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# Study the Effecte of *Oreganum Vulgare L. Extratct Against Some Pathogenic Fungi.*

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## ABSTRACT

The advent of fungi capable of infecting humans is becoming a major public health problem. Fungi with clinical importance can be classified as either primary or opportunistic fungal infections kill about 1,350,000 people per year, and more than 300 million people are infected the present study was conducted to investigate the effect of phytochemical compounds extract from ( *Oreganum vulgareL*. ) by using solvents which is Ethanol on fungal isolated from collected from various and hospitals and the medical province of Babil 2020 in Iraq. Antifungal activity was achieved in vitro by using the food poisoning method against Fungi species by preparing three concentrations for each solvent (5,10 and15 ) mg/ml and compared with positive control represented by anti-fungal ketoconazole 5mg/ml and negative control represented by 10% Ethanol. The aim of this investigation was to control fungi species isolated from various clinical cases by using oregano. The data collected from the study revealed that the Ethanolic extracts of oregano showed a significant reduction at $p < 0.05$ in the growth fungi species especially at 15 mg/ml compared with negative control. We conclude that the alcoholic extract of the oreganum vulgare plant showed high efficiency in inhibiting the growth of the fungi under study.

**Keywords:** Anti-fungal activity, oregano extract, pathogenic fungi.

## Introduction

Oregano the object of our investigations is an aromatic perennial herb that belongs to the Lamiaceae family. Approximately 60 species are known as oregano in the world [1]. It grows in different areas at a wide range of ecological conditions in Armenia: populations are located mostly in the Southern regions (Syunik and Vayots Dzor) [2].O. vulgare has been gathered for its essential oils since ancient times to flavor traditional dishes and to treat a variety of illnesses such as convulsive coughs, colds, skin diseases, and digestive disorders [3].[4].[5].O. Vulgare is now one of the most traded and consumed spice plants due to its importance as a source of oregano[6].

Oregano is a valuable source of natural secondary metabolites that are high in essential oil (EO) and have a wide range of applications in medicine, pharmaceutics, food, and cosmetics. Oregano essential oil is a complex mixture of various compounds, with terpenes as the main constituent (mono- and sesquiterpenes). The presence of thymol and carvacrol as the key components in oregano oil gives it its flavor [7]. However, EO composition varies, and the oregano chemotype is determined by the proportion of compounds within the same genus identified by many chemotypes, each with its own distinct flavor [8].[9]. In 502 plants from 51

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populations from 17 countries, three chemotypes of oregano were discovered, which were categorized based on the content ratios of cymyl, sabinyl, and linalool/linalyl acetate. There are four major chemotypes of oregano, according to other literature sources, based on the proportion of phenolic substances: thymol/carvacrol; carvacrol; thymol; and the chemotype with low phenol and high alcohol content [10].

## Materials and Methods

Plant material:(Oregano) had been purchased from the local market, identified based on the taxonomic features by a botanist. (Table: 1). Materials of these plants were cleaned, dried, and kept according to Von Rudloff, (1975) [11].

**Table 1: Scientific, common, name, Family, and active parts of Medicinal plant**

| Scientific name | Common name | Family | The part use |
|---|---|---|---|
| Oreganum valgare | oregano | Lamiaceae | Leaves |

### Plant materials extraction:

Phytochemicals compounds were extracted by using digestion methods. By using 50 gm of plant materials powder instant in 250 ml of Ethanol solvents separately for oreganum then shake it well for 1 hour, after that leave it for 72 hours in water path at 45C˚ to complete the process of extraction [12], and then dried. A stock solution of 100 mg/ml was prepared in 10% Ethanol then sterilized by a Millipore filter (0.22μm) and stored at (-20C˚) until use [13].

### Isolation and diagnosis of fungi species:

The fungal species used in the present study were *Trichophyton mentagrophytes, penicillium chrychrysogenum, candida Albicans, Asprgillus.flavus* were collected from patients with different clinical cases and different age and gender (wounds, skin). A sample collected by using swab media from the infected area and cultured it in the culture media[14]. Fungal isolated were then diagnose based on the taxonomic key and diagnosed depend on the shape, size, color and growth, SDA and PDA culture planning by the loop method incubated for 5-7 hr. at 28 C [15].

