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Compared between the efficiency of chemotherapy and alcoholic extract of plant leaves *Melia azedarch* L. in the growth of many fungi that cause Tinea capitis in humans in the laboratory

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Abstract
The present study was conducted to proceed a comparison between efficiency of chemotherapy and alcoholic extract of plant leaves *Melia azedarch* L. in the growth of many fungi that cause Tinea capitis in humans using 24 samples belonging to patients with Tinea capitis of Kerion of the children ranged in age (1-18) years old and both sexes dermatophytosis who clinically diagnosed by dermatologist, from Al-Sadder Medical City in Al-Najaf Government, from January to November 2016, by using traditional methods for laboratory isolation and identification. The results shown indicated many fungus *Microsporum canis* and *Microsporum gypseum* and *Trichophyton verrucosum* . the biochemical test refer , *M. canis* featured its growth on the rice media and excellence fungus *M. gypseum* its growth at the corn flour with 80 Tween with Trypan blue The fungus *T. verrucosum* have a clear growth on 1,2 Trichphyton Agar .on the other hands , results indicated in the *M. canis* is the predominant type and cause Tinea capitis within the studied samples as the incidence rate of 42% with this fungus, followed by *M. gypseum* in effect since the infection rate was 38%, and *T. verrucosum* was less influential as the incidence rate of 21%. Results showed the relationship between infection and age and sex ,the males were more susceptible to infection than females especially in ages (12-7) years high infection.
Moreover, in present study found a different concentration of alcoholic extract of leaves *Melia azedarch* L in inhibiting the growth of fungi especially for concentrations (60 and 80%) . on the other hand, when using antibiotics in the treatment of skin infections and compared alcoholic extract leaves *Melia azedarch L.* result appear sensitivity of fungus to antibiotics as it reached the infection ratio (69.7, 40.9, 77.7)% of ketoconazole antifungal *M. canis* , *T .verrucosum, M.gypsem* respectively while fungi tested did not appear affected and clear for antibiotic Flouconazole compared with the rest of antibiotics as it was (10.2, 5.0, 11.5)% respectively.

INTRODUCTION

It is known that fungi adapted to high temperature up to $37C^0$ which infect the skin and spread to humans and animals alike [(1)]. Tinea capitis of the most common skin diseases in humans and that infect the scalp are spread all over the world by a number of factors, most importantly the lack of attention to hygiene and bacterial infections and contact with domestic animals, especially cats, dogs, sheep, pigeons domestic and caused by a number of fungi *M.canis* and *M.gypseum* and *T.verrucosum* and *T.tonsurans* [(2 ; 3).]
In general, the clinical in the form of a circular with the edges of reddish base and the winding covered with white crust dusty patches of symptoms and show the hair area likeness disease fox has many spots on the skin of the head show and talked and frequently in children from age (4-14 years) and rare in adults and be many in males than in females[(4; 5)].
Melia azedarch L. plant (Bead tree) or (China berry) Back to genus *Melia* from *Meliaceae* family, which includes 50 genera and about 800 species live mostly in tropical areas and confined between the tropics and in Iraq and neighboring countries there is one kind is *M. azedarach L.* and the original home of the plant It is the Himalayas and central and western China [(6)]. Using different parts of the plant in cases of fever, colds and skin diseases and as a disinfectant in toothpastes and rheumatism, as well as used to evaluate the speed of blood clotting[(7)].Because the lack of studies on the effect of the plant extract of such genus of plants on the skin fungus considering that extracts natural materials is harmful to human health and animal conducted this study, which included the following:
1. isolate and diagnose the fungi that cause Tinea capitis in humans.
2. Assessment alcoholic extract of plant *M. azedarch L.* efficiency in the inhibition of some fungi that cause head Tinea capitis in humans.

MATERIALS AND METHODS

Specimens collection
Collected 24 samples belonging to patients with Tinea capitis of Kerion of the children ranged in age (1 -18) years old and both sexes clinically diagnosed by doctors jurisdiction and 24 sample as a control.then took scarpping of the affected skin (hair clippings, broken skin) by a scalpel and sterilized the infected area by alcohol 70% before sampling to get rid of bacteria and fungi throw [(8)] , while samples were taken from an infected hair tongs mediated sterile for the purpose of examination as well as scrape the scalp and put the samples in sterile dishes for the purpose of microscopically examined to confirm the presence of fungi and then isolate it.
Culture media used in the study Sabourauds, Dextrose Agar with Chloeamphenicol, Cycloheximide (SDA) prepared by method [(9).]
Corn meal Agar media with Tween 80 with blue Trypan. [(10)] this media prepared:

Corn flour 45 gm
Agar 15 gm
Distilled water 1000 ml
Blue Trypan 1% solution
Trypan blue 0.1 gm
Distilled water 800 ml
80 Tween 200 ml

Put corn flour in a glass beaker with distilled water at the degree of 52 C^0 water bath for an hour and then filtered mediated filter paper and take the filtrate and added a 1.5 ml of blue Trypan 1% solution and 15 ml of 80Tween and Agar and sterilizes the center of closed device for 20 minutes warmly 121 C^0 and pressure 15 psi/$inch^2$.

