The Effect of Aqueous and Alcoholic Extracts of Galls of *Quercus infectoria* on the Growth of Some Pathogenic Fungi

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**ABSTRACT**

The present study was aimed to estimate the antifungal activity of galls of *Quercus infectoria* on the growth of eight species from six different genera of pathogenic fungi, which isolated from different sources, includes: Opportunistic (*Aspergillus flavus, A. fumigates, A. ochraceus, Cladosporium cladosporioides, Penicillium citrinum* and *Stachybotry schartarum*) and dermatophytes: *Microsporum gypseum* and *Trichophyton rubrum*. The Gall extracts were prepared by aqueous and ethanol extraction and two concentrations of each extract were used (2.5 and 5) mg/ml and tested for their activity as antifungal. The results of pour plate method showed that, ethanol extract at both concentrations affected more than aqueous extract on the growth of mycelia, that decreased the growth of mycelia of the pathogenic fungi when compared with control, while the antifungal activity of *Quercus* gall by filter paper disc diffusion method on the radial growth of pathogenic fungi showed that the aqueous extracts of *Quercus* gall have no any effect on the growth inhibition of all studied fungi, while the ethanol extracts of *Quercus* gall have low effect on the studied fungi.

**Keywords:** Pathogenic fungi, Alcoholic extracts, *Quercus infectoria*, dermatophytes

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**1. INTRODUCTION**

The kingdom fungi consist of a variety of species that are associated with a wide spectrum of diseases in animals and humans (Kavanagh, 2007). Pathogenic fungi cause a spectrum of infections from annoying to life-threatening with portals of entry that include skin contact, inhalation, and translocation across physical barriers as a result of host defects or accidental or iatrogenic abridgment of epithelial integrity (Calderone and Cihlar, 2002). The most common diseases of human that caused by fungi are the opportunistic fungal infections that take place in patients with immunity disorder (Kavanagh, 2007). *Aspergillus* sp. is an example of an opportunistic mold; it is responsible for a variety of infections referred to as aspergillosis (Dismukes et al., 2003). The genus *Penicillium* is among the most common contaminant fungi in immunocompromised patients (Zanatta et al., 2006). *Cladosporium* spp., which have a worldwide distribution and are among the most common air-borne fungi, they are the cause of opportunistic mycoses, some species are pathogenic and toxigenic to humans. They cause cerebral and cutaneous phaeohyphomycoses. (Tasic and Tasic, 2007). *Stachybotrys* together with other molds such as *Penicillium, Aspergillus, Alternaria*, and *Cladosporium*, may play a role in the development of sick-building syndrome.
Humans may develop a disease that toxin-related by ingestion of food products contaminated with the *Stachybotrys chartarum* or their toxins, exposure to mycotoxins in building and/or inhalation of conidia (Fung et al., 1998). Dermatophytosis is one of the major public health problems that caused by dermatophytes, it includes three kinds of molds such as *Microsporum*, *Trichophyton* and *Epidermophyton*, which commonly causes humans and animals skin diseases. Dermatophytosis is an infectious disease of hair, skin and nails, which attack the keratinized tissue (Ameen, 2010 and Seebacher et al., 2008).

To treat disease all over the world, there are various medicinal herbs have been used for years in daily life. They have a wide range of usage in folk medicine. The plant extracts and essential oils of many plants have been shown to exert biological activity, which leads to traditional medicine researchers focused on the characterization of antimicrobial activity of these plants (Essawi and Srour, 2000 and Iwu et al., 1999).

Galls of *Q. infectoria* have been documented in pharmacology to possess astringent, antifungal (Digraki et al., 1993) and anti-inflammatory (Kaur et al., 2004) activities. The components of *Quercus infectoria* galls comprise a large amount of syringic acid, tannins, gallic acid, ellagic acid, methylolenatebeta sitosterol, amentoflavone hexamethyl ether, isocryptomerin, methyl betulate and hexagalloyl glucose (Lodhi et al., 2012).

