THE ANTI-DERMATOPHYTE ACTIVITIES OF SOME PLANT EXTRACTS AGAINST *TRICHOPHYTON MENTAGROPHYTES* AND *MICROSPORUM CANIS*

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ABSTRACT

The activity of ethanol and aqueous of plants extracts of (*Quercus aegilops, Hypericum perforatum, Lawsonia inermis and Nicotiana tabaci* plants) were tested on the dermatophytes *Trichophyton mentagrophytes* and *Microsporum canis*.Extract from the galls of *Quercus aegilops* was significantly the most effective in comparison to other plant extracts used. Among the ethanol extract concentration, we found that 5 mg/ml was more potent, that significantly inhibit the mycelium growth of *T. mentagrophytes* and *M. canis* completely. We found the aqueous extract at concentration (10, 15, 20, 25 mg/ml) significantly inhibit the mycelium growth of *T. mentagrophytes* and *M. canis* completely.

Keywords: Dermatophytes, plant extracts, aqueous extract, ethanol extract, *Microsporum, Trichophyton*,

INTRODUCTION

The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Twenty to Fivty % of current pharmaceuticals are derived from plants, none are used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious conditions. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties (Cowan, 1999)**.**Klink (1997) Mentioned that use of plant extracts, as well as other alternative forms of medical treatments, is enjoying great popularity in the late 1990s. It is estimated that there are 250,000 to 500,000 species of plants on Earth **(**Borris, 1996) . A relatively small percentage (1 to10%) of these is used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes (Moerman,1996). The need for new antifungal agents continues. Natural products offer a virtually unlimited source of unique molecules and not only serve as a reservoir for new potential drugs and drug prototypes, but also for probes of fungal biology. Drug companies continue to focus on the development of antimicrobial drugs, especially with the increasing emergence of drug-resistant pathogens. Natural products are just one source of antimicrobial agents among today's world of chemical libraries and combinatorial syntheses, but they offer an almost unlimited reservoir of unique structures (Ernst,and Rogers, 2005).

MATERIALS AND METHODS

Plant Extracts preparation:

The plant samples were collected from Erbil Governorate, and then were cleaned, washed by distilled water, then dried by placing it on filter paper in a shaded, placed at room temperature.

Ethanol extractions of plants

Twenty grams of each plant were weighted, then 200ml of ethanol (95%) were added to it then mixed well in a Shaker (Shaker incubator) for one hour and kept at 4^{o}C for 24 hours, filtered through 4-5 layers of gauze and the 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).

Aqueous extraction of plants:

Forty gram of each plant was weighted, and 160 ml of distilled water were added to it then mixed well in a shaker for one hour and kept at 4^{o}C for 24 hours, filtered through 4-5 layers of gauze and the supernatant was placed in Petri dish to dried out then the powder collected and preserved in vials in a refrigerator (Rashan *et.al*., (1992).

Ethanol plant extracts sterilization and dilution preparation

The stock solution of plant extracts were prepared by adding one gram of ethanol plant extract to 5ml of Di-Methyl Sulf Oxide (DMSO) then this stock solution was sterilized by using (Millipore filters 0.2 µm from Medstore). The concentrations (1, 2, 3, 4 and 5 mg/ml) prepared from the stock solution then added to 500ml of Sabouraud Glucose Agar (SGA) and poured on

to sterilized Petri dishes then inoculated by fungi, a sterilized Petri dish with no addition of plant extract (SGA medium only) used as control was also inoculated by fungi (Rios *et. al*., 1987).

Aqueous plant extracts sterilization and dilution preparation

The stock solution of plant extract were prepared by adding one gram of aqueous plant extracts to 5ml of sterile distilled water (SDW), .The stock solution were sterilized by using (Millipore filter 0.2*M*m) filter, serial concentration (5, 10, 15, 20 and 25 mg/ml) were prepared from the stock solution then added to 500ml of SGA and poured to sterilized Petri dishes then inoculate by fungi, a sterilized Petri dish with no addition of plant extract (SGA medium only) used as control was also inoculated by fungi (Rios et.al.,1987).