### Antifungal activity assay of extract:

SDA medium was prepared and autoclaved after that a known volume (2ml) of each plant extracts is placed in the center of the Petri dishes and complete the volume to 20ml with SDA medium to obtain the required final concentrations ( 5, and 10,15mg/ml) of the medicinal plants after complete solidification of the medium, 5 mm disc of seven days old culture of the test fungus were placed aseptically in the center of the Petri plates and incubated at 28 ± 2C˚ for seven days, and 24-48h of Yeast simultaneously 0.02ml of the antibiotic solution was added to each assay plate to check the bacterial contamination as suggested by [15]. Antifungal ketoconazole2mg/ml [16] was used as positive control and Ethanol as negative control observations were recorded on the seventh day. The colony diameter was recorded in terms of millimeters. For each treatment,

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three replicates were maintained. The fungi toxicity of extracts was calculated in terms of percent inhibition of mycelia growth by using the formula [17].

**Percent Inhibition = (dc- dt/ dc)* 100**

Where:

**dc = Average increase in mycelia growth in control.**

**dt = Average increase in mycelia growth in treatment**

**Statistical analysis**

All statistical calculations were performed by using SPSS software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp. USA) and Microsoft Excel (2010, Microsoft Corp. USA). All the results were expressed as mean. A p. value < 0.05 was considered statistically significant. Analysis of variance was employed to evaluate the presence of significant differences. LSD was carried out to find the significant difference using [18].

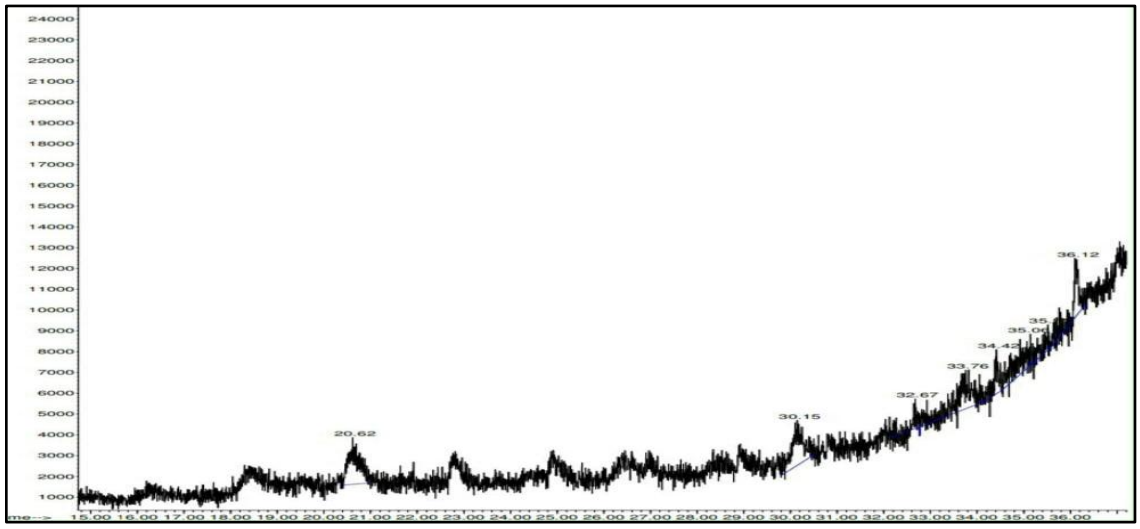
**Results:**

**Fungal isolated**

Fungal isolated from cutaneous infections for spp of fungi were isolated which is *Trichophyton mentagrophytes, Penicillium chrysogenum, candida Albicans, Asprgillus.flavus*

**Gas Chromatography – Mass Spectrum Analysis**

The results of GC-MS analysis of the Bioactives of plant extract that have pharmacological actions are presented in ( figure 1). Bioactives are the chemical compounds often referred to it as secondary metabolites, eight bioactive compounds (Table 3) were produced from Oregnum Vulgare were identified in the Ethanolic extract.




**Figure(1): GC-MS analysis of the Bioactives of oregano extract.**

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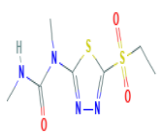
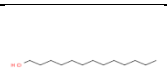
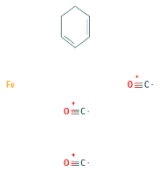
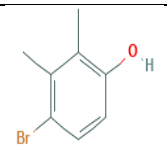
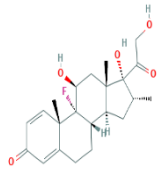
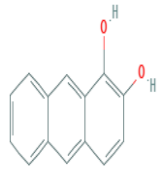
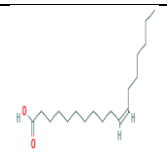
**Table3:The major phytochemical compounds of oregano extract detected by GC-MS analysis that have pharmacological action.**