The objectives of this research are to inquire the activity of gall as herbal medicine and to evaluate the capability of aqueous and ethanol gall extract have by two concentration and two methods to inhibit the growth of some pathogenic fungi such as opportunistic: *A. flavus*, *A. fumigates*, *A. ochraceous*, *C. cladosporioides* *P. citrinum*, and *S. chartarum* and dermatophytes: *M. gypseum* and *T. rubrum*.

2. Materials and Methods
2.1. Collection of Fungi
Different genera and species of fungi were isolated from different samples, such as soil, fruit, vegetables and human skin, which cultures on Potatoes dextrose agar (PDA) with chloramphenicol for opportunistic and Saboraud dextrose agar (SDA) with (chloramphenicol and cycloheximide) for dermatophytes, the grown fungi were mounted on a slide, stained with lactophenol-cotton blue to detect fungal structures, covered with a cover slip, examined under microscope and identified on the basis of their colony morphology and spore characteristics, then preserved in refrigerator in slant tubes until used for studying. Eight species from six different genera of fungi were chosen for this study. The chosen molds were transferred from the slant to Petri dishes for activation, by sub-culturing on PDA and SDA (Rajankar et al., 2007). The spore suspension that used to test the plant aqueous and ethanol extracts were prepared by scraping the spore using sterilized glass rod and placed in a small-sterilized vial containing 10 ml of SDW (William et al., 1976).

2.2. Gall samples collection and preparation
The galls of *Quercus infectoria* that used in this study were obtained from various locations in Erbil city. They cleaned then placed at room temperature, preserved in plastic sacs until used for extraction. After that they were crushed (by using pestle and mortar) to small pieces and powderied in an electric grinder, finally, these powders were stored in plastic containers until use (Basri and Fan 2005).

2.3. Plant extracts preparation
2.3.1. Preparation of aqueous extracts
Forty gram of Gall was weighted, and 160 ml of sterile distilled water (SDW) were added to it then mixed well in a shaker (shaker incubator-4045/gallenkamp-9B/ England) for one hour and kept at 4°C for 24 hours, filtered through 4-5 gauze and supernatant were placed in Petri dish to dried out then the powder collected and preserved in vials in refrigerator (Rashan et al., 1992).
2.3.2. Preparation of alcoholic extracts

Twenty gram of gall samples were weighted, then 200 ml of ethanol (95%) were added to mixed well in shaker for one hour and kept at 4°C for 24 hours, filtered through 4-5 gauze and supernatant were placed in petri dish to dried out at room temperature, then the powder collected and preserved in vials in refrigerator (Grand et al., 1988).

2.4. Aqueous and ethanol plant extracts sterilization and dilution preparation

The stock solution of plant extract was prepared by adding one gram of aqueous plant extracts to 5 ml of SDW, and adding one gram of ethanol plant extract to 5 ml of Dimethyl sulfoxide (DMSO) (Riedel-DeHaen AG-Germany), then this stock solution was sterilized by using (Millipore filters 0.2 µm). The MIC was determined using the two-fold serial dilution technique of concentrations, which were 5mg/ml and 2.5 mg/ml prepared from the stock solution (Rios et al., 1987).

2.5. Determination of antimicrobial activity

2.5.1. Pour plate method:

The prepared concentration of 2.5 and 5 mg/ml were added to 200ml for each of PDA and SDA and poured into sterilized petri dishes then inoculated by one drop of spore suspension of Opportunistic (Aspergillus flavus, A. fumigates, A. ochraceous, Cladosporium cladosporioides, Penicillium citrinum and Stachybotrys chartarum) and dermatophytes: Microsporum gypseum and Trichophyton rubrum, in to the center of the medium, a sterilized petri dishes with no addition of plant extract (PDA and SDA) was used as control and inoculated by fungi (two replication for each concentration), finally incubated at 25°C for 7 days for opportunistic fungi, and at 37°C for 10-15 days for dermatophytes. The diameter of growth (width and length) measured after incubation period (Rios et al., 1987).