RESULT AND DISCUSSION

The results in fig(1 and 5b) showed the ethanol extract of *Q. aegilops* have proven to posses significantly at ($p \leq 0.05$) higher MGI that inhibit the growth of *T.mentagrophytes* completely at concentration (4 and 5 mg/ml) in compareson to other plant extracts that inhibit the mycelium growth (MG) of *T. mentagrophytes* but not significantly, followed by *H. perforatum* that have maximum inhibitory effective at concentration 5mg/ml, while *L. inermis*, and *N. tabacum* respectively have lower inhibition effectiveness on the fungi , and the activity of them were higher at concentration 5mg/ml, as shown in figure (1) the concentration.The galls of *Q. aegilops* have been pharmacologically documented to possess astringent,antifungal activities (Digraki *et.al*.,1999).These extracts may have high total tannin content, the antimicrobial activity seemed to depend on the contents of tannin in the plant extracts, and the galls of *Q. aegilops* are potentially good source of antimicrobial agents (Ikram and Nowshad,1977,Evans,1996.Wiart and Kumar,2001. and Irobi *et.al*.,1994). Implied that tannin in plant extracts may be the active compound which may be responsible for the antibacterial activity (Fenner *et.al*.,2005). The methanol extracts and fractions of seven species of *Hypericum* was test against a panel of standardized and clinical opportunistic pathogenic yeasts and filamentous fungi, including dermatophytes. The chloroform and hexane extract of *Hypericum ternum* showed greatest activity (Tazaki *et.al*.,1991 and Abad *et.al*.,2007).

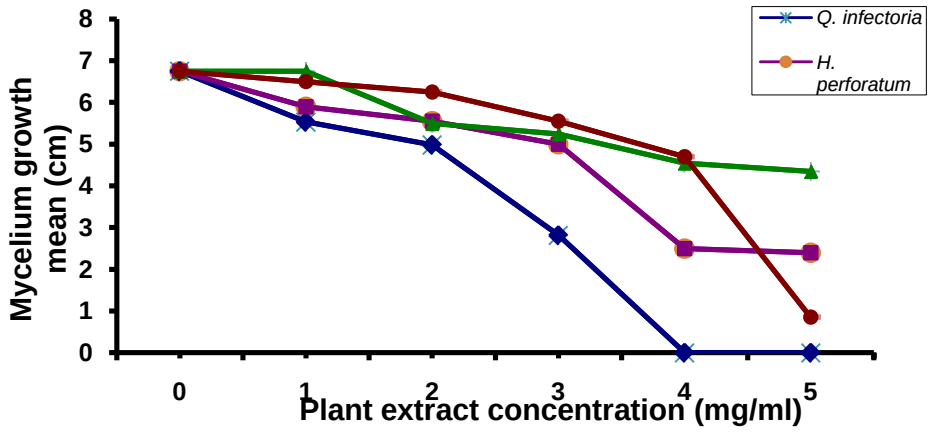

Figure (1): Effect of different concentrations of ethanol plant extracts on mycelium growth mean of *T. mentagrophytes* ($p \leq 0.05$) .

The effect of ethanol plant extract on the growth of *Microsporum canis*:

Figure (2) showed effects of the ethanol extracts of (*Q. aegilops, H. perforatum, L. inermis and N. tabacum*) on the MGI of *M. canis.* Figure (5.a) shows the effect of different concentration of ethanol plant extract on mycelium growth inhibition (MGI) of *M. canis*.

These results show that the ethanol extract of *Q.aegilops* galls at concentration (4 and 5 mg/ml) and *N. tabacum* leaves at concentration (5 mg/ml) significantly ($p \leq 0.05$) have higher MGI that inhibit the growth of *M.canis* completely in compareson with other plant extracts, while *H. perforatum* and *L.inermis*,respectively have lower inhibition

effectiveness on the fungi,and the activity of them were higher at concentration 5mg/ml.

The main constituents found in the galls of *Q. aegilops* are tannin (50-70%) and small amount of free gallic acid and ellagic acid the extracts of the galls of *Q. aegilops* have high potential as antibacterial agent (Ikram and Nowshad,1977,Evans,1996.Wiart and Kumar,2001).

Different isoforms of chitinases and β-1,3-glucanases of tobacco *N. tabacum* were tested for their antifungal activities. The class 1, vacuolar chitinase and β-1,3-glucanase isoforms were the most active against *Fusarium solani* germ lings, resulting in lysis of the hyphal tips and in growth inhibition (Sela-Buurlage *et.al*.,1993).

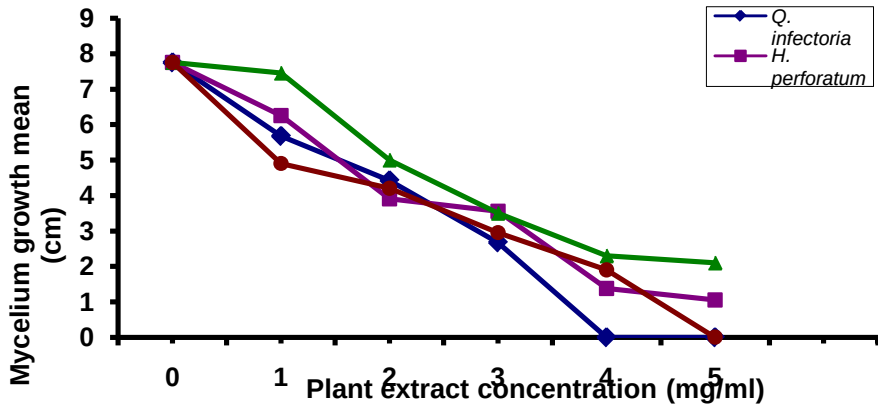

Figure (2): Effect of different concentrations of ethanol plant extracts on mycelium growth (MG) of of *M. canis* (p≤0.05)