| NO. | Compounds | RT (min) | Area % | Molecular Weigh (gm/ml) | Formula | Nature of the compound | Pharmacological actions | Chemical Structure |
|---|---|---|---|---|---|---|---|---|
| **1** | Ethidimuron | 20.62 | 18.38 | 264 | $C_7H_{12}N_{14}O_3S_2$ | Alkaloids | Herbicids Antiviral Antiflmmatray |  |
| **2** | 10-Octadecenoic acid methyl | 30.15 | 7.36 | 282 | $C_{18}H_{34}O_2$ | Unsaturated-fatty acid | Antioxdiant Anti cancer |  |
| **3** | Iron, Tricarbonyl | 32.67 | 4.37 | 220 | $C_9H_8FeO_3$ | Phenols | Anti fungal Anti bacteral |  |
| **4** | Phenol.bromodimethyl | 33.76 | 20.51 | 201.06 | $C_8H_9BrO$ | Phenols | Anti bacteral Anti fungal Anti flammtray |  |
| **5** | Pregna | 34.42 | 7.36 | 374 | C22H30O5 | Steroids | / |  |
| **6** | Anthracenediol | 35.6 | 12.99 | 210.23 | $C_{14}H_{10}O_2$ | Flavonoids | Anti fungal Anti bacteral |  |
| **7** | Cis-Vaccenic acid | 35.51 | 4.37 | 282 | $C_{18}H_{34}O_2$ | Fatty acid | Antivirials, Antipsoria, Antibacteral, |  |
| **8** | Trifluoroacetic acid | 36.12 | 13.30 | 114.02 | $C_2HF_3O_2$ | Organic Citrus | Antiflammtary Antivrial |  |

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The results of antifungal activity of ethanolic extract extracted from against Fungi species isolated in the study are presented in( table 2) activity of (*oreganum vulagre L*) was screened by food poisoning methods.

The result of ethanolic extracts of oregano showed significant reduction at P≤ 0.05 in the growth of *fungi spp* Antifungal activity was applied at (5,10,and15) mg/ml. Mycelial inhibition ranging *A.flavus* (40.7% in 5mg/ml, 69.6% mg/ml, 96.2% in 15) (figure 1), compared with negative control (figure 2), and positive control antifungal ketoconazole 2mg/ml (figure 3), the rate of inhibition was in the *P.chrusogenum* (67.4% in 5 mg/ml ,87.2 in 10 mg/ml, 100% in 15 mg/ml ) (figure 4), and compared with negative control (figure 5) positive control antifungal ketoconazole 2mg/ml (figure 6), the rate of inhibition was in the *Candida albicans* (68.14%, in 5mg/ml and 74.81%, in 10mg/ml and 100%,in 15 mg/ml (figure 7), compared with negative control (figure 8), and positive control antifungal ketoconazole 2mg/ml (figure 9), the rate of inhibition was in the *T.mentagrophytes* (58.7%, in 5mg/ml ,81.7% in 10mg/ml, 100% in 15mg/ml)(figur10) compared with negative control (figur11), and positive antifungal ketoconazole 2mg/ml control (figure 12), were inhibition percentage was (0.00% and 100%) respectively.

**Table 2: Antifungal activity of phytochemical compounds alcoholic extracted from (oreganum vulagreL) against fungi isolated**

| Concentrations Mg/ml | *Aspergillu flavus* | *penicillium chrychrysogenum* | *C.albicans* | *Trichophyton mentagrophytes* |
|---|---|---|---|---|
| Con(-) | 0±0.00 | 0±0.00 | 0±0.00 | 0±0.00 |
| 5mg/ml | 40.7±0.64 | 67.4±0.71 | 68.14±0.64 | 58.7±0.06 |
| 10mg/ml | 69.6±0.64 | 87.2±0.44 | 74.81±0.64 | 81.07±0.06 |
| 15mg/ml | 96.2±3.56 | 100±0.00 | 100±0.00 | 100±0.00 |
| keto +2mg/ml | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 |

***The above results represent and average of three replications / *Conl(-)= negative control/ Keto (+)= Ketoconazol***

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**Fig1:Antifungal activity of Ethanolic extract of (OE.L)15mg/ml against *A.flavus***

