# Genotyping Tinea Capitis Fungi and Evaluate the Antifungal Activity of Plant Extract in Vitro

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## Abstract

Tinea capitis is a prevalent superficial fungal infection seen in developing nations predominantly in child, the aim of this study included isolating and identification of Tinea capitis fungi isolated from clinical samples who attenuate the outpatient clinics and other samples were collected from soils of barber shops, homes and mosques in Babylon / Hilla Province. 120 hairs, scales and soil were 80 clinical samples (hairs and scales), 30 soil samples, and 10 hair samples as control group. The results showed that: numbers of dermatophytes fungi were identified based on microscopic and Molecular criteria. *T. mentagrophytes, T. tonsurans, M. audounii, M. canis*. Also non dermatophytes fungus was *Chaetomium globosum*. Also three yeast species were identified: *Candida albicans, Candida parapsilosis*, *Malassezia* spp. based on molecular assays. The antimicrobial activity of plant extract antifungal shown, *Capparis spinosa* plant has highly antifungal activity shown inhibition on dermatophytes, followed by *Furcraea foetida* shown inhibition compare with activity of Fluconazole in vitro.

***Key wards:*** *Genotyping, Tinea capitis, Dermatophytes, Antifungal activity, Plant extract*

## Introduction

Dermatophytes are known as ring worm, dermatophytosis and Tinea [9,5]. Approximately 10-15% of the world's population has been found to have fungal infections [22]. Tinea capitis is a scalp skin-attacking disease that attacks hair follicles and shafts and causes eyebrow infection. The disease is regarded a shallow mycosis or dermatophytosis form.

Tinea capitis ' epidemiology differs across distinct geographic regions around the globe, and the species may alter in any specified region. Hot tropical humid climates, low socioeconomic status, crowded living conditions, and bad hygiene lead to Tinea capitis ' enhanced incidence. It is highly communicable and may reach epidemic proportions especially in overcrowded setups [16,8]. The aim of study conduct molecular typing of suspected fungi were associated with Tinea capitis samples, and those isolated from patients, and from soils of barber shops, Assessment of the effectiveness of plant extract antifungal activities and assessment of the effectiveness of conventional antifungals.

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## Material and Method

**Collection of Specimens:**

**1. Hair and scalps:** A 80 hair and scalps samples were collected in this study from all patients diagnosed with head lesions (clinical and barber shops).After that, the infection area was sterilized with 70% alcohol to remove the bacteria. Hair samples were collected using forceps and skin scraps from the scaly edge of the area using a surgical blade. The sample was transferred to the laboratory in sterile filter paper.

**2. Soil:** The soil of the barbers shops, house and mosques was collected and transported to the laboratory in sterile paper to be examined.

**3. Cultivation of Samples:**

All clinical samples collected were cultured on SDA agar media that containing chloramphenicol (250mg/l), Streptomycin (150mg/l) and cyclohexomide (250mg/l) then all samples incubated at (28-30°C) for two weeks. Then after the result of culture appeared the samples were examined morphologically from where the color of colony, shape, texture and reverse pigment. Then maintained the samples by using a screw-cup slant on SDA and preserved while the study [20].

All samples that give positive results of Tinea capitis purified by taking a pure colony from culture media that obtain a mixed culture.

**DNA extraction**:

The isolates of yeast and filamentous fungi under interest were subjected to DNA extraction. In brief; a loop full of *yeast* colonies and tiny portion of mycelia were suspended in the lysis buffer supplemented by Favrogen Yeast Extraction Kit (Korea) and following up the extraction steps based on the instruction of this Kit. A dry DNA pellet dissolved in diluted rinse and preserved in - 20 °C until use [13, 12, 1].

