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ANTIFUNGAL ACTIVITY OF *CARUM CARVI* L. EXTRACTION AGAINST *CANDIDIA ALBICAN* AND *ASPERGILLUS NIGER*

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Abstract

The study was carried out in central research laboratories, College of Veterinary medicine, and the laboratory of medical plants college of Pharmacy, University of Basrah-Iraq. Used of volatile oil, water and alcoholic (methanol and ethanol) extraction, the fixed free-oil, volatile free oil and dry leaves of *Carum carvi L.* against two types of fungi (*Candida albican* and *Aspergillus niger*). The results showed that the effectiveness of plant extracts against both types of fungi varied by part of plant and concentration. Oil extract and the methanol extraction of the dry leaves inhibition for both types of fungi in all concentrations, alcoholic extract (methanol and ethanol) of volatile free oil was inhibited at concentrations of (100 and 200) mg/ml, but the aqueous extraction of the fruits of volatile free oil was inhibited fungus *Aspergillus niger* only at Concentration of (200 mg/ ml). Water and alcoholic (methanol and ethanol) extraction of fixed freel-oil showed an inhibitory effect for both fungus at concentrations (100 and 200) mg/ml. The water extract and methanol extraction for dry leaves were also inhibitory the fungus growth at concentrations of (100 and 200) mg / ml. The aimed of study to knowledge fungicidal activity of the three concentrations are (50, 100 and 200) mg/ml of the volatile oil and aqueous and alcoholic (methanol and ethanol) of fixed free oil and volatile free oil and leaves dry plant of caraway *Carum carvi L.* against two fungi *Candidia albican and Aspergillus niger.*

Keywords **:** *Carum carvi* L., Antifungal activity, disc-agar diffusion technique,

Introduction

Caraway fruit is an important medicinal plant as its active compounds have the potential to inhibit microbial and fungal use in large medical fields (Seidler *et al*., 2013). Caraway fruit contains a lot of chemical components, including volatile oils as 30 chemicals were diagnosed which make up 97.58% of the total oil, the most important caravon R-carvone 37.98% and Lemonin D-Iimonene by 26.55% and Alpinin pinene-α by 5.21% and Carviol cis-carveol by 5.01% and beta-myrcene by 4.67% (Fang *et al*., 2010).

The essential oils of Caraway fruit are characterized by their high content of oxygen-containing single turbines with 62.17% monosaccharaides 36.08% and triglycerides 0.41% and also contain saturated and unsaturated fatty acids, ketones, aldehydes and esters (Meshkatalsadata and Salahvarzib, 2012) as well as the fruits contain Resins and 20% fixed oil and 20% carbohydrates and Proteins (Hussain *et al*., 2007).

The plant has many therapeutic uses because it contains many medicinal properties. It is used in traditional Chinese medicine and other popular medicines as a "repellent". Dry fruits are used in the treatment of the digestive system such as loss of appetite, as it increases the secretion of fluids in the large intestine, Appetite and indigestion of adults (Thompson and Ernst, 2002). It was found to have a huge potential for antimicrobial resistance (Iacobellis *et al*., 2005; Simic *et al*., 2008) and an antifungal agent (Iacobellis *et al.,* 2005) and hypoglycaemic (Eddouks *et al*., 2004) and anti-cholesterol (Khayyal *et al.,* 2001) and antacid and tumor (Kumar and Singh, 2006) and the pilot oil is used as an "insect repellent (Rui *et al*, 2010).

Materials and Methods

Collection of samples

Collected leaves and fruits of the Caraway plant from the field. Cleaned the samples of dust and dirt, dried the fruits and leaves in the shade inside the laboratory, and under room temperature and after the completion of the drying was grinded by electric mill.

Fungal isolates

In the experiment, two types of fungal isolates, *Candida albican* and *Aspergillus niger*, were obtained from the central research laboratories, College of Veterinary Medicine, University of Basrah-Iraq.

Preparation of extracts

(a) Volatile Oil Extract: The volatile oil was extracted from the dried material, which was ground to the fruit using a Clevenger device connected to a 1-liter vial. It weighed 100 g of dried dry fruits, grinded with an electric mill, then placed in a special flask and added 500 ml volume of distilled in round-bottom flask connected to a slightly modified Clevenger-head. For two and a half hours for each sample of samples until the extraction of the amount of oil pilot from the sample, as formed two layers water and oil, separated these two layers through the separation bath in the collection of oil, water is below and oil to the top because it is lighter than water. After separating the oily layer, each oil sample was placed in sealed, sealed bottles. The bottles were then kept at a temperature of 4 m until use. (Kapás *et al.,* 2011).

(b) Volatile free-oil Extract: The dried material was used for the fruits after the removal of the volatile Oil according to the (Kapás *et al.*, 2011). Materials were extracted from the volatile Oil extract by Water and alcohol extraction (methanol and ethanol) were made according to the method (Parekh and Chanda, 2007).

