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A STUDY TO DETERMINE TOTAL PHENOLIC CONTENT OF *OPUNTIA FICUS-INDICA* EXTRACTS AND THEIR ACTIVITY AGAINST SOME PATHOGENIC FUNGI

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ABSTRACT

This study was carried out to determine total phenolic content Of cactus plant extracts (*Opuntia ficus-indica*) and their activity against some pathogenic fungi. The results of analysis of total phenolic content of methanolic extracts by using spectrophotometer device showed high content, that was found in fruits extract reached 31.516 mg GAE /g DW, followed by flowers 20.35 mg GAE /g DW and cladodes 14.75 mg GAE /g DW. The effectiveness of the methanolic extracts was determined against two species of fungi (*Aspergillus fumigatus* and *Aspergillus flavus*). The study showed a difference in efficacy of studied extracts in inhibiting the growth of studied fungi, the methanolic extracts of cladodes, flowers and fruits inhibited the growth of *A.fumigatus* by ratio reached (89.57, 100, 78.66) % respectively at concentration 1000 mg/ml, while inhibited the growth of *A.flavus* by ratio reached (85.40, 87.83, 78.10) % at the same of previous concentration. Fluconazole with concentrations (1.25, 2.5, 5, 10) mg/ml was used as a standard drug for antifungal study, which inhibited the growth of *A.fumigatus* and *A.flavus* by ratio reached (75.15, 81.75) % respectively at concentration 10 mg/ml.

KEYWORDS: Opuntia ficus-indica; Methanolic extract; Total Phenolic Content; Fungi; Fluconazole.

1. INTRODUCTION

Cactus plant that returns to *Opuntia* genus exists in several regions occupying around 100,000 ha (Stintzing and Carle, 2005). It is widely distributed in Mexico and in all American hemispheres as well as in Africa and in the Mediterranean basin, especially in the dry and semi-arid regions (Chakraborty, 2009). In South Africa, Mediterranean areas and South American is cultivated for its edible fruit (prickly pear), although in some countries different parts of the plant are utilized in the food and cosmetic industry (Griffith, 2004; Inglese et al., 2002).

The secondary metabolites of cactus serve as plant defence mechanism against predators (microorganisms, insects and herbivores). Chemically, most of secondary metabolites are aromatic substances such as phenols and their oxygen-substituted derivatives; others contribute to plant odour characteristics, such as terpenoids; quinones and tannins are responsible for plant pigmentation (Cowan, 1999).

Opuntia ficus-indica cladodes represent a source of phytochemicals, such as phenolics acids and flavonoids. Especially, Quercetin, kaempferol, isorhamnetin, Nicotiflorin and others (Ramadan and Morsel, 2003; Marizel *et al*., 2015), while fruits contain substantial amounts of ascorbic acid, vitamin E, carotenoids, fibers, amino acids and antioxidant compounds (phenols, flavonoids, betaxanthin and betacyanin) (Osorio- Esquivel, 2011; Schaffer *et al*., 2005), whereas flowers contain different flavonoids notably kaempferol and quercetin (De Leo *et al*., 2010).

The phenolic compounds isolated from parts of the cactus plant have a wide range of therapeutic properties (anti-inflammatory, anti-allergenic, anti-microorganism, cardioprotective effects and others) (Balasundram *et al*., 2006).

Opuntia ficus-indica has been used in traditional folk medicine because of its role in treating a number of diseases and conditions, including anti-inflammatory effects, hypoglycemic effects, inhibition of stomach ulceration and neuroprotective effects. Recently, active compounds derived from plant parts have shown anti-microorganisms effects, including fungi (Belay *et al*., 2015).

In recent years, the rates of infection with invasive fungi have increased significantly Especially the infection has caused by *Aspergillus* genus. That increased had a close

relationship with the extensive use of broad-spectrum antibiotics, especially by immuno-compromised patients (Kousha *et al*., 2011; Walsh *et al*., 2008; Patterson *et al*., 2000).

Aspergillus fumigatus is now the second human pathogenic agent after *Candida albicans*. As well as, it is being the main cause of the most infections of *Aspergillus* and followed by *Aspergillus flavus* (Frisvad and Larsen, 2016; Hedayati et al., 2007; Bertout *et al*., 2001).

A. fumigatus is described as the cause of lung diseases in humans. It often causes a variety of pulmonary syndromes such as allergic bronchopulmonary aspergillosis, chronic pulmonary aspergillosis and invasive pulmonary aspergillosis. Rarely, liver infection (Pyogenic liver abscess, which can be seen in 80% of the patients with liver abscess) (Yu et al.,2016).

