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ASSESSMENT OF ANTIFUNGAL ACTIVITY OF *I.VISCOSA*

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ABSTRACT

Inla viscosa (Asteraceae) air parts ethanolic extract was assessed for its fungal activity against *Mucor* and *Rhizopus*. Growth inhibition percentage ranged between 61-100% in presence of ethanol *I.viscosa* air parts against studied fungi. The extract was more effective against *Mucor* than *Rhizopus* with a statistically significant difference and the effectiveness was concentration dependent. We also found that the MIC of the extract against *Mucor* was 16mg/ml while it was 18mg/ml for *Rhizopus.* Therefore, *I.viscosa* could be an important source of active compounds useful for developing safe antifungal drugs.

KEYWORDS: *I.viscosa*, ethanolic extract, antifungal activity, *Mucor*, *Rhizopus.*

1. INTRODUCTION

Plant products are an ample source of antifungal drugs and are traditionally used for the treatment of various infectious diseases. A vast array of diseases occurs due to the fungal infections such as dermatophytosis, candidiasis, aspergillosis, and mucormycosis. Mucormycosis is an infectious disease caused by a fungus of the class of Zygomycetes and the order of Mucorales including *Rhizopus spp*, *Mucor spp*, and others.[1,2]

In recent decades, the incidence of mucormycosis has increased all over the world, becoming the second most common fungal disease in patients with haematological malignancies and transplant recipients.[3,4,5] Plant products are major sources of therapeutic drugs for infectious disease and commonly harmless or have the least side effects as compared to synthetic drugs.[6,7] Among the large variety of Mediterranean folkloric herbs, *Inula viscosa* belonging to the Asteraceae family, has proven to be a source of natural products forming the basis for alternative medicine and natural therapies.[8,9] *I.viscosa* was studied antifungal activity against *Candida albicans*, *Fusarium* species, and other fungi.[10,11] To the best of our knowledge, reports on antifungal activity of *I.viscosa* against Mucorales are scant.

Thus, this study aimed to determine the phenolic content of ethanolic extract of aerial parts of *I.*viscosa, and evaluate its activities against *Mucor* and *Rhizopus*.

2. MATERIALS AND METHODS

2.1. Materials

Folin-Ciocalteu reagent (Aldrich. Switzerland), sodium carbonate (BDH. England), gallic acid (Biotec LTD), Potato Dextrose Agar (HIMEDIA), Dimethyl sulfoxide (DMSO), ethanol 95% (pharmex), distilled water, Fluconazole (ElSaad pharma), and tween 20.

2.2. Fungal Strains

The studied fungal strains included both *Mucor* and *Rhizopus*, which were identified morphologically as shown in the figure1.

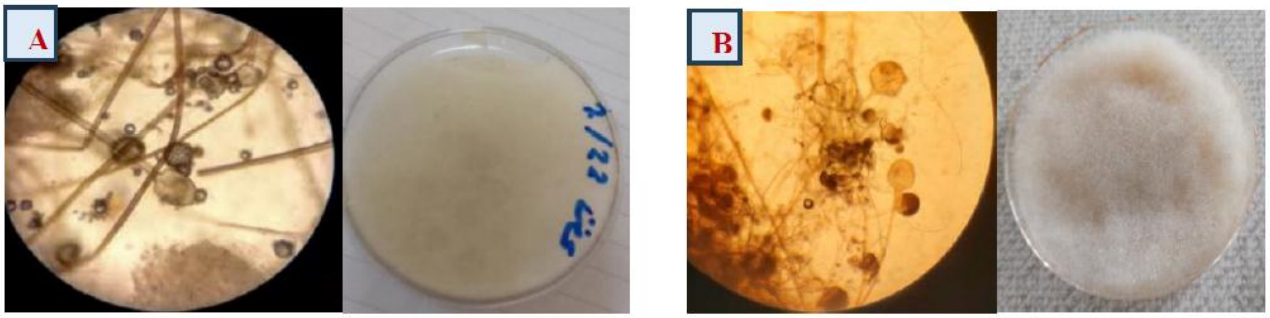

Figure1: culture and microscopic structure (A: *Mucor.* B: *Rhizopus*).

