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التشخيص الجزيئي وتقييم كفائة بعض مركبات الايض الثانوي المستخلصة من نبات الحنظل ضد الفطريات الجلدية

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وهي جزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قبل

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College of Science for Women

Department of Biology

Molecular Identification and Evaluation Efficiency of some Secondary Metabolites Extracted from *Citrullus colocynthis* Against Dermatophytes

A Thesis Submitted to
The Council of the College of Science for Women, University of Babylon in Partial Fulfillment of the Requirements for M.Sc. Degree in Biology
By

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بسم الله الرحمن الرحيم

﴿ قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ ﴾

صدق الله العلي العظيم

سورة البقرة

الايه رقم (٣٢)

Dedication

To …

Great heart .. Dear husband

Great love .. My son and daughter

My family .. Father, Mother and brothers

My second family .. uncle Abu Ali's house

Helper , supporter & friends

Umniyah 2023

Acknowledgments

I would like to express my thanks to "Allah" the Most Gracious and Most Merciful, and to His prophet "Mohammad".

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Conclusions

1- The percentage of clinical cases was heights with Tinea corporirs followed by Tinea capitis and Tinea unguium, and the females were more than of males.

2- Various factors including age, gender, and residency had important effects on the incidence of clinical infections.

3- Seven species have been isolated from dermatophytes, the highest species regarding frequency of infection was *T. mentagrophytes* followed by *T. interdigetale*.

4- Alkaloids, Flavonoids, and Terpenoids extracted from the fruits of (*Citrullus colocynthis* L.) have powerful antifungal activity against dermatophytes species.

5- One of the most effective compounds in inhibiting the growth of dermatophytes is the crude alkaloid extract of *Citrullus colocynthis* L.

Recommendations

1- Study the relationship between the fungal infection with some genetic characteristics.

2- Search for other local plants and study their biological activities to benefit from them as therapeutic alternatives against various diseases.

3- Using modern methods to obtain active substances from plant extracts.

4- Studying the antifungal activity of plant extracts of *Citrullus colocynthis* on other types of pathogenic microorganisms.

5- Combination between antifungal agents and plants extracts and it might be useful to accelerate the clinical and microbiology healing of a superficial infection.

6- Further studies are required to evaluate the effect against dermatophytes in vivo.

الخلاصة

أجريت الدراسة في الفترة من اكتوبر ٢٠٢١ إلى فبراير ٢٠٢٢ ، تم جمع ٦٠ عينة من المصابين بالامراض الفطرية الجلدية من قسم الامراض الجلدية في مستشفى مرجان (مدينة مرجان الطبية) والعيادات الخاصة لاستشاري الامراض الجلدية في محافظة بابل وقد شخصت سريريا من قبل الطبيب، وشملت العينات ٨ عينة أظافر و ١٥ عينة شعر و ٣٧ عينة قشور جلدية ، وأجريت دراسات مختبرية على هذه العينات مثل الزرع المختبري على وسط السابرويد اكار ، للوصول إلى تشخيص دقيق للعزلات باستخدام الطرق التقليدية ومقارنتها بالتقنيات الجزيئية باستخدام بادئات محددة للفطريات الجلدية وتسلسل القواعد النيتروجينية ورسم الشجرة التطورية للأنواع المتقاربة وراثيا.

وأظهرت النتائج أن(٥١.٣٪) من الاختبارات الإيجابية كانت لدى الإناث بالفحص المجهري المباشر ، بينما كانت (٤٨.٧٪) إيجابية عند الذكور. سجلت السعفة الجسدية أعلى نسبة تكرار للفطر الجلدي بنسبة (٤٥٪) ، يليها في المرتبة الثانية سعفة الرأس بنسبة (٢١٪)، بينما احتلت سعفة الأظفر المرتبة الثالثة بنسبة(١٣.٣٪) ، وسعفة اليد كانت أقل تكرارا بنسبة (٣٪). أظهر الفطار الجلدي علاقة معنوية بالجنس لأن الإصابة كانت أعلى في الإناث منها في بعض العزلات بما في ذلك سعفة الأظفر ، سعفة الذقن ، سعفة اليد ، والتي بلغت(١١.٧٪) ، (١٠٪) و (٣.٣٪) على التوالي ، في حين أن نسبة السعفة الجسدية و سعفة الرأس وسعفة القدم ٢٥٪ و ١٦.٦٪ و ٥٪ على التوالي أعلى في الذكور منها عند الإناث.

اظهرت الدراسة أن جميع أنواع السعفات اعطت نسبة اكبر للأصابة في المرضى الذين يعيشون في المناطق الريفية من أولئك الذين يعيشون في المناطق الحضرية ،توزعت الاصابة بالفطريات الجلدية على ما يقرب من ثلاث فئات عمرية المجموعة الأولى من ١-٢٠ سنة ، المجموعة الثانية من ٢١-٤٠ سنة والمجموعة الثالثة من ٤١-٦٥ سنة ، لكن أعلى نسبة للسعفة كانت للمجموعة الأولى (٥٦.٧٪) ، بينما المجموعة الثانية والثالثة كانت (٢٠٪) و (٢٣.٣٪) على التوالي. لم يكن هناك ارتباط معنوي بين الفطار الجلدي و داء السكري فقد كانت نسبة المصابين (٢٣,٣٪) من غير المصابين بمرض السكر (٧٦,٧٪) كما أن نسبة الإصابة لدى المرضى الذين يمتلكون أو يلامسون الحيوانات الأليفة (٤٠٪) بينما أولئك الذين لايمتلكون هذه الحيوانات (٦٠٪).

تم عزل وتشخيص سبعة أنواع من الفطريات الجلدية من الحالات البشرية السريرية ، كان اكثرها تكرارا *Trichophyton. mentagrophytes* بنسبة ٢١,٧٪ يليه *T. interdigetale* بنسبة ١٨,٣٪ ،و *Trichophyton. quinckeanum* ١٦,٧٪ في حين *chrysosporium tropicum* ١٠٪، و *Microsporum. canis* ٨,٣٪ و *T. violaceum* ٦.٧٪ ، و *Epidermophyton . floccosum* ٥٪ .

يشمل التشخيص الجزيئي استخدام زوج من البادئات ITS5 و ITS4 لمنطقة ITS (Internal transcribed spacer region). وتمت مطابقة تتابع القواعد النتروجينية لبعض عزلات أنواع الفطريات الجلدية مع تسلسل العينة المرجعية في بنك الجينات باستخدام برنامج قاعدة بيانات NCBI Blast Nucleotide وكانت النتائج مطابقة للتشخيصات المظهرية لمعظم العينات قيد الدراسة كما نجحت هذه التقنية في تشخيص العزلات التي لم نتوصل الى نوعها

بأستخدام الطرق التقليدية، واظهر تحليل الشجرة التطورية للأنواع قيد الدراسة درجة القرابة الوراثية بين هذه الأنواع.

تم دراسة النشاط المضاد للفطريات لبعض العقارات بما في ذلك Fluconazole (Flu) و Itraconazole (It) و Griseofulvin (Gr) مع سلسلة من التراكيز (٠.٢٥٪ ، ٠.٥٪ ، ١٪) ضد خمسة أنواع من الفطريات الجلدية وهي *Chrysosporium*، *T. mentagrophytes*، *M. canis*، *T. quinckeanum*، *T. interdigitale* وأظهرت النتائج أن أفضل مضاد للفطريات هو (Flu) يليه (Gr) و (It). كانت من أفضل التراكيز التي أثرت على تثبيط نمو المستعمرات الفطرية قيد الدراسة هو١٪ ، وكان أكثر الفطريات حساسية لمضادات الفطريات *M. canis* يليه *T. quinckeanum*.

يتم استخدام النشاط المضاد للفطريات لمستخلصات الأيض الثانوية لنبات الحنظل Citrullus colocynthis منها القلويدات الخام ، و التربينويدات الخام ، و الفلافونويد الخام بثلاثة تراكيز (٥ ، ١٠ ، و ١٥ مجم / مل) ضد خمسة أنواع من الفطريات الجلدية *Chrysosporium* ، *T. mentagrophytes* ، *M. canis*، *T. quinckeanum*، *T. interdigitale* من افضل التراكيز المثبطة لنمو الفطريات هي ١٥٪ ، المستخلص الأكثر فعالية هو القلويد الخام يليه تربينويد الخام والفلافونويد الخام ، وأكثر الفطريات تأثراً في تجربة المستخلصات كانت *M. canis* تليها *T. quinckeanum*.

summary

The study had been conducted in period from October 2021 to February 2022, 60 specimen were collected from people infected with dermatophytosis in dermatology department from Marjan Hosptial (Marjan medical city) and private clinics of Consultant Dermatologists in Babylon province and clinically diagnosed by a physician, the specimen included 8 nails clipping, 15 hair fragment and 37 skin scrapings, laboratory studies were conducted on these specimen such as laboratory culture of SDA (Sabouraud Dextrose Agar), to reach an accurate diagnosis of isolates using the conventional methods and compared with molecular techniques by using specific primers for dermatophytes and a sequence of nitrogenous bases and drawing the evolutionary tree of species converged genetically.

The results showed that 51.7% of the positive tests were in females by the direct microscopically examination, while it was 48.3% positive results in males. Tinea corporis recorded highest percentage frequency of dermatophytoses (45%), followed in second order is Tinea capitis was recorded by (21.7%), while the Tinea unguium came third at (13.3%), and Tinea manuum was lowest frequency (3.3%), the distribution of dermatophytoses showed significant relation to gender because the infection was higher in females than in males in certain isolates including Tinea unguium, Tinea cruris , Tinea manuum , that rated11.7%,10% and 3.3% respectively, while the ratio of tinea corporis, tinea capitis, and tinea pedis 25%,16.6%, and 5% respectively higher in males than in females.

All kinds of tinea were recorded higher occurrence in patients who live in rural areas from those who live in urban areas, The dermatophytoses have distributed to almost three age groups first group from 1-20 years, second group from 21-40 years and third group from

41-65 years, but the highest rate of tinea was of first group (56.7%), whereas the second and third group were (20%) and (23.3%) respectively. There was no significant correlation between Dermatophytosis and Diabetus Mellitus (23.3%) than non- Diabetic patients (76.7%) Also, the percentage of infection in patients who own or live with domestic animals was (40%) while those who did not live with these animals (60%).

Seven species of dermatophytes were isolated and diagnosed from clinical human cases, *Trichophyton. mentagrophytes* was the most (21.7%), followed by *Trichophyton. interdigetale* (18.3%), while *Trichophyton. Quinckeanum* (16.7%), *Microsporum canis* (8.3%) , *Trichophyton. violaceum* (6.7%) and *Epidermophyton floccosum* (5%) as well as *chrysosporium tropicum* (10%).

Molecular diagnosis include using a pair primers ITS5 and ITS4 for ITS region (Internal transcribed spacer region). Sequencing of nitrogen bases for some isolates of dermatophytes species were matched with the reference sample sequence in the gene bank using the NCBI Blast Nucleotide Database program and the results were identical to phenotypic diagnoses for most of the specimen under study.

The antifungal activity are used including Fluconazole (Flu), Itraconazole (It), and Griseofulvin (Gr) with series of concentration (0.25%, 0.5%, 1%) against five species *Chrysosporium, T. mentagrophytes, M . canis, T. quinckeanum, T. interdigitale* The results show that the best antifungal is (Flu), followed by (Gr), and (It). The best concentrations affecting the inhibition of the growth of the fungi colonies under study were 1%, the most fungi that were sensitive to antifungals drug is *M. canis* followed by *T. quinckeanum*.

Antifungal activity of Secondary Metabolites extracts of plant *Citrullus colocynthis* for crude Alkaloid, crude Terpenoid, and crude Flavonoid were studied in three concentrations (5, 10, and 15 mg/ml) are

used gainst five species *Chrysosporium, T. mentagrophytes, M . canis, T. quinckeanum, T. interdigitale* The best concentrations inhibition the growth of the fungi is 15%, the most effective extract is crude Alkaloid followed by crude Terpenoid and crude Flavonoid, and the most affected fungi in the extracts experiment were *M. canis* followed by *T. quinckeanum* .

Chapter One

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Appendix

Introduction

Skin fungal diseases are among the most common diseases in humans, as they have increased recently and are considered one of the most common diseases in the world It affects 10-20% of the world's population (Grumbt *et al*., 2011). Dermatophytes comprise three genera of keratinous fungi and keratolytic fungi. Since dermatophytes are keratinous fungi, they parasitize on corneal structures, such as the skin, hair, or nails. They are highly attracted to keratin-containing tissues, which makes them responsible for superficial dermatophytosis (tinea corporis, tinea capitis, tinea facial, tinea barbie, tinea sciatica or tinea pedis), hair (tinea capitis) and nails (tinea cruris) (Kalenowska *et al*., 2009).

Dermatophytes are equipped with many enzymes, which support their survival on the skin and its appendages, as they possess proteolytic, keratinizing and lipolytic activity. The surface of the epidermis contains proteins, carbohydrates and micronutrients (including iron ions), which may provide substrates for the metabolism and survival of fungi (Kobierzycka *et al*., 2005). The genomes of dermatophytes comprise a broad repertoire of genes encoding hydrolytic enzymes, in particular proteases, which are highly similar from one species to another (Martinez *et al*., 2012).

Dermatophytes (*Trichophyton*, *Microsporum*, *Epidermophyton*) are known in laboratory practice. *Trichophyton* and *Microsporum* genera are the most frequently classified groups, and more than 40 species belong to these two species. Polymerase chain reaction (PCR) is a major advance in the diagnosis of dermatophytosis because results are already available within (1-2) days and also allows detection of multiple pathogens (Gräser *et al*., 2012; Jensen and Arendrup 2012).

Antifungal drugs, primarily topical, oral, and intravenous, are used to treat various types of fungal infections; however, oral antifungal drugs are more toxic to

the human body as compared to topical antifungal drugs. Additionally, commonly used antifungal drugs contain different types of broad categories of components such as azole, echinocandin, and polyenes (Nami *et al*.,2019).

Several azoles are available for topical use. But the drug that commonly use drugs are clotrimazole and miconazole. Both of them used for athlete's foot, otomycosis, oral, cutaneous and vaginal candidiasis, pityriasis versicolor and dermatophytic infections including tinea corporis, tinea pedis and tinea cruris (Weinstein, and Berman, 2002).

Plants are an important source for the discovery of new products of medicinal value for drug development and plants secondary metabolites are unique sources for pharmaceuticals food additives, flavors, and other industrial values. The commercial importance of these secondary metabolites has resulted in a great interest in its production and in exploring possibilities of enhancing its production using tissue culture technology in recent years (Chitra *et al*., 2019).

In plants, terpenes and terpenoids are important mediators of ecological interactions. For example, they play a role in plant defense against herbivory , disease resistance, attraction of mutualists such as pollinators, as well as potentially plant-plant communication (Martin *et al*., 2003; Pichersky, 2006). They appear to play roles as antifeedants, other functions of terpenoids include cell growth modulation and plant elongation, light harvestation and photoprotection, and membrane permeability and fluidity control (Davis and Croteau, 2000).

Flavonoids are the most common and widely distributed group of plant phenolic compounds, occurring virtually in all plant parts, particularly the photosynthesizing plant cells. They are a major coloring component of flowering plants (Koes *et al*.,2005). Alkaloids are pharmacologically active compounds in

that they affect the central nervous system, reduce appetite and act as diuretic (Yadav *et al*., 2014).

Aim of study

Due to the difficulty of treating some skin diseases, especially those caused by dermatophytes, the current study aimed to test the efficiency of some Secondary Metabolites Extracted from *Citrullus colocynthis* Against Dermatophytes and compared with antifungal . This investigation has been done by the following steps:

1- Isolation of dermatophytes from clinical cases with dermatophytoses and take the patients' medical history.

2- Identification of fungal isolates by conventional and molecular methods and draw the phylogenetic tree for fungal isolates based on sequencing analysis.

3- Testing the efficiency of antifungal on isolated fungi.

4- Study the antifungal activity of some plant extracts against dermatophytes.

2.1. Dermatophytes

Dermatophytes are a group of fungi that have the ability to invade keratinized tissues, in humans and animals, causing skin infections called tinea or "ringworm". It mainly relies on keratin as a source of nutrients because it can cause its hydrolysis. Dermatophytes remain in the dead tissue and do not invade the living part of the tissue and only affect the skin, hair and nails (Achterman and White, 2012). The name dermatophye is derived from the Latin words 'derm' which means skin and 'phytes' meaning plants. In fact, the fungi known as dermatophytes are not plants and are not limited to the skin, but can infect hair and nails (Refai *et al*., 2013). Accordingly, the name is incorrect, but was used because it is traditionally applied to this group of fungi and no other name has been proposed. Dermatophytes is a type of fungus that have the ability to attack keratin tissue of animals and humans causing *Dermatophytosis* and includes (*Trichophyton, Epidermophyton, Microsporum*) (Jochen and Yvonne , 2005).

The infections caused by these fungi are usually named after infected part of the body rather than the infecting organism. Fungal infections can cause illness in immunosuppressed persons, mainly those suffering from transplants, chemotherapy, and human immunodeficiency virus-positive patients, which can be cured with suitable antifungal treatment (Rouzaud *et al*., 2015). Dermatophytosis is described either by the unusual range of skin surface part affected by the infection or by the uncommon amount of affected locations. However, the infection remains confined to the epidermis or associated keratinized structures such as nails (Mansouri *et al.*, 2012).

The greatest valuable dermatophytes isolated in Europe are *T. rubrum, M. canis, T. mentagrophytes* var. *granulosum* and *T. verrucosum*. Numerous of these are supposed to have expanse from the Mediterranean Countries. Further dermatophytes such as *M. audouinii*, *T. soudenense* and *T. violaceum* are endemic

in Africa and Asia are now hardly ever isolated in Europe . In Asia *T. rubrum* and *T. mentagrophytes* are the greatest usually isolated pathogens, causing tinea pedis and tinea unguium (Menon and Routray, 2015).

2.2. Importance and Distribution

The group of dermatophytes comprises 52 keratin-degrading species divided into nine genera: *Trichophyton*, *Microsporum*, *Epidermophyton*, *Arthroderma*, *Lopophyton*, *Nannizia*, *Ctenomyces*, *Guarromyces*, and *Paraphyton* (Mercer and Stewart, 2019). Among the fungi causing dermatophytoses in humans, the filamentous fungus *Trichophyton. rubrum* is the main causative agent of cutaneous infections of the feet, nails, and body (Antuori *et al*.,2019). Dermatophytoses affect individuals worldwide but their incidence is higher in tropical countries because of high temperatures and humidity (Taplin, 2001). Factors that influence the development of dermatophytoses include age, sex, season of year, socioeconomic and cultural conditions, and geographic location it estimated the individuals are contaminated with dermatophytes at some point in their life about 10 to 15% (Pires *et al*.,2014).

According to data from the World Health Organization (WHO), dermatophytoses affect about 25% of the world population (Sai *et al*.,2019) and 30 to 70% of adults are asymptomatic carriers of these diseases. In Western countries, 80 to 90% of onychomycosis cases are primarily caused by dermatophytes, with 5–17% being due to yeasts and 2–3% to nondermatophyte molds. In southern European countries, dermatophytes are the causative agents of 40 to 68% of cases, with 21–55% being due to yeasts. In Asian and Middle Eastern countries, dermatophytes account for 40 to 48% of cases, with 43–46% of infections being caused by yeasts and 8–11% by non-dermatophyte molds. Comparatively, in

Africa, onychomycosis-related infections are predominantly caused by yeasts (Gupta and Stec,2019).

The risk of onychomycosis increases with age, probably due to the presence of diabetes, poor peripheral circulation, longer exposure to pathogenic fungi, repeated nail trauma, and suboptimal immune function. A family history of onychomycosis is another risk factor (Gupta and Stec,2019). *Trichophyton rubrum* and *E. floccosum* are dermatophytes found worldwide, with *T. rubrum* being the most common species (Cordeiro,2015).

It is clear that the names of the genera or fungi were coined on clinical basis as in case of *Achorion* or *Epidermophyton* or on the type of hair invasion, as in case of *Microsporum* or *Trichophyton*. The species took the names of scientists as in case of Schoenlein, Megnin, Audouin and Quinck or the host as in gallinae and canis. With the introduction of solid media by Robert Koch in 1880, morphology of dermatophytes on culture led to names as in case of rubrum and gypseum (Refai *et al*.,2013).

2.3. Ecological Classification of Dermatophytes

Dermatophytes have improved to infect animals and humans are therefore grouped into geophilic, zoophilic, and anthropophilic species founded on their foremost habitation or host. Three dermatophytes genera *Trichophyton*, *Microsporum*, and *Epidermophyton*-comprise more than 40 diverse species, animals can be infected by a excessive diversity of dermatophytes, generally zoophilic but likewise geophilic species, and exceptionally anthropophilic dermatophytes (Dimple *et al*., 2016). Geophilic dermatophyte species live in the environment where there is interaction with keratinized tissue, anthropophilic dermatophytes specialist for cause humans infection only, and zoophilic dermatophytes infect animals and, rarely, humans. Arthrospores present in the

environment or shed skin scales are responsible for inoculation (Rouzaud *et al*., 2015).

Table (2-1): The environmental and geographical distribution of dermatophytes .(Howard *et al* 2003).

Anthropophilic	Zoophilic	Geophilic
Cosmopolitan		
E.floccosum	*M. canis*	*M. cookie*
M. audouinii	*var. canis*	*M. fulvum*
T. mentagrophytes var. interdigitale	*M. equinum*	*M. gypseum*
	M. gallinae	*M. nanum*
T. rubrum	*T. equinum*	*M. persicolor*
T. tonsurans	*T. mentagrophytes var. mentagrophytes*	*T. ajelloi*
var. tonsurans		
var. sulfureum	*T. verrucosum*	
	var. album	
	var. ochraceum	
	var. discoide	

Some types of dermatophytes are also very closely geographical spread while other types are universal ,the strain *T. rubrum var. raubitschekii* isolated as

third case of Tinia cruris infection in Spain (Moyano *et al*.,2010). As the fungus *T. vanbreuseghemii* isolated from some forest in Iran which considered as type of geophilic fungi and in rare cases possible to cause Tinea capitis (Moallaei *et al*.,2006). *T. fischeri* Fungus is one strain of *T. rubrum* and considered non-pathogen but was isolated in rare case in patient with AIDS (Rosenthal *et al* .,1998).As the growth of dermatophytes occurs in warm and wet environments so their spread mainly in tropical and semi-tropical areas (Hainer, 2003).

2.4. Genera of dermatophytes

2.4.1. *Trichophyton*

Trichophyton genus, contains the parasitic varieties that occasion tinea, including athlete's foot, ringworm, jock itch, and similar infections of the nail, beard, skin and scalp. *Trichophyton* fungi are molds characterized by the evolution of both smooth-walled macro- and microconidia (Lorch *et al*., 2015). Members of the genus *Trichophyton* are the popular agents of dermatophytoses. They are specially significant in onychomycosis but also invade skin and hair, causing infection associated with substantial morbidity (Scher and Baran, 2003). Though *T. rubrum* and *T. mentagrophytes* are the most frequent pathogens (27 to 76% and 4 to 41%, respectively) (Kardjeva *et al*.,2006), *T. tonsurans, T. violaceum*, and *T. soudanense* are also important. Moreover , there are differences in clinical associations and in geographic distribution between species (Ohst *et al*., 2004).

