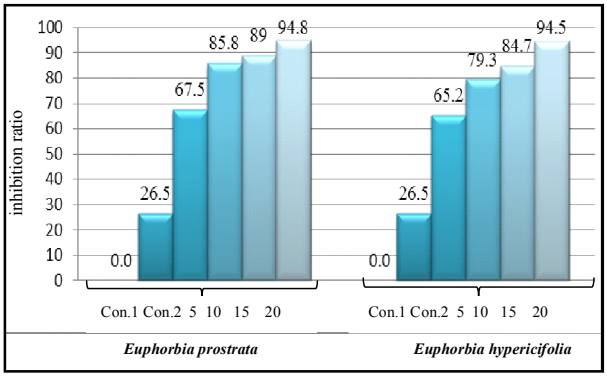

Fig. 1. Inhibition rate of *Fusarium solani* and *Alternaria alternata* by using alcoholic extract of leaves (MIC method).

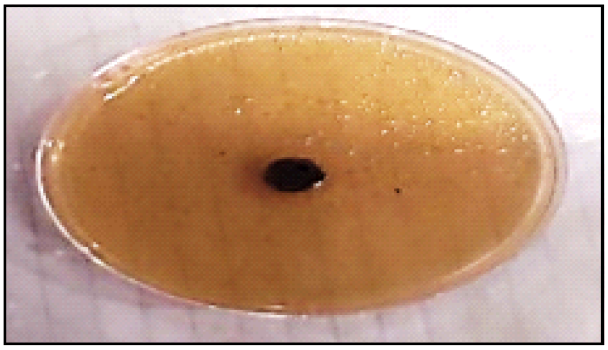

Fig. 2. Effect of alcoholic extract *of E. prostrata* leaves on the radial growth of *A. alternata* at con. of 20 mg/m) by the MIC method.

effect compared to the plant extract. The reason may be due to the dissolution of some biologically active compounds present in the extract, such as resins, which were considered anti-fungal compounds.

Hikmawanti *et al.* (2021) mentioned that

Fig. 3. Effect of alcoholic extract of *E. prostrata* leaves by well method. A: The normal growth of the fungus *F. solani* and B: The effect of the extract at con. of 20 mg/ml.

alcohol could extract effective compounds such as alkaloids, phenols, flavonoids and tannins more than water, as these compounds had anti-fungal effectiveness. In addition, these compounds worked to precipitate cell protein through their ability to unite with it. It changed its nature as it dissolved the membranes of the living cell, being a good solvent for fatty substances, which led to the exit of the contents of the cell to the outside, and the fungal cell died.

According to Chouhan *et al.* (2017), the inhibitory action of some plant extracts against microorganisms may be due to the presence of essential oils rich in effective antifungal compounds that dissolved in alcohol but not in water, as evidenced by the detection of chemical plant extract, which contained active substances of great importance.

The percentages of inhibition of *A. alternata* at concentrations of 5, 10, 15 and 20 mg/ml were 72, 82.7, 100 and 100, respectively (Table 2; Fig. 4). As for the percentages of inhibition of the same fungus using methyl alcohol, it was 89%. Similarly, *F. solani* showed inhibition percentages of 80.3, 80.7, 83.7 and 95.5% at concentrations of 5, 10, 15 and 20 mg/ml, respectively, when the percentage of inhibition of the same fungus using methyl alcohol was 78.8. There was non-significant difference between 15 and 20 mg/ml in *F. solani.* Further, *A. alternata* was more sensitive to the plant extract, as the inhibition rate reached 100% at a concentration of 15 mg/ml, while the inhibition rate for *F. solani* was 84% at the same concentration.