The genomic DNA was extracted according to [13], The PCR procedure was as previously described by [13,12,1]. The universal primer pair used in this study was ITS5/ITS4 Forward: (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and Reverse (5′-TCCTCCGCTTATTGATATGC-3′), the specific primer pairs for *Malassezia:* Malup (5′-AGCGGAGGAAA AGAAACT-3′), Mal down primer (5′-GCGCGAAGGTGTCCGAAG-3′) [17],

**Sequencing of Selected PRC Product**

A 10 dermatophyte isolates PCR products were sent to Korea's Macrogen Laboratory and obtained the positive sequence information for various species. The sequencing findings were subjected to those deposits in GenBank for sequence species identification for alignment with reference strains [11].

The Plant Extract Samples

Plant samples that used in this study, which comprised plant parts from the local markets for center of Babylon province. Then cleaned the plant from dust and impurities, Put in a flat bowl and leave to dry at room temperature, take a while, after the plants completely dry, then crashed by electric grinder well crashes [1].

## Static Analysis

Statistical analysis was performed with the use of SPSS version20.ANOVA test was used to compare distinctions between variables, at a probability level (≤ 0.05).

## Results and Discussion

Prevalence of dermatophytes among Tinea capitis sufferers:

A total of 120 samples, 110 samples were (80 hairs and scales and 30 soils) were collected from different age and genders of patients with Tinea capitis and 10 samples were collected by the same techniques from like healthy persons (as control samples), the clinical specimens were collected from private clinics in Babylon province and from barbers shops, the soil specimens were collected from the dust of the ground shops of barbers, houses and mosques. Identification results based on cultural and microscopic methods showed that 48 (60%) of 80 clinical samples were positive cases. The majority of the patients were males 38.75 %( 31⁄ 80) while, 21.25 % (17⁄ 80) specimen from females. The samples of soils were 30 samples (27.27%), the results were 1(3.33%) out of 30 samples were positive case. While only one specimen shown positive out of 10 samples from control group.

**The relations between the infection and age for both genders:**

It had been registered the number and percentage of infected patients by Tinea capitis , depending on gender and age ,table (1) had shown the significant difference between genders and infection with this disease , the number of males (31)(64.58%) and the number of females reached (17)(27.08%) to the consultative of the dermatology .

It was clear from the current study, males infection with Tinea capitis was (64.58%) while in females (27.08%) it was found existence of significant differences of the infection in both sexes agreed with results of other researchers outside of Iraq, including [15] who concluded that the Tinea capitis is a disease common in children

and occurs in males more than females , and [19] in the city of New Delhi, India, [3] in Kenya and [2] in Palestine and [18] in South Africa , [23] in Kashmir, who found that Tinea capitis affects males more than females in their studies.

**Table (1) Distribution of age and gender among patients of Tinea capitis.**

| Age/year | Gender | | | | Infection | |
|---|---|---|---|---|---|---|
| | Male | | Female | | | |
| | Number | percentage | Number | percentage | Number | percentage |
| 1 – 10 | 18 | 37.5% | 13 | 27.08% | 31 | 64.58% |
| 11 – 20 | 5 | 10.41% | 0 | 0% | 5 | 10.41% |
| 21 - 30 | 2 | 4.16% | 2 | 4.16% | 4 | 8.33% |
| 31 – 40 | 3 | 6.25% | 1 | 2.08% | 4 | 8.33% |
| 41 – 50 | 0 | 0% | 0 | 0% | 0 | 0% |
| 51 – 60 | 3 | 6.25% | 1 | 2.08% | 4 | 8.33% |
| Total | 31 | 64.58% | 17 | 35.41% | 48 | 99.99% |

Males' sensitivity to tinea capitis. The elevated rate of tinea capitis in males can also be ascribed to simple sporal implantation owing to short hair and frequency sharing of comb, brushes and caps [10].

**The Identified fungi:**

A total of 11 fungal isolates have currently been identified based on evidence of morphological, microscopic, and molecular analysis. The dermatophytes were: *T. mentagrophytes, T. quinckeanum, T. tonsurans, M. audonii, M. persicolor, M. canis,* and non dermatophytes was*: Chaetomium globosum*. Also, two *Candida* spp. were *C. albicans, C. parapsilosis,* and *Malassezia* spp.