(c) Fixed free-oil Extract: Take 100 g of grounded seeds and place in a suction flask for the Soxhlet, which is connected to the receiver with a pint size. Use 300 ml of the petroleum spirit (Distillation range 40-60^{o} C) to

separate the oil for 48 hours. Then, evaporate the solvent from the oil using the evaporator Rotary evaporator rotor at a temperature of 60 m to the stage of complete evaporation of the solvent. The fruits were taken from the fixed oil distillation and water and alcohol extracts (methanol and ethanol) were used according to (Parekh and Chanda, 2007) method.

Dry leaf extraction

(a) Water extract of dry leaves

According to method of (Parekh and Chanda, 2007) take about 100 g of crushed dry leaves and placed in a electric mill. Add 375 ml of distilled water for 15 minutes and stir in a hot magnetic magnetic stirrer for 48 hours at 45-50 m and then put the solution in the centrifuge at 3000 cycles. 1-min for 30 minutes. The precipitate was neglected and the filtrate was taken. The process was repeated three times to ensure disposal of the sediments and was nominated using the filter paper Whatman No. 1 and drain the leachate using a water bath at a temperature of 60 m and put the extract in sterile bottles and kept in the refrigerator at 5 m until use.

(b) Ethanol extract for dry leaves

Ethanol extraction process was performed as in the steps of the water extract with the water replaced with ethanol alcohol. The extract was then placed in sterile bottles and stored in the refrigerator at 5° C Until use.

(c) Methanol extract for dry leaves

Extraction process was performed with methanol, 90% methanol, as in the steps of the water extract, with water replaced with methanol. The extract was then placed in sterile bottles and stored in the refrigerator at 5° C until use.

Anti-fungal activity of *Carum carvi* L.

Carum carvi L. extraction was screened in vitro for their fungicidal activity against *Aspergillus niger and Candida albican* using the disc-agar diffusion technique (Soňa *et al.*, 2015).

The disc agar diffusion test was performed using Sabouraud dextrose agar (SDA, Hi Media, Bombay,India). The inoculum was prepared using the fungi from seven days culture on Sabouraud dextrose agar and the suspension was made in a sterile saline solution. The turbidity of the suspension was adjusted with a spectrophotometer at 530 nm to obtain a final concentration to match that of the 0.5 McFarland standard. Briefly, 100 µL of spore suspension (0.5 McF) was spread thoroughly all over the surface onto Sabouraud dextrose agar plates. The plates were dried in an air-dry stiller at 60°C until evaporation of residual water. Whatman no.1 filter paper disks (6mm in diameter) (Oxoid, Cambridge, UK) impregnated with Recommended concentration (50, 100 and 200 mg/ml) of the test samples in DMSO as a solvent. Antibiotic drugs Ecantrazole (7mg) was used as control. Petri plates containing 20 ml of Sabouraud dextrose agar for *Aspergillus flavus* and *Candida albican* strains were cultivated in. A paper disc impregnated with dimethyl sulfoxide (DMSO) was used as negative control. The plates were incubated for 72 at 28°C. The inhibition zone diameters were measured in millimeters using a caliper vernier. All of microorganisms were isolated in central research laboratories, College of Veterinary medicine, University of Basrah-Iraq. (Al-Thaher *et al.,* 2015)

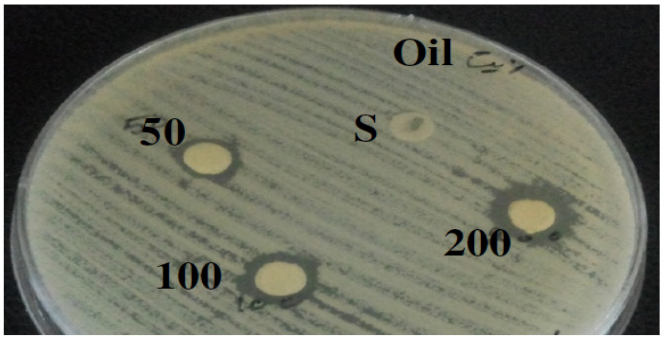
Results and Discussion

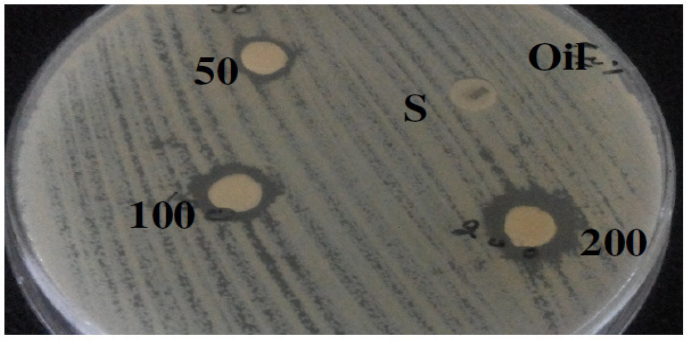
The results showed that the volatile oil extract was significantly effective against the two fungal isolates under study. It was observed that all concentrations of (50, 100 and 200) mg / ml gave inhibitory effect, but highest inhibition at 200mg/ml concentration that indicated in Table 1 and Figure 1, so this was because volatile oils Contained and their compounds with the ability to inhibit both pathogenic fungus and bacteria (Bonyadian and Karim, 2002; Seidler-Łożykowska *et al*., 2013 ; Grigore *et al*., 2012).