2.2. METHODS

2.2.1. Preparation of Extract

Dried and ground aerial parts (10g) were macerated in ethanol 95% (10:100 w/v) for 15 days with shaking at room temperature. The extracts were filtered and the filtrate evaporated to dryness using a rotary evaporator. In order to test the antifungal activity, the sample was solubilized in dimethyl sulfoxide (DMSO 2%).

2.2.2. Determination of Total Phenol Content (TPC)

The TPC was determined using the Folin-Ciocalteu method [12]. Briefly, 0.1 mL of dissolved extract was mixed with 0.1 mL of Folin-Ciocalteu reagent in test tube. After 5 min, 2 mL of saturated sodium carbonate (Na2CO3) solution (2%) was added to the mixture. The reaction mixtures were incubated for 30 min. All assays were conducted in triplicate and the results were averaged. The TPC was calculated from a calibration curve using gallic acid (GAE) as the standard and the results were expressed as grams of gallic acid equivalent per kilogram of extract (g GAE/kg).

2.2.3. Determination of Antifungal Activity of *I.viscosa* Extract

The fungal inoculum was prepared from fresh culture of about 7 days for *Rhizopus*, and *Mucor* following a procedure based on the EUCAST with minor changes.[13] Briefly, 5 mL of sterile water with Tween 20 (0.1% v/v) was added to the culture. To promote conidial suspension, the culture was gently scraped using a sterile cotton swab. The obtained suspension was recovered, shaken for about 15 s with a vortex and filtered to remove hyphae and clumps. The inoculum was adjusted with a hemocytometer to a range of 2×10^{6} to 3×10^{6} CFU/ml. Testing media were obtained by adding different amounts of extracts to PDA to obtain final concentrations of 12, 14,16, 18, 20mg/ml. A total of 20 µL of conidial suspension was inoculated in the center of the agar plate. Plates were incubated at 25 ◦C and growth was observed daily until the mycelium of the negative control (PDA only) touched the edge of the plate. Growth inhibition (GI), expressed as a percentage, was calculated by measuring the colony diameter and using the following equation:

GI(%) = [(dc − dt)/dc] × 100 where dc is the mean diameter of the negative control (PDA only) and dt is the mean diameter of the treatment.

2.2.4. Statistical Analysis

Data analysis was performed using IBMM SPSS Statistic 20 program. To determine the differences between treatments, a one-way analysis of variance (ANOVA) was performed with significance level set at p = 0.05. The Post hoc tests were conduced to determine the statisfical difference between treatment.

3. RESULTS AND DISCUSSIONS

3.1. Total Phenol Content

The TPC amount of *I,viscosa* extract was 169.271±6.785g GAE/kg. Similar results were reported by Salim and his colleagues when studying ethanol extracts of leaves and flowers respectively. The results showed that the leaves had the highest phenolic content, 149.8mg GAE/g, while the flowers contained 99.1mg GAE/g of phenols.[14]

Another study of *I.viscosa* by chami et al. indicated that the total phenolic content of the ethanolic extract was 274.39mg GAE/g, wich was higher than in previous studies.[15]

The difference in phenolic content between our study and other studies is due to many reasons, including differences in geographical location, climatic conditions, extraction method, plant harvest season, part used, and other factors that can play a major role in the difference in phenolic content.

3.2. Antifungal Activity

The data of the antifungal activity of I.viscosa extract against Mucor and Rhizopus are reported in table1 and figure2,3,4,5.