2.5.2. Antimycotic sensitivity test:

Fungal sensitivity for aqueous and ethanol plant extract was tested by using filter paper disc diffusion method, the filter paper disc prepared by using ordinary office two-hole puncture, paper discs with approximate diameter of 6mm. were punched out one by one from a sheet of filter paper, the disks placed in vial, sterilized by oven and allowed to cool. The concentration 2.5 and 5 mg/ml of aqueous and ethanol plant extracts were prepared from the stock solution, blank discs were soaked in a known concentration of plant extract; another filter paper disc was soaked with SDW and DMSO used as negative control. Fungal spore suspension prepared from 5-7 day, were inoculated on PDA and SDA then spread by using sterilized glass rod, the discs were soaked with known concentration placed in petri dish. Finally, incubated at 25°C for 7 days for opportunistic fungi, 37°C for 10-15 days for dermatophytes. Zone of inhibition was obtained by measurement the radius growth of fungal colony from the center of the disc to the edge of the inhibition then measured both sides of the slope and their average accepted (Al-Refai, 2006).

2.6. Experimental microorganisms

Different genera and species of opportunistic fungi (Aspergillus flavus, A. fumigates, A. ochraceous, Cladosporium cladosporioides, Penicillium citrinum and Stachybotrys chartarum) and dermatophyte (Microsporum gypseum and Trichophyton rubrum) were isolated from different sources and diagnosed under microscope on the basis of their colony morphology and spore characteristics and used in this research to show the activity of plant extracts as an antifungal (Rajankar et al., 2007).

2.7. Statistical analysis

Data entry and statistical analysis were performed using IBM Corp. Released for Windows: IBM SPSS software version 20. All values have been expressed as mean ± standard error (Mean±SE), that used for statistical comparison and the Results were considered to indicate statistically significant difference at P value ≤ 0.05.
3. Results

The results of aqueous and ethanol extract of Quercus infectoria gall were studied and evaluated for their antifungal activities against some of the human pathogenic fungi such as Opportunistic (A. flavus, A. fumigates, A. ochraceous, C. cladosporioides, P. citrinum and S. chartarum) and Dermatophytes (M. gypseum and T. rubrum). In general, the research indicated that the ethanolic extract of gall has more effects as antifungal on the growth of dermatophytes than opportunistic fungi.

Antifungal activities of both aqueous and ethanol extracts of galls on the inhibition of mycelium growth of pathogenic fungi showed in (Table 1: a and b) in the concentration 2.5 and 5mg/ml by pour plate method. It has found that ethanol extract of oak galls at both concentrations affected more than aqueous extract on the growth of mycelia of fungi and reduced the mycelia growth compared with the control.

In aquatic extract the diameter of colony growth of all fungi at the concentration of (2.5 and 5) mg/ml were shown as opportunistic: A. flavus (2 and 1) cm, A. fumigates (2.25 and 1) cm, A. ochraceous (5.75 and 3) cm, C. cladosporioides (3.5 and 2) cm, P. citrinum, (2 and 1) cm and S. chartarum (2.25 and 0.7) cm and Dermatophytes: M. gypseum (1.75 and 0.1) cm and T. rubrum (2 and 0) cm, respectively at both concentration. While in ethanol extract the growth of all fungi in the concentration 2.5 and 5mg/ml were as shown, opportunistic: A. flavus (1.25 and 0.5) cm, A. fumigates (1.75 and 0.8) A. ochraceous (3.75 and 2.25) cm, C. cladosporioides (2.75 and 1.5) cm, P. citrinum (1.25 and 0) cm and S. chartarum (2 and 0) cm, respectively, while in both dermatophytes: M. gypseum and T. rubrum, there were no any growth at both concentration.

The higher effect of ethanol plant extract as shown in figure 1 (a and b), at the concentration 2.5 and 5 mg/ml was on inhibition the growth of both Microsporum gypseum and Trichophyton rubrum, followed by P. citrinum and S. chartarum, while the lower effect of ethanol extract was found on the growth of A. ochraceous.

Antifungal activities of galls aqueous and ethanol extracts on the inhibition of mycelium growth of pathogenic fungi as shown in (Table 2: a and b) in the concentration (2.5 and 5mg/ml), by using filter paper disc diffusion method. It has revealed that ethanol extract of oak galls at both concentrations had more inhibition zone than aqueous extract and reduced the mycelia growth when compared with control.