**Fig.2:-growth A.flavas in negative control(10% Ethanol)**

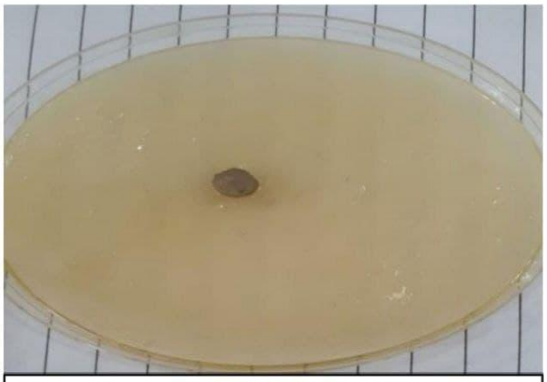

***Fig3:growth A.flavas in positive control***

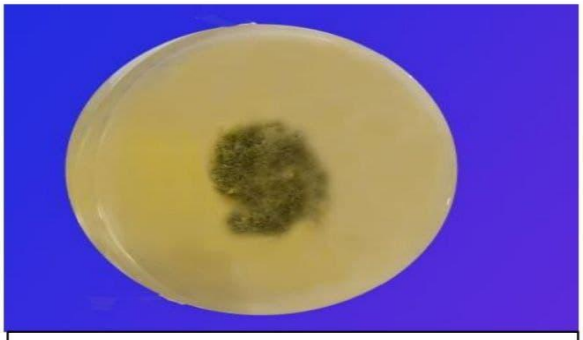

**Fig4:Antifungal activity of Ethanolic extract of (OE.L)15mg/mol against *penicillium chrysogenum***

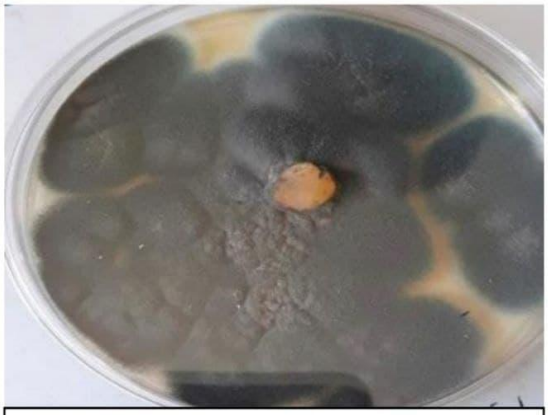

**Fig.5:-growth of *penicillium chrysogenum* in negative control (10% Ethanol)**

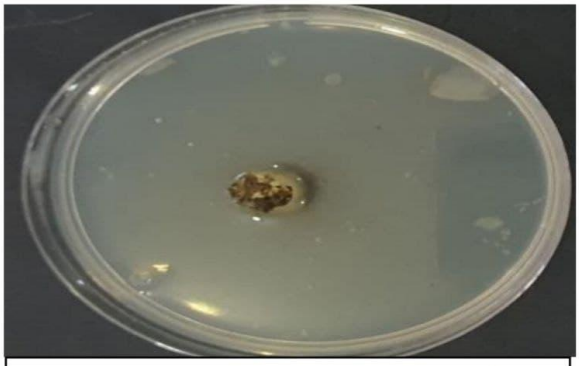

**Fig6:growth of positive *penicillium chrysogenum* in positive control (ketoconazole 2mg/ml ) treatment**

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**Fig7:Antifungal activity of Ethanolic extract of (OE.L15mg/ml) against *candida albicans***