The risk of *A. flavus* lies in being produced a group of harmful toxins to humans and animals known as aflatoxins, the most important of them (B1 , B2, G1, G2). These toxins occur on the consumer a range of chronic effects such as immune suppression, impaired child growth, abnormal foetal development, and cancer, and acute effects such as hepatitis and jaundice, abnormal swellings, and even death (Amaike and Keller, 2011; Cotty and Mellon, 2006; Yu *et al*.,2002).

Due to the lack of studies available on the efficacy of cactus extracts against fungi, this study is complementary to previous studies by obtaining effective extracts compared to antibiotics.

In the following study, the total phenolic content of methanolic extracts for three parts of *Opuntia ficus-indica* (cladodes, flowers, and fruits) was determined because its importance in inhibiting the growth of fungi, while the efficacy of these extracts was tested against fungal isolates returned to *A.fumigatus* and *A.flavus* compared with antibiotic (fluconazole).

2. MATERIALS AND METHODS

2.1 Plant material

The three plant parts of *Opuntia ficus-indica* collected from several areas of east of Lattakia city, the samples of cladodes collected during April 2017, while flowers collected during June, whereas fruits collected during August at the same year.

The samples were brought to the Graduate Laboratory at the Faculty of Science, Department of Plant Biology in Tishreen university, cleaned well from the thorns and the soil, washed with distilled water several times, cut the samples of the cladodes and fruits into slices by

using a sterile sharp knife, Leave to dry in the shade for several days, then put in oven at Temperature 35^0C until the weight stability, then grind by using the electric mixer to get a fine powder, kept in the refrigerator in sealed and sterile glass containers until use.

2.2 Preparation of crude extract

20 g of dired powder was soaked in 200 ml of methanol 95%, the flask was covered with aluminum foil and then placed on a magnetic stirrer for half hour, left for 7 days into dark, stirring occasionally, then filtered with Whatman No.1., concentrated in a rotary vacum evaporator at 40°C. The crude extract kept in the refrigerator in sealed and sterile glass containers until use.

2.3 Determination of Total phenolic content

The total phenolic content was determined by the Folin–Ciocalteu method (Singleton et al., 1999). The dissolved extract (100 µL) was mixed with 6 mL of distilled water and 500 µL of Folin–Ciocalteu reagent. After 1 min, 1.5 mL of Na_2CO_3 (20%) was added and the volume was adjusted to 10 mL with distilled water. After 30 min of incubation at room temperature, the absorbance was measured at 760 nm. Gallic acid was used for the calibration curve. The results were expressed as Gallic acid equivalents, mg GAE/g dry weight of the plant extracts and calculated as meanvalue ± SD (n = 3).

2.4 Isolated the pathogenic fungi

Aspergillus isolates were obtained from Tishreen Hospital in Lattakia, then planted on the Saubourads Dextrose Agar culture (SDA) and incubated at 30^0C for a week, after that the studied fungi (*A.fumigatus* and *A.flavus*) isolated and purified, then stored in the refrigerator at 4^0C in tubes contain SDA culture.

2.5 Antifungal activity

The effect of methanolic extract of plant extracts and water solution of fluconazole were examined in inhibiting the growth of fungi (*A. fumigatus* and *A.flavus*) by the Poison Food Method (Nene and Thapilyal, 2002) With some appropriate modifications. The extracts prepared at concentratios (125,250, 500, 1000) mg/ml, while the fluconazole solution was prepared at concentrations (1.25, 2.5, 5, 10) mg/ml.

Then added 1 ml of each of the concentrations to 10 ml of the SDA culture and stirred well, then poured in plastic petri plates, left to harden at the laboratory temperature, then took a

disc with a diameter of 5 mm from the sides of Colony of the studied fungus by using a sterilized needle and placed in the middle of each plate. The control plates were made by Cultivate the studied fungus on the SDA culture without adding any extract, the plates incubated at 28 ± 2^0C for 7 days (Belay *et al*., 2015).

The experiment was performed with three replicates for each extract and each concentration alonly and the control plates, then the colony diameter was measured in the middle of each dish, took the average growth rate of the fungal colonies of the three replicates and then calculated the inhibiting percentages according to the following equation (Yigit and Korukluoglu, 2007).