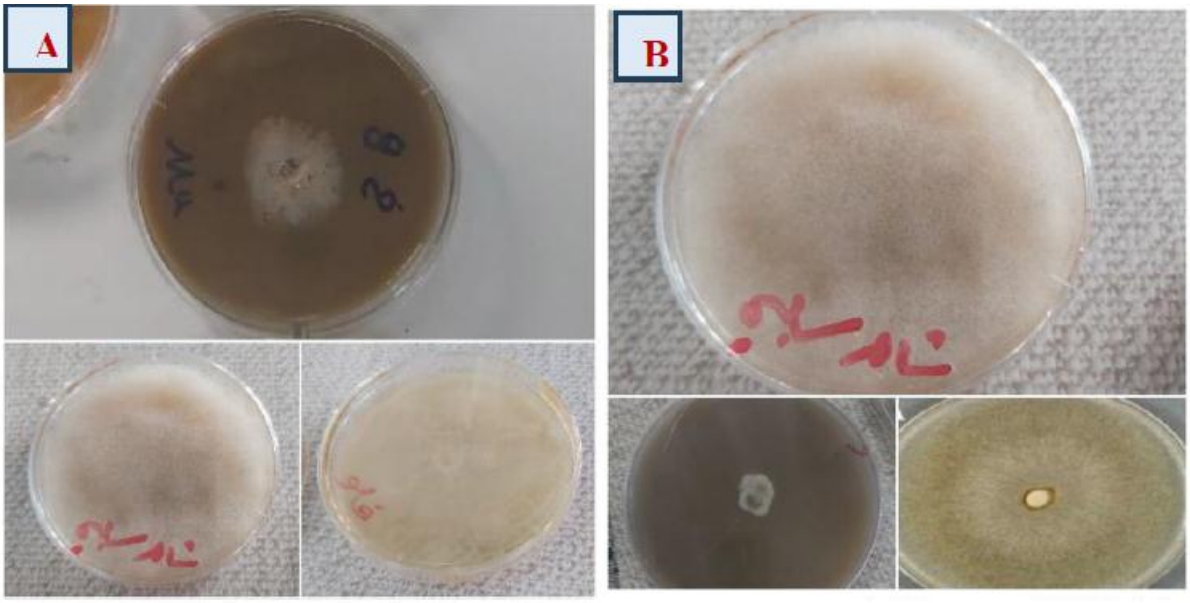

Figure2: Antifungal activity of *I.viscosa* against Mucorales (A: *Mucor,* B: *Rhizopus*).

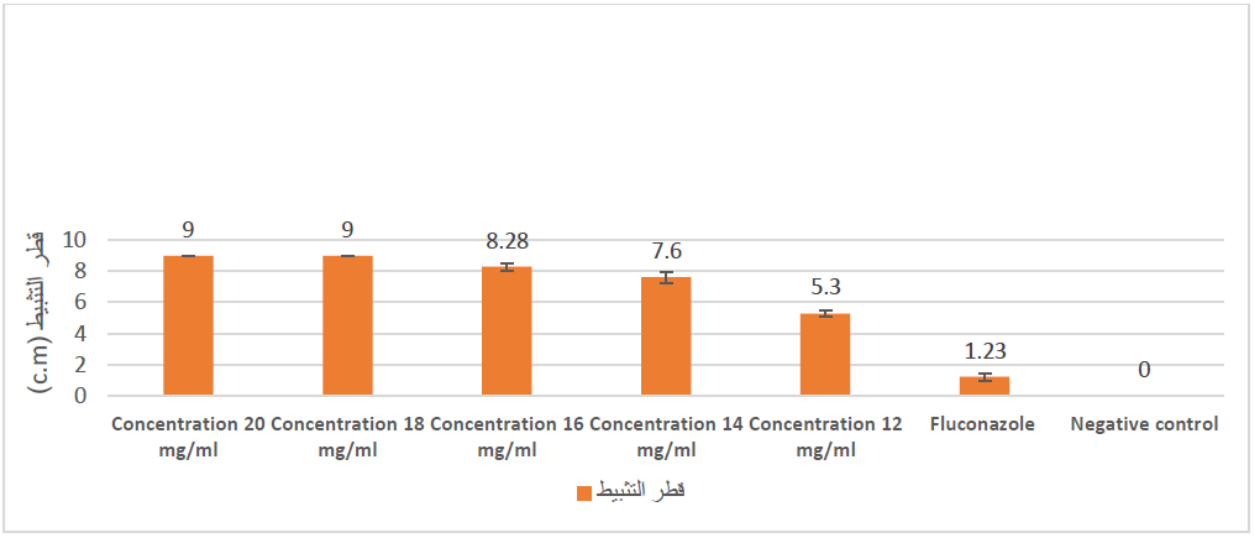

Figure3: Effect of ethanolic extract of I.viscosa against Rhizopus.