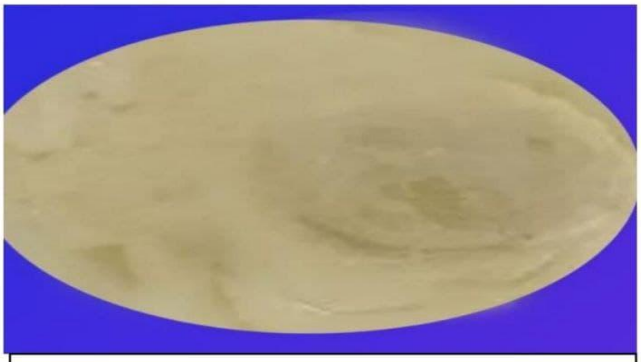

**Fig.8:-growth of *candida albicans* in negative control (10% Ethanol**

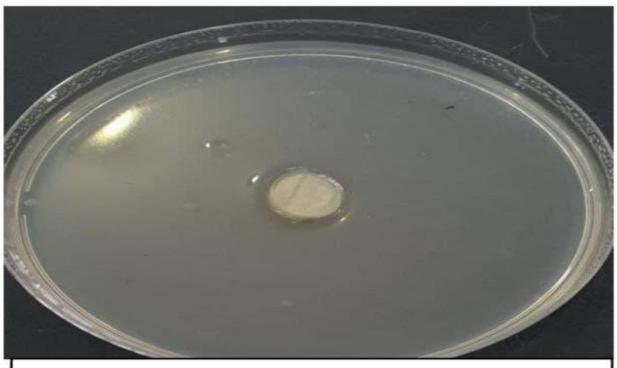

**Fig9:growth of *candida albicans* in positive control (ketoconazole 2mg/ml ) treatment**

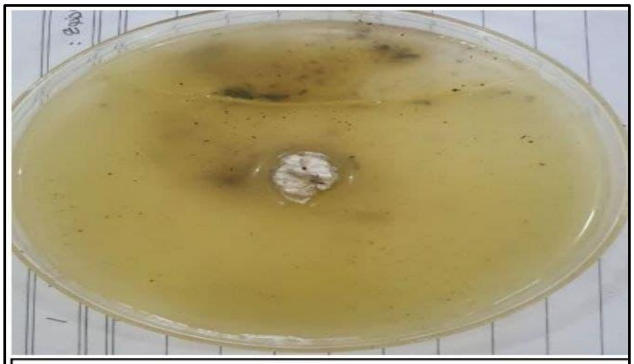

**Fig 10 : Antifungal activity of ethanolic extract of oregano15mg/ml against *Trichophyton mentagrophytes***

**Fig 11: Growth of *Trichophyton mentagrophytes* in negative control (10% ethanol)**

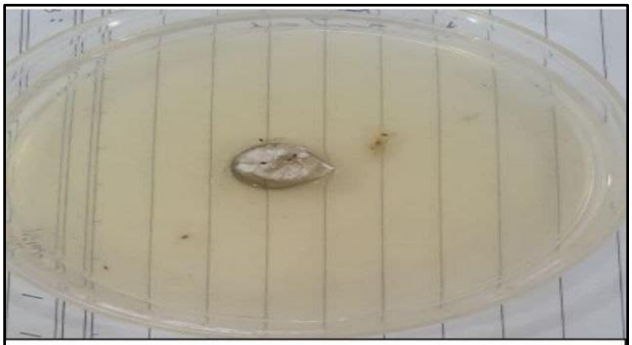

**Fig12 :growth of *Trichophyton mentagrophytes* in positive control (ketoconazole 2mg/ml ) treatment**

**Discussion:**

The results showed that the inhibitory effect of plant extracts the tested fungi depended on the type of extract (alcoholic) and its concentration in addition to the type of fungal isolate, as the

alcoholic extract had a high inhibitory activity and it was evident that the rates of fungal colony diameters increased with the increase in the concentration of the extract used at the time in which the inhibition percentages were directly proportional to the increase in the concentration of the extract. This present study is consistent with a numerous studies, Akgula and Kivanc [19] found that, among ten tested spices, only oregano exerted antifungal activity against nine tested fungi. A study by Paster et al. [20] demonstrated the antifungal activity of oregano at concentrations of 2.0 and 2.50 μL/L on mycelium and spores of *A. niger, A. flavus*. Baratta et al. [21], Bouchra et al. [22] and Vuida-Martos et al. [23] implied that essential oil of oregano possesses stronger antifungal activity against *A. niger and A. flavus* in comparison to those of rosemary, sage, thyme and clove. A study by [24] revealed that the oregano extract has the ability to reduce mould growth at all applied concentrations. Stronger inhibitory effect on the growth of *Penicillium species*, contrary to *Fusarium*, was determined. A similar another study conducted by [25] which found that the extracts of *Origanum vulgare* possess compounds with antimicrobial and antifungal properties. Yotova and Ts, 2015 [26], who demonstrated that oregano represents an economic source of natural mixtures of antifungal compounds that can be as effective as modern medicine to combat pathogenic microorganisms and safe alternative to treat infectious diseases. Also, a study by [8] which found that the existence of antifungal activity of solutions of oregano towards various pathogenic eukaryotic microorganisms. According to the studies conducted by [27] and [28] which revealed that flavonoids inhibit fungal spore germination and have been proposed to control fungal pathogens. A study by [29], who found that some flavonoids have been isolated from mango (Mangifera indica) leaves, and they inhibit the growth of *Alternaria alternata, Aspergillus fumigatus, Aspergillus niger, Macrophomina phaseolina, and Penicillium citrii.*

**Conclusions:**

1- The alcoholic extract of the oreganum vulgare plant showed high efficiency in inhibiting the growth of the fungi under study.
2- It was found through the detection with the GC-MS technology that Oreganum vulgare contains a range of active compounds Important as phenols, flavonoids, steroids, and fatty acids.

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