The difference in the degree of an inhibitory effect of methanolic extract of the studied

Table 2. The effect of different concentrations of the methanolic extract of *E. prostrata* leaves on the growth of *Alternaria alternata* and *Fusarium solani* by Well method

Concentration (mg/ml)	Fungi species		Average (%)
	Alternaria alternate	*Fusarium solani*	
	(Average %±Standard deviation)		
Control (1)	0.0±0.0	0.0±0.0	0.0
Control (2) methanol	89.0±0.0	78.8±9.1	83.9
5	72.0±1.0	80.3±3.2	76.2
10	82.7±4.5	80.7±2.1	81.7
15	100.0±0.0	83.7±6.0	91.8
20	100.0±0.0	95.5±1.5	97.8
Average	73.9±4.1	69.8±3.3	

Fungi species = 2.067, Concentration = 3.581 and Concentration x Fungi species = 5.064.

plants on microorganisms may be due to different factors such as the type of plant extract or the nature of the polar solvent used as well as the method of extraction or to the inability of the cell membrane to permeability to those substances present in the extract and thus led to a weakness in the effective effect on the enzymes and proteins present inside the cell (Jafer and Naser, 2020).

A study of the antifungal activity using the methanolic extract of the leaves of *E. postrata* against each of the two fungal strains *A. alternata* and *F. solani* showed that it had a medium activity as an antifungal using the disc diffusion method (MIC), while Well method showed good activity in inhibiting fungal growth itself. Also, it appeared that *A. alternatea* had a higher sensitivity to the plant

Table 3. Effect of methanolic extract of *E. hypericifolia* leaves in inhibiting the radial growth of *F. solani* and *A. alternata* using the MIC method

Concentration (mg/ml)	Fungi species		Average (%)
	Alternaria alternate	*Fusarium solani*	
	(Average %±Standard deviation)		
Control (1)		0.0±0.0	0.0
Control (2) methanol	39.0±5.0	14.0±3.0	26.5
5	69.3±4.5	61.0±4.0	65.2
10	81.8±1.2	76.7±1.5	79.3
15	85.3±6.3	84.0±0.0	84.7
20	89.0±0.0	87.7±0.0	94.5
Average	60.8±2.9	55.9±3.7	

Fungi species = 1.752, Concentration = 3.053 and Concentration x Fungi species = 4.317.

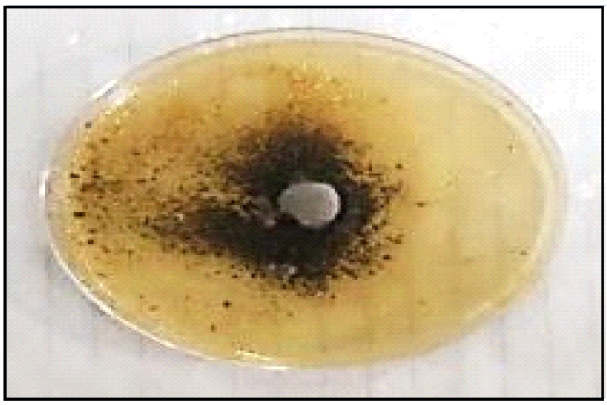

Fig. 4. Inhibition by concentration (10) mg/ml to *E. hypericifolia* of fungi *A. alternata* by MIC method.

extract than that of *F. solani*, there were saponions, alkaloids, flavonoids, polyphenols, glycosides and tannins, many of which had antifungal activity (Jassim, 2017).

The percentage of inhibition of *F. solani* was 61, 76.7, 84 and 87.7 at concentrations of 5, 10, 15 and 20 mg/ml, respectively (Table 3; Fig. 4) when the percentage of inhibition by methyl alcohol was 14. However, the percentages of inhibition of the *A. alternata* were 69.3, 81.8, 85.3 and 89 at the same concentrations, respectively, when the percentage of inhibition of the fungus by methyl alcohol was 39. Thus, there were significant differences between all the different concentrations, between the fungal types, as well as between the type of fungi and the concentration used in the study. The methanolic plant extract and the inhibitory effect of methyl alcohol showed a weak inhibitory effect compared with the inhibitory effect of different concentrations of the plant extract.