**Table (2): Fungal isolates from hair infected with Tinea capitis.**

| Fungal isolates | No. of isolates | Percentage of frequency | Males (%) | Females (%) |
|---|---|---|---|---|
| Chaetomium globosum | 3 | 6.52% | 2(7.14%) | 1(5.55%) |
| M. audounii | 21 | 45.65% | 12(42.85%) | 9(32.14%) |
| M. canis | 2 | 4.345 | 2(7.14%) | 0(0%) |
| M. ferrugineum | 1 | 2.175% | 1(3.57) | 0(0%) |
| M. persicolor | 9 | 19.56% | 4(14.28%) | 5(27.77%) |
| T.mentegrophytes | 1 | 2.17% | 1(3.57%) | 0(0%) |
| T. quinckeanum | 1 | 2.17% | 0(0%) | 1(5.55%) |
| T. tonsurans | 5 | 10.86% | 4(14.28%) | 1(5.55%) |
| Unidentified fungi | 3 | 6.52% | 2(7.14%) | 1(5.55%) |
| **Total** | **46** | **100%** | **28(60.86%)** | **18(39.13%)** |

Table 2 shows the types of dermatophytes isolated from the human, the most common isolate predominant was *M. audonii which* represent 21 (45.65%) of cases followed by *T. persicolor* 9 (19.56%). It showed the presence of differences between sex and species of the fungi isolated.

**Molecular identification**

**A. Molecular typing by ITS region**:

The particular results were 12 dermatophytes and non-dermatophytes based on the rDNA region ITS1-5.8S-ITS2, 28S partial sequence). The dermatophytes were: *Microsporum* spp*; Trichophyton* spp.; *Cheatomum* spp., *Candida* spp.; *and Malassezia* spp. Also, some isolates of molds Figure (1).

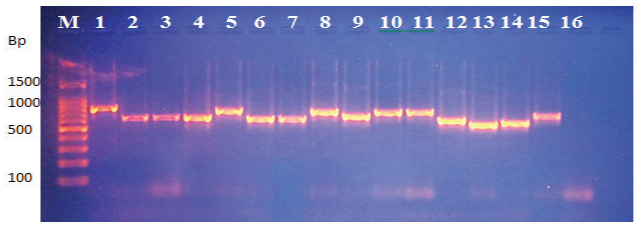


**Figure (1):** Profile gel electrophoresis of PCR products of 12 dermatophyte and non-dermatophyte isolates of fungi amplified by ITS5/ITS4 primer: Lanes (left to right): isolate of 1- *M. audouinii (*800 bp); 2, 6, 7- *Chaetomium globosum* ; 3- ,4 unidentified fungi (600 bp) ; 5- *T. mentagrophytes*(780 bp ) ; 8, 9 - *T. queckanum* (700 bp) ; 10- unidentified fungi ; 11- *T. tonsurans* ( 700 bp ) ; 12 - *M. persicolor* ( 700 bp ), these results according to sequence analysis ; 13 , 14 *Candida albicans* (500, 510 bp ) ; 15 - *Candida parapsilosis* Molecular M (100bp for each step.). 1.6% agarose gel at 70 volt for one hour.

There are high variations in PCR products among of fungi under interest as in used ITS5 / ITS4 as primer pair for amplification of ITS region. ITS region consider good barcoding region due to high variation for another ward the amplification of this region show high polymorphism in PCR product. (*Chaetomium globosum , M. audouinii , M. canis , M. ferrugineum , M. persicolor, T. mentagrophytes , T. quinckeanum , T. tonsurans* ) these range not always require for other specific genetic analysis. [6]

Shown that all *Malassezia* spp., shown monomorphic bands, approximate 605bp of PCR products. Figure (2), these results consistent with [17].