Effects of aqueous plant extracts on the growth of *T. mentagrophytes*:

The results in figure (3) showed the effects of aqueous extracts of *Q. aegilops, H. perforatum, L.inermis and N.tabacum* on the MGI of *T. mentagrophytes.* And figure (6.b) shows effects of concentrations of aqueous plant extracts on MGI mean of *T. mentagrophytes.*

From these results appear that *Q. aegilops* have proven to posses significantly (p≤0.05) higher MGI that inhibit the growth of the fungus completely at all concentration except at concentration 5mg/ml in compareson to other aqueous plant extracts. Then *H. perforatum* at the second rank that significantly (p≤0.05) inhibit the growth of *T. mentagrophytes* completely at concentration 25mg/ml and in other concentration inhibit the mycelium growth but not significantly, while *N. tabacum* and *L. inermis,* respectively have lower inhibition effectiveness on *T. mentagrophytes,* and the activity of them were higher at concentration 25mg/ml.

The galls of *Q. aegilops* have been pharmacologically documented to possess astringent, antifungal activities (Digraki *et.al*.,1999). It is well known that tannin is a phenolic compound which is soluble in water, alcohol and acetone, and gives precipitates with protein (Leache,1986). These extracts may have high total tannin content. The antimicrobial activity seemed to depend on the contents of tannin in the plant extracts (Irobi *et.al*.,1996). The aqueous and acetone extracts displayed similarities in their antimicrobial activity on the bacterial species and as such, the galls of *Q. aegilops* are potentially good source of antimicrobial agents (Dijipa *et.al.*,2000).

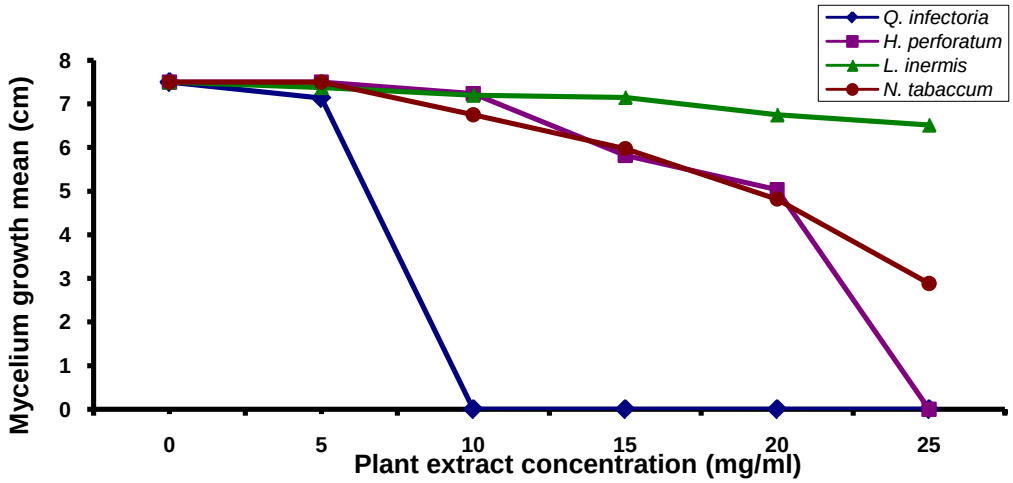

Figure (3): Effect of different concentrations of aqueous plant extracts on mycelium growth mean of *T. mentagrophytes* (p≤0.05) .

Effects of aqueous plant extracts on the growth of *M. canis*:

The result in figure (4 and 6. a) showed that the aqueous extract *Q. aegilops* galls was significantly (p≤0.05) has higher effects on the MGI that inhibit the growth of *M. canis* completely at all concentration except at (5gm/ml) comparing with other aqueous plant extracts. The aqueous extracts of *L. inermis* and *N. tabacum* have inhibitory effect on the MG of *M. canis* but not significantly their higher inhibitory effect was at 25mg/ml. While th aqueous extract of *H. perforatum* leaves has the lower inhibitory effect on the MG of *M. canis*. This result is in agreement with that found by (Digraki *et.al*.,1999) that the galls of *Q. aegilops* have antifungal activities. The similarity in the antimicrobial activity of both the aqueous and acetone extracts suggests that these extracts may have high total tannin content. The antimicrobial activity seemed to depend on the contents of tannin in the plant extracts (Irobi *et.al*.,1994).