This agree with (Lixandru *et al.,* 2010) in terms of containing Caraway oil contains a mixture of active compounds with the inhibitory effect, the most important Alcarvon and limonene, and also agrees (Begum *et al.,* 2008). In their study, the minimum inhibitory concentration of the volatile oil for Caraway at a concentration of (50 - 500) mg /ml for a number of fungi and bacteria indicated that the oil showed efficacy against all species and the inhibitory concentration of oil was (200-400) mg/ml.

Table 1 : The antifungal activity of the volatile oil extract of *Carum carvi* L. Against the *Candidia albican* and *Aspergillus niger*

Fungus	50mg/ml	100mg/ml	200mg/ml	Standard
Candida albican	8mm	10mm	12mm	7mm
Aspergillus niger	15mm	15mm	18mm	7mm





a- *Aspergillus niger* **b- *Candida albican***

Fig. 1 : The inhibitory effect of volatile oil extract of *Carum carvi* L. against fungal growth: *a- Aspergillus niger* b- *Candida albican*

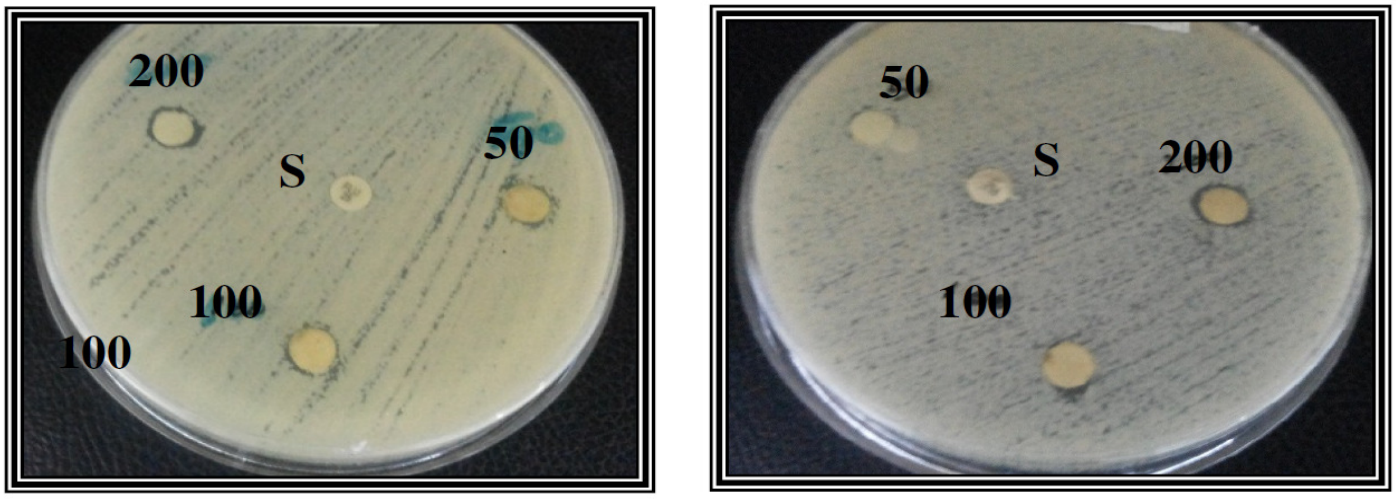
Table and Figure (2) show that the water extraction of the volatile free-oil of Caraway with a concentration of (200mg/l) only inhibited the growth of *Aspergillus niger* and the diameter of the inhibition was (8 mm). The other concentrations did not effect on growth of the same fungus, but *Candidia albican* show resistance for all concentration of water for the fruits of the volatile free-oil.

In the same table and Figure (3,4) the methanolic and ethanolic extracts the fruits of the volatile free-oil showed that the growth of *Aspergillus niger* was inhibited at all concentrations and the growth of *Candida albicans* was inhibited at concentrations of (100 and 200) mg /l. The inhibitory effect of fungi growth was due to the presence of these extracts on proteins (Rich in amino acid cysteine) containing about (45-54) amino acids that have antifungal activity (Broekaert *et al*., 1995).