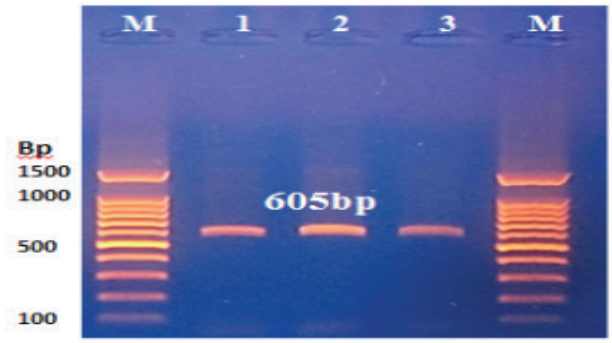


**Figure (2): Electrophoresis gel profile of PCR amplification products. All samples yielded a single band of approximately 605 bp.:1-3 isolates of *Malassezia* spp. Molecular marker: M1= (100bp).**

The current study agreed with [7] concluding that M. Globosa appears to be the most prevalent pathogen responsible for Tinea capitis, as well as , also *M.furfur* identified one of causative agent to cause Tinea capitis [21].

**DNA sequence analysis:**

About16 µl of PCR products were sent to Macrogen Laboratory in Korea for sequencing analysis. After the sequence results had been obtained, each sequence alignment with the sent isolate's nitrogen bases sequence. They correspond to reference sequencing samples in the gene bank using NCBI Blast Nucleotide.

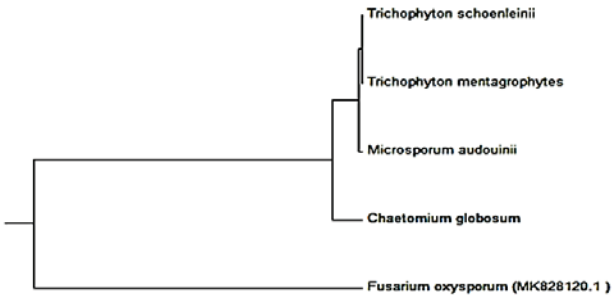


**Figure (3): Phylogeny construction for all identified isolates based on sequence charts with one reference strain (marked with the accession number, while our isolates have full names).**

**Antifungal Activity of Some Plant aqueous extracts against dermatophytes spp.**

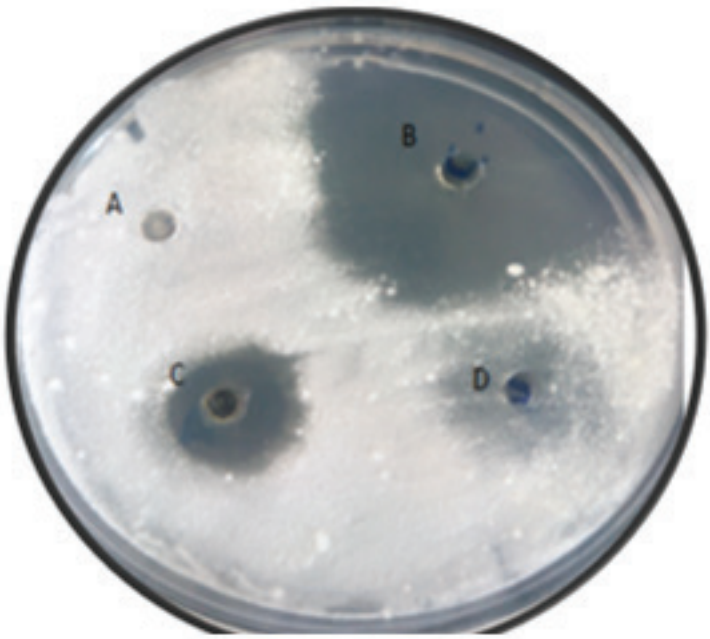


**Figure (4): Inhibition growth of aqueous plant extracts compared with the antifungal fluconazole, A. Water as control treatment, B. Effect of *Capparis* plant, C. effect of *Furcraea* plant, D. effect of the antifungal Fluconazole.**

In recent study 3 isolates of dermatophytes are used to study the effectively of this plants extracts, the concentration of plant aqueous extracts was (5%).The plant extracts showed inhibition of dermatophyte growth, the aqueous plant extract from Capparis gives the highest inhibition zone respectively followed by plant extract from Furcraea, while the antifungal fluconazole was less inhibited compared to those plants. The sensitivity of fungus Figure (5).