2.4.2. *Microsporum*

The hair and skin affects by 16 species such as *M. canis* and *M. gypseum Microsporum canis* is a filamentous fungus that can source superficial fungal infections in animals and humans (Moriello,2014). This Species of dermatophytes places veterinarians, animal care staff, and owners at risk of infection. Although dermatophytosis is generally self-limiting in immunocompetent individuals, the

zoonotic and contagious nature of the infection and its propensity to infect children and medically underserved populations categorize it as an agent of worry (Mushtaq *et al*.,2020).

M. gypseum is a geophilic dermatophyte, it worldwide in the soil in its natural reservoir, where it colonizes keratinous substrates as a Saprophyte (Ginter,2009). in the natural environment It is a part of the diverse fungal microflora . The complex of *M. gypseum* combine of three anamorphic species, *M. gypseum*,*M. fulvum* and *M. incurvatum* (Rezaei *et al*.,2017).

2.4.3. *Epidermophyton*

It is effects on the skin and nails and does not affect hair. It loss small spores and produces only large spines with smooth, swollen walls at the top. It consists of 1-4 cells. These spores appear bundled and colonies take a yellowish green powder (Tortora *et al.*, 1998; Rook and Ebling, 1998).

2.5. Clinical Types of Dermatophytoses

The infection of dermatophytes can appear in different dissemination areas of the body for this classified according to the area :

2.5.1. Tinea capitis: is the infection of the scalp and may be classified as scaly, black-dot , kerion and favus. Infection with *M. audouinii* and *M. canis* is characterized by small-spore ectothrix hair invasion, where the spores are surround the hair shaft, and the hairs fluoresce green under the U.V. light (Glaven *et al*., 2013). Large-spore ectothrix hair invasion is seen in case of infection with *M. gypseum, T. verrucosum and T. mentagrophytes*. Infection with *T. violaceum* and *T. tonsurans* is characterized by endothrix hair invasion, the inside of the hair may fully filled with spores and the hair may be break and the remaining part appears as a black dot. In case of favus wide hyphae and air spaces are seen (Refai *et al*., 2013).

2.5.2. Tinea pedis: also called foot ringworm is an infection of the feet affecting soles, interdigital clefts of toes, and nails with a dermatophyte fungus. It is also called athlete's foot ,The infection is caused by the dermatophyte, *Trichophyton rubrum* which was once endemic to numerous parts of Africa, Asia, and Australia (Rajagopalan *et al*,.2018). Fundamentally, *T. rubrum* causes tinea pedis, *T. interdigitale* and *E. floccosum* are also involved. Other occasional agents include *T. violaceum*. *T. rubrum* counting for about 70% of the cases (Lipner and Scher,. 2019).

2.5.3. Tinea corporis : is a disease that affects the smooth areas of the skin as the trunk , shoulders or extremities and sometimes face ,this type can cause all kinds of dermatophytes, the lesions caused by humans species (Anthropophilic) and be round or oval which diffusion is peripheral , it is a mild inflammation with redness and slightly (Sahoo and Mahajan, 2016). The infections caused by species of animal origina (zoophilic) its characterized by acute inflammation and a large flaking separating the area of injury from the skin is healthy and oftentimes accompanied by itching (Kalinowska, 2012).

2.5.4. Tinea Unguium: Onychomycosis is caused by dermatophytes, yeasts or non-dermatophyte molds; when caused by dermatophytes, it is called tinea unguium. The main etiological agents are *T. rubrum* and *T. interdigitale*. The most frequent types are distal and lateral subungual onychomycosis. Diagnosis usually requires mycological laboratory confirmation. Dermoscopy can be helpful and also biopsy is an excellent diagnostic method in uncommon cases or when mycological test is negative. Treatment must be chosen according to clinical type, number of affected nails and severity. The goal for antifungal therapy is the clearing of clinical signs or mycological cure (Daniel *et al*,.2017).

2.5.5. Tinea cruris: are the infections of the thigh areas, around the anus, and sometimes the upper thighs. It is commonly caused by *E. floccosum, T. rubrum and T. violaceum* (Nejad *et al* ., 2007; Dogra and Uprety, 2016).

2.5.6. Tinea faciei: is a relatively uncommon superficial dermatophyte infection that occurs on the smooth regions of the face and affects both sexes and all age groups (Ebrahimi et al., 2019). the causative agents of the infection are mainly *M. canis, T. mentagrophytes* and *T. rubrum* (Ansari *et al*., 2021).

2.5.7. Tinea barbae: is a rare dermatophyte infection affecting the skin, hair, and hair follicles of the beard and mustache, Tinea barbae is also known as tinea sycosis, as one of the clinical manifestations is inflammation of the hair follicles (Furlan *et al*.,2017).it is exclusively caused by zoophilic and anthropophilic dermatophytes, *T. verrucosum, T. mentagrophytes*, and *T. rubrum* are the most commonly reported causative organisms (Duarte *et al*., 2019).

2.5.8. Tinea manuum: the disease may occurs in one or both hands, which is less spread than the foot often cause the dryness, itching and scaling on the soles of the hand especially when the cause is the *Trichophyton* species of Anthropophilic (Perez-Gonzalez *et al* ., 2009).

2.5.9. Tinea imbricate : They appear in the form a series of concentric rings, covering the upper body and causing severe scalying of affected areas, which are uncommon cases caused by fungus of *T. concentrium* (Arenas, 2002).

2.6. Skin Immunity to Dermatophytes

The skin is the most extensive organ of the body, is an ecological niche for microbiota and the first barrier against aggression of environmental noxa and pathogenic microorganisms. It is not only a physical barrier but also a dynamic system constituted by the skin resident immune system that is critical to control an infection, resolve damage, or maintain tissue homeostasis. Among the generality

frequent human skin infections, dermatophytoses (ringworms) represent the fourth cause of disease with a global incidence estimated in 20 to 25% within the healthy population (Rouzaud *et al*., 2018). Anthropophilic species (*T. rubrum, E. floccosum*) are well acclimatized to humans and often cause chronic infections with mild clinical symptoms. In contrast, dermatophytes from animals (*M. canis, Arthroderma*/ *T. benhamiae, T. mentagrophytes*, etc.) or soil (*Nannizzia gypsea*/ *M. gypseum*) are frequently isolated from patients suffering from mild to highly inflammatory dermatophytosis but with lesions that are prone to spontaneous resolution (de Hoog *et al*, 2017).

In contrast, the immunosuppressed population (especially cell-mediated immunity deficiency settings such as HIV-AIDS, transplant, neoplasia, diabetes, or corticosteroid therapy) is particularly oversensitive to these infections showing extensive superficial lesions that are often unresponsive to conventional antifungal treatment (Verma,2018). This was newly observed in India where there was a significant increase in treatment- recalcitrant, recurrent and chronic dermatophytosis probably due to indiscriminate use of antibiotics and corticosteroid drug combination (Bishnoi *et al*., 2018).

2.7. Efficiency of Antifungal Agents Against Dermatophytes

One of the most vastly used antifungal groups is the azole group. They have wide spectrum antifungal activity covering dermatophytes, deep mycoses, Nocardia and few bacteria too. Azoles fundamentally include two subclasses based on the number of nitrogen atoms in a ring; The first class includes imidazoles which consist of miconazole, oxiconazole, econazole, ketoconazole, tioconazole, and clotrimazole with two nitrogen atoms in an azole ring, while another class includes triazoles such as fluconazole , posaconazole, itraconazole, terconazole, and voriconazole which contain three nitrogen atoms in a cyclic ring. Imidazoles

are mainly used for the mucosal fungal infections while triazoles are recommended for the systemic also for the mucosal infections (Vandeputte *et al*. 2012). Depletion of membrane ergosterol due to the utilize of azoles are also shown to disrupt vacuolar ATPase functions resulting in an impairment of the vacuolar acidification and ion homeostasis (Zhang *et al*. 2010). azoles are fungistatic, their prolonged utilize poses greater threat of emergence of drug resistance among the surviving fungal population (Shapiro *et al*. 2011).

The other group that the most widely used as antifungal antibiotic is Polyene. Polyenes are the amphipathic organic natural molecules called macrolides and are generally produced by Streptomyces (Vandeputte *et al*. 2012). Polyenes fundamentally include amphotericin B (AmpB), natamycin and nystatin. AmpB is mostly effective in systemic invasive fungal infections and is used generally against *Cryptococcus*, *Candida* and *Aspergillus* species (Sanglard *et al*. 2009) while nystatin and natamycin are preferred for topical infections due to their weak absorption (Vandeputte et al. 2012).

Azoles suppress the oxidative enzymes present in the fungal cell membrane, which prevents the cell wall of the fungus from forming sterol (ergosterol), and due to incomplete synthesis, cells become permeable. furthermore, echinocandins inhibit the synthesis of important polysaccharides (1,3-β-glucan) responsible for developing the cell wall, whereas polyenes directly bind to the ergosterol and move inside the cell through the cell membrane by creating pores, and through these pores, cellular organelles come out that cause the death of the cell (Marek *et al*.,2019). While the local antifungal drugs act on the different sites to target the molecules for the treatment of fungal infections, they show various side effects on the application site, such as burning, redness, and some allergic reactions (Girois *et al*.,2006).

2.8. Molecular Methods of Dermatophytes Identification

Diagnostis of pathogenic dermatophytes is an important factor not only in determining the epidemiology of disease but also in order to access successful treatment (Sahgal and Magan, 2008), In addition to that type of infection in the correct diagnostic of the infection (skin or nails) and also determine the cause of relapse which may be either due to failure treatment or infection of new strain (Baeza & Giannini, 2004). Also, local patterns of dermatophytes isolated from clinical specimens may change with time and geographical area so it is important to know local species in order to improve the diagnostic of these species (Prasad et al., 2013).

In recent years, Molecular methods such as conventional polymerase chain reaction (PCR) technique, Real-time (RT-PCR) technique, polymerase chain reaction Enzyme immune assay (PCR-EIA) , Nested- PCR, Restraction Fragment Length Polymorphism (PCR-RFLP) and Random Amplified Polymorphic DNA (RAPD-PCR) techniques have been widely used for identification of dermatophytes (Rezaei- Matehkolaei et al., 2012). Some molecular methods have many advantages such as rapid identification of dermatophytes at genus and species level either directly in clinical samples or in young nonreproductive fungal colonies (Kanbe et al.,2003). The ITS regions of ribosomal DNA gene (rDNA) in the dermatophyte species were used as a reliable marker for species identification. Analysis of the ITS regions by PCR-RFLP has provided a simple and accurate method for dermatophyte species identification (Rezaei-Matehkolaei et al., 2012). Some studies have also used Real-time polymerase chain reaction technique to detect species Trichophyton and has given good result compared to traditional method to diagnose fungus such as microscopic examination and planting on cultivation and physiological tests (Wisselink et al.,2011).

Almost all researches depend on the reliance on the Internal Transcribed Spacer regions (ITS) as amolecular marker shows successes at high rates and critical differences among dermatophytes species and this has been adopted most of the studies of molecular diagnosis in dermatophytes on the sequence of nitrogen bases in ITS1 and ITS2 of the rDNA (Putignani et al., 2010). Symones et al. (2013) explained the sequence of nitrogen bases for a number of isolates T. mentagrophtes complex, isolated from human and animales by using primer pair ITS1 and ITS2. There are a number of studies that have used ITS1 ,ITS2 and ITS4 primers in molecular diagnostic of dermatophytes (Alaa et al., 2012).

2.9.1.Antifungal activity of medical plant extracts

Plants have been applied therapeutically for thousands of years everywhere in the world. Medicinal plants incorporate herbs, herbal materials, and items that contain various pieces of plants or other plant materials that have traditionally been utilized to combat health disorders (Petrovska , 2012). Essential oils and herbal extracts have antifungal properties because their phenolic groups act as the primary antimicrobial bioactive composition (Socaciu *et al*.,2020).

Phenolic groups are complex, volatile, aromatic compounds with different chemical structures and are stored in diverse parts of the plant, in particular tissue such as glandular hairs, oil cells, and oil ducts (Fajinmi *et al*., 2019). They are now well recognized due to their antimicrobial, germ-killing, anti- inflammatory, and antioxidant properties (Swamy *et al*., 2016).

However, the synthesis of an antimycotic drug involves a diversity of processes, including high-heat and high-temperature treatments, and as a result of these treatments, the structure of the phytochemicals in the herbal extract is disrupted, leading to the epimerization process. diverse studies have found that combining high temperatures and an alkaline state causes structural changes in polyphenolic components (Kothe *et al*.,2013).

2.9.2 Secondary metabolites in plants

Plants possess capacity to synthesize different organic molecules called secondary metabolites. Unique carbon skeleton structures are basic properties of plant secondary metabolites. Secondary metabolites are not necessary for a cell (organism) to live, but play a role in the interaction of the cell (organism) with its surroundings, ensuring the continued existence of the organism in its ecosystems. Formation of SMs is generally organ, tissue and cell specific and these are low molecular weight compounds, These compounds often differ between individuals from the same population of plants in respect of their amount and types(Croteau *et al*.,2000). They protect plants against stresses, both biotic(bacteria, fungi, nematodes, insects or grazing by animals) and abiotic (higher temperature and moisture, shading, injury or presence of heavy metals). SMs are used as especially chemical such as drugs, flavours, fragrances, insecticides, and dyes by human because of a great economic value. In plants, SMs can be separated into threegroups (Terpenoids, Polyketides and Phenypropanoids) based on their biosynthesis origin (Verpoorte and Alfermann *et al*.,2000 ;Hussein and Al-Marzoqi, 2020).

1.Terpenes: are a class of natural products consist of compounds with the formula (C5H8) . Comprising more than 30,000 compounds , these unsaturated hydrocarbons are produced predominantly by plants particularly conifers(Alipour *et al*.,2014;Aiyaz *et al*.,2015) Terpenes are furthermore classified by the number of carbons : monoterpenes (C10) , sesquiterpenes (C15) , diterpenes (C20) , etc (Eberhard and Breitmaier,2006) .

Terpenes involve the biggest group of secondary metabolites and are free by their common biosynthetic origin from acetyl-coA or glycolytic intermediates. An immense bulk of the diverse terpenes structures produced by plants as secondary metabolites that are assumed to be concerned in defense as toxins and

feeding deterrents to a large number of plantfeeding insects and mammals. Terpenes aredivided into monoterpenes, sesquiterpenes, diterpene, Triterpenes and polyterpenes. Thepyrethroid (monoterpenes esters) occur in theleaves and flowers of Chrysanthemum speciesshow strong insecticidal reactions to insects like beetle, wasps, moths, bees, etc and a popular ingredient in commercial insecticides because of low persistence in the environment and low mammalian toxicity (Berli *et al* ., 2010; Hussein *et al*.,2018).

2. Flavonoids: Flavonoids (or bioflavonoids; from the Latin word flavus, meaning yellow, their color in nature) are a class of polyphenolic secondary metabolites found in plants, and thus commonly used in diets (Delage, 2015). Chemically, flavonoids have the general structure of a 15-carbon skeleton, which consisting of two phenyl rings (A and B) and a heterocyclic ring (C, the ring containing the embedded oxygen) (de Souza *et al*.,2021). This carbon structure can be brief C6-C3-C6. According to the IUPAC nomenclature they can be classified into :

(1) flavonoids or bioflavonoids , (2) isoflavonoids , derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure (3) neoflavonoids, derived of 4-phenylcoumarine (4-phenyl-1,2-- benzopyrone) structure (Nič *et al.*,2009).

The three flavonoid classes above are all ketone containing compounds and as such, anthoxanthins (flavones and flavonols) . This class was the first to be termed bioflavonoids. The expression flavonoid and bioflavonoid have also been more loosely used to describe non-ketone polyhydroxy polyphenol compounds, which are more specifically termed flavanoids. The three cycles or heterocycles in the flavonoid backbone are mostly called ring A, B, and C (de Souza *et al*.,2021).

Flavonoids are an complementary part of human and animal diet and cannot be synthesized by humans and animals. It is group of natural substances with

variable phenolic structures, are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. These natural products are well known for their useful effects on health and efforts are being made to isolate the ingredients so called flavonoids. Flavonoids are now considered as an fundamental component in a variety of pharmaceutical, medicinal and cosmetic applications, This is attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function (Panche *et al.*,2016) .

3. Alkaloids: They are diversefied low-molecular-weight, cyclic organic colorless and odorless and have the solubility properties of organic solvents such as alcohol and ether and do not dissolve in water compounds containing nitrogen in a negative oxidation state, Alkaloids belong to the broad category of alkaline secondary metabolites that constitute the pharmacologically bioactive principles that are predominant, but not exclusively existing, in flowering plants (Pelletier, 1983; Kutchan, 1995).

Alkaloids are synthesized from decarboxylation of amino acids to produces amines, which interact with amine oxides to form aldehydes (Tadele, 2015). Chemo-taxonomical survey showed that alkaloids are exceedingly distributed in higher plants belonging to Apocyanaceae, Ranunculaceae, Papaveraceae, Solanaceae and Rutaceae in Africa as well as in lower plants, insects, marine organisms and microorganisms (Kutchan, 1995; Kuete, 2014).

2.9.3 Toxicity Effects of Medicinal Plants Extracts

Toxic effects of medicinal plants extracts are interested with assessing the damaging of chemical, biological and physical substances on biological systems of live organisms, The combination between dosage and its effects on the exposed organism is of high implication in toxicology, Greatest of these studies are leading to evaluate the grade to which materials are toxic (poisonous) for humans, animals or the environment, to explore the mechanism of poisonous chemicals, or to develop new tests for precise kinds of chemically induced effects (Vikram and Kaushal,2015).

Usually, a lot of people disbelieve that therapeutic herbs being natural are harmless and free from undesirable materials, failing to distinguish that herbs are composed of bioactive chemicals some of which may be poisonous, In fact, the adversarial effects of phytomedicines, furthermore its contamination, toxicity, and drug interface are common problems reviled to communal health, Toxic effect due to herbal medicine maybe clear in a number of organs such as kidney, liver, stomach, nervous system and blood, liver is a vital organ for preserving of metabolic service and detoxification from exogenous and endogenous substances like xenobiotics, drugs and viral infections (Debelo *et al*.,2015).

2.10. The plant used in the study

2.10.1. Colocynth

common name: Indrayan ,Tumba.

The scientific name: *Citrullus colocynthis Schrad*

family name: Cucurbitaceae

Citrullus colocynthis, a beneficial cucurbit plant, is widely distributed across the dry regions of the planet. According to Coffey *et al*. (2015), these plants are mostly found in the deserts of Arabia, the Sahara, and southern Asia, which primarily

consists of India, Pakistan, and southern islands. Colocynth, bitter cucumber, bitter melon, egusi melon, Citrullus colocynthis lanatus, and the bitter vine of Sodom are all frequent names for the bitter apple fruit. It is a perennial plant that grows wild in dry regions all over the globe and has potential applications in food, nutraceuticals, and medicine (Asyaz *et al*. 2010).

Jaundice, diabetes, and asthma are just a few of the illnesses that may be treated using various portions of these plants (Li *et al*. 2021). The *Citrullus colocynthis* fruit is spherical in form, has a smooth and fine texture, and has a bitter flavor. Each gourd has 200–300 seeds and is solid with a crust surrounding it (Uma & Sekar 2014). These plants are often perennial vines that produce extremely tiny blooms that are fragrant in nature and have mesocarp thickness, density, and seed-fruit proportion. Fruit mass, seed and pulp content, volume, epicarp, and other physical characteristics of bitter apples are listed below. The fruit has a 506 g average mass in grams. About half of the fruit's bulk is made up of pulp. According to Hussain *et al*. (2014), the fruit's seeds make up around 75.1 g of its weight.

Protein, vital minerals, and oil content are claimed to be rich in egusi melon seed kernels. Egusi oil is a viable alternative to most vegetable oils because to the low cost of melon growing and the plant's drought tolerance when compared to other oil crops (Olubi *et al*. 2019).

Bitter apple is used to cure a variety of ailments, including joint discomfort, leprosy, bronchitis, cancer, and mastitis (Heydari *et al*. 2016). Furthermore, these plants have therapeutic properties that aid in the prevention of gut-related illnesses such as dysentery and indigestion. Because it has anti-inflammatory effects, it is often used as a traditional medication for inflammation (Kuralkar & Kuralkar 2021). Decoction, emulsion, liniments, electroactive, and powder are common medicinal plant therapy ingredients (Rashid *et al*. 2021). The components of such plants are also employed in a range of sectors, primarily the cosmetic, fragrance,

and food industries. Bitter apple active components are extracted using various methods before being used in the development of medications and the production of functional food items (Mubeen et al. 2022).

3. Materials and Methods

3.1 Equipment of laboratory.

Table (3-1): Equipment and instruments which were used in the study

No.	Equipment	Manufacturing company
1.	Autoclave	Memmert /Germany
2.	Automatic micropipette(different volumes)	Germany
3.	Benzene burner	Iraq
4.	Centrifuge	Labcco /Germany
5.	Compound microscope	Olympus /Japan
6.	Digital camera	Sony /Jaban
7.	Electrophoresis apparatus	Shandon /England
8.	Electrophoresis meter	Mupid-one /Japan
9.	Filter paper	BioBasic Inc./ Korea
10.	Hood	Lab TECH
11.	Hot plate	Heidoph MR Hei-Standerd /England
12.	Incubator	Memmert /Germany
13.	Light microscope	Olympus/ Japan
14.	Micropipetter	Germany

15.	PCR – Machine	Labnet /USA
16.	Sensitive balance	Melter /Switzerland
17.	Slides and cover slip	Witeg/ Germany
18.	Sterile test tube	Superestar/ India
19.	Tips (different volumes)	China
20.	UV- Spectrophotometers	EMC lab/Germany
21.	UV- Tran illuminator	Desktop Gel image /scope-21/European
22.	Vortex mixture	Griffin /Germany
23.	Watch stop	Damon/ Turkey
24.	Water distillatory	Gallenkamp /England
25.	Water bath	Taffa hanover

3.2. Chemicals and Materials

Table (3-2): Chemicals and Biological Materials used in study.

No.	Chemical Materials	Manufacturing company
1.	Agar	Hi-media/ India
2.	Agarose	Shenzhen/China
3.	Cyclohexemide	Hi-media / India
4.	Chloramphenicol	Iraq
5.	Ethedium bromide	Promega/ USA
6.	Ethanol	BDH / England
7.	Isopropanol	BDH/ England
8.	Lacto phenol cotton blue stain	Fluke / Switzerland
9.	Ladder	Korea/ Intron
10.	Master Mix	Promega/ USA
11.	Phenol	BDH/ England
12.	Primers	Bioneer/ Korea
13.	TBE buffer	Promega/ USA
14.	Sodium chloride	BDH/ England

3.3. Culture media

Table (3-3): Culture media used in the study

No.	Culture	Manufacturing
1.	Sabouraud Dextrose Agar	Hi-media /India
2.	Potato dextrose agar	Hi-media /India

3.4. The DNA Extraction Kit

Table (3-4):The Contents of the DNA Extraction Kit (FAVORGEN).

No.	Material	Volume
1.	FA Buffer	120 ml
2.	FB Buffer	65 ml
3.	TG1 Buffer	45 ml
4.	TG2 Buffer	30 ml
5.	W1 Buffer	44 ml
6.	Wash Buffer	20 ml
7.	Elution Buffer	15 ml
8.	Proteinase K	11 mg
9.	Lyricas solution	550 µl x 5
10.	Bead tube	50 pcs
11.	TG mini column	10 pcs x 5

12.	Collection tube	100 pcs
13.	Elution tube	50 pcs

3.5. Primers used in DNA amplification

Table (3-5): the Primers used in the study.