And that the effects of optimal medicinal plants may not be due to one active substance but rather to the interplay of the effect of several compounds with each other (Kianbakht and Jahaniani, 2003)

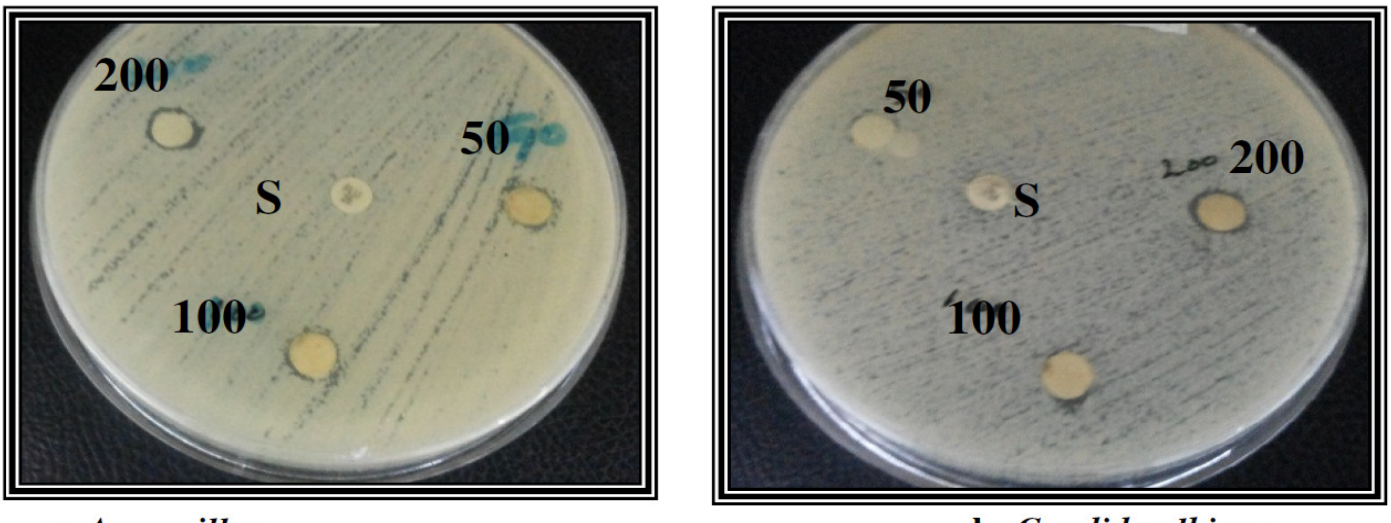
Table 2 : The antifungal activity of water and alcohol extraction (methanol and ethanol) extract of the volatile-free oil for *Carum carvi* L. against *Candidia albican* and *Aspergillus niger.*

Microorganism	Concentrations	Watery	Methanol	Ethanol
Aspergillus niger	50 µg/ ml	-	7mm	-
	100 µg/ ml	-	7mm	7mm
	200 µg/ ml	8mm	8mm	8mm
	Standard	-	-	7mm
Candida albicans	50µg/ml	-	-	-
	100µg /ml	-	7mm	7mm
	200µg/ ml	-	8mm	9mm
	Standard	-	-	7mm



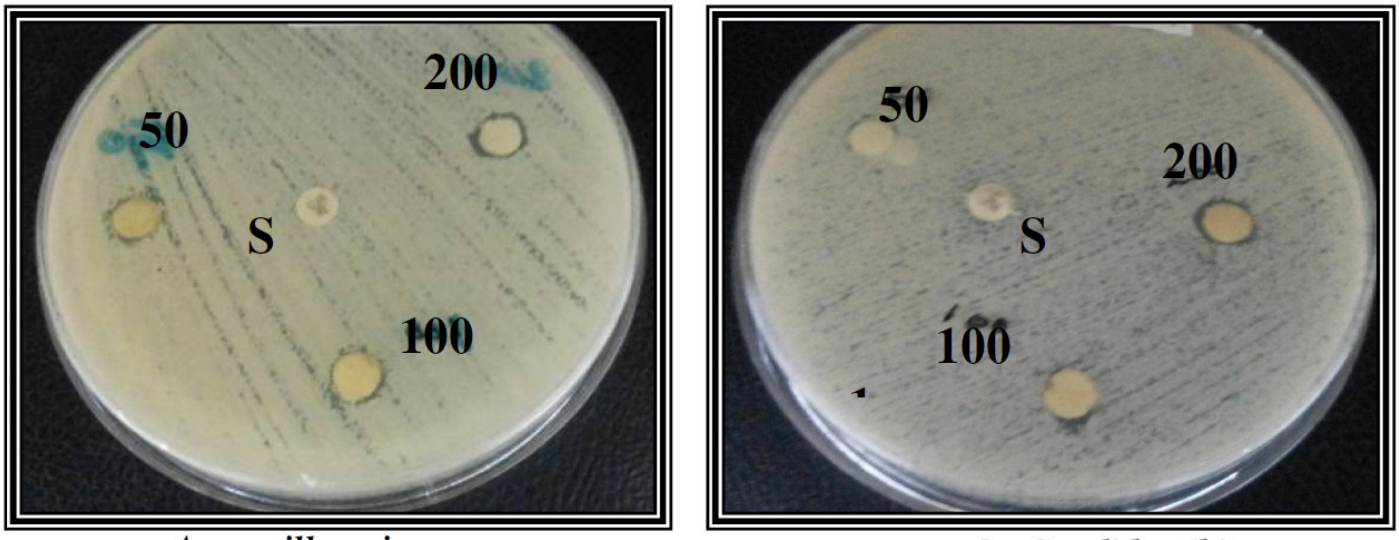
a-*Aspergillus niger* **b- *Candida albican***