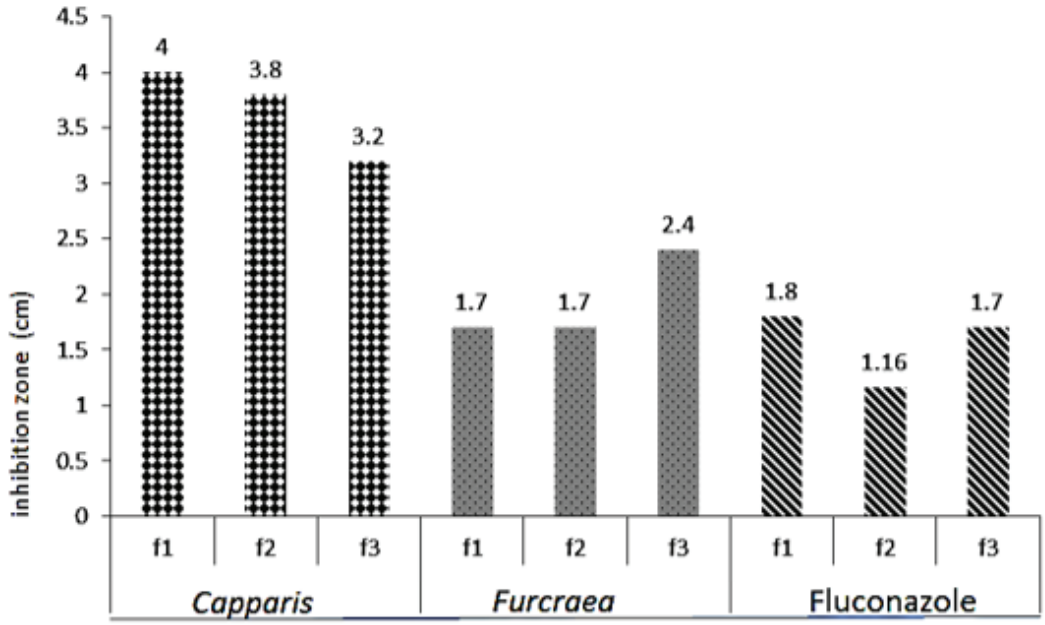


**Figure (5) Comparison of antifungal agents activity between plant type (*Capparis*, *Furcraea*) and Fluconazole dermatophytes growth. The species of dermatophytes were (f1: *T. tonsurans,* f2: *M. ferrugineum ,* f3: *M. audonii*),**

Figure (5) shown that the dermatophytes strains shown significant difference of response, the highest effect of inhibition was *Capparis* and *T. tonsurans* the most fungus in response to the extract was the mean (4 cm) followed by *M. ferrugineum* (3.8 cm) and *M. audonii* (3.2 cm). *Furcraea* was less inhibited than *Capparis*; *M. audonii* was the highest fungus in response to (2.4 cm) of the plant while *T. tonsurans* and *M. ferrugineum* equal response (1.7 cm). Fluconazole was the least fungal response compared to plants and *T. tonsurans* was the highest fungus in response to antifungal (1.8 cm), followed by *M. audonii* (1.7 cm) *M. ferrugineum* the lowest fungus (11.6 cm). There were significant differences between *Capparis* plant and *Furcraea* and Fluconazole, while *Furcraea* showed no significant differences between it and the antifungal.

The conclusion of this study gave attention that particular study about Tinea capitis more producible in their result compared with survey studies of dermatophytes and non dermatophytes fungi in whole human body. The results of our study after isolated and identified 8 dermatophyte and non-dermatophyte fungi and 3 yeasts. The antifungal activity of plant extract of *Capparis* and *Furcraea* plants shown highly inhibition compared with Fluconazole, this result was light on uses natural product more effective and revealed rise of Fluconazole resistance by dermatophytes.

**Ethical Approval :** Both authors hereby declare that all actions have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

**Conflict of Interests** : The authors did not declare any conflict of interest.

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