Test growth on the grain rice media

Examination was conducted for the purpose of distinguishing between *M . canis* and *M. gypseum* by taking 8 grams of beans and rice are placed in a beaker contain, 25 ml of distilled water and then vaccinated infertility center fungal thoughtful and incubated 26 $C^0$for 10-14 days. It was observed heavy growth of the *M. canis* on grain rice. While *M. gypseum* doesn't growth of the media.

Urease test medium attended by methods [9].

Trichophyton Agar [9]

A series of Trichophyton agars (No. 1-7) were prepared from the following ingredients to differentiate among the Trichophyton species through the differing needs of this genus of growth factors which vitamins sterilized media and incubated 26 $C^0$for 10-14 days.

Sampling

Microscopic examination by Direct examination taking a small sample part mediated needle vaccination in a drop of KOH solution concentration of 10% subject to a glass slide and put the slide cover, then drained a little flame is weak and examined microscopically to note fungus in skin scaling of the hair[11].

Culture of Specimens [12]

After direct microscopic samples examination were culture planting of specimens by taking a small part of the sample-mediated needle vaccination implanted on the surface of a glass container dish on (SDA) with the antibiotic chloramphenicol Chloeamphenicol (0.5 g \ L) and Cycloheximide (0.5 g \ l) to reduce contamination with fast growth Bacteria and opportunistic fungi respectively. Then dishes were incubated highly cultivated 35ºC for 14-21 days and fellow dishes were examined daily after 7 to14 days growth of fungi and diagnosed fungi isolated based on the phenotypic characteristics of the colony, such as shape and color and diameter of those colonies, as well as microscopic properties such as size, shape and color condia adopted as well as the adoption of some biochemical tests to diagnosis and with the help of the following sources: [13; 14; 15].

Extract the organic solvent (alcohol)

Attended the organic solvent extract (ethyl alcohol) to *Melia azedarch L.*plant leaves by the way[16] and [17]. Attended series concentrations from *Melia azedarch L.* extract (0, 20.40, 60.80%) that using in his study.

Efficient *Melia azedarch L.* extract in inhibiting the growth of fungi that cause tinea capitis

Four concentrations of each extract (20.40, 60.80)% were mix with the SDA, after cooling vaccinated all the dishes 0.5 cm from the colony each fungus in the dish Center [18] and the dishes were incubated at a temperature of 30 ° C for 7 days and with four replicates per treatment with a comparative treatment of each fungus. After the arrival of the colonies to the edge of the dish was the damping rate is calculated in the radial growth of the fungus to take diagonals perpendicular rate according to the amount of damping according equation [19] as follows:

$$\text{Inhibition} = \frac{R_1 - R_2}{R_1} \times 100$$

R_1 maximum radial growth of pathogenic fungus colony (treatment comparison).

R_2 maximum radial growth of pathogenic fungus colony in the dishes extract container.

The sensitivity antibiotics Test of the study

Used group of antibiotics processed from a company (Oxoid) to test the sensitivity of fungi her as she followed the diffusion method in Agar by NCCLS, 1984 Disc diffusion method)) have been deployed airborne fungal Comparative a roll McFarland at the SDA left dishes to dry for 15 minutes, then put antibiotic tablets (Teraconazole, Terbinafine, Ketonazole, Itraconazole, Flouconazole).

Then dishes were incubated 30 C^0 for 7 days after it was measured area inbibition zone by the ruler [20]. Then the results were compared with the results of *Melia azedarch L.*extract

Statistical Analysis

All laboratory results were analyzed according to (C.R.D) Design and averages were compared by test teams less moral L.S.D and at the level of probability of 0.05.