Fig. 2 : The inhibitory effect of water extraction of volatile free- oil for *Carum carvi* L. against fungal growth*:* a- *Aspergillus niger* b- *Candida albican*



a-*Aspergillus* **b- *Candida albican***

Fig. 3 : The antifungal activity of the ethanolic extraction of volatile free- oil for *Carum carvi* L. against a- *Aspergillus niger* b- *Candida albican*



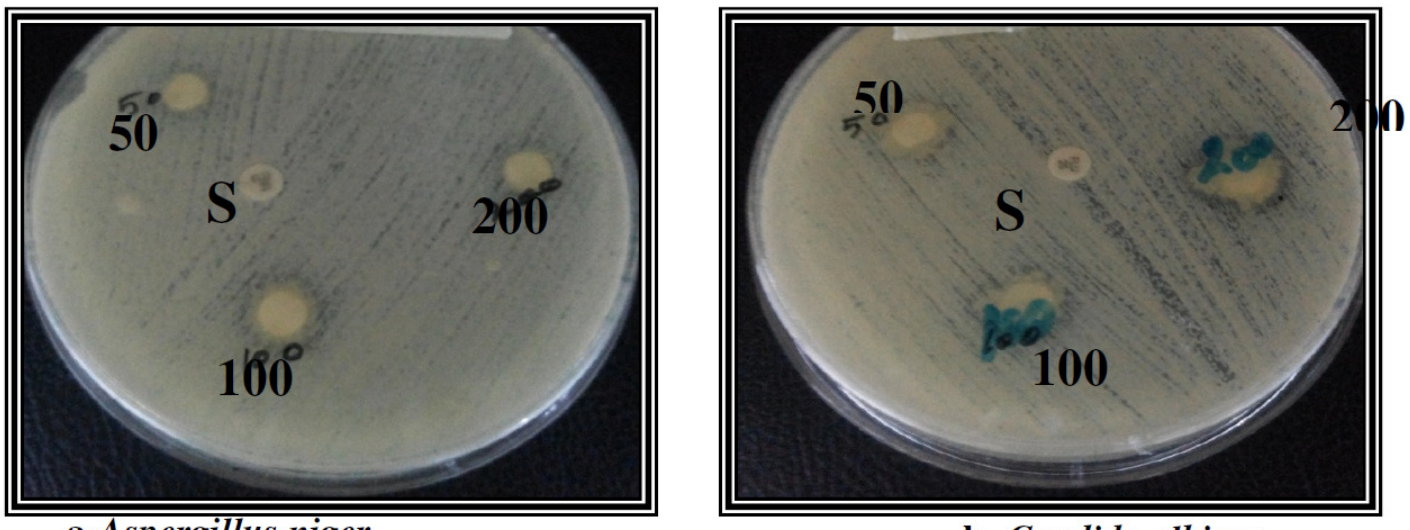
a-*Aspergillus niger* b- *Candida albican*

Fig. 4 : The antifungal activity of the methanolic extraction of volatile free-oil for *Carum carvi* L. against a- *Aspergillus niger* b- *Candida albican*

In water and alcohol extracts (methanol and ethanol) of the fixed free-oil of the Caraway show in table (3) and Figure (5, 6 and 7) that indicated the inhibited growth of the two fungus at concentrations (100 and 200) mg /l for all extraction, while no inhibitory growth at concentration of (50 mg /l) due to presence of alkaloids, especially aromatic alkaloids, that having ability to inhibit microorganisms. This is in line with previous studies showing that this inhibition is due to the presence of alkaloids as well as the effect of the active groups of other such as glucosides, flavonoids, resins, phenolic compounds and tannins make alcohol and water extraction more effective for experimental microorganism (Draughon, 2004). The difference in the effect of secondary metabolites on the effect of inhibition is due to the different types and quantities of these active substances (Nweze and Njoku, 2004). This is in line with (Saumendu *et al.,* 2012) that work on methanol extraction of Caraway fruits of fixed free-oil.

Table 3 : The antifungal activity of water and alcohol extraction (methanol and ethanol) of the hard oil free-oil for *Carum carvi* L. against *Candidia albican* and *Aspergillus niger.*

Microorganism	Concentrations	Watery	Methanol	Ethanol
Aspergillus niger	50 µg/ ml	-	-	-
	100 µg/ ml	8mm	8mm	7mm
	200 µg/ ml	8 mm	9mm	8mm
	Standard	-	-	-
Candida albicans	50µg/ml	-	-	-
	100µg/ml	8mm	9mm	8mm
	200µg/ ml	9mm	8mm	8mm
	Standard	-	-	-



a-*Aspergillus niger* b- *Candida albican*

Fig. 5 : The antifungal activity of Water extraction of hard free-oil for *Carum carvi* L. against a- *Aspergillus niger* b- *Candida albican*

