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CALENDAR OF TREATMENT FUNGAL ANTIBIOTIC, PHARMACEUTICAL AND ALCOHOLIC EXTRACT OF PROPOLIS ON SOME FUNGI THAT CAUSE TINEA CAPITIS IN CHILDREN

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Abstract

This study was conducted to evaluate antibiotic fungal drug and alcoholic extract of Propolis on some fungi that cause skin infections when children that therapy was isolated a number of fungi that cause disease Tinea capitis included fungus *Microsporum canis*, *Microsporum gypseum*, *Trichophyton verrucosum.* As indicated the results of biochemical and physiological isolated fungi from head checks that fungus *M. canis* characterize its growth on the grain rice by observing the intensive growth The fungus *M .gypseum* was widely growth on corn flour and *T. verrucosum* was a clear growth on Trichophyton Agar No. 3. On the other hand the results showed that the fungus *M. canis* is the predominant type that causes tinea capitis infection rate has reached to 60% and then followed by two fungi *M. gypsem, T. verrucosum* reached incidence ratio (22.5 to 17.5)%. The results of the study when examining the relationship between infection and the age and sex that males were more likely than females since its arrived in the incidence of male to 75% in age7-12 year old and the female has reached the 25% mentioned in the same ages. The relationship between the season and the spread of infection have proven results of the study that the spring season in March recorded the highest rate of injury as it reached 25.9%, followed by months in April and May and June reached incidence ratios of (17.7, 12.2, 12.8)% respectively, compared months in January and February were the incidence ratio (0.2 and 0.4)%, respectively. Moreover The results of the statistical analysis that the use of alcoholic extract of Propolis was effective and all concentrations in reducing the incidence of fungal skin as it was *M. gypseum* most affected as the inhibition zone amounted to 0% and all concentrations compared with control group which reached 80% of the other seemed All fungi sensitivity to concentrations of 5% of the extract reached inhibition zone to 0%. in the other hand, was conducted by examining the sensitivity of fungi studied test within a number of fungal antibiotics as results showed that the fungus has affected all antibiotics inhibition zone reached (35.7, 39.8, 33.4)% to the antibiotic Terbinafine on *M. canis* and *M. gypseum* and *T. verrucosun* respectively. While the tested fungi did not appear for the impact of antibiotic Flouconazole it was the inhibition zone 0%.

Keywords : Fungal Antibiotic, pharmaceutical and alcoholic extract of propolis, tinea capitis

Introduction

For thousands of years they killed microbes in human life through their effects pathogenesis and transmission through contact with infected persons. It is a skin filamentous fungi of the most prevalent of these microbes comes after the bacteria as skin lesions among school students and military forces deployed by more than 65% (Zuber and Baddan, 2001). The majority of skin infections caused by a fungus usually is an isolated episode of infection linked to the development of immune status of various environmental factors, as these fungi are similar looking food and requirements that would enable them to be a specialist infecting the host (Jawetz *et al.,* 1998). using of chemical agents in the treatment of skin lesions lead to a break down body object exhibition without changes occurring in the host body and, more recently resorted most countries of the world to the medicinal plants as a major source for the pharmaceutical and described as anti against many microbiology bacteria, fungi, viruses (Scheller *et al.,* 1999) and other plant-based compounds used in the treatment of many medical conditions, including organic and microbial which propolis which is one of the active compounds against a large number of microbiology compound. Use the compound propolis popular for therapeutic purposes and is the ancient Greeks and Romans first used propolis in the treatment of other skin lesions and skin diseases The Egyptians and Africans are still used by reducing the present day in the treatment of diseases as a medicine in the treatment of boils and burns with pus and called Avicenna in his book, The Canon of Medicine as a black wax used in the treatment of skin diseases (Graham, 1992; Abd-El-Salam *et al.,*1989 and Al-hakeim *et al.,*2012)

That is an anti-vital natural where to stop the growth of bacteria and eliminate them because they contain flavonoids, especially Alvalengin which is found in the poplar buds were tested on bacteria *Bacillus spp*, *Salmonella spp, Staphelococcus spp*. Its also antibiotic-fungal and resistant to oxidation and has no side effects to humans. In view of the large number of infections tinea capitis in children in recent times, especially the type of acute and festering infections caused by these complications are usually secondary infections, we suggest this study, which included:

- Isolate and diagnose many fungi that cause tinea capitis in children.
- Preparation of alcoholic extract of propolis.
- The use of different concentrations of the extract against some fungi tested.
- Test of some antibiotics against fungal studied.