RESULTS AND DISCUSSION

Biochemical tests and physiological fungi studied

Results shown indicated in the table (1) The fungus *M. canis* featured its growth on the rice media by observing the intensive growth as diameter was colonized 7 cm yellow in color tannic pale to yellow, orange, and large conidia spindle (10-24 × 35-90) Micron, either small conidia and be caraway shape. As and excellence fungus *M. gypseum* its growth at the corn flour with 80 Tween with Trypan blue as the diameter colony 4.5 cm white cardigan shape and large conidia no clear many of elliptical numbers, the summit is found in large conidia base Annular Frill and small conidia Mace shaped peripheral or side. The fungus *T. verrucosum* have a clear growth on 1,2 Trichphyton .Agar colonies diameter of 2.7 cm white Sporadtha type Chlamydospors not contain large or small conidia .

Table 1: shows the biochemical and physiological tests of fungi studied

Fungal species	urea test	Rice media test	Corn flour with Tween80 Trypan blue	Test Trichophyton agars (No. 1-7)
M,canis	_	++	_	_
M.gypseum	_	_	+	_
T.verrucosum	_	_	_	+NO.3

++ Test positive (confirmed test)
+ Test positive - Test negative

Table 2: shows the fungal species isolated from the head and the number and percentage of infection.

Type fungal species	Number of fungal species	percentage of infection%
M,canis	10	42
M.gypseum	9	38
T.verrucosum	5	21

On the other hand, The results indicated in the in table (2) to the *M. canis* is the predominant type and cause Tinea capitis within the studied samples as the incidence rate of 42% with this fungus, followed by *M. gypseum* in effect since the infection rate was 38%, and *T. verrucosum* was less influential as the incidence rate of 21%.

This is due to the majority of the population infected with tinea capitis are rural residents in order to provide the appropriate conditions and catalysts for growth and reproduction of fungus as well as the infection moves as a result of their upbringing, domestic animals, dogs and cats that are the cause of the transmission as a result of contact the children with some of them during the play or in school as well as reduced the cultural level of health awareness [21].

The relationship between infection and age and sex.

Results of the current study are described in the figure (1) refer the males were more susceptible to infection than females which reached the percentage of infected to 75% for ages (12-7) years exclusively the female has reached incidence to 25% in the same ages. This is due to medical negligence and cases of poverty reduction cultural awareness and congestion in schools or clubs in addition to the absence of fatty acids and saturated in the hair of the head when the kids and the short hair and the vulnerability and lack of personal hygiene factors conducive to the spread of infection in children males more than females [22].

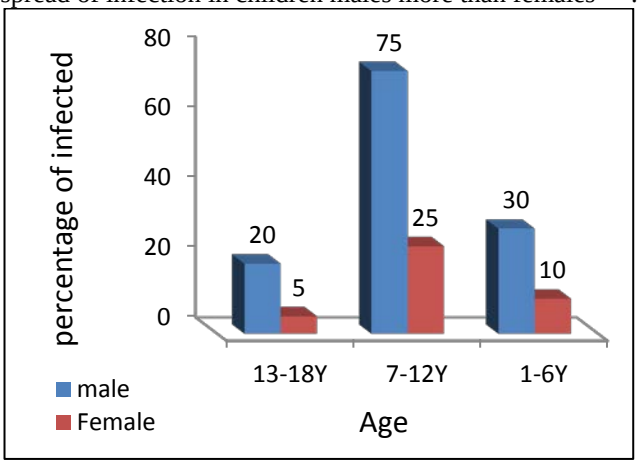

Figure 1: The relationship between infection and age and sex.

Effect different concentration of alcoholic extract of leaves *M. azedarch* L in inhibiting the growth of fungi in study .

In may study observed all concentrations was affected significant inhibition of radial growth isolated fungi especially for concentrations (60 and 80%) were inhibition (90,76, 92) and (98, 85,100) % to fungi *M. canis* and *M. gypseum* and *T. verrucosum*, respectively, compared with control group. figure (2)

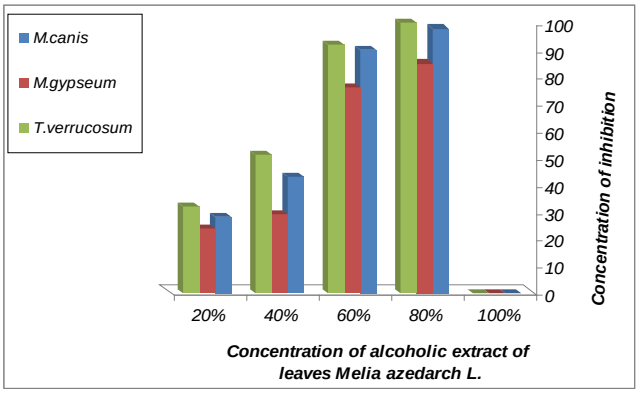

Figure 2: Different concentration of alcoholic extract of leaves *Melia azedarch* L in inhibiting the growth of fungi in study.