Type of primer	DNA sequence	Size bp
ITS5 (F) *	5-AAGTAAAAGTCGTAACAAGGTTTCCG -3	550-900
ITS4 (R)	5-TCCTCCGCTTATTGATATGC-3	550-900

*** Brilhante *et al*., 2006.**

3.6. Antifungals

Table (3-6): Antifungals .

Seq.	Antifungal	Abbreviation	Dosage/ disc	Manufacture
3	Fluconazole	FLC	150 mg	Lincoln /India
4	Itraconazole	IT	100 mg	Lincoln /India
5	Griseofulvin	GR	500 mg	Lincoln /India

3.7. Preparation of Culture Media

All media were prepared according to the manufactures instructions fixed on their containers, they were sterilized by autoclave at 15 psi/inch2 in 121ºC for 15 min., 20 ml of sterilized media were poured in disposable Petri dishes and they were incubated at 30 ºC for overnight to ensure sterility, and stored at 4ºC until used.

1- Sabouraud Dextrose Agar (SDA) Medium: SDA medium was prepared according to the manufacturer's instructions, by dissolved 65 gm of SDA in 1000 ml of distilled water, then sterilized by autoclave, this medium was used for culturing the dermatophytes isolates. It was prepared with addition of 0.05 gm/L Cychlohexamide added to prevent growth of saprophytic fungi and 250mg/L of chloramphenicol to prevent growth of bacteria. This medium was used for primary isolation of dermatophytes (Kwon-Chung and Bennett , 1992).

2- Potato Dextrose Agar medium: According to the manufacture's instruction, this medium is prepared by suspending 39 gm of medium in 1000 ml of distilled water with added 250 mg of chloramphenicol that prevent growth of bacteria and sterilized by autoclave. This medium is used to sub culturing for fungi.

3.8. Preparation of Solutions and Stains:

1. Lacto phenol cotton blue stain: is prepared by using 20g of phenol nitrate, 20ml of lactic acid and 40ml of glycerol mixing with 20 ml of distilled water. By using water bath, the stain mixed well and added (0.05g) of a blue cotton stain. It is used to stain and distinguish fungal structures as hyphae and chlamydospores under microscopic (Koneman and Roberts, 1985).

2. Sodium hydroxide solution: is used to adjust the pH of the medium.

3.9. Collection and cultivation of Specimens

During the study period from October 2021 to February 2022, a total of 60, scraps of skin scales, hair parts and nail. samples were collected from patients with dermatophytosis and kept in sterile containers. For identification of dermatophytes, those specimens were cultured on Sabouraud`s Dextrose agar (SDA) with chloramphenicol and cycloheximide and cultured at 25-30˚C for up to 4 weeks.

3.10. Diagnosing fungal colonies

1- Morphological examination

After appearance growth as well as examining colonies of fungi from respect colony color, shape and texture (Powdery, Granular, Cottony) as recorded pigments is examined on foundation at surface of colony, appearance.

2- Microscopy

Fungi isolates were examined microscopically, taken the fingerprint of the fungus in the colony by Adhesive tape touching with the surface of the fungal colonies and then paste the tape on a glass slide containing a drop of lacto phenol cotton blue. Slides examined under magnification 10X, 40X and 100X as described by Astrid (1999).

3.11. Molecular Identification

3.11.1. DNA Extraction from Fungi (Favorgen kit)

1. Tiny portion of small colony of fungi was transferred to a 1.5 ml microcentirfuge tube.
2. One ml of FA buffer was added to the cells and resuspend the cells by pipetting.
3. The cells were descended by centrifuge at 5,000 rpm for 2 min and discarded the supernatant completely.
4. The cells were resuspend in 550 µl of FB buffer and 50 µl of lyticase solution were added , mixed well by vortexing. the samples were incubated at 37 ^{o}C for 30 min.
5. A 8µl of 50 mg\ml RNase A was added and incubated for 2 min at room temperature .
6. The cells were descended by centrifuge at 5,000 rpm for 10 min . The supernatant were removed completely.

7. A 300 µl of TG1 buffer was added and mixed well by pipetting , the sample mixture was transferred to bead tube, and mixed well by plusvortexing for 5 min.

8. A total of 20 µl was added of Proteinase K (10 mg/ml) and mixed well by vortexing. Incubated at 55 ^{o}C for 15 min, vortex 30 seconds for every 5 minutes incubation.

9. The cells were descended by centrifuging at 5,000 rpm for 1 min and 200 µl of supernatant were transferred to a new 1.5 ml microcentrifugetube.

10. A 200 µl of TG2 Buffer was added and mixed well by pipetting.

11. A 200 µl of ethanol (96-100%) was added and mixed well by pulsevortexing for 10 seconds.

12. A TG Mini Column was placed in Collection Tube. The sample mixture (including any precipitate) was transferred carefully to TG Mini Column. Centrifuged at 11,000 rpm for 30 second then the TG Mini Column was placed to a new Collection Tube.

13. A total 400 µl of W1 Buffer was added to the TG Mini Column. Centrifuged at 11,000 rpm for 30 seconds and discarded the flowthrough.
The TG Mini Column was placed back to the Collection Tube.

14. A 750 µl of Wash Buffer was added to the TG Mini Column. Centrifuged at 11,000 rpm for 30 seconds and discarded the flowthrough,
and the TG Mini Column was placed back to the Collection Tube, centrifuged at full speed (12000 rpm) for an additional 3 min to dry the column.

15. The TG Mini Column was placed to a Elution Tube.

16. A 50 ~100 µl of Elution Buffer or ddH2O was addedto the membrane center of the TG Mini Column. Stranded TG Mini Column for 3 min. centrifuged at full speed (12000 rpm) for 1 min to elute total DNA.

17. Total DNA was stored at 4^{o}C or -20^{o}C.

3.11.2. DNA electrophoresis:

1- At first, 100 ml of the T.B.E buffer is placed in a beaker.

2- Then 1 g weight of agarose is added to the buffer.

3- the buffer with the agarose is heated on a hot plate to boiling point so that all of its components are solvent.

4- The agarose mixture is cooled by leaving it between 50-60˚C.

5- Ethidium Bromide dye is added at 0.5 µl to the agarose before solidification of the liquid and mixed it well.

6- The comb is put into one of the ends of the agarose gel template.

7-Agarose is poured into the template to prevent the formation bubbles and left to cool at room temperature for 30 minutes.

8-The migration electric basin is filled with the T.B.E buffer solution so that it rose from the gel surface.

9-The samples are placed in the pits with the addition of the agarose gel loading dye so that the dye was linked to the DNA.

10- The electrophoresis is performed in 70 V to 45 min.

11-The agarose gel is exposed to UV trans illuminator for DNA bands visualized and documented.

3.11.3. Chemical material used in PCR

Table(3-7): Chemical material and volumes used in PCR

No.	Chemical materials	Volumes (µl)
1.	Master Mix	12 µl
2.	Forward Primer	1 µl
3.	Reveres Primer	1 µl

4.	DNA	1-2 µl
5.	Deionizer D. W	Adjusted the volume to 25 µl
	Total mix	25 µl

3.11.4. PCR assay

ITS5/ITS4 was universal primer couple that targeted the sequences place of the ITS1-5.8S-ITS2 gene of the dermatophyte isolates was used. The PCR mixture was prepared according to the table (3-8), and augmented on the current System of cycler PCR (Labnet, USA) by the following conditions:

Table(3-8) : Steps of PCR assay

No.	Steps	Temp.	Time	cycles	Cool stage
1.	Primary denaturation	95 C	Min. 5		
2.	Denaturized Annealing Extension	95 C 56 C 72 C	30 sec 1.5 Min 1 Min	30	
3.	Final extension	72 C	10 Min		4C

The products of PCR were run on 1.5% agarose gel and electrophoresis was made at 70 V for 30 min. The gel was pre-stained with 0.05% ethidium bromide. The PCR bands were noticed by using ultraviolet trans illuminator.

3.11. 5. PCR gel Electrophoresis:

The amplified PCR products were detected by agarose gel electrophoresis which was visualized by staining the Ethidium bromide. The electrophoresis result was detected by using gel documentation system. The positive result was distinguished when the PCR band base pairs of samples equal to the target product size (Bartlett and sterling, 2003), or the size of amplified DNA fragments which were identified by a comparison with molecular size marker DNA (1500 - bp DNA ladder).

3.11.6. Sequencing analysis:

Twenty isolates of dermatophytes species from this study were subjected for sequencing analysis. Direct sequencing analysis was performed to the 20 µl PCR product of ITS region which were sent to Macrogen Laboratory in Korea. the received sequencing data were compared with gene bank by using NCBI Blast nucleotide database.

3.11.7. Phylogenetic tree:

The phylogenetic tree was analyzed by using Mega 6 software program with un weighted pair group method with arithmetic mean (UPGMA) tree type based on sequence data of ITS region amplified by ITS5/ITS4 primer pair for 20 isolates.

3.12. Plant Materials

3.12.1.Colocynth (*Citrullus colocynthis*):

Fresh fruit of *Citrullus colocynthis* were collected from countryside close to Hilla city in period from November 2021 to March 2022, After cleaning, it was left to dry, then crushed and stored in plastic bags.

Table(3-9): Scientific, Local, English name, Family, and active parts

Scientific name	Local name	English name	Family	Active part used
Citrullus colocynthis	Indrayan ,Tumba	Colocynth	Cucurbitaceae	fruit

3.13. Plant materials extraction

3.13.1. Alkaloid determination: 20 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 24 hrs. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide (NH_4OH) was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute 1% ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Harborne, 1973).

3.13.2. Flavonoid determination : 20 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight Boham and Kocipai Abyazan(1994).

3.13.3. Extraction of crude terpenoid compounds: Crude terpenoids compounds were extracted according to(Harborne;1984). 20 gm of plant powder for fruits were extracted by 200 ml of chloroform . The solvent then evaporated in rotary

evaporator. The samples were placed in clean dark vials and kept in refrigerator at 4°C until use.

3.14. Poisoned Food Technique

A series of dilutions of the plant extracts under study were prepared (5%, 10%, 15%) mixed with molten SDA medium at 45 °C by poisoned food technique. After pouring the medium into petri dishes, allowed to solidify at room temperature for thirty minutes. A mycelial disc 5 mm diameter, cut out from periphery of 7 day old cultures, was aseptically inoculated onto the agar plates containing the plant extract. Positive and negative control was also taken (Trivedi and Singh, 2014).

3.15. Antifungal activity assay of extract

PDA medium was prepared and autoclaved after that a known volume (2ml) of the each plant extracts concentration is placed in the center of the petri dishes and complete the volume to 20ml with PDA medium to obtain the required final concentrations (5, 10, and 15 mg/ml) of the medicinal plant after complete solidification of the medium, 5 mm disc of 7-10 days old culture of the test fungus were placed aseptically in the center of the Petri plates and incubated at 25-30 ± 2C˚ for 28 days, simultaneously 0.02ml of antibiotic solution was added to each assay plate to check the bacterial contamination as suggested by(Gupta and Banerjee,1970). dimethyl sulfoxide was used as a negative control. Observations were recorded on twenty eighth day. The colony diameter was recorded in terms of millimeters. PDA medium devoid of extract served as control. For each treatment three replicates were maintained. The fungi toxicity of extracts was calculated in terms of percent inhibition of mycelia growth by using the formula (Singh and Tripathi ,1999)

Percent Inhibition = (dc- dt/ dc) * 100

Where:
dc = Average increase in mycelia growth in control.
dt = Average increase in mycelia growth in treatment.

3.16. Antifungal Activity Against Growth of Dermatophytes

In the same way as the previous paragraph, SDA plates with Fluconazole, Itraconazole and Griseofulvin were used as positive control. While the series of dilutions used for the antifungals under study are(0.2%, 0.5%, 1%)**.**

3.17. Statistical Analysis

All data of treatments were dictated by three replicates data were subjected to an analysis of variance by using SPSS 16.0 program, a completely randomized design was used and least significant difference (L.S.D) was performed at $P \leq 0.05$.

4. Results and Discussion

4.1. Collection of specimens

A total of 60 specimens were collected from dematophytosis patients diagnosed clinically by a specialist, and included 37 (61.7%) sample of skin scales, 15 (25%) samples of hair parts and 8 (13.3%) nail samples. All specimens consisted of male 25 (41.7%) and females 35 (58.3%). Table (4-1) shows the type and number of specimens in both males and female. This result was agreed with (Alatbee & Obaid, 2022) shows that the rates of infection with dermatophytes in males 30% Were less than females 70%. But not agreed with (Aref *et al*., 2022) in Tehran_ Iran where the males (58.4%) was higher than that of females (41.6%). Also in Kuwait the Higher rates of infection were seen in males compared to females (Al-Aryan *et al*., 2022).

Table (4-1): The percentages of Dermatophytosis samples types according to gender.

Type of specimens	Males	Females	Total no. (%)
Skin scales	**17**	**20**	**37 (61.7)**
Hair parts	**5**	**10**	**15 (25)**
Nail samples	**3**	**5**	**8 (13.3)**
Total no. (%)	**25 (41.7)**	**35 (58.3)**	**60 (100)**

4.2. Clinical cases of dermatophytosis

In this study showed six types of clinical cases of dermatophytosis patients under study. The highest infection percentage was tinea corporis 27 (45%) and the lowest infection percentage was tinea manuum 2 cases (3.3%).The other types of infection were varied as (tinea capitis, tinea unguium, tinea cruris and tinea pedis (21.7%,13.3%, 10%, and 6.7%) respectively as shown in the figure (4-1). These results are similar to the researches that indicated that tinea corporis occupies the first place in terms of prevalence among the types of dermatophytes (Sharquie and Jabbar, 2021). These results were consistent with the results obtained by the study of (Alatbee & Obaid, 2022) on dermatophytes in Basra–Iraq , where the higher rate of tinea infections was tinea corporis 50% , which ranked first among the types of Tineas and in North India Tinea corporis was most common clinical type where the percentage was 41.6% (Sharma *et al*., 2020) , but this study did not agree with the (Cortez *et al.*, 2012) where ranked second place with 22.9% in a study of dermatophytes for children under 12 years of age in Brazil.

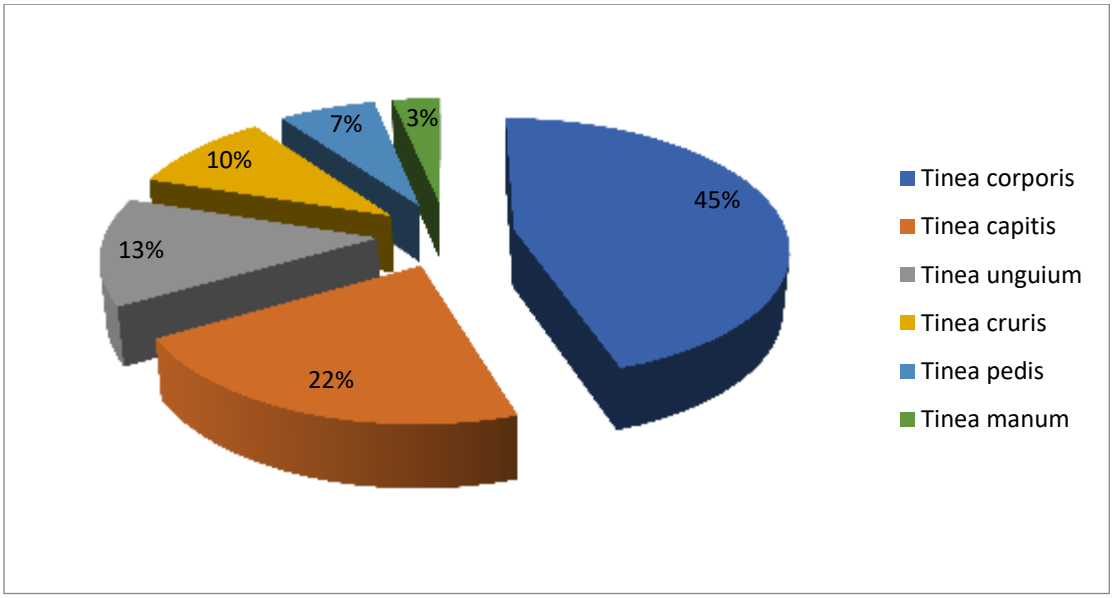


Fig.(4-1): Percentage of clinical cases of dermatophytes.

4.3. Distribution of dermatophytosis according to the risk factors

The present study, table (4-2) showed that there were significant effect of risk factors on clinical types of dermatophytosis such as gender, age, residency, found of domestic animals and chronic diseases as the following:

Table (4-2): Distribution of dermatophytosis according to the risk factors.

Clinical cases	Gender		Age			Residency		Domestic animals		Diabetus Mellitus		Total no. (%)
	Female	Male	1-20	21-40	41-65	Urban	Rural	Yes	No	Yes	No	
Tinea corporis	12	15	18	4	5	10	17	14	13	7	20	27 (45)
Tinea capitis	3	10	2	7	4	8	5	4	9	2	11	13 (21.7)
Tinea unguium	7	1	6	0	2	3	5	5	3	2	6	8 (13.3)
Tinea cruris	6	0	3	1	2	5	1	0	6	2	4	6 (10)
Tinea pedis	1	3	3	0	1	2	2	1	3	0	4	4 (6.7)
Tinea manuum	2	0	2	0	0	1	1	0	2	1	1	2 (3.3)
Total no. (%)	31 (51.7)	29 (48.3)	34 (56.7)	12 (20)	14 (23.3)	29 (48.3)	31 (51.7)	24 (40)	36 (60)	14 (23.3)	46 (76.7)	60 (100)

4.3.1. Distribution of dermatophytosis according to the gender

This results showed a difference in the distribution of clinical cases of dermatophytosis between males and females. Some types of tineas such as tinea corporis, tinea capitis, and tinea pedis had higher percentage of infection in males than females (25%,16.6%, and 5%) respectively, while the other types (tinea unguium, tinea cruris, and tinea manuum) were higher rate of infection in females than male (11.7%,10% and 3.3%) respectively, as shown in the figure (4-2). These

results were consistent with the results of the researchers (Saxena et al.,2022) where they are found tinea capitis in male is 62.5% while in female 37.5% . In addition, male also have a higher risk of developing tinea pedis than female, which also may be due to their more common exposure to moist environments due to wearing of occlusive footwear and more frequent physical exercise. These aforementioned lifestyle characteristics more commonly associated with male sex are thought to be responsible for the high prevalence of dermatophytosis in men (Son *et al.*,2022).

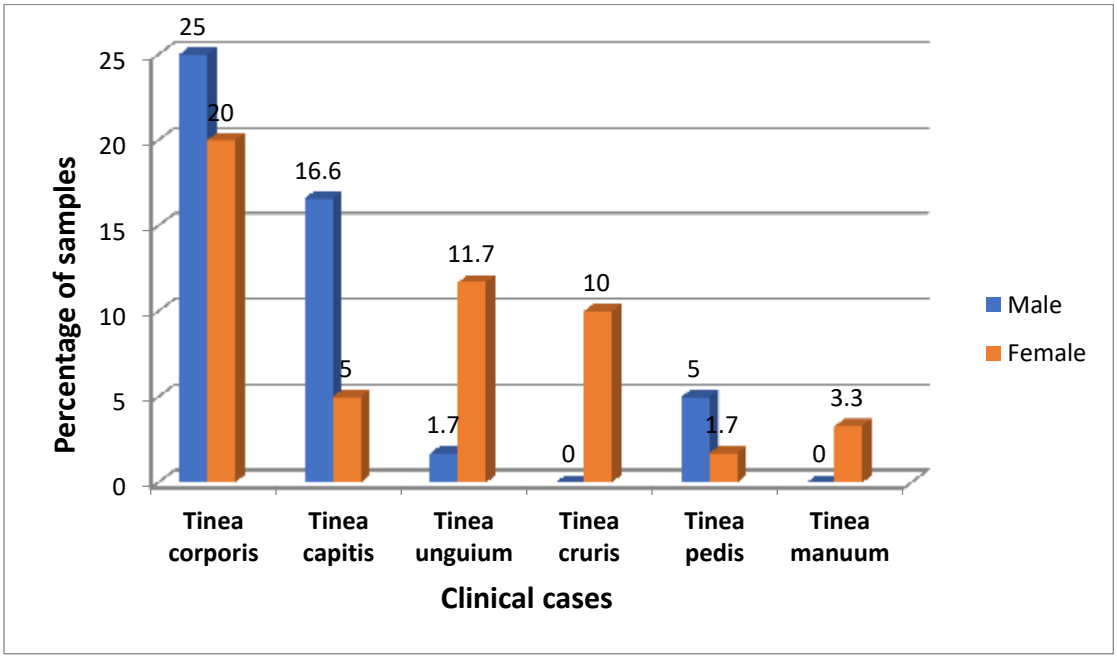


Fig.(4-2): Distribution of dermatophytosis according to the gender.

4.3.2. Distribution of dermatophytosis according to the age

According to age of patients, the infection of dermatophytes was distributed to almost three age groups first group from 1-20 years, second group from 21-40 years and third group from 41-65 years. But the highest incidence of infection was of first group (56.7%), whereas the second and third group were (20%) and

(23.3%) respectively. The figure (4-3) shows that the highest percentage of tinea corporis was in first group (30%), while the highest percentage of tinea capitis was in second group (11.7%). All of tinea unguium, tinea cruris, tinea pedis and tinea manuum were highest percentage of first group (10%, 5%, 5% and 3.3%) respectively. Different researches were exploring the relationship of dermatophytosis and age. This study was showed Most of the cases were in the age group of (11-20) years Where the percentage of infection was in the ages of less than or equal to 10 years (Nawfal & Zghair, 2022). In the study by (Abourghib & Almskat ,2021) the persons of all age groups were susceptible to dermatophytosis but it appeared to be less common in age group over 61 years (11.4 % of all cases). However, this results does not agree with the study conducted by (Kromer *et al*,2021) in Germany where The age distribution shows a linear increase above approximately 20 years of age with a peak at around 70 years of age.

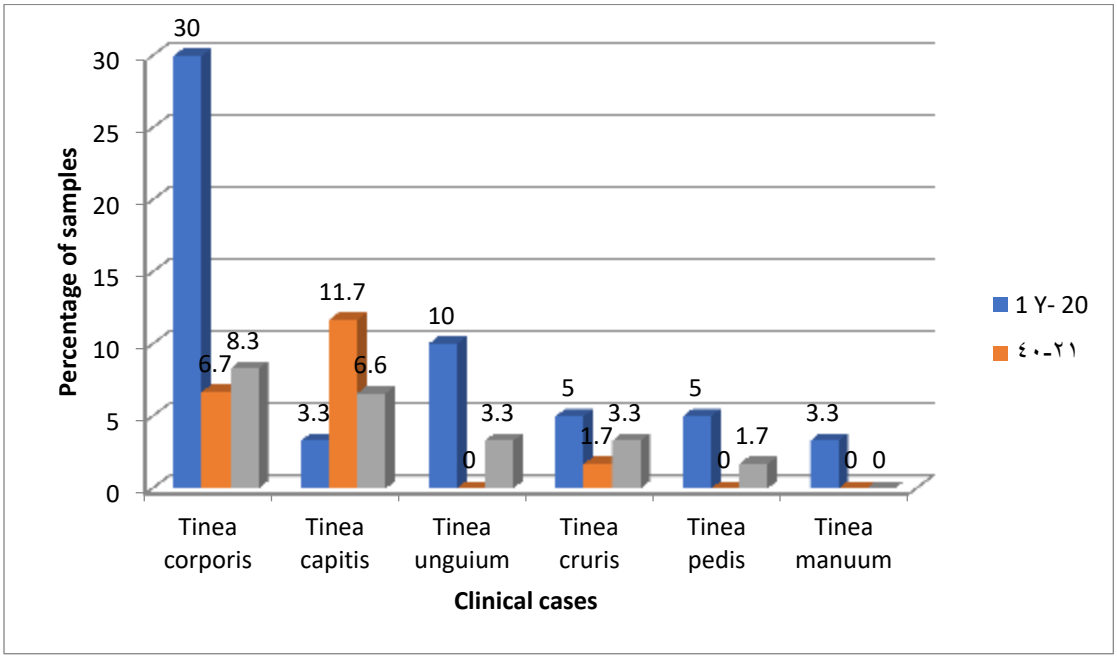


Fig. (4-3): Distribution of dermatophytosis according to the age.