Materials and Methods

Biological Methods

1. Specimens Collection by took 40 samples belonging to patients with tinea capitis kind of sharp festering Kerion of children aged (4 -14) years and for both sexes clinically diagnosed by doctor's competence in medical clinics and 40 sample as a control. of the affected skin Scarpping (hair clippings, broken skin) with a scalpel and the area sterilized by alcohol 70% ethanol before sampling to get rid of bacteria and fungi throw (Rook and Machail, 1986). While the samples were taken from an infected hair tongs mediated for the purpose of examination as well as scrape the scalp and samples and placed in sterile dishes for the purpose of microscopically examined to confirm the presence of fungi and isolate.

2. Culture media used in the study.

- Sabourauds Dextrose Agar with Chloramphenicol and Cycloheximide SDA

  Its attended as by prepared by method (Kwon-Chung and Bennett, 1992).

  This is the media and used for the purpose of isolating and diagnosing fungi studied.

- Corn meal Agar media with Tween 80 with blue Trypan. (Koneman *et al.*, 1978) using this medium for the purpose of discrimination two fungi *T. rubrum, T. mentagrophytes.*

- 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* the central grain rice. While *M. gypseum* doesn't growth of the media.

- Urease test medium attended by methods (Burns *et al.*, 2010)

- Trichophyton Agar (Kwon-Chung and Benntt, 19 : 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 ℃ for 10-14 days.

3. Sampling

- Direct Microscopic Examination: Taking part mediated inoculation needle in a drop of KOH solution concentration of 10% subject to a glass slide and put the slide cover, then drained a bit on the weak flame and examined microscopically to note fungus in skin scaling of the hair.

- Planted specimens Direct examination / taking a small sample of part-mediated inoculation needle implanted on the surface of a glass container dish on (SDA) and after the dishes were incubated cultivated a degree of 30 °C for 10 days and when the fungal growth has been the emergence of samples diagnosed.

- Identification and diagnosis of pathological fungi

- fungi isolated depending on the phenotypic characteristics of the colonies, such as the shape and color of these colonies and diameter, as well as microscopic characteristics such as shape, color, size conidia adopted as well as the adoption of some biochemical tests in the diagnosis and with the help of the following sources : (Kwon-Chung and Bennett, 1992; Collee *et al.*, 1996; DeHoog *et al.*, 2002; Burns *et al.*, 2010).

Biochemical Tests

(A) Test penetrate the hair perforation : Conducted this test as stated in (English, 2005) by taking parts of the hair and put in a glass sterile dish and then add to it ml 15 distilled water and (3-2) drop of yeast extract 10% sterile filtration of each dish and then vaccinated hair part of the colony fungal incubated 14-10 day at 30 °C and examined after the addition of dye Lactophenol blue as the *T. mentagrophytes* happen noticeable holes in the infected hair at the same time *T. rubrum* any effect it does not happen and this is a recipe differential.

(B) Decomposition of urea Hydrolysis : Examination conducted for the purpose of identifying the viability of fungi isolated on urea analysis was done inoculating tube container Millipore filter sterilized urea agar base was mixed with sterile agar and allowed to set. The medium is then inoculated with the tested organism. After incubation at of 4 weeks was recorded no change in the middle color from two to four weeks in the case of complete decomposition or full of urea becomes the center color from yellow to dark red or pink. In the case of partial decomposition change their minds middle yellow color to pale pink. In the absence of any

decomposition of the inability of the fungus on the analysis of urea yellow color remains unchanged. (Burns *et al.*, 2010).

(C) Growth on rice grains was conducted for the purpose of this test is to distinguish between the two fungi *M. canis, M. gypseum* by taking 8gm of rice seed and placed in 25 ml of distilled water and infertility center and then vaccinated fungal thoughtful and incubated 2–3 weeks' incubation at 26ºC.. As a thick growth of the fungus was observed *M. canis* the center while there is growth in the second of the fungus.

3. Preparation of alcoholic extract of propolis Ethanol extracted.

Attended the organic solvent extract (ethyl alcohol) to Propolis by the way (Nasseem and Patil 1998; Ladd *et al.,* 1987). were taken 5gm of dry matter were extracted material extraction device which sequentially Soxholate by melt ml 100 of organic solvent (ethanol). For 24 hours after it was learned the concentration of the rotor evaporator degree 45-40 ºC for the purpose of estimating biological activity of the extract solvent. In order to prepare the concentrations required. The weight of dry matter and melted in 3 ml of the same solvent used in the preparation (ethyl alcohol) or finished size to ml 100 to be the main solution concentration of 1%, and it came to focus (1, 3, 5)%.