Percentage of inhibition = (The average diameter of the control colony ‗ The average diameter of the treated colony) x100 / The average diameter of the control colony.

RESULTS

3.1 Total phenolics contents in *Opuntia ficus-indica* extracts

Table (1) shows a difference in total phenolic content between the extracts of the studied parts. The fruits extract showed the highest phenolic content reached 31.516 mg GAE /g DW followed by the extracts of flowers and cladodes with a content reached (20.35, 14.75) mg GAE /g DW respectively.

Table 1: Total phenolic content in *Opuntia ficus-indica* extracts.

Opuntia ficus-indica extracts	Total phenolic content (mg GAE /g DW)±SD
Cladodes	14.75±0.46
Flowers	20.35±1.01
Fruits	31.516±0.09

3.2 The activity of *Opuntia ficus-indica* extracts against *A.fumigatus*

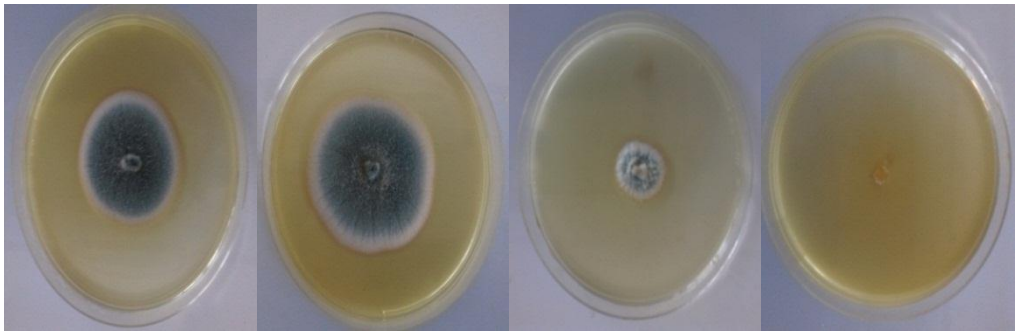
Table (2) shows that, the methanolic extract of flowers was the most effective against *A.fumigatus* with inhibiting ratio reached 100% at concentration 1000 mg / ml, followed by cladodes and fruits extracts by inhibiting ratios (89.57, 78.66)% respectively at the same of previous concentration.

Figure 1. shows colonies of *A.fumigatus* treated by different concentrations of methanolic extract of cactus flowers compared to control sample

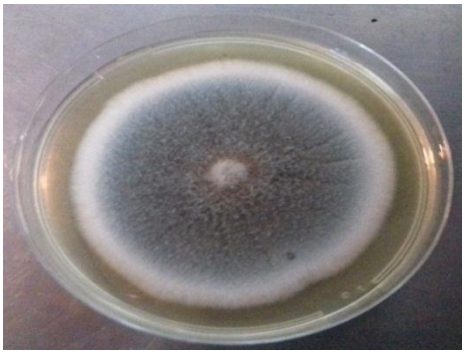
Table 2. The activity of *Opuntia ficus-indica* extracts against *A.fumigatus*.

Cactus plant	Control	125	250	500	1000
	% DIZ	% DIZ	% DIZ	% DIZ	% DIZ
cladodes	0 8.25	24.48 6.23	50.90 4.05	2.06 75.03	89.57 0.86
flowers	0 8.25	40 4.95	51.15 4.03	1.86 77.45	100 0
fruits	0 8.25	18.06 6.76	30.54 5.73	54.66 3.74	1.76 78.66

DIZ= Diameter of inhibition zone; %= inhibiting ratio.



1000 mg/ml **500 mg/ml** **250 mg/ml** **125 mg/ml**



Control sample

Figure 1: Colonies of *A.fumigatus* were treated by different concentrations of methanolic extract of cactus flowers compared to control sample.

3.3 The activity of *Opuntia ficus-indica* extracts against *A.flavus*

Table (3) also shows that, the methanolic extract of flowers was the most effective against *A.flavus* with inhibiting ratio reached 87.83% at concentration 1000 mg / ml, followed by cladodes and fruits extracts by inhibiting ratios (85.40, 78.10)% respectively at the same of previous concentration.

Figure 2. shows colonies of *A.flavus* treated by different concentrations of methanolic extract of cactus flowers compared to control sample.