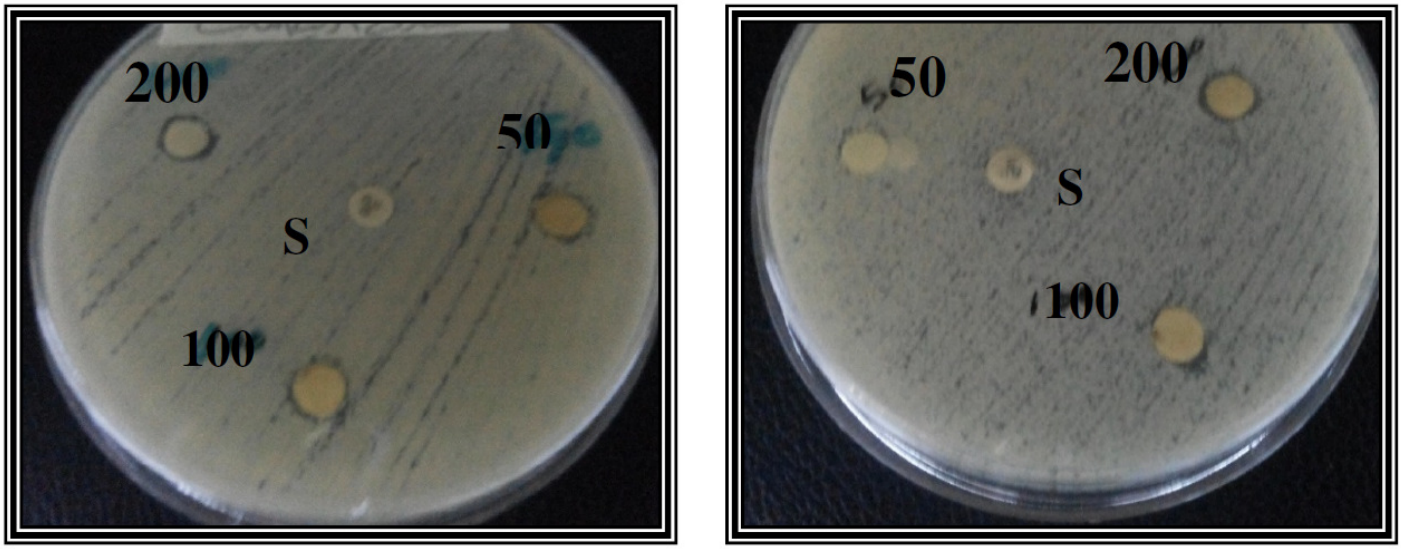


Fig. 6 : The antifungal activity of methanolic extraction of hard free-oil for *Carum carvi* L. against a- *Aspergillus niger* b- *Candida albican*