4. Test alcoholic extract of propolis efficiency in the inhibition of fungi in this stud

Used in this experiment alcoholic extract of Propolis and by three concentrations (1,3,5)% and mix with the SDA after cooling to within all the dishes incolum 0.5 cm colony each fungus in the dish center (Maurhofer *et al.*, 1994) and then the dishes were incubated degree of 30 ° C for 7 days and by three replicates per treatment with a comparative treatment of each fungi. After the arrival of the colonies to the edge of the dish was the damping rate of expense growth radiographic fungicide. Taking diagonals perpendicular rate according to the amount of inhibition Equation Abbot (1925), as it 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 containing the extract.

5. Test studied the sensitivity to antibiotics.

Used group of antibiotics processed from a company (Oxoid) to test the sensitivity of fungi have since used the diffusion method in agar by the way(N.C.C.L.S; 1984) was published stuck innate Comparative a roll McFarland at the center of SDA steel left dishes to dry for 15 minutes, then put antibiotic tablets vital (Teraconazole, Terbinafine, Ketonazole, Itraconazole, Flouconazole).

Then dishes were incubated 30 °C for 7 days after it was measured inhibition zone by the ruler (Saxena *et al.*, 1995) and then the results were compared with the results of proplis extract.

6. Statistical Analysis

All carried the laboratory results by randomized complete design model of randomization Completely. (C.R.D) were compared by testing less significant difference (L.S.D) and at the level of probability of 5.0. (SAS, 2010)

Results and Discussion

(1) Biochemical and physiological tests fungi isolated from the head. results shown in Table (1) The fungus *M. canis* its growth at the rice grains by observing the hyper growth that distinguish on fungus *M. gypseum* its growth amid the corn flour with Tween-80 and Blue Trypan on the other hand, fungus *T. verrucosum* a clear growth in the middle of Trichophyton 1-7.

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

Fungal species	urea test	Rice media test	Corn flour with Tween 80 Trypan blue	Test Trichophyton agars (No. 1-7)
M, canis	–	++	–	–
M. gypseum	–	–	+	–
T. verrucosum	–	–	–	+NO.3

++Test positive (confirmed test¶[

+ Test positive ¶[

- Test negative

On the other hands, the results referred to in the table (2) to the fungus *M. canis* is the predominant type that causes tinea capitis within the studied samples as infection rate stood at 60%, followed by *M. gypsesm* reached infection rate to 22.5%, while the *T. verrucosum* was the least impact which reached infection rate of 17.5 %.

This study agreed with the findings of the (Matsumoto, 1996) of the fungus *M. canis* is the predominant type that causes tinea capitis which reached the percentage of infected to 45% of cases, followed by two fungi *T. rubrum* and *M. gypseum* reached infection rate to 21 and 8%.

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	24	60
M. gypseum	9	22.5
T. verrucosum	7	17.5

In study (Abass, 1995) that the fungus *M.canis* had recorded for the first time in Iraq in perpetuating patients for tinea capitis. In a study conducted by (Abid Ali, 2009). in Al-Qadisia, the type of tinea capitis erythemal festering is the most widespread of tinea capitis clinical type accounted for 41% and *M. canis* is causal accounted for 55.1%, followed by *T. verrucosum* 26.1% and *T. violaceum*, *T. mentagrophyt* accounted for 13 and 5.8%.

2. The relationship between infection, age and sex.

The results of the current study set out in the Figure (1) that males were more likely to develop the disease than females which reached the percentage of infected to 60% for ages (12-7) years exclusively the female has reached incidence to 35% in the same ages.