Table 3. The activity of *Opuntia ficus-indica* extracts against *A.flavus*:

Cactus plant	Control	125	250	500	1000
	% DIZ	% DIZ	% DIZ	% DIZ	% DIZ
Cladodes	08.22	56.20 3.6	68.97 2.55	2.15 73.84	1.2 85.40
Flowers	08.22	63.50 3	70.55 2.42	1.95 76.27	1 87.83
Fruits	08.22	39.78 4.95	45.01 4.52	3.23 60.70	78.10 1.8

DIZ= Diameter of inhibition zone; %= inhibiting ratio.

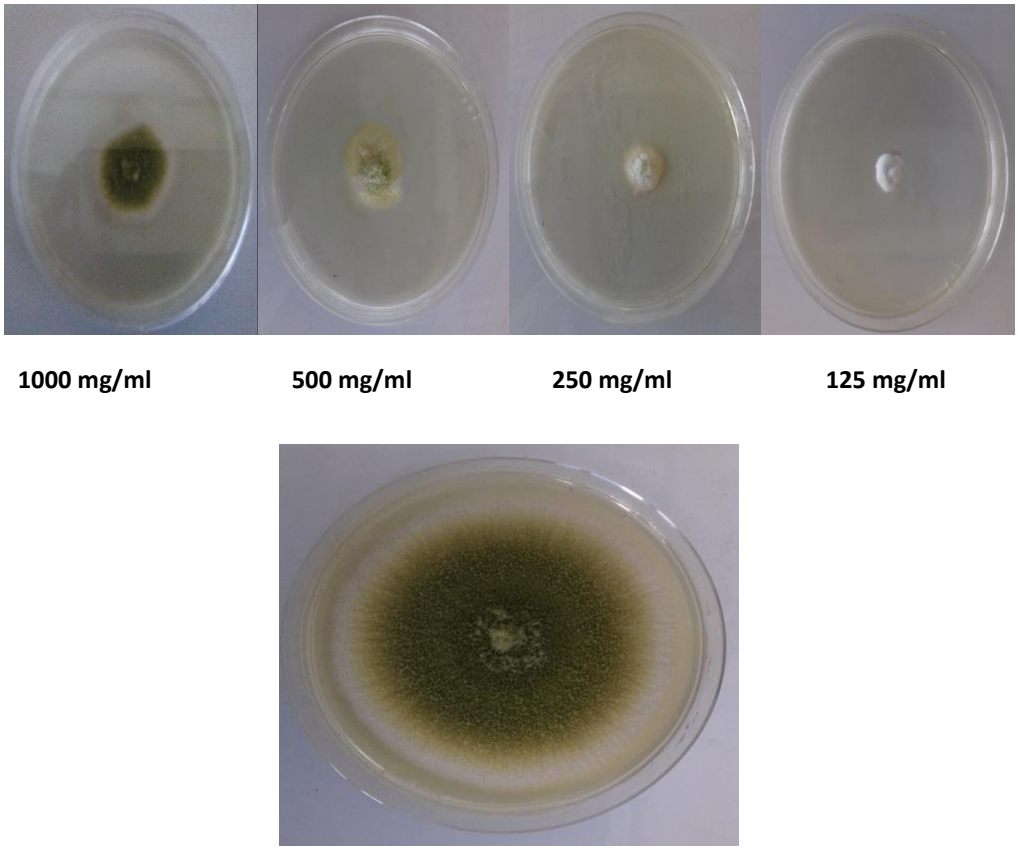


Figure 2: Colonies of *A.flavus* were treated by different concentrations of methanolic extract of cactus flowers compared to control sample

3.4 The activity of fluconazole against (*A.fumigatus* and *A.flavus*)

Table (4) shows that, fluconazole inhibited the growth of both fungus (*A.fumigatus* and *A.flavus*) with different percentages increased with the increase of concentration, the highest inhibitory ratio reached (75.15, 81.75) % respectively at concentration 10 mg / ml.

Figure 3. shows colonies of *A.fumigatus* treated by different concentrations of fluconazole.

Figure 4. shows colonies of *A.flavus* treated by different concentrations of fluconazole.

Table 4. The activity of fluconazole against (*A.fumigatus*, *A.flavus*).

The Studied Fungi	Control	1.25	2.5	5	10
	% DIZ	% DIZ	% DIZ	% DIZ	% DIZ
A.fumigatus	0 8.25	52.72 3.9	60.48 3.26	67.03 2.72	75.15 2.05
A.flavus	08.22	36.73 5.2	47.68 4.3	3.25 60.46	1.5 81.75

DIZ= Diameter of inhibition zone; %= inhibiting ratio.