In aquatic extract the diameter of inhibition zone of all fungi at the concentration of (2.5 and 5) mg/ml were shown as opportunistic: Aspergillus flavus ranging from (0.2 and 0.5) mm, while there had no inhibition zone in each of A. fumigates, A. ochraceous, C. cladosporioides and P. citrinum and the diameter of inhibition of S. chartarum was (0.25 and 1) mm and dermatophytes: M. gypseum (0 and 1.25) and T. rubrum had no inhibition zone. While in ethanol extract the diameter of inhibition zone of all fungi at the concentration of (2.5 and 5) mg/ml were shown as opportunistic: A. flavus ranging from (0.15 and 0.45) mm, Aspergillus fumigates (1.25 and 1.5) mm, A. ochraceous (1 and 1.5) mm, Cladosporium cladosporioides (0.5 and 1) mm, Penicillium citrinum (1 and 1.5) mm Stachybotrys chartarum (0.45 and 0.85) mm and dermatophytes: Microsporum gypseum (0.75 and 1.25) mm and Trichophyton rubrum (1 and 1.5) mm, at both concentration respectively.

Aqueous extracts of Quercus gall have no significant effect on the growth of all studied fungi as shown in figure 2 (a and b), while the effect of the ethanol extracts of Quercus gall have significant effect on the studied fungi.

4. Discussions

In the present study the ethanol extract of oak galls were more effective on controlling all studied fungi than aqueous extract and caused significant reduction in mycelial growth for the fungi, this may be due to the differences between the chemical components of the plant extract and most of the biologically active chemicals are soluble in ethanol more than water such as volatile oils, tannin and glycosides and these components are physiologically active against fungi (Paaverurve and Raal, 2010). Oak galls
containing tannins, which have inhibitory effects on fungi (Vonshak et al., 2003).

The results of the current study are in agreement with those found by Al-Refai, (2006), who demonstrated that the aqueous extract of galls of Quercus had no effect on C. albicans, C. krusi, C. pseudotropicalis and Rhodotorula sp., and also coincided with the results of Gulluce et al., (2004), who have found that galls of Quercus have inhibitory effects on the growth of all Candida albicans isolates and also another study suggested that Quercus possesses compounds with antibacterial and antifungal properties (Nair et al., 2007). These results are in agreement with Farahmand et al., (2016), who have evaluated the antifungal activities of methanolic extract of plants against pathogens (Trichophyton mentagrophytes, Epidermophyton floccosum, Microsporum canis) with pour plate method; they have determined that plant extracts inhibited the growth of mentioned fungi at all studied doses. Suleiman and Omafe, (2013), have demonstrated that anti-fungal activities of three medicinal plants such as: Lemon grass (Cymbopogon citrates), Morinda (Morinda lucida) and castor oil (Ricinus communis) extracts were more on Penicillium digitata than on Aspergillus niger, especially at higher concentrations by serial dilution method. These extracts on Aspergillus niger showed progressive retardations on the vegetative growth of Zea Mays, the inhibitory action of the extracts on the growth of mycelium increased with increase in concentrations. Ahmed and Kadhim, (2007), who evaluated two kinds of plant extracts for their antimicrobial activity against two molds (Aspergillus flavus and Penicillium sp.). The plant ethanol extracts were prepared from leaves and concentrated after that the two concentrations of each extract were prepared (1, 0.5) mg/ml and tested their activity as an antifungal. The results revealed that both extracts at both concentrations inhibited the growth of tested fungi as compared with control. Sarmany et al., (2011), who determine the effects of aqueous and ethanol extracts of pomegranate and oak gall to control Penicillium spp. and Aspergillus niger, the results revealed that all the plant extract concentrations used were effective against the two fungi. Ethanol extracts were more efficient against the growth of mycelia of the two fungi than aqueous extracts. Penicillium spp. was more sensitive to the plant extracts of pomegranate and oak gall more than Aspergillus niger.