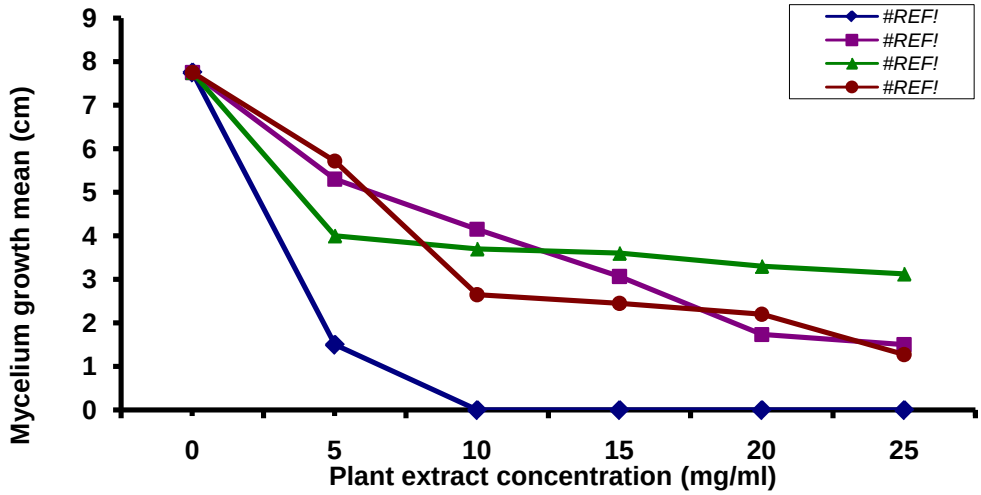

Figure (4): Effect of different concentrations of aqueous plant extracts on mycelium growth mean of *M. canis* (p≤0.05) .

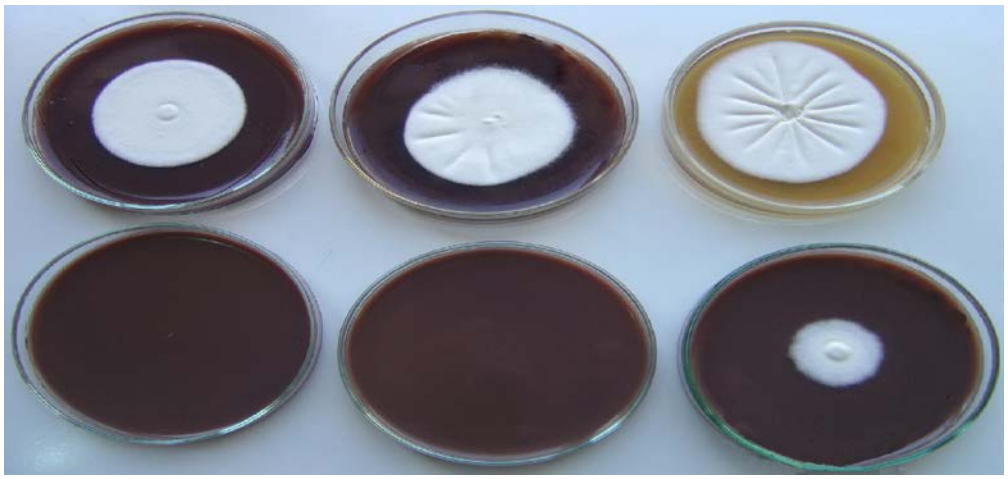

Figure (5): a: Shows effect of ethanol extract of *Q. aegilops* galls on the MGI on *M. canis*, (C: control, 1, 2, 3, 4 and 5 mg/ml) plant extract.
b: Shows effect of ethanol extract of *Q. aegilops* galls on the MGI on *T. mentagrophytes* (C: control, 1, 2, 3, 4, 5 mg/ml) plant extract.

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التاثير التضادي لمستخلصات بعض النباتات في الفطريات الجلدية

الخلاصة

درس تاثير المستخلصات الكحولية و المائية لنباتات (تورمات اشجار البلوط و *Hypericum perforatum* و *Lawsonia inermis* ونبات التبغ) في الفطريات الجلدية وهي *Trichophyton mentagrophytes* and *Microsporum canis*وكان لمستخلصات تورمات البلوط تاثير معنوي مقارنة مع بقية مستخلصات النباتات الاخرى . وجد ان لتركيز 5 ملغم / مل من المستخلص الكحولي تاثير واضح ومعنوي في تثبيط نمو غزل الفطريات *T. mentagrophytes* and *M. canis* كليا . تراكيز المستخلص المائي 10 و15 و 20 و25 ملغم / مل ثبطت نمو غزل الفطريات السابقة كليا.

الكلمات المفتاحية : الفطريات الجلدية ، المستخلصات النباتية ، المائية،الكحولية ، *Microsporum* , *Trichophyton*, .