4.3.3. Distribution of dermatophytosis according to the residency

It was clear from the results of the figure (4-4) that all of tinea corporis and tinea unguium gave a higher rate of infection in patients living in rural areas than those living in urban areas as 28.4% and 8.3% respectively, while all of tinea capitis and tinea cruris gave a higher rate of infection in patients living in urban areas as 13.4% and 8.3% respectively. This result was consistent with the findings In Baghdad City by researchers (Alhamdani & Ali, 2021) rural areas recorded a high rate (66.08%) compared with urban areas (33.91%). These results was consistent with the results of (Nawfal & Zghair, 2022) which recorded high cases of skin infection in a rural area (63.5%) compared to an urban area (36.5%). this study was also consistent with the results of (Abourghib & Almskat, 2021) the distribution of infection in rural areas was higher than those of urban areas. Although there was no significant association between infection and residence, more people in rural areas seemed to be infected than those in urban areas which may be due to prolonged exposure to soil in rural areas and have frequent encounter with animals.

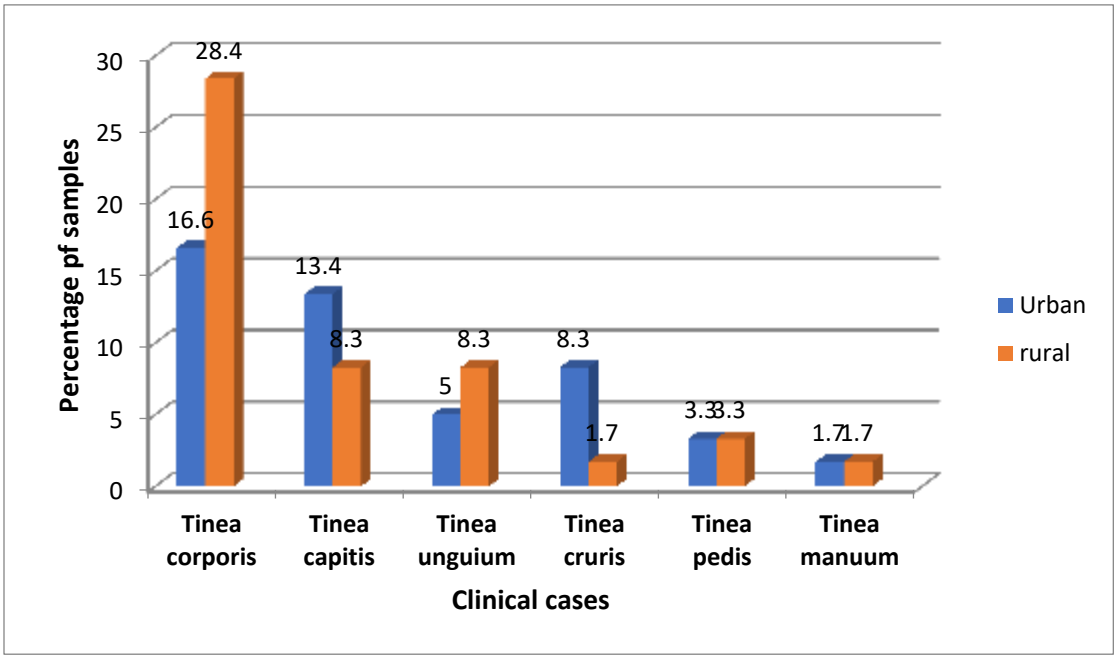


Fig. (4-4): Distribution of dermatophytosis according to the residency.

4.3.4. Distribution of dermatophytosis according to the domestic animals

It is clear from the results of the figure (4-5) that almost all types of tinea gave a higher no. of infection in patients who do have or live with domestic animals 24 cases (40%) than patients who were not living with these animals 36 cases (60%), the highest incidence that associated with the presnce of domestic animals tinea corporis was 23.3% followed by tinea unguium was 8.3%. This result did not correspond with the findings of (Dawa *et al*, 2021), where the history of animal contact had the increased odds of being infected with the dermatophytosis. On the other hand This study was consistent with the results of (Sharma *et al*, 2022), where almost 20–50% human skin infections were from zoonotic dermatophytes mainly found in pet animals which can be easily spread to other animals and humans also. The pet owners are more susceptible to get this infection from their pets, because of the close contact with them as dermatophytosis is very much prevalent in those pets.

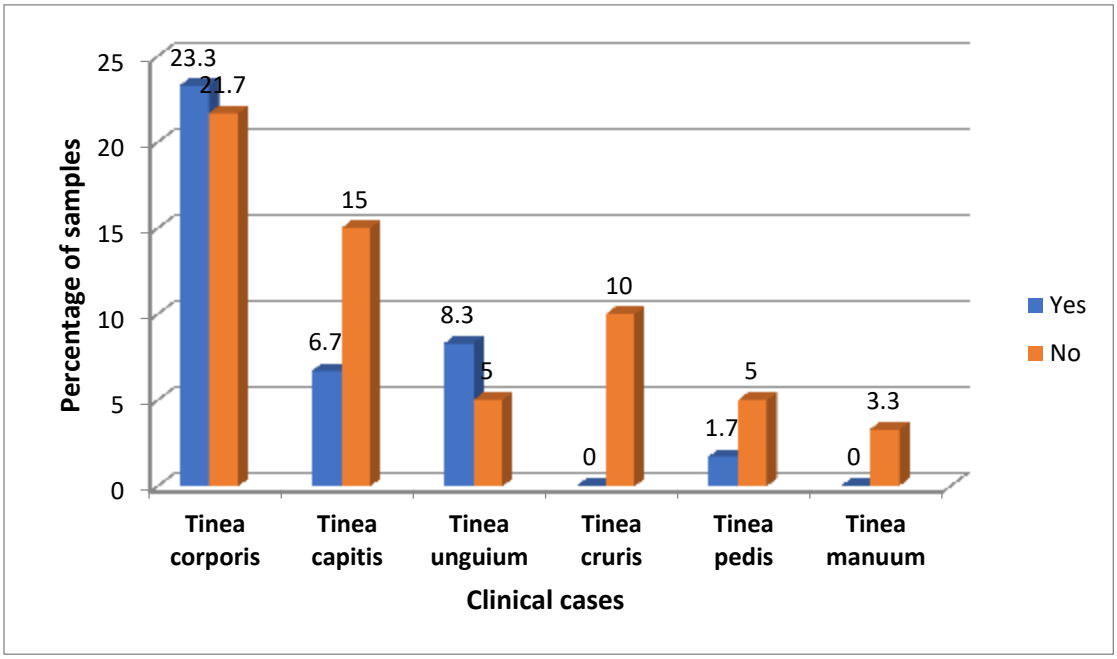


Fig. (4-5): Distribution of dermatophytosis according to the domestic animals.

4.3.5. Distribution of dermatophytosis according to the Diabetus Mellitus

All of cases of dermatophytosis under study, only 14 (23.3%) cases were affected with Diabetus Mellitus**,** while the rest of the cases, which amounted to 46 (76.7%) were not suffering from any Diabetus Mellitus (figure 4-6). This results refer to the disease with dermatophytes infected both immunocompetent or immunocompromised patients. These results were in parallel with that obtain by (Al-Jobory et al,2020) which showed that cases were not suffering from any Diabetus Mellitus (81.4%) compared with cases that suffering from Diabetus Mellitus (18.5%). Belmiloud et al., 2022 found the presence of Diabetus Mellitus led to a twofold increase in the odds of having dermatophyte infection among patients.

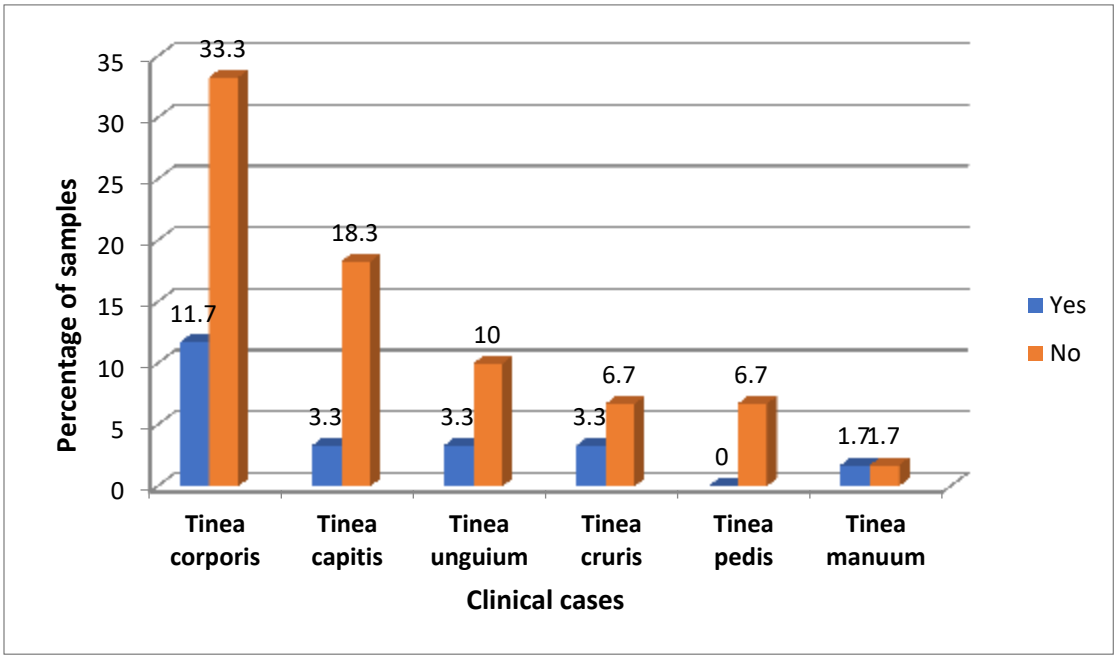


Fig. (4-6): Distribution of dermatophytosis according to the chronic diseases.

4.4. Isolation and identification of dermatophytes

Seven species of dermatophytes belonging to the four genera *Trichophyton*, *Microsporium* and *Epidermophyton* as well as *Chrysosporium* sp. were isolated and diagnosed under study (Fig. 4-7). The results showed that *T. mentagrophytes* was the highest frequency 13 (21.7%) cases followed by *T. interdigetale* 11 (18.3%) cases, and *T. quinckeanum* 10 (16.7%) cases, while *M. canis* 5 (8.3%) cases, *T. violecum* 4 (6.7%) cases, and *E. floccosum* 3(5%) cases, as well as *Chrysosporium tropicum* 6 (10%) cases, while there were 8 (13.3%) negative cases. These species were diagnosed according to the phenotypic and microscopic features, as well as molecular diagnostic. These results were consistent with the other studies (Ahmad, 2022 ; Correia *et al*., 2022).

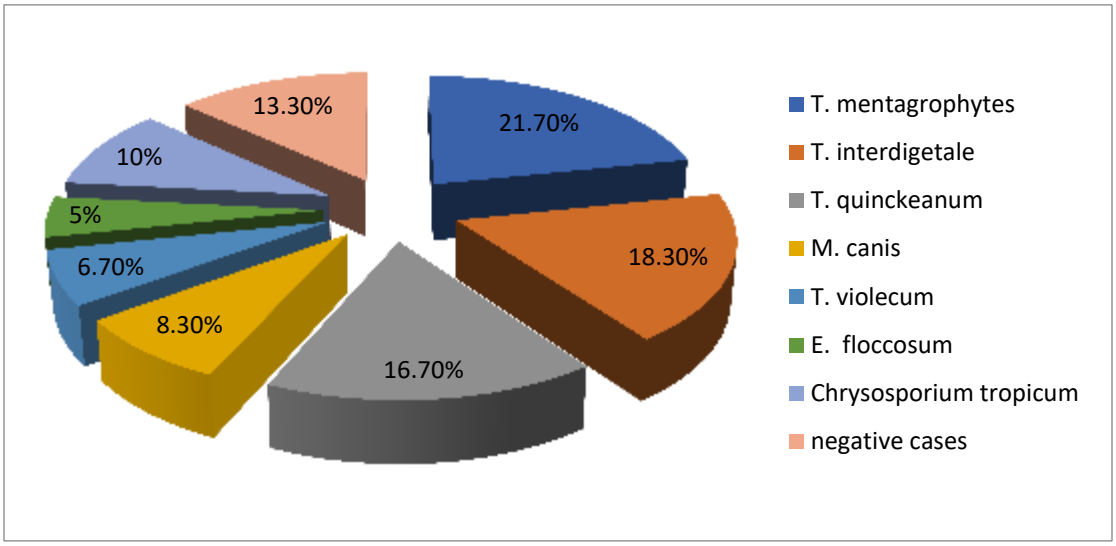


Fig. (4- 7): Percentage of fungal species from dermatophytosis.

4.4.1. Phenotypic diagnosis of dermatophytes

Dermatophytes colonies isolated in the study on SDA medium containing antibiotics Chloramphenicol and Cyclohexamide at 28–30 ° C for 2–4 weeks are varied considerably at the genera and species and sometimes at isolates level. Figures (4-8 to 4-14) shows isolated fungi from phenotypic and microscopic and these species were diagnosed based on the following reference (Refai *et al*., 2013).

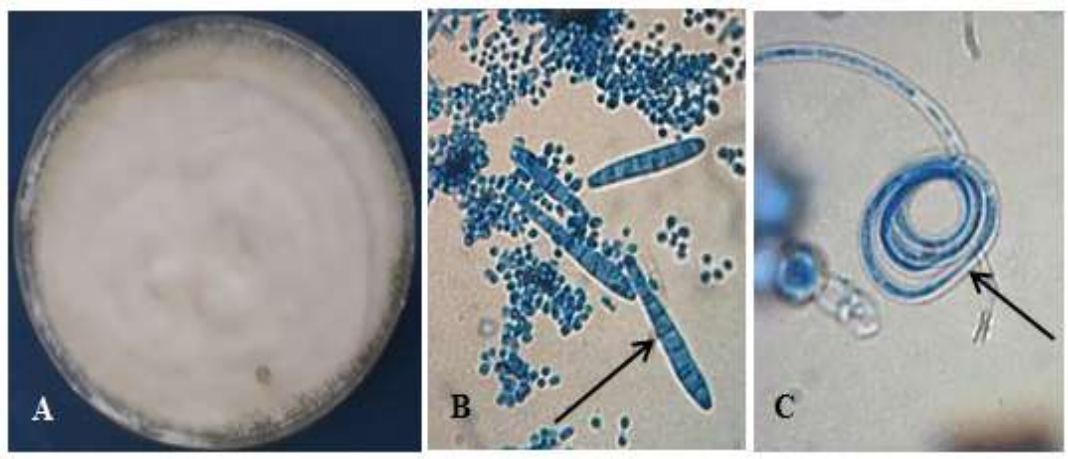


Fig. (4- 8): *T. mentagrophytes*, (A) Colony morphology, (B) Macroconidia and microconidia (C) Spiral hyphae (40X magnification). incubated at 25-30 ± 2C° for 28 days.

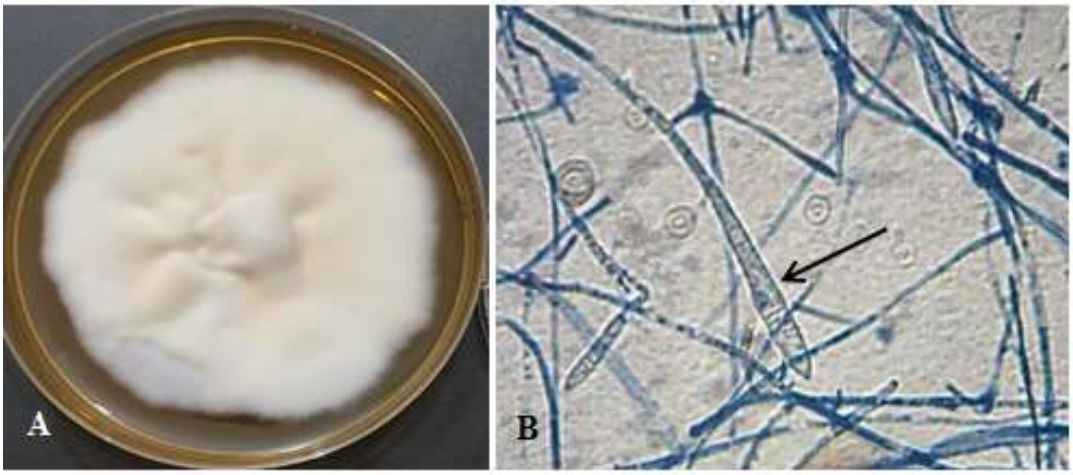


Fig.(4- 9): *T. interdigetale*,(A) Colony morphology,(B) Macroconidia(40X magnification). Incubated at 25-30 ± 2C° for 28 days.

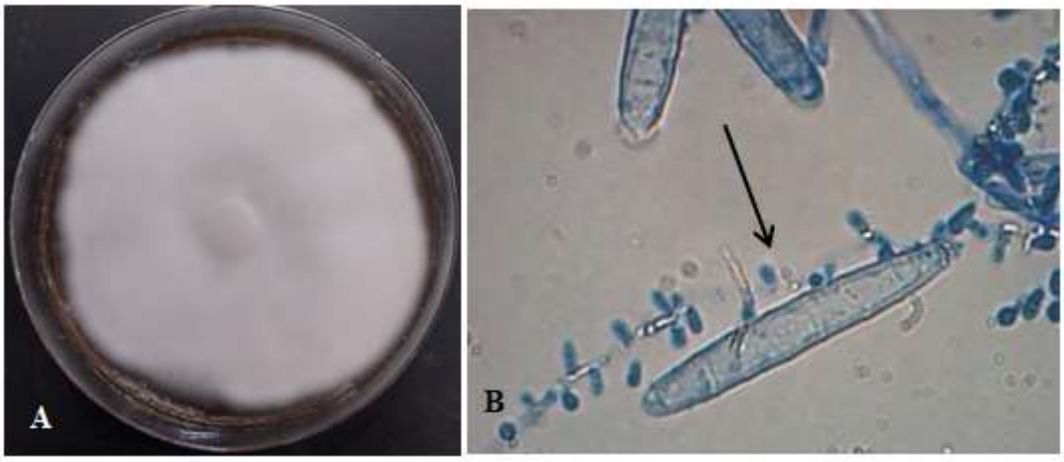


Fig. (4- 10): *T. quinckeanum*, (A) Colony morphology, (B) Macroconidia and Microconidia (40X magnification). incubated at 25-30 ± 2C° for 28 days.

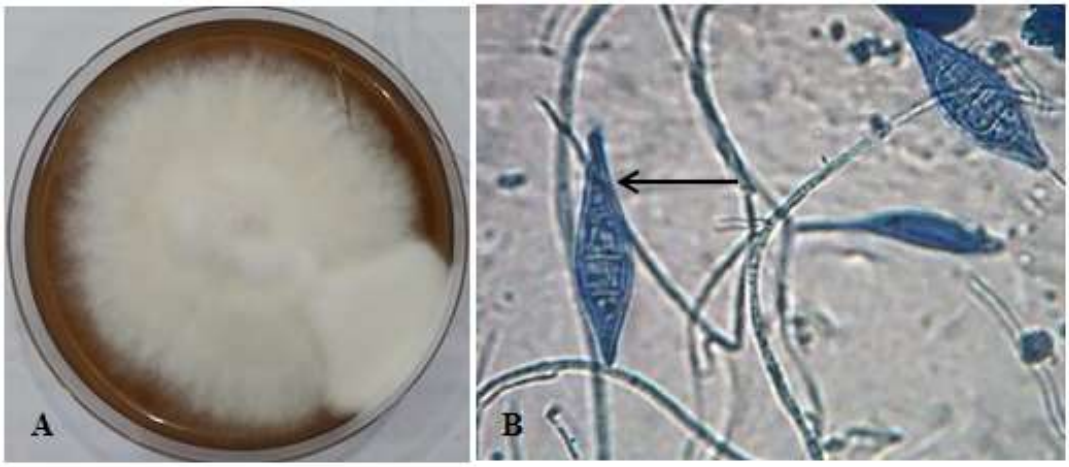


Fig. (4- 11): *M. canis*, (A) Colony morphology, (B) Macroconidia (40X magnification). incubated at 25-30 ± 2C° for 28 days

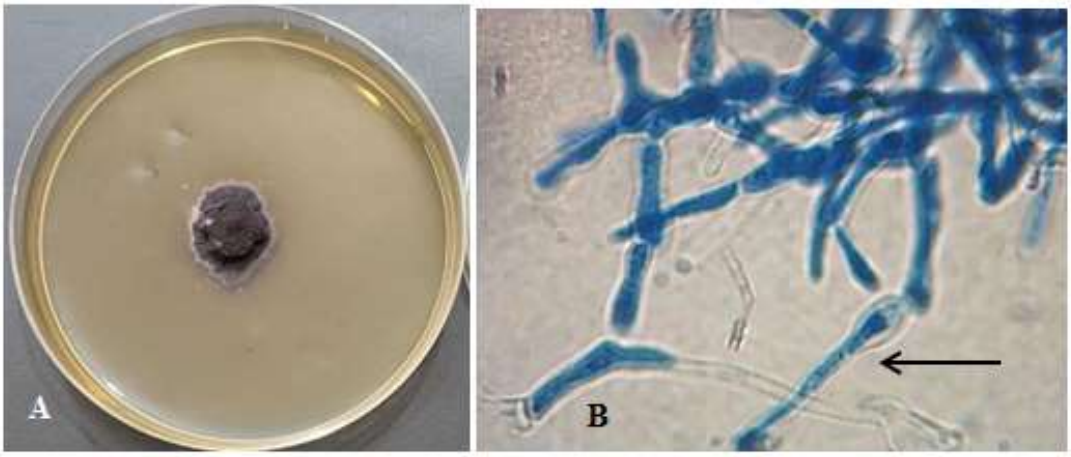


Fig.(4-12):*T. violaceum* (A) Colony morphology (B) hyphae and chlamydospores. (40X magnification) incubated at 25-30 ± 2C° for 28 days.