The methanolic extract of *E. hypericifolia* had activity in inhibiting the growth of the tested fungi. It depended on the concentration of the plant extract in addition to the type of fungal isolate, where the extract showed inhibitory activity in percentages directly proportional to the increase in the concentration of the plant extract and inversely with the rate of growth diameters of fungal colonies. The percentages of inhibition of the growth of *F. solani* were 54.9, 56.9, 60.5 and 64.9 at concentrations of 5, 10, 15 and 20 mg/ml, respectively, when the percentage of inhibition for methyl alcohol was 78.8 (Table 4; Fig. 5). For the fungus *A. alternata*, the growth inhibition percentages

Table 4. Effect of different concentrations of methanolic extract of *E. hypeircifolia* leaves on the growth of fungi *A. alternata* and *F. solani* by Well method

Concentration (mg/ml)	Fungi species		Average (%)
	Alternaria alternate	*Fusarium solani*	
	(Average %±Standard deviation)		
Control (1)	0.0±0.0	0.0±0.0	0.0
Control (2) methanol	88.8±0.0	78.8±8.1	82.8
5	67.4±1.1	54.9±5.5	62.4
10	70.9±1.2	56.9±4.4	65.3
15	74.4±0.6	60.5±3.1	68.8
20	75.7±5.4	64.9±2.3	71.4
Average	61.3±6.3	50.7±2.9	

Fungi species = 2.044, Concentration = 3.131 and Concentration x Fungi species = 4.062.

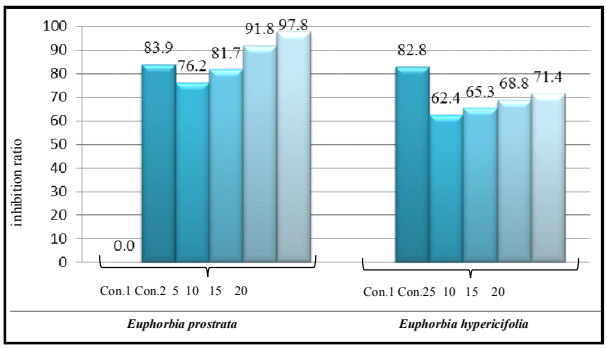

Fig. 5. Inhibition rate of *F. solani* and *A. alternata* by using alcoholic extract of leaves by Well method.

were 67.4, 70.9, 74.4 and 75.7 at the same concentrations, respectively, when the inhibition percentage of fungal growth using methyl alcohol amounted to 88.8 (Figs. 6 and 7).

The methanolic extract of *E. prostrata* leaves had a higher ability to inhibit the growth of the two pathogenic fungi *A. alternata* and *F. solani* by using the MIC and Well methods. The fungal inhibition rate of the methanolic extract of the leaves of *E. prostrata* by MIC method for *F. solani* was 56.7% and for *A. alternata* was 64.4%, while using the Well method, the fungal inhibition rate for *F. solani* 69.8 and 73.9% for *A. alternata*, while the rate of fungal inhibition of *E. hypericifolia* leaf extract using the MIC method was 55.9% for *F. solani* and 60.8% for *A. alternata*, but using the Well method, the inhibition rate for the growth was 50.7% for *F. solani* , whereas for the fungus *A. alternata,* it reached 61.3%.

Fig. 6. The percentage of inhibition at a con. of 20 mg/ml of E. *hypericifolia* in *F. solani*.

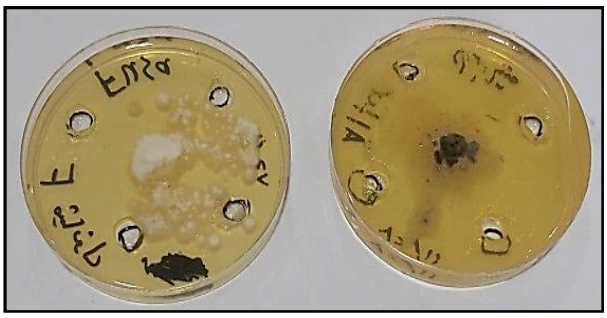

Fig. 7. The inhibition rate of methanol on the *A. alternata* and *F. sola*ni.

CONCLUSION

It was concluded that the percentage of inhibition was directly proportional to the increase in the concentration of the plant extract, and the *E. prostrata* plant had the most inhibition of pathogenic fungi by Well method.

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