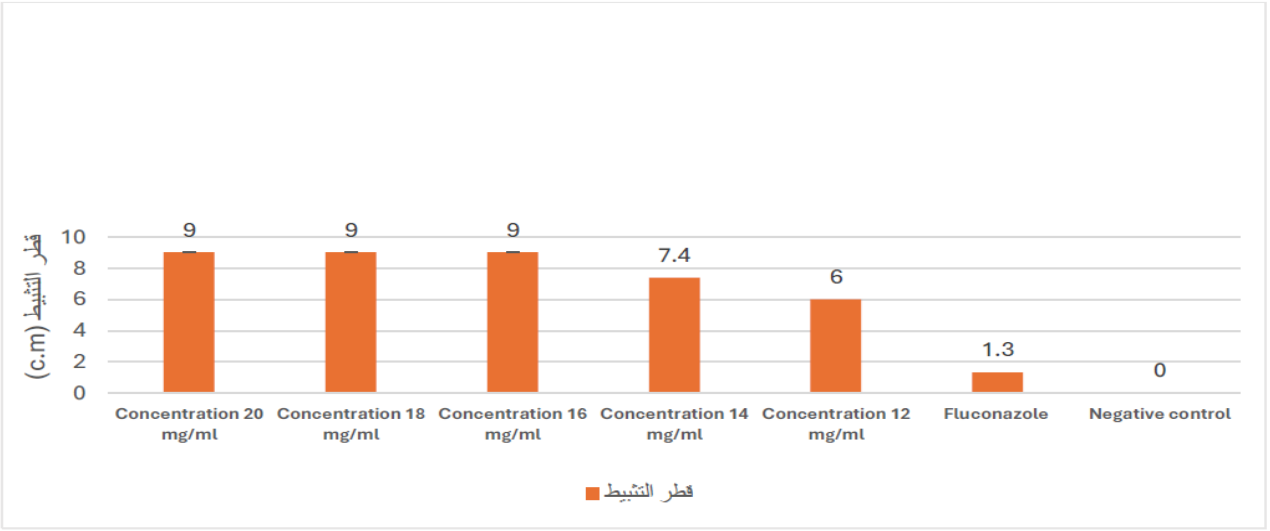

Figure4: Effect on ethanolic extract of I.viscosa against Mucor.

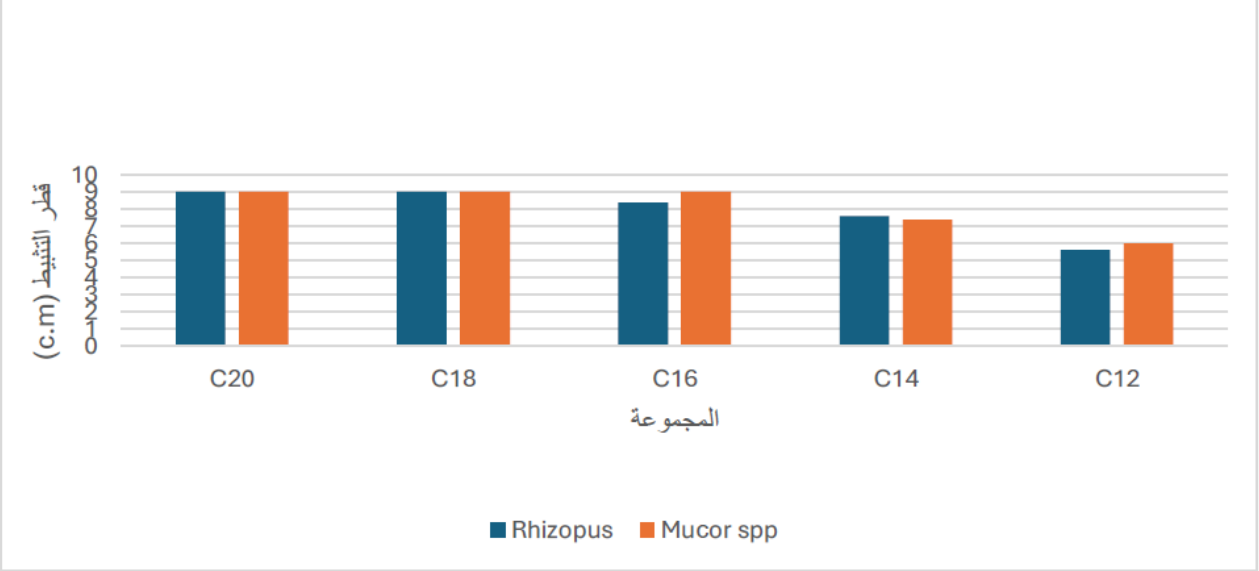

Figure5: Comparison of antifungal effect of ethanolic extract of I.viscosa.