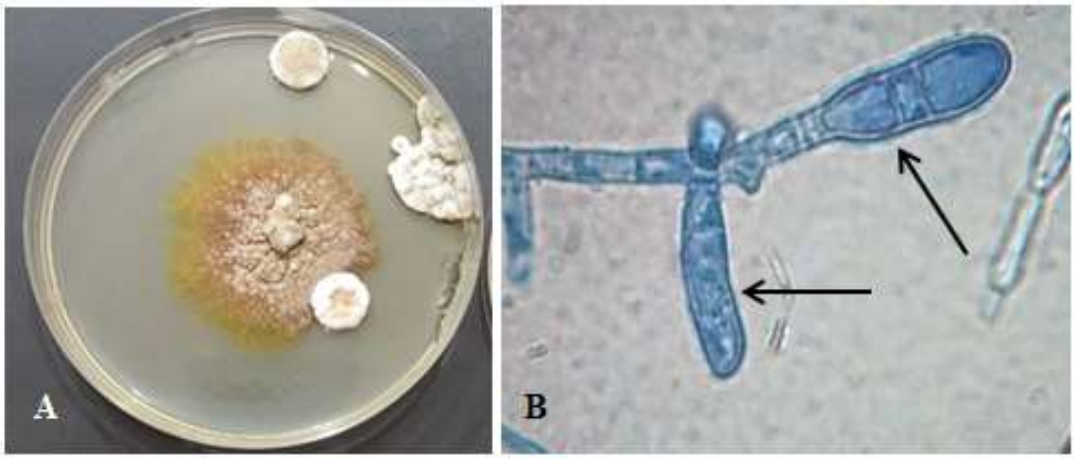


Fig. (4- 13): *E. floccosum*, (A) Colony morphology, (B) Macroconidia (40X magnification). incubated at 25-30 ± 2C° for 28 days.

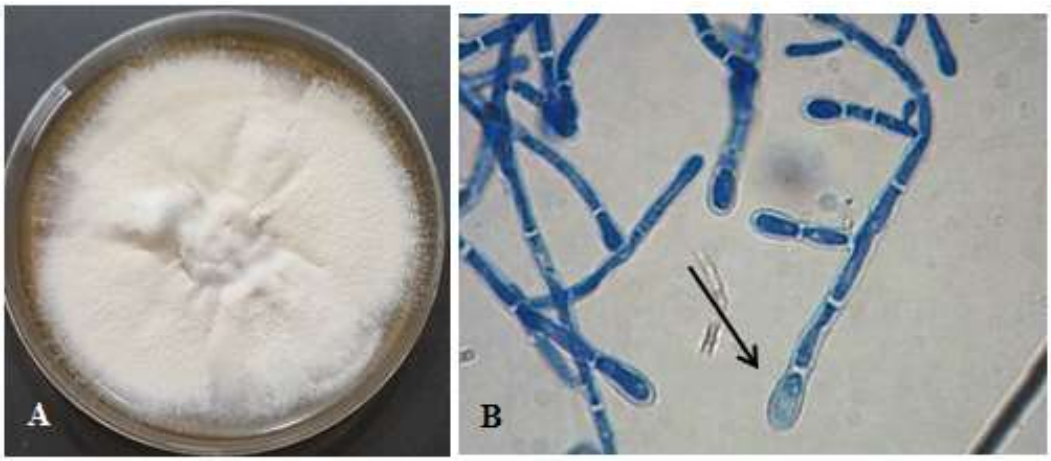


Fig. (4- 14): *C. tropicum*, (A) Colony morphology, (B) Macroconidia (40X magnification). incubated at 25-30 ± 2C° for 28 days.

4.4.2. Molecular diagnosis of dermatophytes

4.4.2.1. DNA extraction and PCR assay

Twenty isolates of dermatophytes species of this study were subjected for DNA extraction . ITS5/ITS4 are universal primer pair that targeted the sequences place of the ITS1-5.8S-ITS2 region of the dermatophytes isolates was used to discrimination of fungi to the species level. Fig. (4-15) shows agarose gel electrophoresis of PCR products for dermatophytes species.

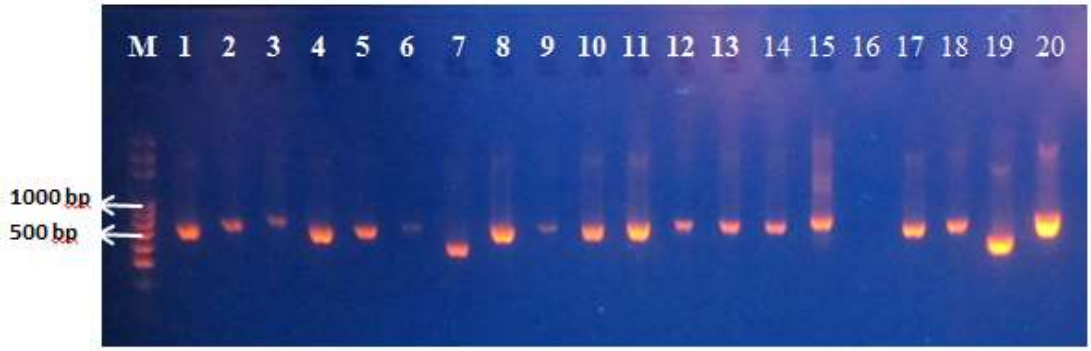


Fig. (4-15): Agarose gel electrophoresis of PCR products for ITS1-5.8S-ITS2 region of dermatophytes species. Lane M= molecular marker 1500 bp.

The molecular weights of the PCR products for the dermatophytes isolates under study ranged from 500-800 bp., as there are clear differences in the molecular weight of dermatophytes species, this agree with most references that used ITS5 and ITS4 primers (Abd Alla *et al*,. 2013). Al-Kafaji, (2014) identified three species of dermatophyte, *T. rubrum* isolates which their molecular weights were from 720-800 bp., *T. mentagrophyte*s isolates from 690-720 bp. and *E. floccosum* isolates was 780 bp.

Identification for clinical isolates of *Arthroderma otae* complex which include three species of *Microsporum* (*M. canis, M. audouinii,* and *M. ferrugineum*) that identified by ITS5 and ITS4 primers for sequencing analysis, the

molecular weight of ITS region of *A. otae* ranged 780-820 bp. (Hasan, 2018). Diongue *et al*., (2019) ITS-based PCR and DNA sequencing were applied for identification of the isolated dermatophytes that identification revealed *Trichophyton soudanense* (*n*= 13), *T. interdigitale* (*n*= 10), *Microsporum audouinii* (*n*= 5), and one strain for each of the following species: *T. rubrum*, *T. mentagrophytes*, and *M. canis* and one unidentified strain.

While Pospischil *et al*., (2022) used LSU1 and LUS2 primers for amplification of 28S ribosomal DNA region of dermatophyte species and non-dermatophyte in Onychomycosis. Also, Abdel-Fatah *et al*., (2013) were able to distinguish between dermatophytes species with molecular weights from 670-780 bp. and *Candida* species with molecular weights from 510-550 bp. The amplification of ITS regions was successful in certain dermatophyte species by using the fungus-specific universal primers (ITS1 and ITS4) and PCR-RFLP technique, Ayah *et al.* (2022) used restriction enzyme *BstN1*for digestion several dermatophyte species isolated from Palestine. Lavari *et al*., (2022) also used primers (ITS1 and ITS4) for 30 isolates of *M. canis* and their sexual stage (teleomorph) was *Arthroderma otae* isolated from dogs and cats with skin lesions.

4.4.2.2. Sequencing analysis

PCR was done for Twenty dermatophytes isolates for ITS region, Direct sequencing analysis is performed to the 20 µl PCR product of ITS region were sent to Macrogen Laboratory in Korea. After obtaining the sequence of the nitrogenous bases of the sent isolates, they are matched with the sequence of reference samples in the gene bank using the NCBI Blast Nucliotide Database to confirm the highest proportion of the genus and species name for each isolates. Appendix (1) shows the nitrogenous bases sequence for some of the isolates under study and the ratios of their corresponds with the reference isolates in the gene

bank. These corresponds between the results of the sequence of the nitrogenous bases of the isolates under study and the sequence of reference samples in the gene bank showed that the point mutation which represents miss matching and genetic gaps .

Table (4-3) showed a comparison of the results of the phenotypic diagnosis for dermatophytes isolates under study with the results of DNA sequencing of the ITS regions for identification of dermatophyte species. The table showed that the molecular diagnosis using the nitrogenous bases sequence of some samples were identical to the phenotypic diagnosis using traditional laboratory methods except several isolates, some have not been diagnosed with traditional methods to species level, which are diagnosed by molecular methods as *Trichophyton* sp. (isolates no. 3, 4, 10, 11, 12, 16, 17 and 18). PCR sequencing provided an excellent tool for identifying dermatophyte strains that do not present typical morphological characteristics. It was also able to give correct identification of an atypical strain of *T. quinckeanum* responsible of dermatophytosis (Diongue *et al*., 2019).

Some studies were using sequence analysis of ITS region by using ITS5 and ITS4 primers for identification of *Trichophyton* species such as *T. verrucosum, T. interdigetale, and T. mentagrophytes* and other species(Hasan and Al-Shibli 2015).Or for identification of *Microsporum* species by sequence analysis of ITS region (Hasan, 2018). Other studies Jang *et al*., (2012) were using 47 clinical isolates due to dermatophytes and non-dermatophytes species to identification by sequence analysis of three genetic region ITS, D1-D2, and ß-tubulin regions. Rezaei-Matehkolaei *et al.,* (2012) were differentiated between *Trichophyton tonsurans* and *T. equinum* and in both studies used nucleotide sequencing of three of genetic loci ITS1, *BT2* and *TEF1* and determined SNP among species studied.

DNA extraction followed by the application of deferent methods for molecular diagnosis has advanced significantly in the last 15 years (Begum *et al*.,

2020). DNA sequences are very useful for this purpose and permit an accurate identification. The internal transcribed spacer polymorphisms ITS1 and ITS2 that flank the region encoding the 5.8S rDNA show adequate and reliable sensitivity in distinguishing different species. In addition, the 28S rDNA sequences and genes encoding topoisomerase II and chitin synthase I are used for the identification of dermatophyte species (Petrucelli *et al*., 2022).

Table (4-3): Comparison between the phenotypic and molecular diagnosis results for dermatophytes isolates under study in reference to NCBI.

No.	Phenotypic diagnosis	Molecular diagnosis		Reference strain	Final diagnosis
		NCBT strain	Identities		
1.	*Chrysosporium* sp.	*Chrysosporium* sp.	99%	MH864428.1	*Chrysosporium* sp.
2.	*Chrysosporium* sp.	*Ch. tropicum*	99%	OW988053.1	*Ch. tropicum*
3.	*Trichophyton* sp.	*T. interdigitale*	100%	ON059700.1	*T. interdigitale*
4.	*Trichophyton* sp.	*T. interdigitale*	99%	MH517552.1	*T. interdigitale*
5.	*T. interdigitale*	*T. interdigitale*	99%	MH517547.1	*T. interdigitale*
6.	*T. interdigitale*	*T. interdigitale*	99%	KT963003.1	*T. interdigitale*
7.	*T. interdigitale*	*T. interdigitale*	99%	MN885443.1	*T. interdigitale*
8.	*T. mentagrophytes*	*T. mentagrophytes*	99%	MT261767.1	*T. mentagrophytes*
9.	*T. mentagrophytes*	*T. mentagrophytes*	99%	Z97995.1	*T. mentagrophytes*
10.	*Trichophyton* sp.	*T. quinckeanum*	100%	OP821484.1	*T. quinckeanum*
11.	*Trichophyton* sp.	*T. quinckeanum*	99%	OP391642.1	*T. quinckeanum*
12.	*Trichophyton* sp.	*T. quinckeanum*	99%	KJ606088.1	*T. quinckeanum*
13.	*T. mentagrophytes*	*T. mentagrophytes*	99%	MT261766.1	*T. mentagrophytes*
14.	*Epidermophyton*	*E. floccosum*	99%	MN966495.1	*E. floccosum*
15.	*Chrysosporium* sp.	*Ch. tropicum*	99%	MW616914.1	*Ch. tropicum*
16.	*Trichophyton* sp.	/	/	/	/
17.	*Trichophyton* sp.	*T. quinckeanum*	100%	OP419586.1	*T. quinckeanum*
18.	*Trichophyton* sp.	*T. quinckeanum*	98%	OL798098.1	*T. quinckeanum*
19.	*Epidermophyton*	*E. floccosum*	100%	OW988452.1	*E. floccosum*
20.	*T. interdigitale*	*T. interdigitale*	99%	ON059700.1	*T. interdigitale*

4.4.2.3. Phylogenetic tree

The phylogenetic tree was analyzed by using Mega 6 software programe with unweighted pair group method with arithmetic mean (UPGMA) tree type based on sequences data of ITS region amplified by ITS5/ITS4 primers pair for 20 isolates. The results of phylogenetic tree analysis for dermatophytes species were observed five clade. *T. interdigitale* shown in clade 1, *T. quinckeanum* in clade 2, *Epidermophyton* in clade 3, *T. mentagrophytes* in clade 4, and *Chrysosporium* sp. in clade 5 (Fig., 4-16).

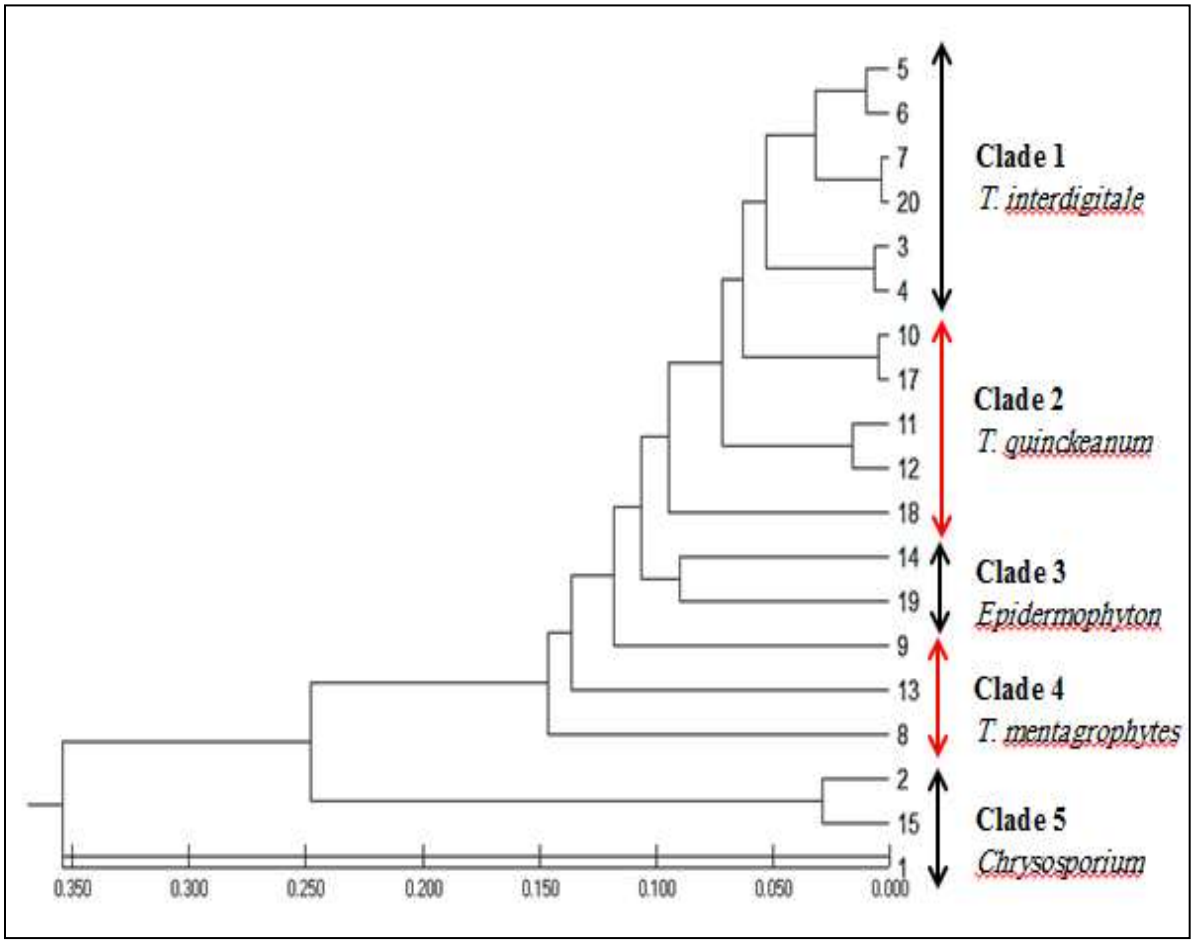


Fig. (4-16): Phylogenetic tree based on ITS1-5.8S-ITS2 region sequences for Dermatophytes isolates under studies.

By drawing the phylogenetic tree of the ITS region sequences for the isolates under study that show a high degree of similarity between species ranging 99.65-100 %. The difference in sequence among any clades shows approximately 0.0-0.35 %.

Un weighted pair group method with arithmetic mean (UPGMA) tree type was a common type of phylogenetic tree to determinate diversity of pathogenic fungi. Therefore, there were many studies that have used this type of tree. Al-Amari and Mohammed (2015) in Iraq, used 18S rDNA gene to diagnosis several species of *Trichophyton* and *Microsporum* by UPGMA tree type analysis and showed a clear convergence of fungal isolates.

Results coincided with several recent studies which show a high degrees of similarity and homogeneity in several genetic regions among dermatophytes according to ITS region (Ahmadi *et al*., 2016; de Hoog *et al*., 2017; Zhan *et al*., 2018) or whole genome sequences (Alshahni *et al*., 2018). While Ungo-kore *et al*., (2021) used analysis of 28S rRNA gene sequences for Phylogenetic tree of 32 isolates representative eight dermatophyte species. A major taxonomic problem, frequently encountered in environmental fungi in general, is unexpected phylogenetic diversity of groups that previously seemed to be phenotypically monomorphic, this shared phylogeny has been explained by their keratinophilic character, which is a rare property in the fungal kingdom. (de Hoog *et al*., 2017).

4.5. Antifungal Activity Against Growth of Dermatophytes

In this study, the antifungal activity are used including Fluconazole (Flu), Itraconazole (It), and Griseofulvin (Gr) by using poisoned food technique or agar dilution methodes against growth of dermatophytes species under study with series of concentration (0.25%, 0.5%, 1%). Table (4-4) shows the activity of variation type of antifungal drugs of dermatophytes species by effected of growth rate

measured with cm, The highest antifungal activity appears with first Fluconazole against all dermatophytes species used followed by Griseofulvin and Itraconazole respectively. As for the dermatophytes species, the most fungi that were sensitive to antifungals drug and gave the largest percentage of inhibition is *M. canis* followed by *T. quinckeanum* (Table 4-4). These results prove that there are significant differences between dermatophytes species used and significant differences between antifungal drugs used in this study.

Table (4-4): Growth of dermatophytes species with different concentration of antifungal measured with cm.

Fungal sampls		***Chrysosporium***	***T. mentagrophytes***	***M . canis***	***T. quinckeanum***	***T. interdigitale***
antifungal	**control C**	**8**	**8.5**	**7**	**8**	**7.5**
Flu	**(0.25%)**	**0.5**	**0.5**	**0**	**0**	**0.5**
	(0.5%)	**0**	**0.5**	**0**	**0**	**0.5**
	(1%)	**0**	**0**	**0**	**0**	**0**
Gr	**(0.25%)**	**2**	**1.5**	**1.6**	**1.3**	**1.1**
	(0.5%)	**1.3**	**1.2**	**1.5**	**1.2**	**1**
	(1%)	**0**	**0**	**0**	**0**	**0**
It	**(0.25%)**	**2.5**	**1.8**	**1.8**	**2.3**	**2.7**
	(0.5%)	**1.4**	**1.7**	**1.7**	**1**	**2**
	(1%)	**0**	**0**	**0**	**0**	**0**

According to this results, the antifungal drug that gave the largest percentage of inhibition for the studied dermatophytes is Fluconazole with rate of inhibition 94.4-100% followed by Griseofulvin with rate of inhibition 77.7-100% and finally Itraconazole with rate of inhibition 70-100% respectively (Figure 4-17, 4-18, and 4-19).

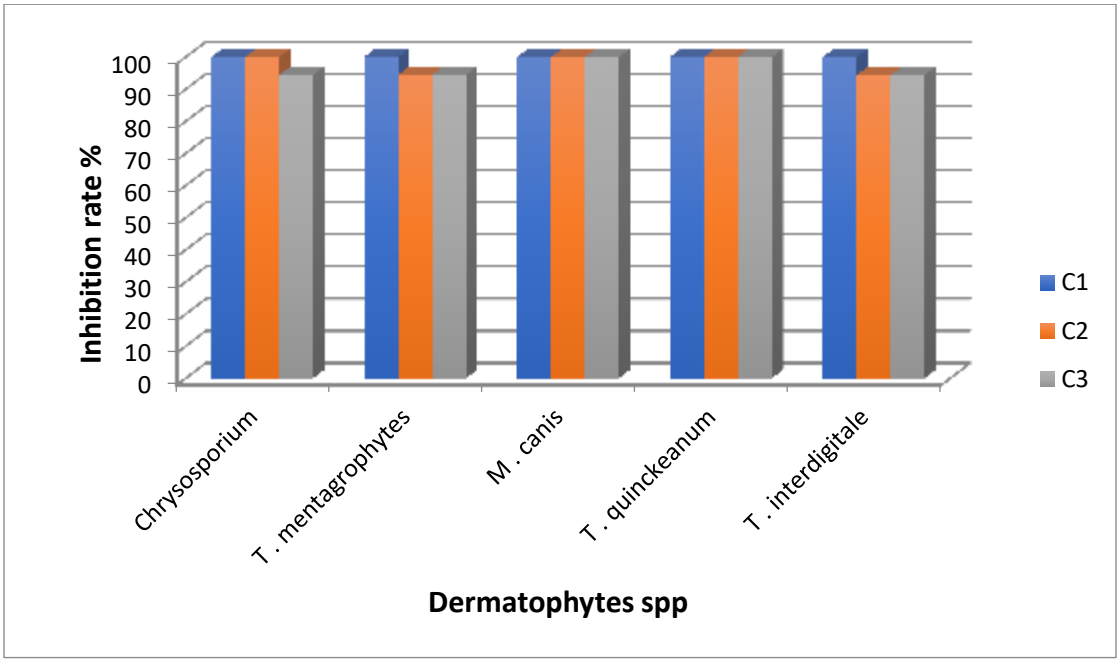


Fig. (4-17): Percentage of Inhibition of Dermatophytes spp. effected by antifungal Fluconazole.