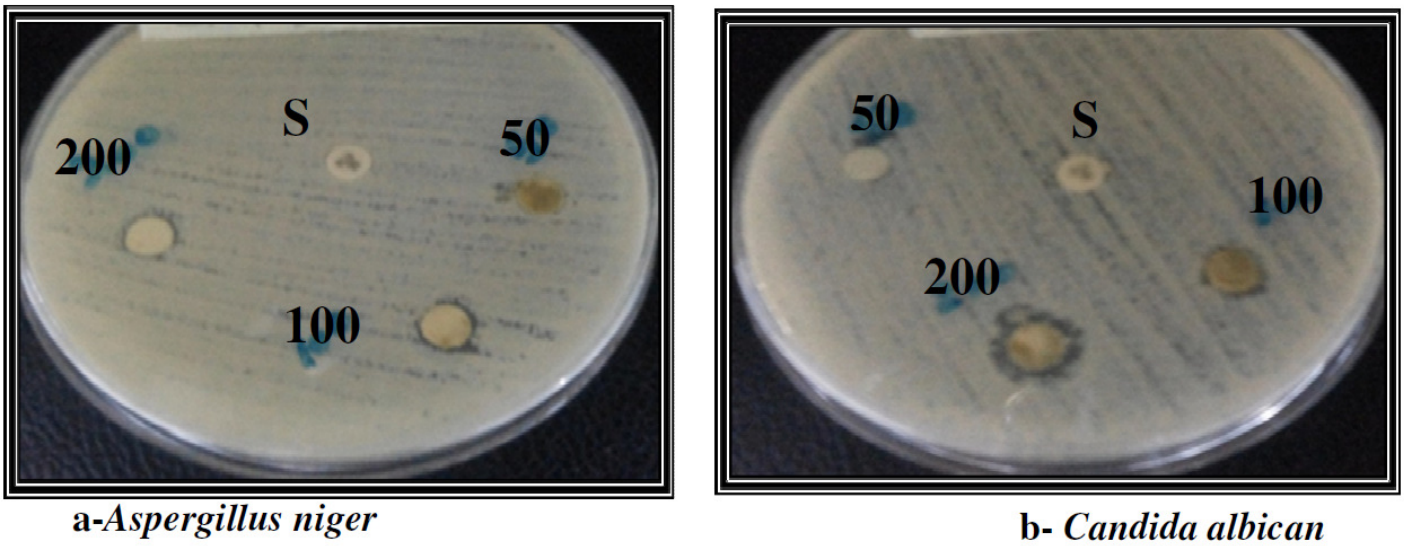
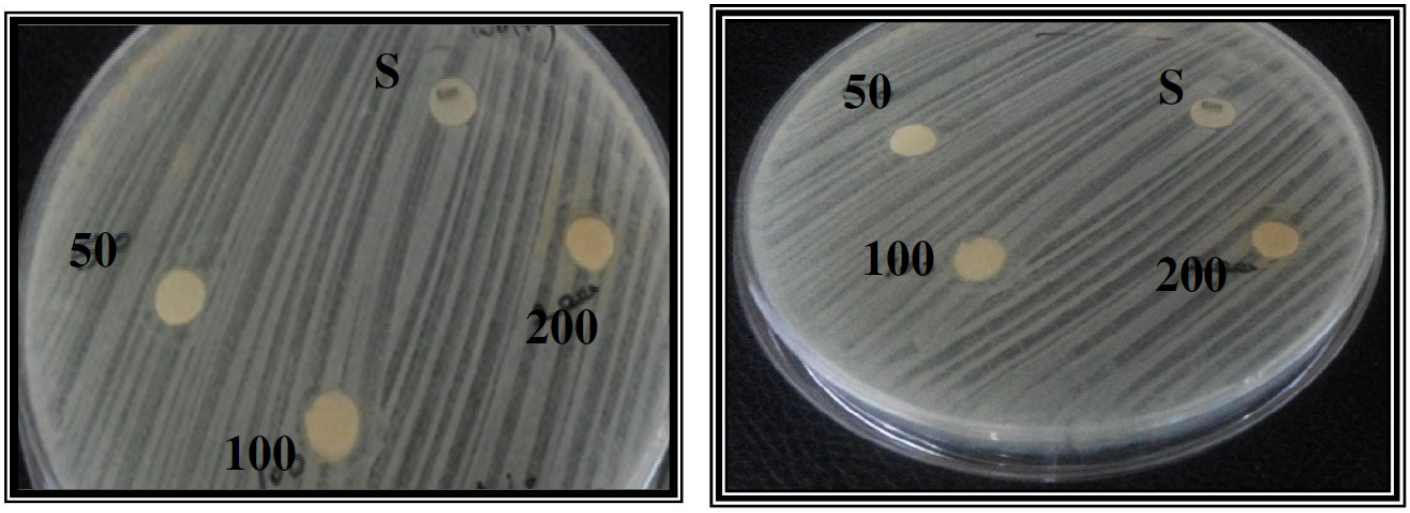


Fig. 7 : The antifungal activity of ethanolic extraction of hard free-oil for *Carum carvi* L. against a- *Aspergillus niger* b- *Candida albican*

Table (4) and Figure (8, 9 and 10) showed that the water and methanol extraction for the dry leaves of the Caraway plant inhibited the growth of the two fungus under study at concentrations of (100 and 200) mg/ l while there was no inhibiting at concentration of (50mg/l) while the ethanol extraction showed fungal activity at all concentrations. The reason for the leaf extracts was more effeteness against two fungal (***Aspergillus niger and Candida albican***) that contain in its different parts all active compounds with the inhibitory effect to fungus. The appropriate concentration of the extract is closely related to the amount of active substances with the inhibitory effect of fungal activity and this is in line with what was found (Khalil *et al*., 2005). These results support what we obtained from the fact that the active ingredients in medicinal plants or in oils are affected by several genetic factors, Or environmental conditions that cause variation in impacts against these microorganisms (Tepe *et al*., 2004).