This may be belong to contain the plant *Melia azedarch* L alkaline toxic substances in the leaves and fruits and plant lignans called (Tazetin), which is the active substance powerful and influential in the inhibition of fungi, as well as plant *Melia azedarch* L contains many of the compounds that were used in the fight against insect pests [23 ; 24].

On the other hand [25], found that the alcoholic extract of the leaves Elias possessed highly effective against bacteria, yeasts and found that the alcoholic extract of the leaves Elias has proven highly effective against bacteria, *Pseudomonas spp* that have exceeded [26].

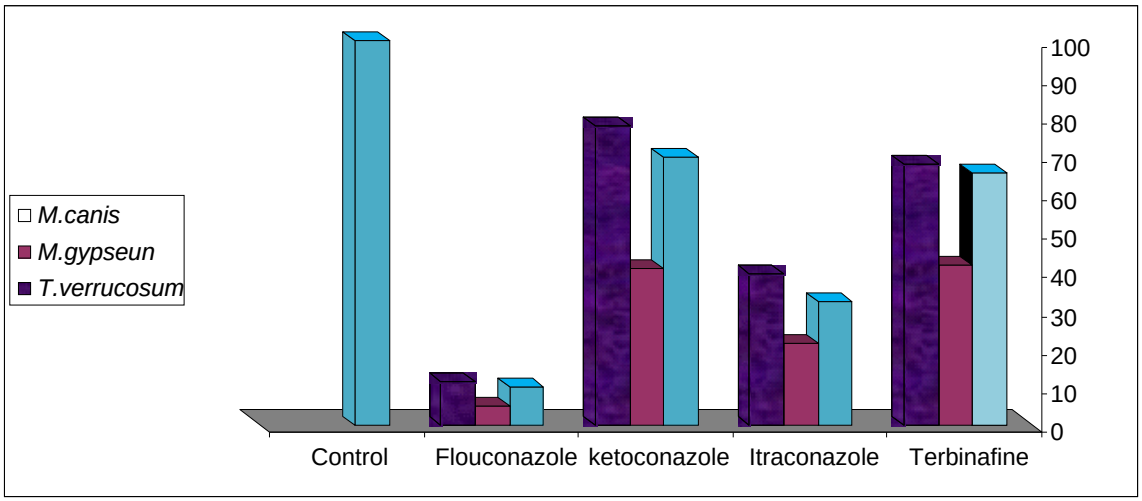

Figure 3: Using some antibiotics in the treatment of skin infections

Using some antibiotics in the treatment of skin infections and compared alcoholic extract leaves *Melia azedarch L* in laboratory.

Testing in figure (3) included the sensitivity of fungi to a number of antibiotics fungal included Itraconazok, Terbinafin, Flouconazole, Ketoconazole) that the fungus may be affected by antibiotics used as it reached the infection ratio (69.7, 40.9, 77.7)% of ketoconazole antifungal *M. canis* , *T .verrucosum*, *M.gypsem* respectively while fungi tested did not appear affected and clear for antibiotic Flouconazole compared with the rest of antibiotics as it was (10.2, 5.0, 11.5)% respectively.

It is known that the treatment of fungal infections are generally less successful than the treatment of bacterial infection to a large extent because the real organic cell nucleus which is more similar to cells in the human bacteria (27, 28).

On the other hand , antibiotic-derived compounds Azole compould include Ketanozole and Clotrimzole and mincanzole and Econzole and others taken by mouth because it is easily absorbed in the intestine either clotrimzole used ointment of Dermatology and he also cannot be given by mouth .The Oxiconazole external ointment is easily absorbed through the Katarina layer of the skin down to the deep layers of the skin and uses of Dermatology and yeasts. The fungus resistance arising from the possession of these fungi different mechanisms that help to resist many of the antibiotics of life such as the production of enzymes capable of editing molecule antibiotic substance to be ineffective (29)

On the other hand found that Terbinafine and, Naftifine affect the effectiveness of enzyme-fungal skin and can be taken by mouth and easily absorb in the intestines (30 ,29)

The overall treatment of skin diseases is extremely difficult because most do not have the only stance Fungistatic as well as its characteristic high for a person of Like a lot of reason between the cellular and molecular characteristics between them and the human (31, 32). As well as other resistance mechanisms which are considered the target site, which is running on an antibiotic because of a genetic mutation in the opposite correlation vital sites occurs with its place with fungi and thereby lose the antibiotic familiarity.

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