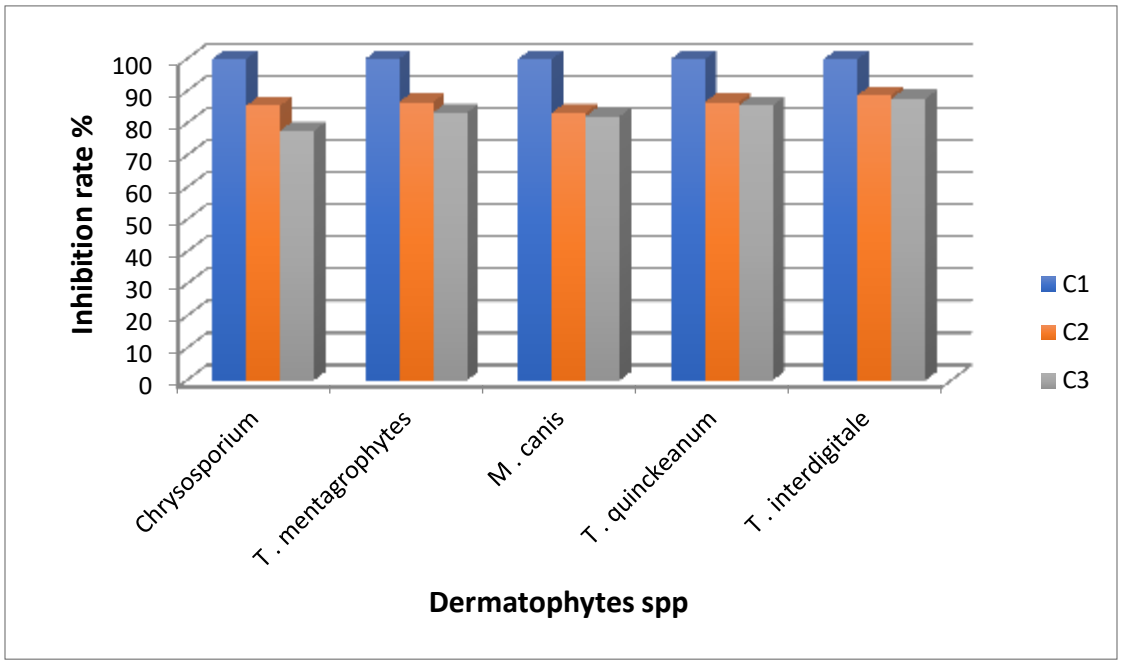


Fig. (4-18): Percentage of Inhibition of Dermatophytes spp. effected by antifungal Griseofulvin.

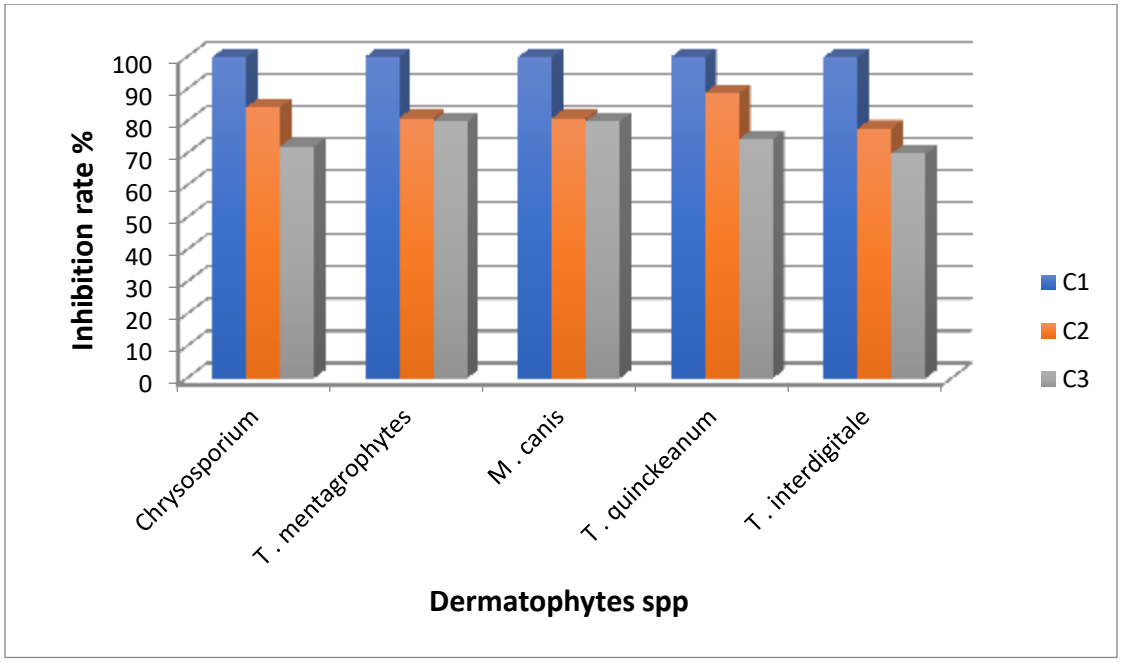


Fig. (4-19): Percentage of Inhibition of Dermatophytes spp. effected by antifungal Itraconazole.

Fig. (4-20 , 4-21, 4-22, 4-23, and 4-24) show growth of dermatophytes spp. with 0.25% of Itraconazole, and Griseofulvin.

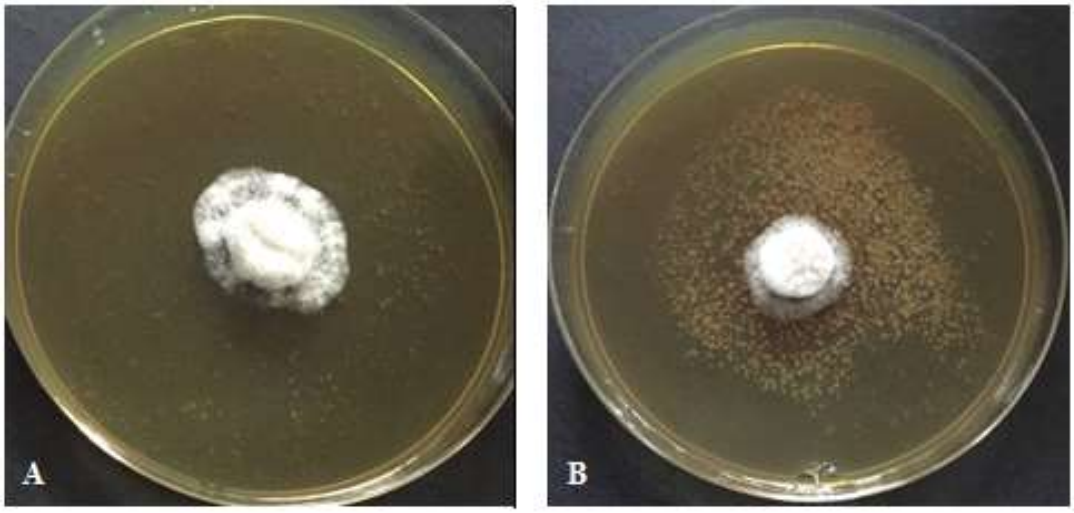


Fig. (4-20): Growth of *Chrysosporium* sp. with 0.25% of (A): Itraconazole, (B): Griseofulvin.

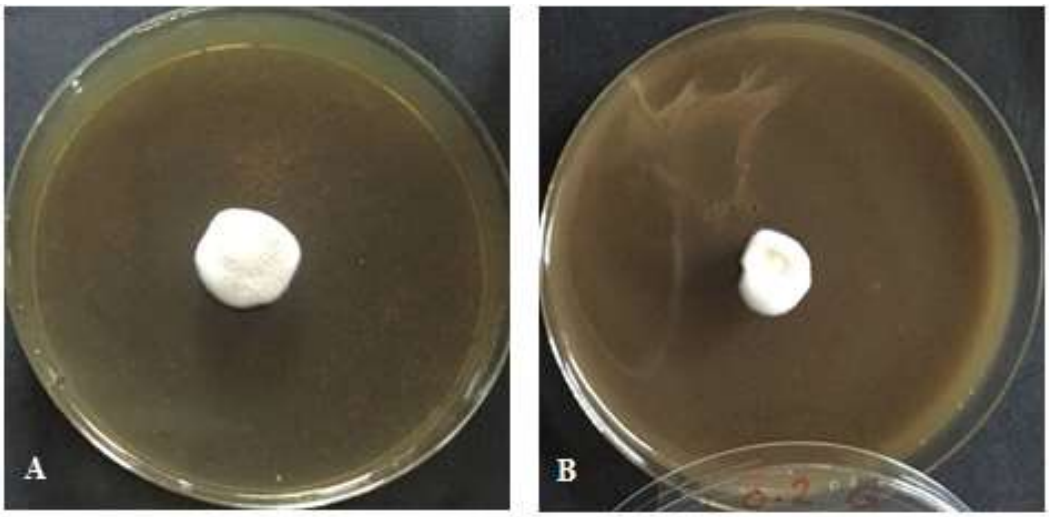


Fig. (4-21): Growth of *T . mentagrophytes* with 0.25% of (A): Itraconazole, (B): Griseofulvin.

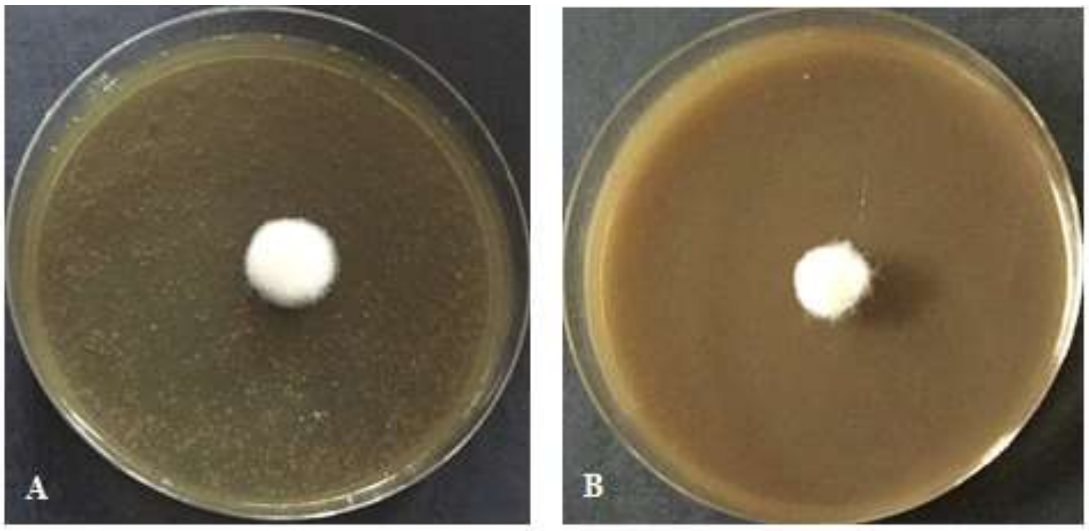


Fig. (4-22): Growth of *M . canis* with 0.25% of (A): Itraconazole, (B): Griseofulvin.

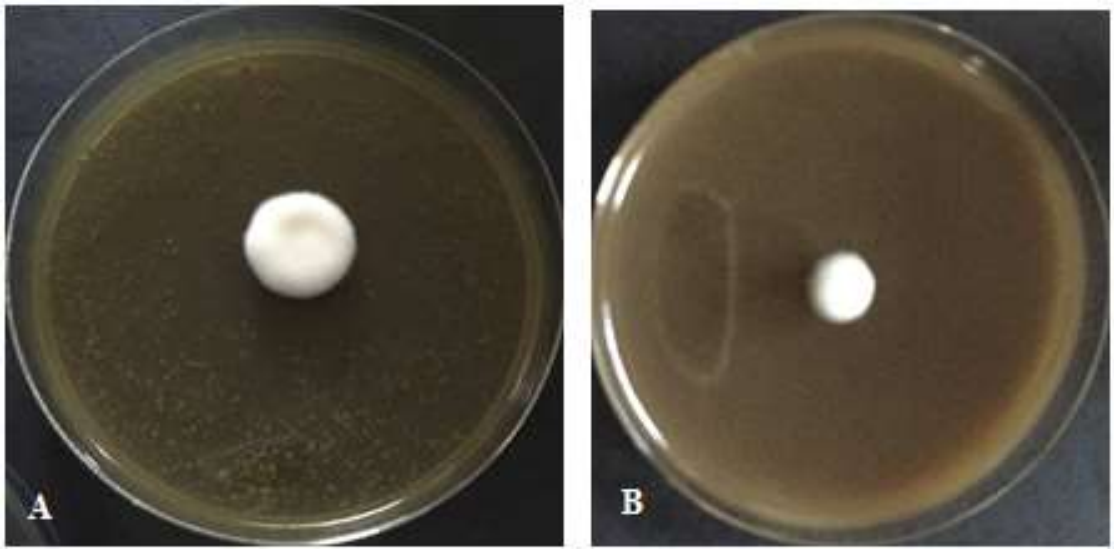


Fig. (4-23): Growth of *T . quinckeanum* with 0.25% of (A): Itraconazole, (B): Griseofulvin.

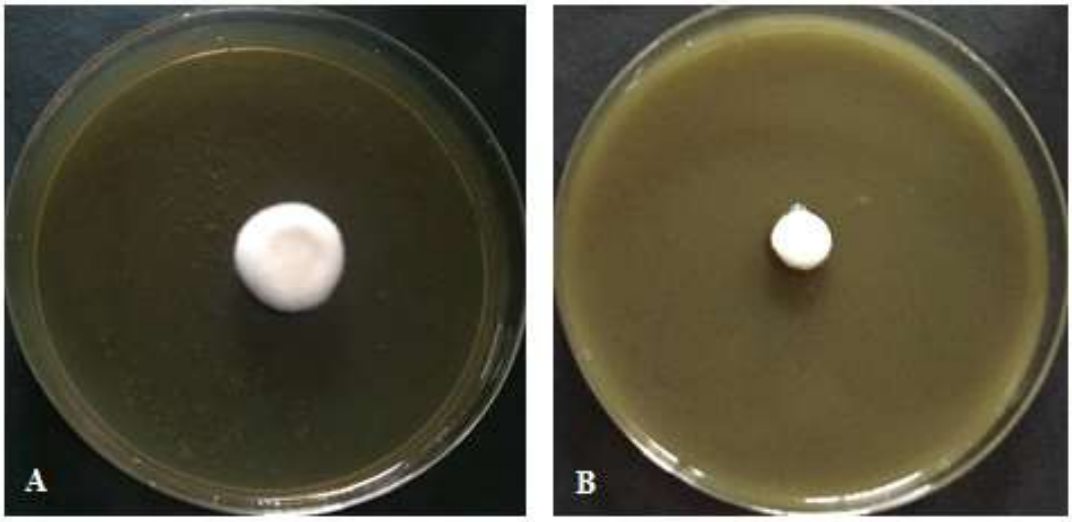


Fig. (4-24): Growth of *T . interdigitale* with 0.25% of (A): Itraconazole, (B): Griseofulvin.

These results agree with Norris *et al*., (1999) tested four antifungal drugs such as griseofulvin, itraconazole, terbinafine and fluconazole against 18 dermatophytes isolates, there was no difference in the MICs of itraconazole and terbinafine with higher inoculums size but higher MICs were observed for fluconazole and griseofulvin.

Brescini *et al*., (2021) referred that combinations included two antifungal agents or an antifungal agent plus another chemical compound including plant extracts or essential oils, calcineurin inhibitors, peptides, disinfectant agents, and others, clinical results indicate that association of antifungal agents is effective. Sugiura *et al*., (2021) tested combinations between two or more antifungal agent against *T. rubrum* and *T. interdigitale* and indicated the results of synergy between these agents.

Dogra *et al*., (2019) referred that standardization of antifungal susceptibility testing for dermatophytes is generally difficult as there are many variable critical parameters that needs to be considered while performing the test such as inoculum size (i.e., number of conidia/spores), incubation temperature and duration, media to be used, and time and percentage of growth inhibition for end point detection.

These results were consistent with The three antifungal susceptibility patterns were studied by Taher, (2022) for dermatophytes such as fluconazole, itraconazole and terbinafine and it was found that 10% of isolates were sensitive to fluconazole and 90% were resistant to fluconazole, 20% of isolates were sensitive to itraconazole and 80% were resistant to itraconazole, and 26% of isolates were sensitive to terbinafine and 74% were resistant to terbinafine. This results not agree with findings of other studyin were performed on 58 clinical isolates of dermatophytes using four antifungals (fluconazole, itraconazole, terbinafine, and griseofulvin). They showed only six strains resistant to fluconazole, five resistant to terbinafine, and five, four, and three strains were found intermediate sensitive to fluconazole, itraconazole, and griseofulvin respectively Nasir *et al*., (2014).

This study was also similar with the results of Baghi *et al*., (2016) where antifungal agents, that is, butenafine, , anidulafungin, caspofungin, econazole, miconazole, leoconazole, fluconazole, luliconazole, and lanoconazole, against Iranian clinical isolates of dermatophyte was investigated, Where this study showed that more than 90% of *T. interdigitale* isolates are sensitive to all factors, but they showed low sensitivity to miconazole and fluconazole , and the MIC range was narrow for leoconazole and wider for miconazole and fluconazole.

In a study by Adimi *et al*., (2013) in Iran, itraconazole and terbinafine showed the lowest MIC values, while fluconazole displayed the highest MIC. These results are inconsistent with Sooriya *et al*., (2021) where antifungal susceptibility testing was performed against three commonly used antifungals, fluconazole, itraconazole, and terbinafine, to analyze the role of antifungal resistance as a cause for clinical nonresponsiveness and chronicity. Antifungal resistance was observed in 77.4% of isolates 73.6% resistant to terbinafine and 3.8% resistant to fluconazole.

Also the results not agree with Soodan & Kaur, (2022) where they found in their research which they conducted that Itraconazole was found to be the best drug for the treatment of dermatophytes among the tested antifungals. The second most sensitive drug was found to be Terbinafine. Griseofulvin was the least effective drug among the tested antifungals.

In search by Vanapalli *et al*., (2022) they found The overall mean minimum inhibitory concentration values for itraconazole were low when compared to griseofulvin and terbinafine. Combination of itraconazole and griseofulvin achieved highest clinical and mycological cure rates (93.1%). Griseofulvin is still used to treat tinea, especially in liver function problems Mariyani, (2022).

4.6 Antifungal activity of plant (*Citrullus colocynthis*) against Dermatophytes.

The results of antifungal activity of the crude Alkaloid compounds extracted from the fruits of *Citrullus colocynthis* against Dermatophytes such as *Chrysosporium, T. mentagrophytes, M. canis, T. quinckeanum*, and *T. interdigitale* isolated from clinical samples are presented in (table 4-5). The antifungal activity of Alkaloid secondary metabolites with three concentrations (5, 10, and 15 mg/ml) was screened by food poisoning methods. The results revealed that, the crude Alkaloid compounds extracted from the fruits of *Citrullus colocynthis* showed significant reduction at $P \leq 0.05$ in the growth of Dermatophytes species. Antifungal activity was applied at (5, 10, and 15) mg/ml. Inhibitory Percentage% of Alkaloid ranging from 54.4% in 5 mg/ml, 73.7% in 10 mg/ ml, and 100% in 15 mg/ml when applied of *Chrysosporium* sp and 52.5% to 100% when applied of *T. mentagrophytes*, and ranging from 73.3% to 100% when applied of *M. canis* and also Inhibitory Percentage reached to 100% when applied upon *T. quinckeanum* and *T. interdigitale* at 15 mg/ml of Alkaloid extract (Fig. 4-17) compared with

negative control and positive control where inhibitory percentage was 0.00% for negative control (Fig. 4-21), and 100% for positive control (Figure: 4-20). In the same context, the crude Terpenoid compounds showed 43.3% growth inhibition at (5 mg/ml) and 60.7% at (10 mg/ml), and 100% at (15 mg/ml) concentration when applied of *Chrysosporium sp* and from 47.7% at 5 mg/ml to 100% at 15 mg/ml when applied of *T. mentagrophytes*, and also Inhibitory Percentage effect reached to 100% when applied upon *M. canis*, *T. quinckeanum* and *T. interdigitale* at 15 mg/ml of Terpenoid extract respectively (table 4-6), Thus, it differed significantly compared to the control treatment (Fig. 4-18).

Table(4-5): Antidermatophytal efficacy of the crude Alkaloid compounds extracted from *Citrullus colocynthis* fruits against *Dermatophytes* isolated from clinical samples

Concentration (mg/ml)	Pathogenic Fungi				
	Chrysosporium	*T. mentagrophytes*	*M. canis*	*T. quinckeanum*	*T. interdigitale*
	Inhibitory Percentage% of Alkaloid				
Control negative (DMSO 10%)	0± 0.0	0± 0.0	0± 0.0	0± 0.0	0± 0.0
5 mg/ml	54.4± 1.1	52.5± 1.6	73.3± 1.1	55.9± 1.7	55.5± 1.1
10 mg/ml	73.7± 2.7	62.6± 1.7	77.4± 1.6	65.1± 1.6	64.4± 2.2
15 mg/ml	100± 0.0	100± 0.0	100± 0.0	100± 0.0	100± 0.0
Control positive	100± 0.0	100± 0.0	100± 0.0	100± 0.0	100± 0.0
L.S.D	2.445	1.950	1.648	1.951	2.191

***Mean± standard deviation**

Mean difference is significant at 0.05 level

Table (4-6): Antidermatophytal efficacy of the crude Terpenoid compounds extracted from *Citrullus colocynthis* fruits against Dermatophytes isolated from clinical samples

Concentration (mg/ml)	Pathogenic Fungi				
	Chrysosporium sp	*T. mentagrophytes*	*M. canis*	*T. quinckeanum*	*T. interdigitale*
	Inhibitory Percentage% of Terpenoid				
Control negative (DMSO 10%)	0± 0.0	0± 0.0	0± 0.0	0± 0.0	0± 0.0
5 mg/ml	43.3± 1.1	47.7± 1.1	47.4± 0.6	50.3± 1.2	53.7± 0.7
10 mg/ml	60.7± 1.7	61.1± 1.1	71.1± 1.1	57.4± 0.6	58.8± 1.1
15 mg/ml	100± 0.0	100± 0.0	100± 0.0	100± 0.0	100± 0.0
Control positive	100± 0.0	100± 0.0	100± 0.0	100± 0.0	100± 0.0
L.S.D	1.652	1.277	1.043	1.170	1.966
*Mean± standard deviation Mean difference is significant at 0.05 level					

In addition to that, the crude Flavonoid compounds showed significant activity at three concentrations (5, 10, and 15 mg/ml) compared with negative control against *Dermatophytes* species isolated from clinical samples (Table 4-7). The highest percentage of inhibition (100%) was recorded at 15 mg/ml when applied upon *T. mentagrophytes*, *T. quinckeanum*, and *T. interdigitale* (Fig. 4-19). On the other hand, the effect of secondary metabolites compounds extracts extracted from *Citrullus colocynthis* was equal to the effect of the antibiotic, which confirms the effectiveness of the *Citrullus colocynthis* against *Dermatophytes* under study.