5. Conclusion

In this study it found that Q. infectoria is an important medicinal plant to estimate there antifungal activity on the growth of some pathogenic fungi and it revealed that ethanol extract of affected more than aqueous extract on the growth of mycelia and reduced the mycelia growth of the pathogenic fungi as compared with control.

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Table (1a): Effect of aqueous and alcoholic extracts of oak galls extracts by pour plate method on the growth of opportunistic fungi

| Opportunistic Fungi           | Plant extract | Concentration | The average diameter of mycelial growth (cm) | Mean ± SE   |
|-------------------------------|---------------|---------------|---------------------------------------------|-------------|
|                               |               | Control (D.W) |                                             |             |
| Aspergillus flavus            | Aqueous       | 8             | 8.5                                         | 8.25±0.0121 |
|                               |               | 2.5           | 2                                           | 2.00±0.013  |
|                               |               | 5             | 1                                            | 1.00±0.0899 |
|                               | Ethanol       | Control (DMSO)| 8                                           | 8.00±0.0097 |
|                               |               | 2.5           | 1                                           | 1.25±0.0772 |
|                               |               | 5             | 0.5                                         | 0.50±0.0985 |
| A. fumigatus                  | Aqueous       | Control (D.W) | 8.5                                         | 8.50±0.1114 |
|                               |               | 2.5           | 2.5                                         | 2.25±0.1383 |
|                               |               | 5             | 1                                           | 1.00±0.0989 |
|                               | Ethanol       | Control (DMSO)| 8                                           | 8.50±0.1567 |
|                               |               | 2.5           | 1.5                                         | 1.75±0.0745 |
|                               |               | 5             | 0.6                                         | 0.80±0.0563 |
| A. ochraceous                 | Aqueous       | Control (D.W) | 9                                           | 9.00±0.0344 |
|                               |               | 2.5           | 5.5                                         | 5.75±0.0448 |
|                               |               | 5             | 4.5                                         | 3.00±0.1455 |
|                               | Ethanol       | Control (DMSO)| 9                                           | 9.00±0.1844 |
|                               |               | 2.5           | 3.5                                         | 3.75±0.0994 |
|                               |               | 5             | 2,5                                         | 2.25±0.0712 |
| Cladosporium cladosporioides  | Aqueous       | Control (D.W) | 7                                           | 6.50±0.1156 |
|                               |               | 2.5           | 3                                           | 3.50±0.0557 |
|                               |               | 5             | 2                                           | 2.00±0.0332 |
|                               | Ethanol       | Control (DMSO)| 7                                           | 6.50±0.1777 |
|                               |               | 2.5           | 3                                           | 2.75±0.0666 |
|                               |               | 5             | 1                                           | 1.50±0.0224 |
| Penicillium citrinum          | Aqueous       | Control (D.W) | 9                                           | 8.50±0.0334 |
|                               |               | 2.5           | 2                                           | 2.00±0.0339 |
|                               |               | 5             | 1                                           | 1.00±0.0098 |
|                               | Ethanol       | Control (DMSO)| 9                                           | 8.50±0.0332 |
|                               |               | 2.5           | 1                                           | 1.25±0.0656 |
|                               |               | 5             | -                                          | -            |
| Stachybotrys chartarum        | Aqueous       | Control (D.W) | 3.5                                         | 3.75±0.0444 |
|                               |               | 2.5           | 2                                           | 2.25±0.0378 |
|                               |               | 5             | 0.6                                         | 0.70±0.0007 |
|                               | Ethanol       | Control (DMSO)| 4                                           | 3.50±0.0346 |
|                               |               | 2.5           | 2                                           | 2.00±0.0556 |
|                               |               | 5             | -                                          | -            |
**Table (1b):** Effect of aqueous and alcoholic extracts of oak galls extracts by pour plate method on the growth of Dermatophytes

| Dermatophytes | Plant extract | Concentration | Average diameter (cm) | Mean±SE |
|---------------|---------------|---------------|-----------------------|---------|
| *Microsporum gypseum* | Aqueous | Control (D.W) | 6.5 | 6.5 | 6.50±0.1678 |
| | | 2.5 | 1.5 | 2 | 1.75±0.0676 |
| | | 5 | - | 0.2 | 0.10±0.0007 |
| | Ethanol | Control (DMSO) | 6.5 | 6 | 6.25±0.0675 |
| | | 2.5 | - | - | - |
| | | 5 | - | - | - |
| *Trichophyton rubrum* | Aqueous | Control (D.W) | 7 | 7 | 7.00±0.1222 |
| | | 2.5 | 2 | 2 | 2.00±0.0444 |
| | Ethanol | Control (DMSO) | 7 | 7 | 7.00±0.1547 |
| | | 2.5 | - | - | - |
| | | 5 | - | - | - |