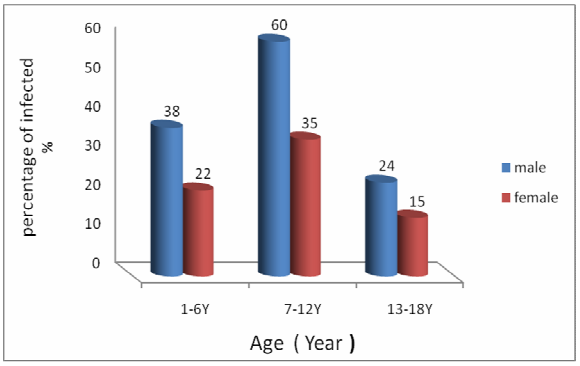

Fig. 1 : The relationship between infection, age and sex

These results coincided with the findings of a number of researchers (Abass, 1995; Hassan, 2007). In Baghdad and by (Abid Ali, 2009) in Al-Qadisia, since pointed out that tinea capitis disease is common in children and rarely occurs in adults and in males than in females. As the disease is spreading among school children due to medical negligence, poverty or congestion in the classroom. On the other hand the lack of saturated fatty acids in the head hair in children and the short hair male and decency of immunity factors helps incidence among children.

On the other hand, found The incidence rate common in the age group (3-12 years), and concluded The rate of infection was at the male children in the 6-9 years age group (Abass, 1995).

3. The relationship between the spread of infection and incidence season.

This study proved and shown in Figure (2). The head injury in children occur in the warmer months of the year and warm. It recorded the highest rate of rise of the injury in March as the infection rate reached 25.9%, followed by months of April and May and June in which the incidence rate reached 17.7, 12.2 and 12.8%, respectively, and in July and August and September infection rate reached 10.2, 10.4 and 3.6% respectively. In January and February the injury rate is very low and amounted to 0.2 and 0.4% respectively.

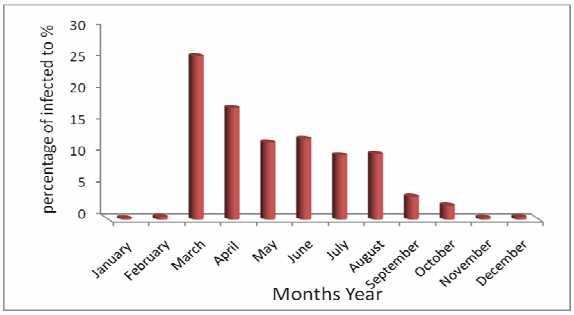

Fig. 2 : The relationship between the spread of infection and incidence season.

These results proved that the infections occur frequently during the warm months. In the cold months shall be very few. This is due to rising temperatures and increased humidity to 95% in the summer and high temperatures to more than 50 ^{0}C in Iraq.

These results agreed with the findings of the (Maraki and Tselentis, 2000) were found that the infection had spread in the spring and summer season, more than other months and reached the infection rate to (24.4, 18.8, 10.7)% in the months of March and April and May while she was in January and February less than the rest of the months and reached incidence ratios (7.1, 9.6)%, respectively (Hijazi, 1998) reach The high temperatures to more than 35 ^{0}C and humidity of 90% was enough to increase injuries fungi that cause tinea capitis rate and (Maraki, and Tselentis, 2000) he pointed out that skin infection, especially tinea capitis be many deployment during summer and autumn. The (Zuber, and Baddan, 2001) has found that the most important factors helping to increase the incidence of infection tinea capitis is the warm weather and high humidity especially in the spring and summer.

4. The effect of different concentrations of the alcoholic extract of propolis on the growth of some fungi isolated that causing tinea capitis.

The results described in a Table (3). All concentrations of the extract was effective in reducing

the rates of incidence of fungal skin as it was *M. gypseum* most affected as the inhibition ratio amounted to 0% in all the concentrations used in comparison with control which reached 80% On the other hand showed all fungi sensitivity to the concentration of 5% of the extract inhibition ratio to reached 0%. The concentrations were 1, 3 % also have a role in reducing the percentage of inhibition, but with less emphasis 5% compared to the control of the transactions fungi tested the inhibition ratio reached 80%.

Table 3 : Different concentrations of the alcoholic extract of Propolis the growth of some fungi isolated and that causes tinea capitis.

Fungi	Concentration %			
	0	**1**	**3**	**5**
M,canis	80	18	5	0
M.gypseum	80	0	0	0
T.verrucosum	80	10	3	0