Table(4-7): Antidermatophytal efficacy of the crude Flavonoid compounds extracted from *Citrullus colocynthis* fruits against *Dermatophytes* isolated from clinical samples

Concentration (mg/ml)	Pathogenic Fungi				
	Chrysosporium sp	*T. mentagrophytes*	*M. canis*	*T. quinckeanum*	*T. interdigitale*
	Inhibitory Percentage% of Flavonoid				
Control negative (DMSO 10%)	0± 0.0	0± 0.0	0± 0.0	0± 0.0	0± 0.0
5 mg/ml	42.2± 1.1	38.4± 0.6	56.9± 1.6	46.2± 1.6	38.8± 1.1
10 mg/ml	53.3± 2.2	48.1± 1.7	60.3± 4.2	53.3± 1.1	51.1± 1.1
15 mg/ml	98.5± 0.6	100± 0.0	97.7± 1.1	100± 0.0	100± 0.0
Control positive	100± 0.0	100± 0.0	100± 0.0	100± 0.0	100± 0.0
L.S.D	2.852	2.228	3.832	1.634	1.295

***Mean± standard deviation**

Mean difference is significant at 0.05 level

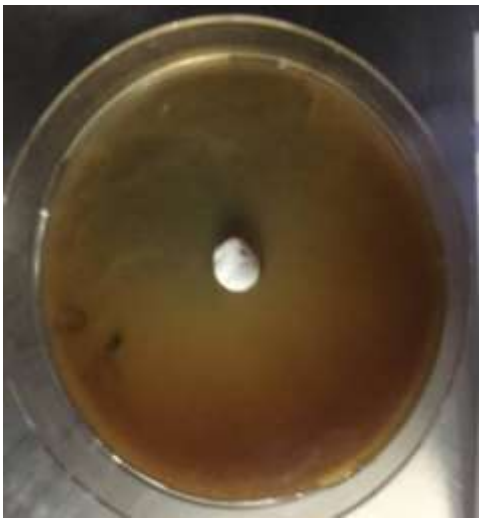
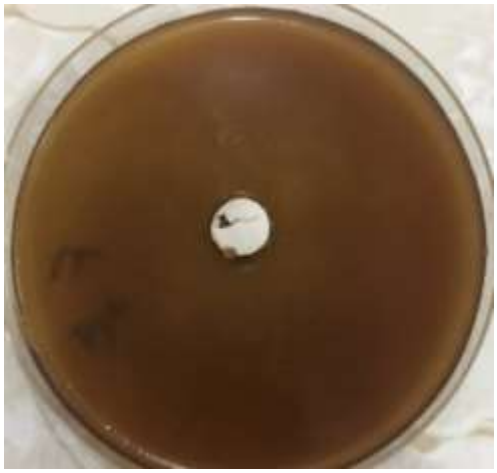


Figure: (4-25).(A) Anti-dermatophytal Efficacy of the crude Alkaloid compounds at 15 mg/ml against dermatophyte species

(B) Anti-dermatophytal Efficacy of the crude Terpenoid compounds at 15 mg/ml against dermatophyte species



(C) Anti-dermatophytal Efficacy of the crude Flavonoid compounds at 15 mg/ml against dermatophyte species

Figure: (4-26).(A) the growth of *chrysosporium* in control negative treatment

(B) Growth of dermatophyte species in antibiotic control positive

Although the resistance of dermatophytes to antifungals and cytotoxic drugs in different ways such as modifications of target enzymes, over-expression of genes encoding ATP-binding cassette transporters and stress-response-related proteins, the present study was proved that, the secondary metabolites include Alkaloids, Terpenoids, and Flavonoids extracted from the fruits of *Citrullus colocynthis* L. have powerful antifungal activity against *Dermatophytes* species isolated from clinical samples. The plant kingdom provided and is still providing endless sources of medicinal plants of various uses for example, secondary metabolites extracted from different active parts of numerous medicinal plants such as (*Lactuca serriola* leaves; *Lepidium sativum* leaves; *Myrtus Communis* leaves; *Cassia senna* leaves; *Ricinus communis* leaves; *Cassia didymobotrya* leaves; *Melia azedarach* leaves; *Dianthus caryophyllus* flowers bud; and *Salvia hispanica* seeds), possess ability of antibacterials for controlling several pathogenic microorganisms isolated from different clinical samples (Hussein & Al-Marzoqi, 2020).

Hussein *et a*l. (2018) reported that, phytochemical compounds extracted from the unicellular primitive plant like *Chlorella vulgaris* possess ability of antibacterial counter to pathogenic bacteria. (Kamal *et al*., 2019) used phytochemical compounds extracted from *Hibiscus sabdarifa* for controlling Enterobacteriaceae (Kamal *et al*., 2020) used phytochemical compounds extracted from of *Ficus carica* L. for controlling *E. coli* and *Pseudomonas aeruginosa*. AL-Masoodi *et al*., (2020) used phytochemical compounds extracted from *Boswellia carteri* and *Curcuma longa* for controlling *Fusarium* spp. isolated from seeds of maize. Hussain *et al*., (2021) used terpenoids compounds extracted from *Carthamus tinctorius* seeds and flavonoid compounds extracted from *M. Communis* leaves against *Aspergillus* species isolated from stored medicinal plant seeds. Secondary metabolites represented by Alkaloids and Flavonoids compounds extracted from *M. Communis* leaves respected a worthy source for controlling pathogenic microorganisms segregated from hemodialysis fluid specimens (Sharara *et al*.,2021).

Terpenoids and Flavonoids compounds is most effective in controlling *Candida* species (Mohammed *et al*., 2023). The extracts of *Citrullus colocynthis* seeds used at low concentration may have significant potential for biological control of fungi and theirs toxins (Gacem *et al*., 2013). *Citrullus colocynthis* seeds have inhibitory effect against *Rhizopus* spp (Prasad, 2014). *Citrullus colocynthis* fruits have inhibitory effect against different *Candida* and *Aspergillus* strains (Eidi *et al*., 2015).

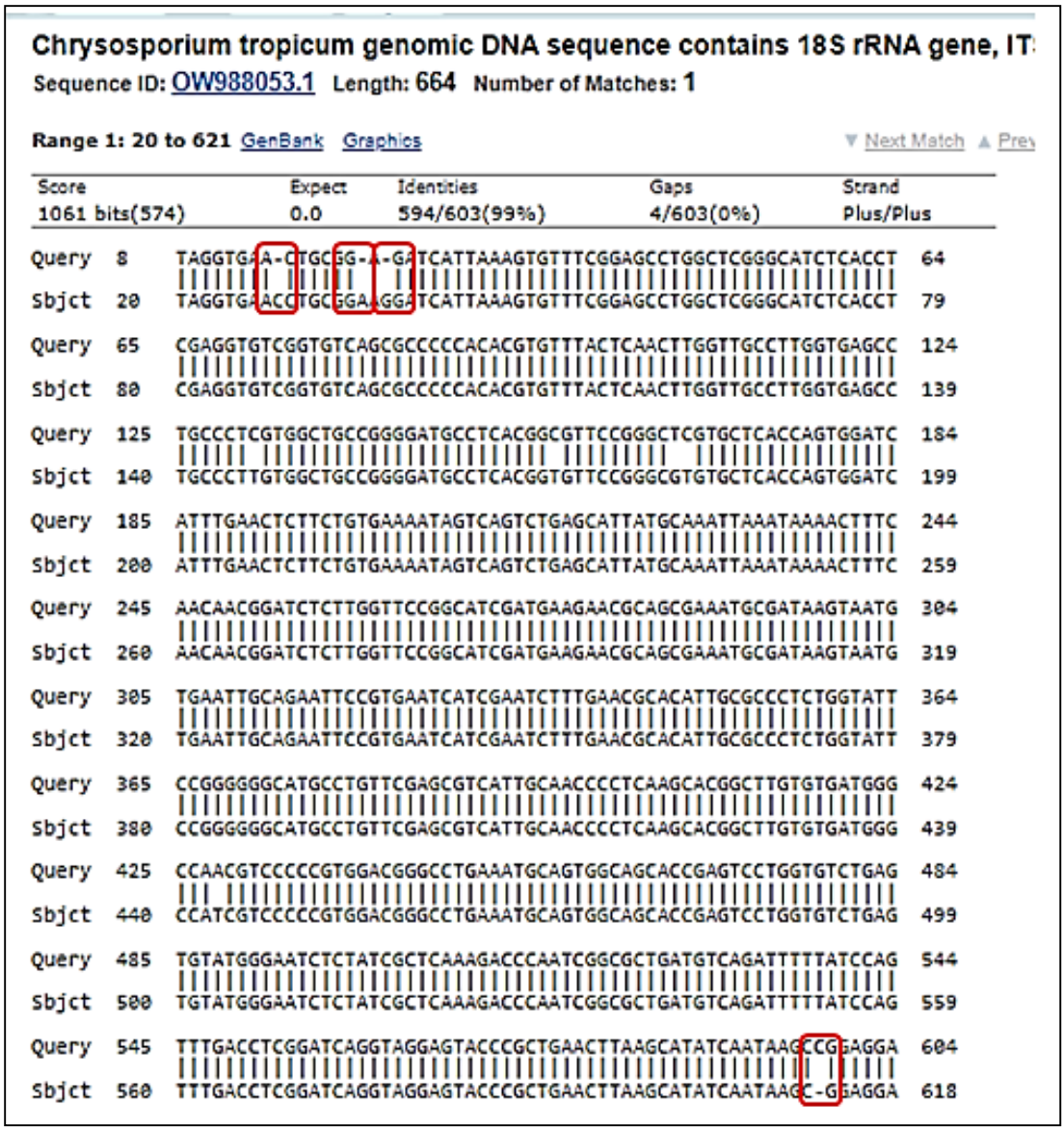
The leaves of *Citrullus colocynthis* have antifungal properties against resistance *Candida* spp (Alsubhi *et al*., 2019). *Citrullus colocynthis* has an inhibitory effect on bacteria and *Candida albicans* (Tahmasebi *et al*., 2022). *C. colocynthis* seed methanol extract from Sinai desert combined with 0.5 µg/ml of the

antifungal drug fluconazole was more effective against dermatophytes than the extract or fluconazole each on its own (Ouf *et al*., 2022). On the other hands, the mode of the antifungal action of the Alkaloids is usually pleiotropic, where protein synthesis is inhibited, and the fungal DNA is intercalated or by boosting the development of fungi inhibitors (Arif *et al*., 2009).

Terpenoids reduced the mitochondrial content, thus modified the level of reactive oxygen species (ROS) and ATP generation. It is also reported that triterpenoid possesses more potent antifungal activity as compared to the tetraterpenoid (Haque *et al*., 2016). Terpenoids and flavonoids make their effects by disruption of microbial membranes (Okusa Ndjolo *et a*l., 2009). Medicinal plant possessed antifungal effects by many mechanisms, they caused membrane disturbance resulting in the loss of membrane integrity, inhibited DNA transcription and reduced the cell populations, inhibited the activity of fungal antioxidant enzymes and inhibited fungal biofilm formation (Wu *et al*., 2013). Alternatively, the presence of alkaloids compounds such as colocynthidin and colocynthin may be disrupt cytoplasmic membrane of the microorganisms through their action on lipids and protein (Anthony ,1976). Finally, antifungal activity of *Citrullus colocynthis* L. might be belonging to secondary metabolites like Alkaloids, Flavonoids, and Terpenoids and their effect in proteins and DNA synthesis and disruption in membranes permeability or disturbance in metabolic activity.

Appendix (1): shows the nitrogenous bases sequences for some of the isolates under study and the ratios of their corresponds with the reference isolates in the gene bank and the sites of genetic gaps (red circles).

Isolate no. 2



Chrysosporium tropicum genomic DNA sequence contains 18S rRNA gene, IT

Sequence ID: OW988053.1 Length: 664 Number of Matches: 1

Range 1: 20 to 621 GenBank Graphics ▼ Next Match ▲ Prev

Score	Expect	Identities	Gaps	Strand
1061 bits(574)	0.0	594/603(99%)	4/603(0%)	Plus/Plus

```
Query  8    TAGGTGAA-CTGCGG-A-GATCATTAAAGTGTTTCGGAGCCTGGCTCGGGCATCTCACCT  64
            ||||||||| ||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  20   TAGGTGAACCTGCGGAAGGATCATTAAAGTGTTTCGGAGCCTGGCTCGGGCATCTCACCT  79

Query  65   CGAGGTGTCGGTGTCAGCGCCCCCACACGTGTTTACTCAACTTGGTTGCCTTGGTGAGCC  124
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  80   CGAGGTGTCGGTGTCAGCGCCCCCACACGTGTTTACTCAACTTGGTTGCCTTGGTGAGCC  139

Query  125  TGCCCTCGTGGCTGCCGGGGATGCCTCACGGCGTTCCGGGCTCGTGCTCACCAGTGGATC  184
            |||||| ||||||||||||||||||||||||| |||||||||  ||||||||||||||||||
Sbjct  140  TGCCCTTGTGGCTGCCGGGGATGCCTCACGGTGTTCCGGGCGTGTGCTCACCAGTGGATC  199

Query  185  ATTTGAACTCTTCTGTGAAAATAGTCAGTCTGAGCATTATGCAAATTAAATAAAACTTTC  244
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  200  ATTTGAACTCTTCTGTGAAAATAGTCAGTCTGAGCATTATGCAAATTAAATAAAACTTTC  259

Query  245  AACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG  304
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  260  AACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG  319

Query  305  TGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATT  364
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  320  TGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATT  379

Query  365  CCGGGGGGCATGCCTGTTCGAGCGTCATTGCAACCCCTCAAGCACGGCTTGTGTGATGGG  424
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  380  CCGGGGGGCATGCCTGTTCGAGCGTCATTGCAACCCCTCAAGCACGGCTTGTGTGATGGG  439

Query  425  CCAACGTCCCCCGTGGACGGGCCTGAAATGCAGTGGCAGCACCGAGTCCTGGTGTCTGAG  484
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  440  CCATCGTCCCCCGTGGACGGGCCTGAAATGCAGTGGCAGCACCGAGTCCTGGTGTCTGAG  499

Query  485  TGTATGGGAATCTCTATCGCTCAAAGACCCAATCGGCGCTGATGTCAGATTTTTATCCAG  544
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  500  TGTATGGGAATCTCTATCGCTCAAAGACCCAATCGGCGCTGATGTCAGATTTTTATCCAG  559

Query  545  TTTGACCTCGGATCAGGTAGGAGTACCCGCTGAACTTAAGCATATCAATAAGCCGGAGGA  604
            ||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  560  TTTGACCTCGGATCAGGTAGGAGTACCCGCTGAACTTAAGCATATCAATAAGC-GGAGGA  618
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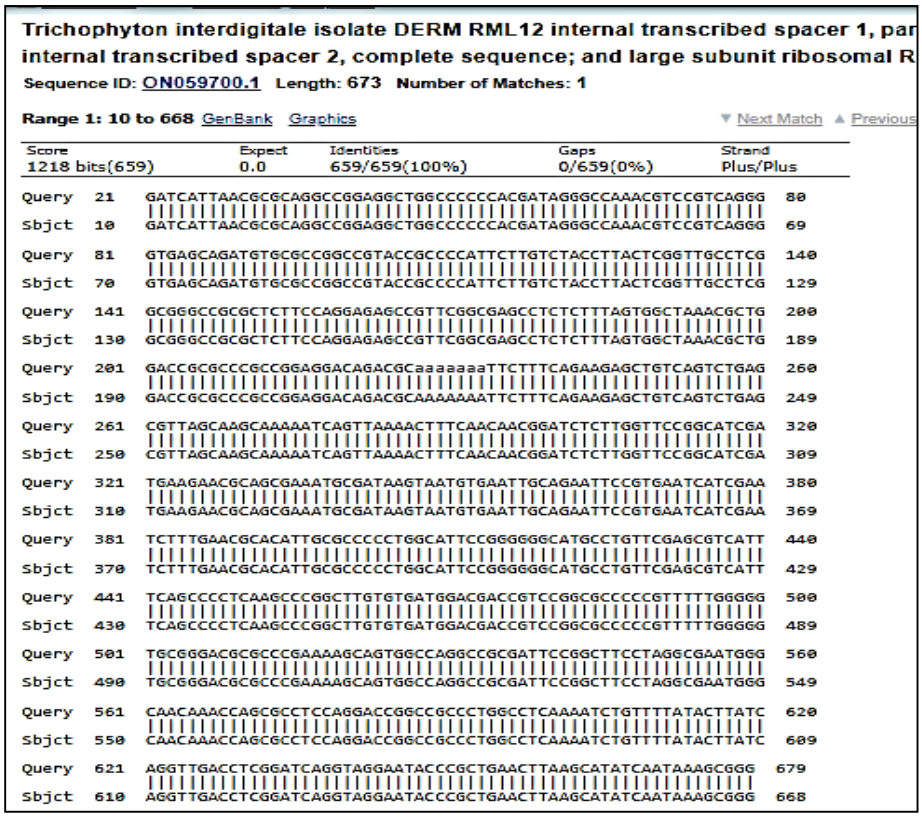
Isolate no. 3



Trichophyton interdigitale isolate DERM RML12 internal transcribed spacer 1, par
internal transcribed spacer 2, complete sequence; and large subunit ribosomal R

Sequence ID: ON059700.1 Length: 673 Number of Matches: 1

Range 1: 10 to 668 GenBank Graphics ▼ Next Match ▲ Previous

Score	Expect	Identities	Gaps	Strand
1218 bits(659)	0.0	659/659(100%)	0/659(0%)	Plus/Plus

```
Query  21   GATCATTAACGCGCAGGCCGGAGGCTGGCCCCCCACGATAGGGCCAAACGTCCGTCAGGG  80
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  10   GATCATTAACGCGCAGGCCGGAGGCTGGCCCCCCACGATAGGGCCAAACGTCCGTCAGGG  69

Query  81   GTGAGCAGATGTGCGCCGGCCGTACCGCCCCATTCTTGTCTACCTTACTCGGTTGCCTCG  140
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  70   GTGAGCAGATGTGCGCCGGCCGTACCGCCCCATTCTTGTCTACCTTACTCGGTTGCCTCG  129

Query  141  GCGGGCCGCGCTCTTCCAGGAGAGCCGTTCGGCGAGCCTCTCTTTAGTGGCTAAACGCTG  200
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  130  GCGGGCCGCGCTCTTCCAGGAGAGCCGTTCGGCGAGCCTCTCTTTAGTGGCTAAACGCTG  189

Query  201  GACCGCGCCCGCCGGAGGACAGACGCaaaaaaaTTCTTTCAGAAGAGCTGTCAGTCTGAG  260
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  190  GACCGCGCCCGCCGGAGGACAGACGCAAAAAAATTCTTTCAGAAGAGCTGTCAGTCTGAG  249

Query  261  CGTTAGCAAGCAAAAATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGA  320
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  250  CGTTAGCAAGCAAAAATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGA  309

Query  321  TGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTGAATCATCGAA  380
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  310  TGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTGAATCATCGAA  369

Query  381  TCTTTGAACGCACATTGCGCCCCCTGGCATTCCGGGGGGCATGCCTGTTCGAGCGTCATT  440
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  370  TCTTTGAACGCACATTGCGCCCCCTGGCATTCCGGGGGGCATGCCTGTTCGAGCGTCATT  429

Query  441  TCAGCCCCTCAAGCCCGGCTTGTGTGATGGACGACCGTCCGGCGCCCCCGTTTTTGGGGG  500
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  430  TCAGCCCCTCAAGCCCGGCTTGTGTGATGGACGACCGTCCGGCGCCCCCGTTTTTGGGGG  489

Query  501  TGCGGGACGCGCCCGAAAAGCAGTGGCCAGGCCGCGATTCCGGCTTCCTAGGCGAATGGG  560
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  490  TGCGGGACGCGCCCGAAAAGCAGTGGCCAGGCCGCGATTCCGGCTTCCTAGGCGAATGGG  549

Query  561  CAACAAACCAGCGCCTCCAGGACCGGCCGCCCTGGCCTCAAAATCTGTTTTATACTTATC  620
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  550  CAACAAACCAGCGCCTCCAGGACCGGCCGCCCTGGCCTCAAAATCTGTTTTATACTTATC  609

Query  621  AGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAAGCGGG  679
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  610  AGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAAGCGGG  668
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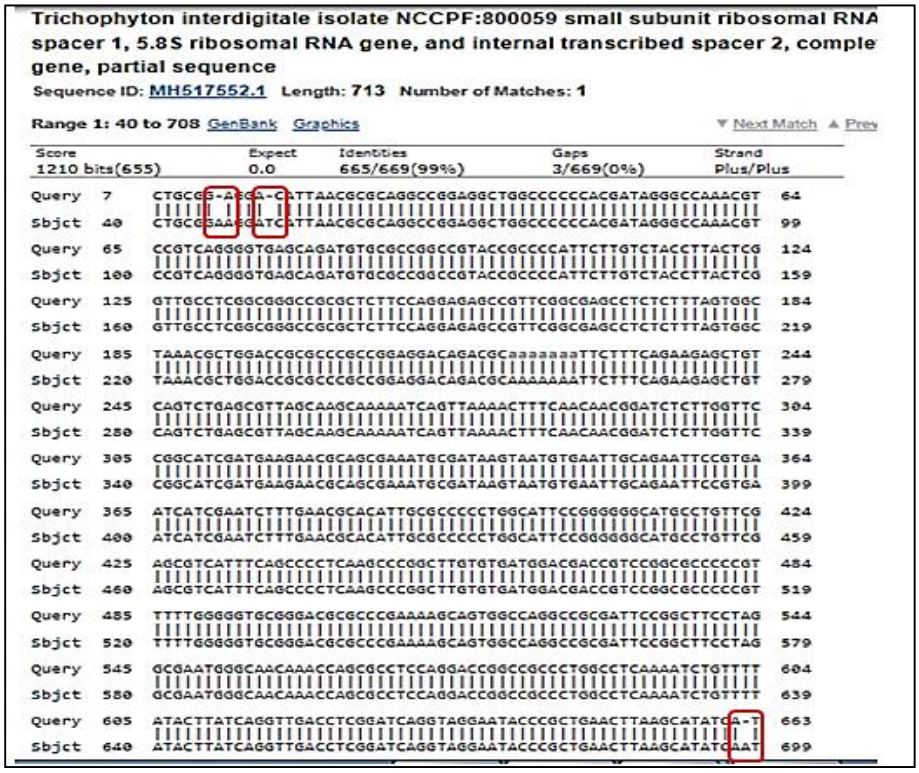
Isolate no. 4



Trichophyton interdigitale isolate NCCPF:800059 small subunit ribosomal RNA
spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, comple
gene, partial sequence