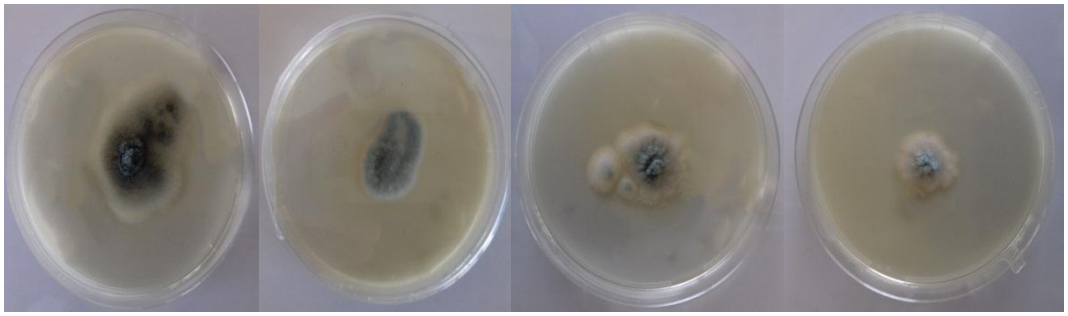


Figure 3: Colonies of *A.fumigatus* were treated by different concentrations of fluconazole.

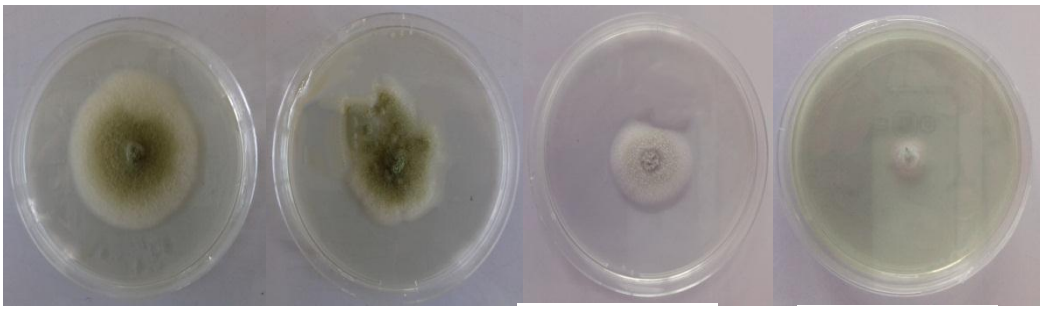


Figure 4: Colonies of *A.flavus* were treated by different concentrations of fluconazole

4. DISCUSSION

Phenolic compounds exhibit the fungal growth by forming hydrogen bonds with the wall or cell membrane of the fungal cell. They can also spread through the cell membrane and interfere with many metabolic methods which demand for synthesis of argostirol, glucan, chitin, proteins, and glucosamine (Brul and Klis, 1999).

The difference of the phenolic content between the extracts of cactus plant returns to the variety of chemical composition of the studied parts, when the recovery of phenolic compounds affects in the solubility of these compounds in the used solvent during extraction, the polarity of the solvent also plays a main role in increasing the solubility of phenolic compounds (Naczk and Shahidi, 2006; Prior and Cao, 1999).

The effectiveness of phenolic compounds relates to kind of microorganisms and the amount of effective antimicrobial compound in the extracts (Harborne and Williams, 2000).

Although the methanolic extract of fruits showed the highest phenolic content, it was the least effective in inhibiting the fungal growth (A.fumigatus, A.flavus) compared to the cladodes and flowers extracts, This is may return to the quality of the phenols in the fruits which not insufficient to give high efficiency, this result agreed with (Rabhi *et al*., 2013).

The flowers of cactus plant contain a variety of phenolic compounds that possess antioxidant and antimicrobial properties such as (gallic acid, quercetin and kaempferol), while the cladodes contain (coumaric acid, syringic acid, and salicylic acid) (Teresita *et al*., 2010; De Leo *et al*., 2010).

The methanolic extract of the studied parts showed high efficacy compared with fluconazole, and this was agreed with several studies that showed the efficacy of cactus plant extracts compared to many antibiotics (amphotericin.B, fluconazole) (Belay *et al*., 2015; Kumaar *et al*., 2013).

5. CONCLUSION

This study showed antifungal properties of cactus plant. so that, we suggest to do further studies to determine and isolate some of phenolic compounds which may play a role in inhibiting the growth of fungi and we also recommend to make clinical studies to investigate of the activity of cactus plant extracts in animal models.

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