The results showed that the alcoholic extract of Propolis highly efficient in its effect on the growth of fungi laboratory with increased concentration because that the properties of therapeutic and biological Propolis back to contain on a highly effective compounds against microbes (Husseini, 2008). This can be attributed obvious effect of Propolis is an effective compounds with medical impact towards the aetiology most important phenolic compounds, phenolic acid. Caffic Acid, Ferulic Acid, Coumaric Acid and organic acids such as acid, gallic compounds aldehydes, aromatic benzoic acid, aldehydes included Vanlen, Azovanlen. and Coumaric like Askoblitol, Skobolitol, and Flavonoid included Akasetin, Krayizen and Bactulnaregin, Pinnocmbrin, and Flavonlate included Gelngen, Kimpferid, Korosten, Ramnoctren, Flavonot, Benustrobin, Sackorantin and Flavonte pinobanksin. In addition to a number of vitamins, especially B, D_3, A and other and other components included Zantoreol and Petrostelin lactones and complex sugars and amino acids do not dissolve in cold water and dissolve partially in acetone, alcohol and gasoline (Salah, 2000; Mahasneh,1996) refer to that Filavonat especially Erapzin, Akasetin effective materials touching her role in the treatment given to the complex physiological effects and are of yellow color Propolis source. Add to that the propolis is a natural antibiotic has proven therapeutic efficacy bestow experiments conducted especially Filanin found in sprouts leaf poplar trees, these molecules have no side effects when used medically.

On the other hand found (Assiouti, 2008) the propolis contain active substances included Sinamic Acid, Kimpferid, AzovielicAcid and others. chemotherapy for Propolis very complex and that he was considered an anti vital because it may be due to its ability to deform the proteins of microorganisms, it has an effect to stop the microorganism growth and thus stop the sporulation process, especially fungi *M. canis, M. gypsenm*, because these two fungi possess virulence factors more than others opportunistic fungi that infect the skin tissue and its accessories (Haddadand Omar 2007).

Attributed inhibition processes for vehicles phenolic in microbiology on the basis of its ability to deform proteins Denaturation and stop doing the enzymes responsible for a series of metabolic reactions and thus the object loses its ability to growth and stability and this study agreed with the findings of the (Bankova *et al.*, 1987). It noted that the use of alcoholic extract of Propolis was effective in the treatment of many skin diseases, especially eczema and tinea capitis and psoriasis, especially fungi *M. canis, M. audouinil, T. verrucosum, T. shocnleinii.* In study conducted by (Shoaiband Marwan, 1997; Aboud 2001) in Sanaa as the use of ethanolic extract of Propolis has had a inhibition effect for the growth of skin fungus and private *M. canis, M. gypseum, T. verrucosrm, Epdermophyto* spp. were all affected by the concentrations of the extract alcohol except *T. verrucosrm* less affected by this our study supports current.

5. Using some of antibiotics in the treatment of skin infections and compared alcoholic extract of Propolis in laboratory.

For the purpose of testing the sensitivity of fungi against a number of antibiotics fungal included Itraconazok, Terbinafin, Flouconazole, Ketoconazole. It was chosen as the sensitivity of fungi to antibiotics such as diffusion method in agar by discs (N.C.C.L.S; 1984). She Results described in a Table (4) that the fungus may be affected by antibiotics used as it reached the inhibition ratio (35.7, 39.8, 33.4%) antibiotic Terbinafin-fungal, *M. canis, M. gypseun, T. verrucosum,* respectively while the fungus did not show affected by the tested for antibiotic Flouconazole, if the inhibition ratio of 0%. 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 fungal cell nucleus which is more similar to cells in the human bacteria (Myrvik and Weiser, 1988; Patel *et al.,* 2000) show that antibiotic-derived compounds, Azole compould include Ketanozole and Clotrimzole and mincanzole and Econzole and other because it is easily absorbed in the intestine taken from oral and clotrimzole either using external ointment of Dermatology and as he cannot be given by mouth because it is toxic. The Oxiconazole external ointment is easily absorbed through the keratin layer of the skin

down to the deep layers of the skin and uses of Dermatology and yeasts (Harjan and Al-Khafaji, 2018).

On the other hand it found that Terbinafine and Naftifin affect the effectiveness of enzyme-fungal skin and can be taken by mouth and easily absorb in the gut (Brinboum, 1995; Degreef and DeDoncker, 1994) the treatment of skin diseases in general is extremely difficult because most do not have the only position Fungi static as well as high toxicity to humans of Like a lot of reason between the cellular and molecular characteristics between them and human (Mc Ginnis1980).

Table 4 : The use of some antibiotics in the treatment of some skin fungus

Antibiotic / fungi	Terbinafine	Itraconazole	ketoconazole	Flouconazole	Control
M. canis	35.7	29.7	29.9	0	0
M. gypseun	39.8	28.9	29.3	0	0
T. verrucosum	33.4	29.3	28.2	0	0

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