**Figure (1a):** Effect of aqueous and alcoholic extracts of oak galls by pour plate method on the growth of opportunistic fungi
Figure (1b): Effect of aqueous and alcoholic extracts of oak galls extracts by pour plate method on the growth of dermatophytes

Table (2a): Inhibitory activity of gall extracts against growth of opportunistic fungi by using disc diffusion method

| Opportunistic Fungi | Plant extract | Concentration | Inhibition diameter (mm) | Mean±SE  |
|---------------------|---------------|---------------|--------------------------|----------|
| Aspergillus flavus   | Aqueous       | Control (D.W) | 0 | 0 | 0 |
|                     |               | 2.5           | 0.2 | 0.2 | 0.20±0.0004 |
|                     |               | 5             | 0.5 | 0.5 | 0.50±0.0011 |
|                     | Ethanol       | Control (DMSO)| 0 | 0 | 0 |
|                     |               | 2.5           | 0.1 | 0.2 | 0.15±0.0032 |
|                     |               | 5             | 0.4 | 0.5 | 0.45±0.0034 |
| A. fumigatus        | Aqueous       | Control (D.W) | 0 | 0 | 0 |
|                     |               | 2.5           | 0 | 0 | 0 |
|                     |               | 5             | 0 | 0 | 0 |
|                     | Ethanol       | Control (DMSO)| 0 | 0 | 0 |
|                     |               | 2.5           | 1.5 | 1 | 1.25±0.0364 |
|                     |               | 5             | 2 | 1 | 1.5±0.0221 |
| A. ochraceous       | Aqueous       | Control (D.W) | 0 | 0 | 0 |
|                     |               | 2.5           | 0 | 0 | 0 |
|                     |               | 5             | 0 | 0 | 0 |
|                     | Ethanol       | Control (DMSO)| 0 | 0 | 0 |
|                     |               | 2.5           | 1 | 1 | 1.00±0.0065 |
|                     |               | 5             | 2 | 1 | 1.50±0.0088 |
| Cladosporium cladosporioides | Aqueous | Control (D.W) | 0 | 0 | 0 |
|                     |               | 2.5           | 0 | 0 | 0 |
|                     |               | 5             | 0 | 0 | 0 |
Table (2b): Inhibitory activity of gall extracts against growth of dermatophytes by using disc diffusion method

| Dermatophytes         | Plant extract | Concentration | Inhibition diameter (mm) | Mean±SE  |
|-----------------------|---------------|---------------|--------------------------|---------|
| Microsporum gypseum   | Aqueous       | Control (D.W) | 0                        | 0.50±0.0223 |
|                       | 2.5           | 0             | 1                        | 1.00±0.0564 |
|                       | 5             | 1             | 1                        | 1.50±0.0879 |
|                       | Ethanol       | Control (D.MSO) | 0                        | 0.25±0.0032 |
|                       | 2.5           | 1             | 1                        | 1.00±0.0017 |
|                       | 5             | 1.5           | 1.5                      | 0.85±0.0044 |
| Trichophyton rubrum   | Aqueous       | Control (D.W) | 0                        | 0.75±0.0065 |
|                       | 2.5           | 1             | 0.5                      | 1.25±0.0143 |
|                       | 5             | 1.5           | 1                        | 1.50±0.0073 |
Figure (2a): Inhibitory activity of gall extracts against growth of opportunistic fungi by using disc diffusion method

Figure (2b): Inhibitory activity of gall extracts against growth of Dermatophytes by using disc diffusion method
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