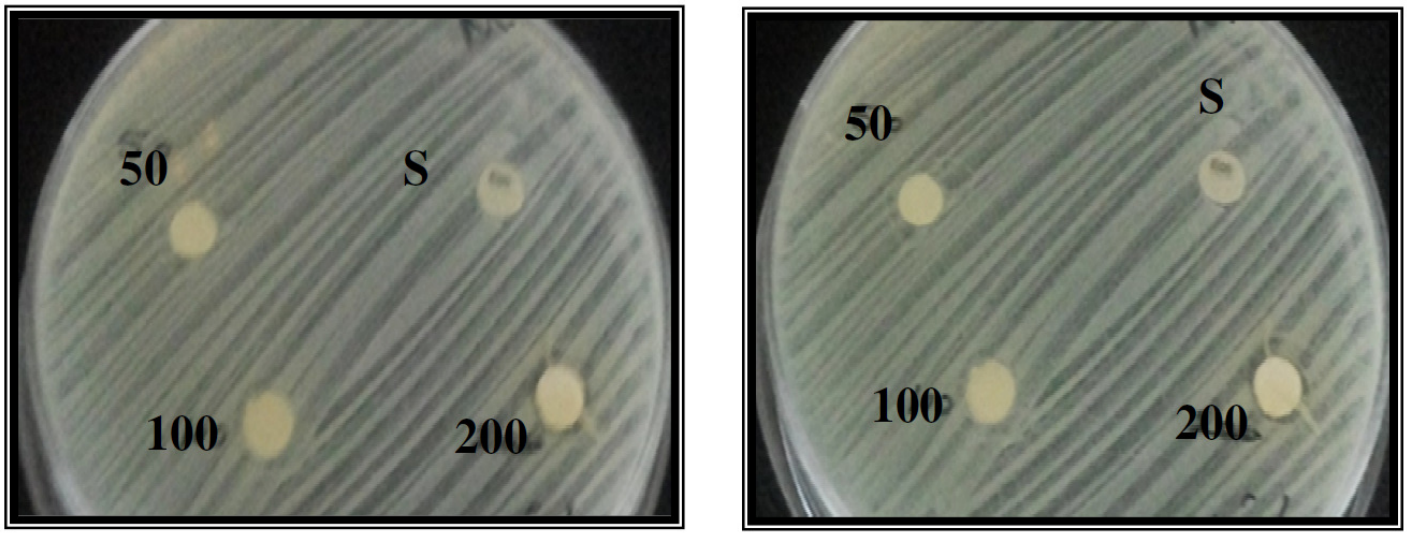
Table 4 : The antifungal activity of water and alcohol extraction (methanol and ethanol) of the dry leaves of the for *Carum carvi* L. against *Candidia albican* and *Aspergillus niger.*

Microorganism	Concentrations	Watery	Methanol	Ethanol
Aspergillus niger	50 µg/ ml	-	-	7mm
	100 µg/ ml	7mm	7Mm	7mm
	200 µg/ ml	7mm	8mm	9mm
	Standard	-	-	-
Candida albicans	50µg/ml	-	-	7mm
	100µg/ml	7mm	7mm	7mm
	200µg/ ml	7mm	8mm	9mm
	Standard	-	-	-



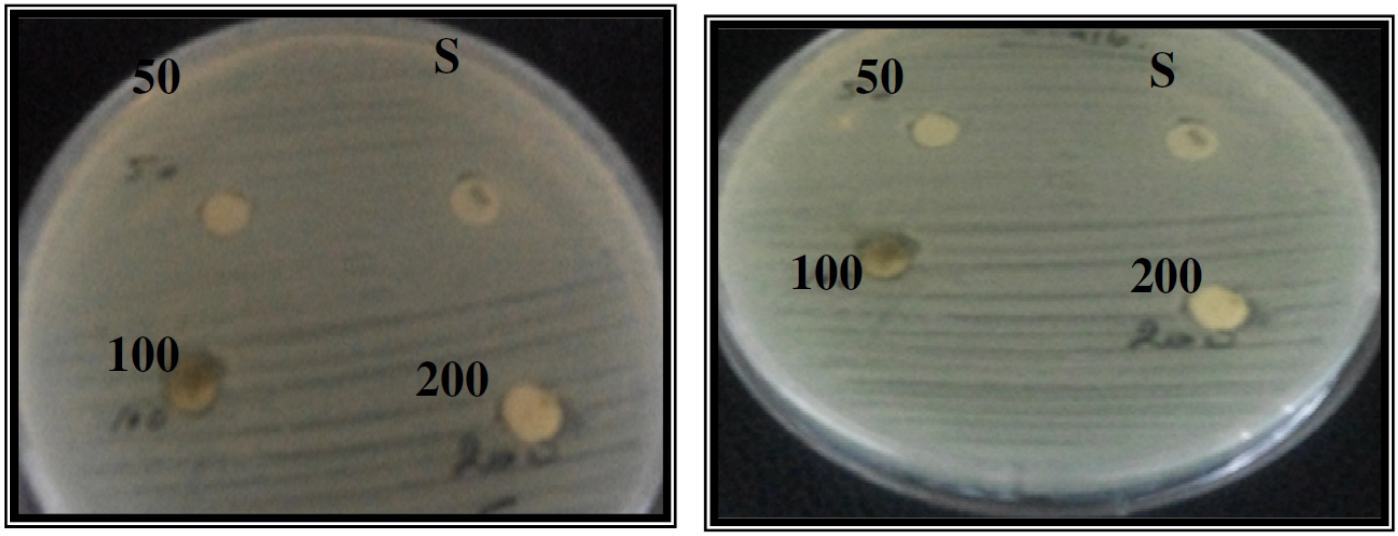
a-*Aspergillus niger* b- *Candida albican*

Fig. 8 : The antifungal activity water extract of the dry leaves for *Carum carvi* L. against a- *Aspergillus niger* b- *Candida albican*



a-*Aspergillus niger* b- *Candida albican*

Fig. 9 : The antifungal activity methanol extraction of the dry leaves for *Carum carvi* L. against a- *Aspergillus niger* b- *Candida albican*



a-*Aspergillus niger* b- *Candida albican*

Fig. 10 : The antifungal activity ethanol extraction of the dry leaves for *Carum carvi* L. against a- *Aspergillus niger* b- *Candida albican*

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