Table1: Growth Inhibition rate of ethanolic extract on fungal growth.

Extract mg/ml Fungi	12	14	16	18	20
Mucor	66.66%	82.22%	100%	100%	100%
Rhizopus	61.11%	84.44%	93.11%	100%	100%

The ethanolic extract of *I.viscosa* was sensitive to broth mucor and Rhizopus, but more sensitive to mucor than to Rhizopus with a statistically significant difference (p-value=0.035) at the extract concentration of 12mg/ml. The activity was concentration dependent, as the increase in activity was accompanied by a significant increase in the diameters of inhibition with a statistically significant difference (p-value=0.000). The minimum inhibitory concentration (MIC) of ethanolic extract of *I.viscosa* values were 16mg/ml, 18mg/ml for *Mucor* and *Rhizopus*, respectively.

The effect of ethanolic extract of *I.viscosa* may be due to its high phenolic content, especially phenolic acids and flavonoids such as caffeoylquinic acid, catchin, apigenin, and quercetin derivatives, according to previous studies.[16,17] Our study also showed a strong correlation between the effectiveness and phenolic content of the ethanolic extract, where the Pearson correlation coefficient for *Rhizopus* (r=0.941) and *Mucor* (r=0.927) where they were statistically significant as shown in figure6. Although the extract mechanism of the antifungal effect of *I.viscosa* has not been precisely determined, many studies have indicated that many phenolic compounds have a destructive effect on both the cell wall and cell membrane of the fungal cell, wich results in disturbance of the cellular ionic balance, in addition to their ability to inhibit cellular respiration and inhibit ATP, thus leading to the death of the fungal cell.[18.19]

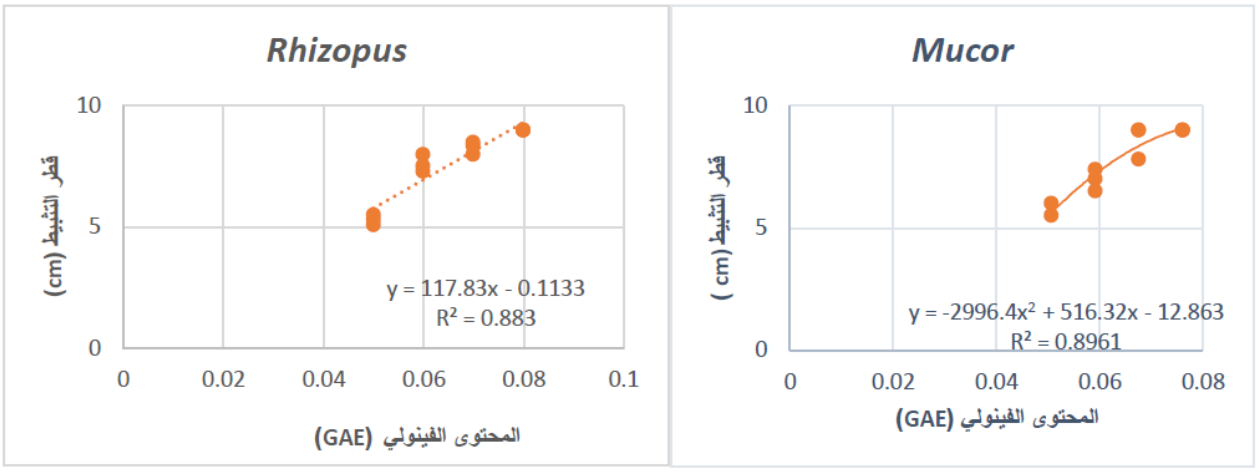

Figure6: Relationship between phenolic content and effectiveness.

4. CONCLUSION

In this study, we found that the ethanolic extract of *I.viscosa* plant available in syria had antifungal activity against both *Mucor* and *Rhizopus*, where the activity was concentration dependent and there was a strong correlation between the activity and phenolic content of the extract. *I.viscosa* is a promising new source for the treatment of mucormycosis with lower cost and side effects.

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