Sequence ID: MH517552.1 Length: 713 Number of Matches: 1

Range 1: 40 to 708 GenBank Graphics ▼ Next Match ▲ Prev

Score	Expect	Identities	Gaps	Strand
1210 bits(655)	0.0	665/669(99%)	3/669(0%)	Plus/Plus

```
Query  7    CTGCGS-AGGA-CATTAACGCGCAGGCCGGAGGCTGGCCCCCCACGATAGGGCCAAACGT  64
            |||||| |||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  40   CTGCGSAAAGGATCATTAACGCGCAGGCCGGAGGCTGGCCCCCCACGATAGGGCCAAACGT  99

Query  65   CCGTCAGGGGTGAGCAGATGTGCGCCGGCCGTACCGCCCCATTCTTGTCTACCTTACTCG  124
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  100  CCGTCAGGGGTGAGCAGATGTGCGCCGGCCGTACCGCCCCATTCTTGTCTACCTTACTCG  159

Query  125  GTTGCCTCGGCGGGCCGCGCTCTTCCAGGAGAGCCGTTCGGCGAGCCTCTCTTTAGTGGC  184
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  160  GTTGCCTCGGCGGGCCGCGCTCTTCCAGGAGAGCCGTTCGGCGAGCCTCTCTTTAGTGGC  219

Query  185  TAAACGCTGGACCGCGCCCGCCGGAGGACAGACGCaaaaaaaTTCTTTCAGAAGAGCTGT  244
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  220  TAAACGCTGGACCGCGCCCGCCGGAGGACAGACGCAAAAAAATTCTTTCAGAAGAGCTGT  279

Query  245  CAGTCTGAGCGTTAGCAAGCAAAAATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTC  304
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  280  CAGTCTGAGCGTTAGCAAGCAAAAATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTC  339

Query  305  CGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTGA  364
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  340  CGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTGA  399

Query  365  ATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGCATTCCGGGGGGCATGCCTGTTCG  424
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  400  ATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGCATTCCGGGGGGCATGCCTGTTCG  459

Query  425  AGCGTCATTTCAGCCCCTCAAGCCCGGCTTGTGTGATGGACGACCGTCCGGCGCCCCCGT  484
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  460  AGCGTCATTTCAGCCCCTCAAGCCCGGCTTGTGTGATGGACGACCGTCCGGCGCCCCCGT  519

Query  485  TTTTGGGGGTGCGGGACGCGCCCGAAAAGCAGTGGCCAGGCCGCGATTCCGGCTTCCTAG  544
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  520  TTTTGGGGGTGCGGGACGCGCCCGAAAAGCAGTGGCCAGGCCGCGATTCCGGCTTCCTAG  579

Query  545  GCGAATGGGCAACAAACCAGCGCCTCCAGGACCGGCCGCCCTGGCCTCAAAATCTGTTTT  604
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  580  GCGAATGGGCAACAAACCAGCGCCTCCAGGACCGGCCGCCCTGGCCTCAAAATCTGTTTT  639

Query  605  ATACTTATCAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCA-T  663
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  640  ATACTTATCAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAAT  699
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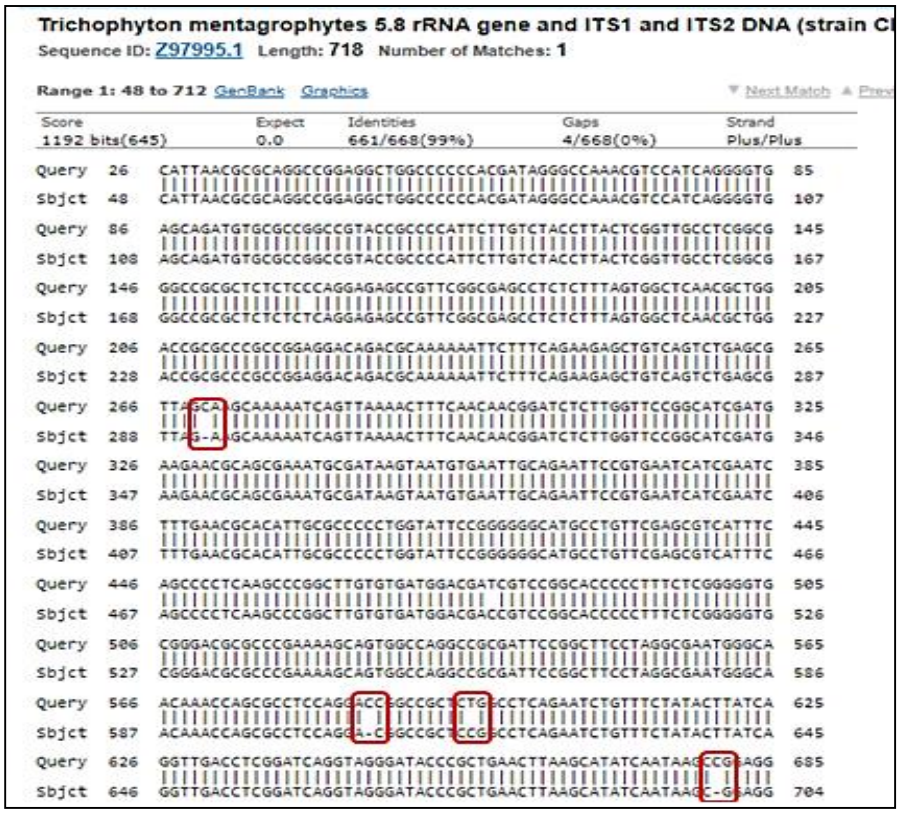
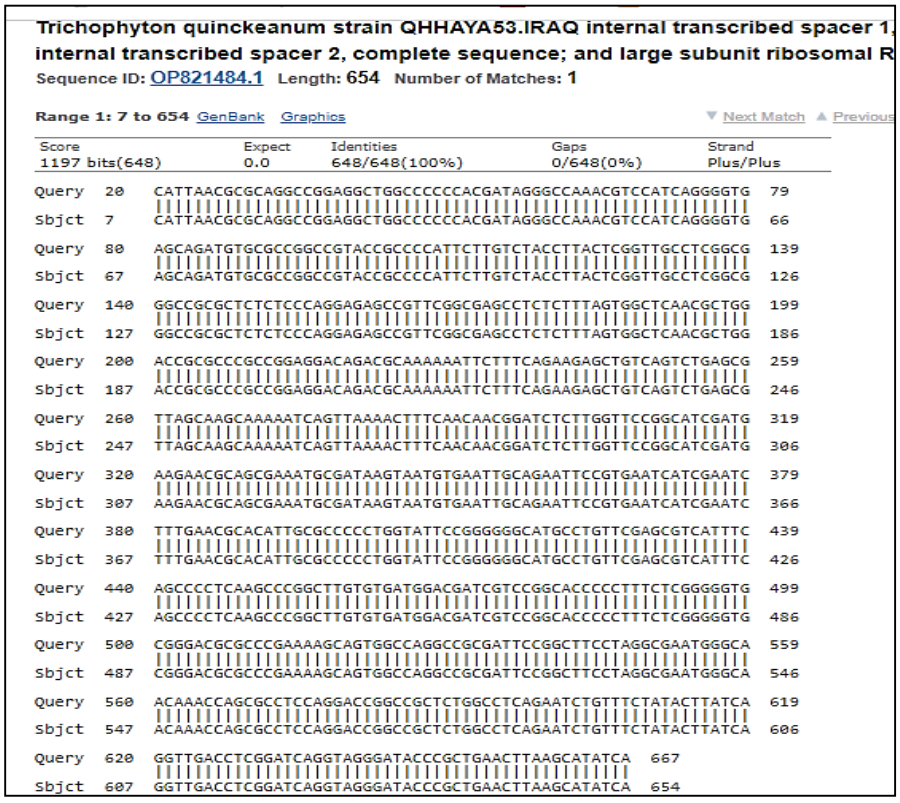
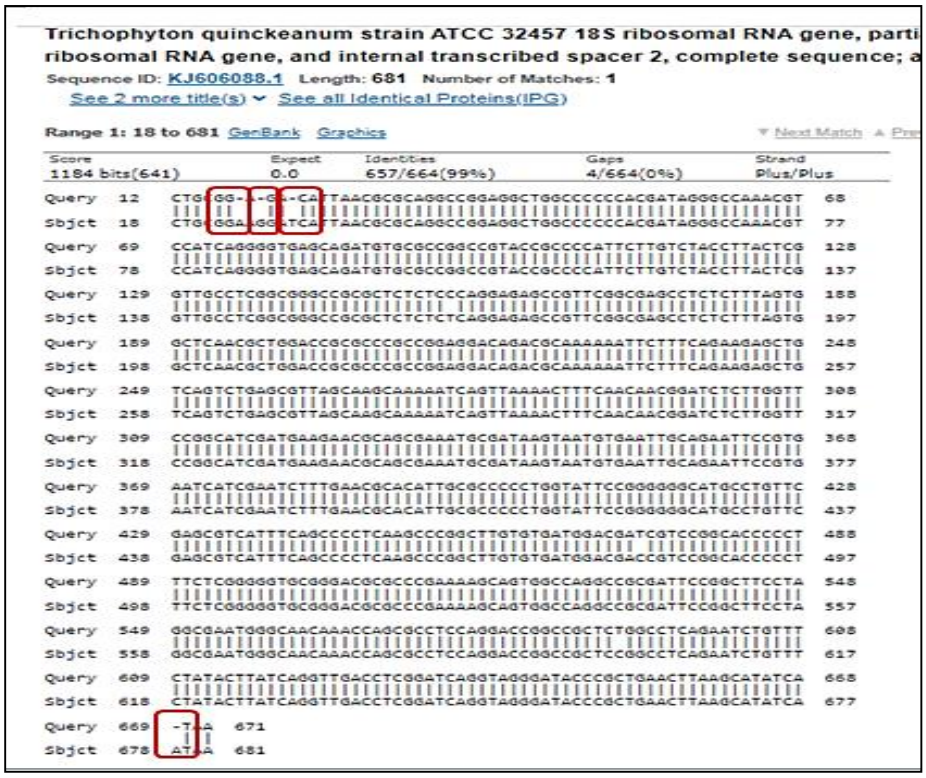
Isolate no. 9



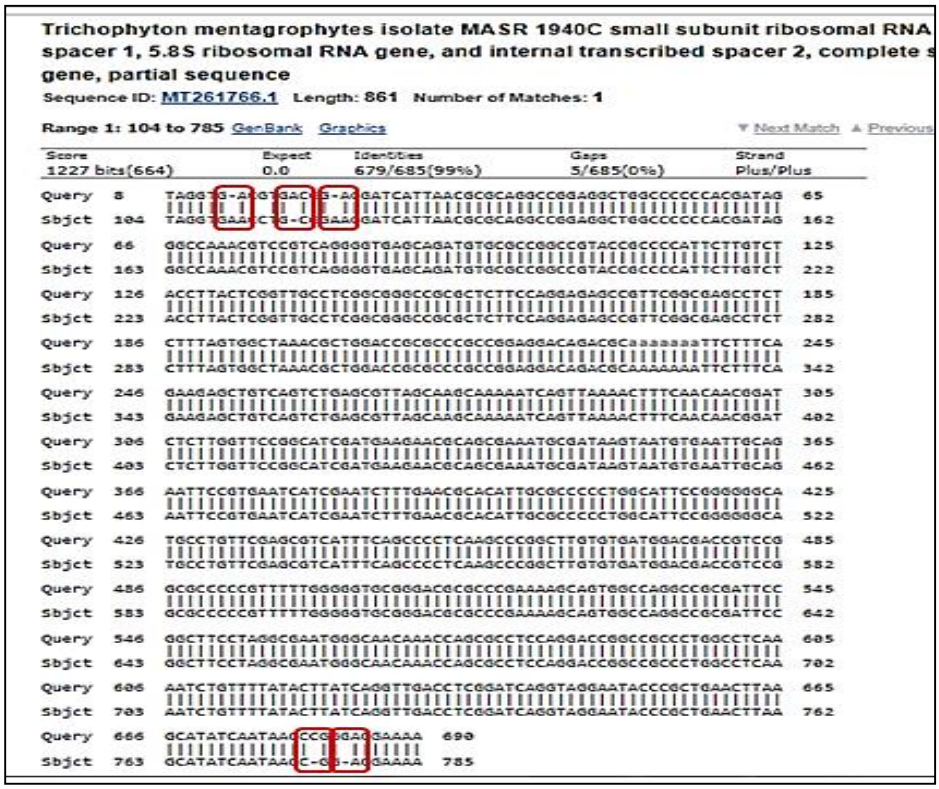
Isolate no. 10



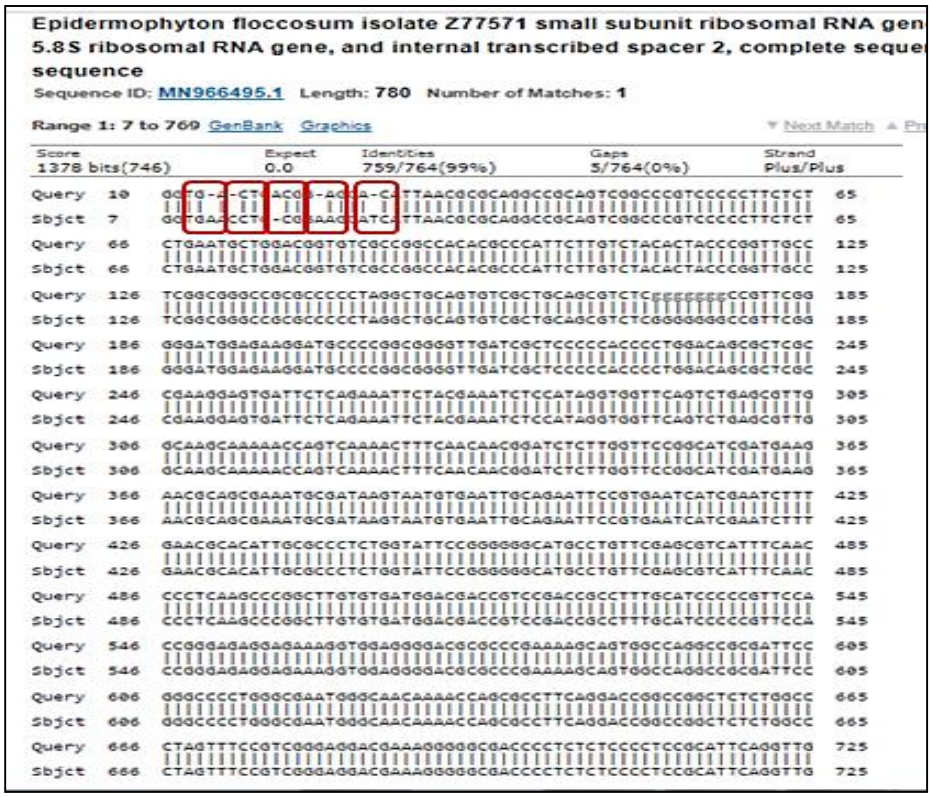
Isolate no. 12



Isolate no. 13



Isolate no. 14



Epidermophyton floccosum isolate Z77571 small subunit ribosomal RNA gen
5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete seque
sequence

Sequence ID: MN966495.1 Length: 780 Number of Matches: 1

Range 1: 7 to 769 GenBank Graphics ▼ Next Match ▲ Pr

Score	Expect	Identities	Gaps	Strand
1378 bits(746)	0.0	759/764(99%)	5/764(0%)	Plus/Plus

```
Query  10   GCTG-A-CT-AACG--AGGA-CA TTAACGCGCAGGCCGCAGTCGGCCCGTCCCCCTTCTCT  65
            |||| | || |  |  |||| | ||||||||||||||||||||||||||||||||||||||
Sbjct  7    GCTGAACCTT-CGTAAGTATCATTAACGCGCAGGCCGCAGTCGGCCCGTCCCCCTTCTCT  65

Query  66   CTGAATGCTGGACGGTGTCGCCGGCCACACGCCCATTCTTGTCTACACTACCCGGTTGCC  125
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  66   CTGAATGCTGGACGGTGTCGCCGGCCACACGCCCATTCTTGTCTACACTACCCGGTTGCC  125

Query  126  TCGGCGGGCCGCGCCCCCTAGGCTGCAGTGTCGCTGCAGCGTCTCgggggggCCGTTCGG  185
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  126  TCGGCGGGCCGCGCCCCCTAGGCTGCAGTGTCGCTGCAGCGTCTCGGGGGGGCCGTTCGG  185

Query  186  GGGATGGAGAAGGATGCCCCGGCGGGGTTGATCGCTCCCCCACCCCTGGACAGCGCTCGC  245
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  186  GGGATGGAGAAGGATGCCCCGGCGGGGTTGATCGCTCCCCCACCCCTGGACAGCGCTCGC  245

Query  246  CGAAGGAGTGATTCTCAGAAATTCTACGAAATCTCCATAGGTGGTTCAGTCTGAGCGTTG  305
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  246  CGAAGGAGTGATTCTCAGAAATTCTACGAAATCTCCATAGGTGGTTCAGTCTGAGCGTTG  305

Query  306  GCAAGCAAAAACCAGTCAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAG  365
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  306  GCAAGCAAAAACCAGTCAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAG  365

Query  366  AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTT  425
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  366  AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTT  425

Query  426  GAACGCACATTGCGCCCTCTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAAC  485
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  426  GAACGCACATTGCGCCCTCTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAAC  485

Query  486  CCCTCAAGCCCGGCTTGTGTGATGGACGACCGTCCGACCGCCTTTGCATCCCCCGTTCCA  545
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  486  CCCTCAAGCCCGGCTTGTGTGATGGACGACCGTCCGACCGCCTTTGCATCCCCCGTTCCA  545

Query  546  CCGGGAGAGGAGAAAGGTGGAGGGGACGCGCCCGAAAAGCAGTGGCCAGGCCGCGATTCC  605
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  546  CCGGGAGAGGAGAAAGGTGGAGGGGACGCGCCCGAAAAGCAGTGGCCAGGCCGCGATTCC  605

Query  606  GGGCCCCTGGGCGAATGGGCAACAAAACCAGCGCCTTCAGGACCGGCCGGCTCTCTGGCC  665
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  606  GGGCCCCTGGGCGAATGGGCAACAAAACCAGCGCCTTCAGGACCGGCCGGCTCTCTGGCC  665

Query  666  CTAGTTTCCGTCGGGAGGACGAAAGGGGGCGACCCCTCTCTCCCCCTCCGCATTCAGGTTG  725
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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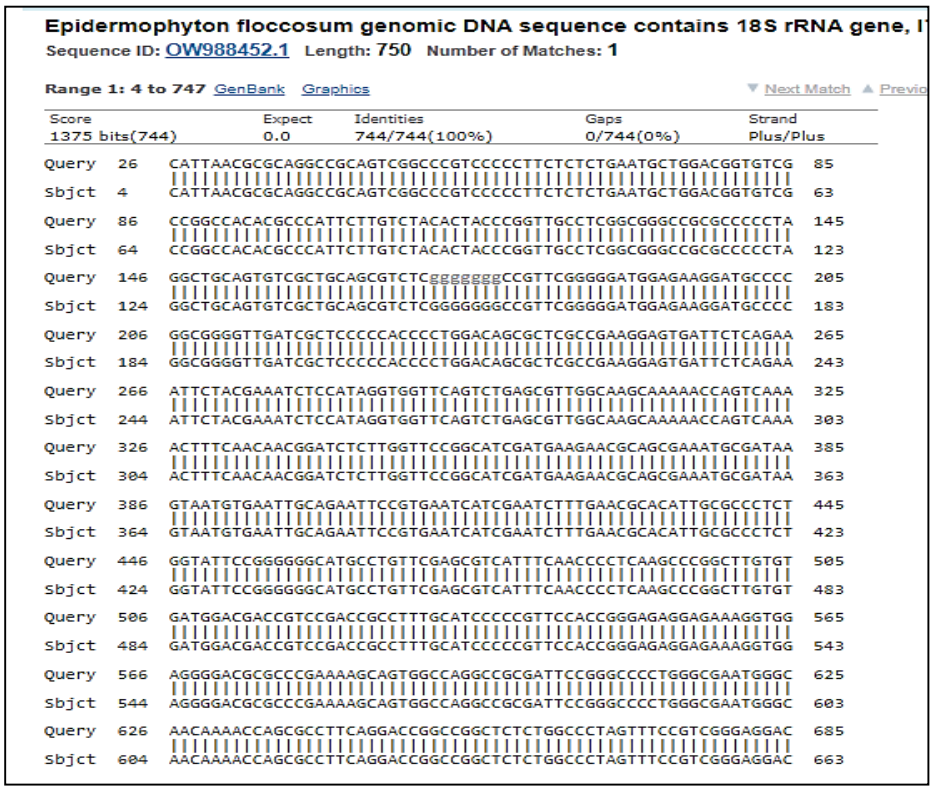
Isolate no. 19



Epidermophyton floccosum genomic DNA sequence contains 18S rRNA gene, I

Sequence ID: OW988452.1 Length: 750 Number of Matches: 1

Range 1: 4 to 747 GenBank Graphics ▼ Next Match ▲ Previo

Score	Expect	Identities	Gaps	Strand
1375 bits(744)	0.0	744/744(100%)	0/744(0%)	Plus/Plus

```
Query  26   CATTAACGCGCAGGCCGCAGTCGGCCCGTCCCCCTTCTCTCTGAATGCTGGACGGTGTCG  85
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4    CATTAACGCGCAGGCCGCAGTCGGCCCGTCCCCCTTCTCTCTGAATGCTGGACGGTGTCG  63

Query  86   CCGGCCACACGCCCATTCTTGTCTACACTACCCGGTTGCCTCGGCGGGCCGCGCCCCCTA  145
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  64   CCGGCCACACGCCCATTCTTGTCTACACTACCCGGTTGCCTCGGCGGGCCGCGCCCCCTA  123

Query  146  GGCTGCAGTGTCGCTGCAGCGTCTCgggggggCCGTTCGGGGGATGGAGAAGGATGCCCC  205
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  124  GGCTGCAGTGTCGCTGCAGCGTCTCGGGGGGGCCGTTCGGGGGATGGAGAAGGATGCCCC  183

Query  206  GGCGGGGTTGATCGCTCCCCCACCCCTGGACAGCGCTCGCCGAAGGAGTGATTCTCAGAA  265
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  184  GGCGGGGTTGATCGCTCCCCCACCCCTGGACAGCGCTCGCCGAAGGAGTGATTCTCAGAA  243

Query  266  ATTCTACGAAATCTCCATAGGTGGTTCAGTCTGAGCGTTGGCAAGCAAAAACCAGTCAAA  325
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  244  ATTCTACGAAATCTCCATAGGTGGTTCAGTCTGAGCGTTGGCAAGCAAAAACCAGTCAAA  303

Query  326  ACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAA  385
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  304  ACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAA  363

Query  386  GTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCT  445
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  364  GTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCT  423

Query  446  GGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCCTCAAGCCCGGCTTGTGT  505
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  424  GGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCCTCAAGCCCGGCTTGTGT  483

Query  506  GATGGACGACCGTCCGACCGCCTTTGCATCCCCCGTTCCACCGGGAGAGGAGAAAGGTGG  565
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  484  GATGGACGACCGTCCGACCGCCTTTGCATCCCCCGTTCCACCGGGAGAGGAGAAAGGTGG  543

Query  566  AGGGGACGCGCCCGAAAAGCAGTGGCCAGGCCGCGATTCCGGGCCCCTGGGCGAATGGGC  625
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  544  AGGGGACGCGCCCGAAAAGCAGTGGCCAGGCCGCGATTCCGGGCCCCTGGGCGAATGGGC  603

Query  626  AACAAAACCAGCGCCTTCAGGACCGGCCGGCTCTCTGGCCCTAGTTTCCGTCGGGAGGAC  685
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  604  AACAAAACCAGCGCCTTCAGGACCGGCCGGCTCTCTGGCCCTAGTTTCCGTCGGGAGGAC  663
```

Recommendations

1- Study the relationship between the fungal infection with some genetic characteristics.

2- Search for other local plants and study their biological activities to benefit from them as therapeutic alternatives against various diseases.

3- Using modern methods to obtain active substances from plant extracts.

4- Studying the antifungal activity of plant extracts of *Citrullus colocynthis* on other types of pathogenic microorganisms.

5- Combination between antifungal agents and plants extracts and it may be useful to speed up a superficial infection's clinical and microbiological healing.

6- Further studies are required to evaluate the effect against dermatophytes in vivo.

Supervisors Recommendation

We certify that this thesis entitle (**Molecular Identification and Evaluation Efficiency of some Secondary Metabolites Extracted from Citrullus colocynthis Against Dermatophytes**) was prepared under our supervision at the Department of Biology, College of Science for Women, University of Babylon as a partial requirements of the degree of Master in **MICROBIOLOGY**.

Signature
Dr.**Kawther mohammed ali hasan**
Scientific order: Prof.
Address: University of Babylon / College of Science for Women
Date: / /2023

signature
Dr. **Hussein Jebur Hussein**
Scientific order: Prof.
Address: University of Babylon/ College of Science for Women
Date: / /2023

Department Head of Biology Recommendation

In view of the available recommendation, I forward this thesis for debate by the examining committee.

Signature of head Department
Dr. **Ekhlas M. Ali AL Shareefi**
Scientific order: Assist. Prof.
Address: Head of biology Department
University of Babylon/ College of Science for Women
Date: / / 2023

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