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Isolation and Identification of Some Dermatophytes and study its sensitivity to antifungal and Ago, Zno nanoparticles

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ABSTRACT

This study aimed to determine the main species of dermatophytes which caused skin infection and effect of antifungal and Ago and Zno nanoparticles on them. The result of this study showed that out of 80 sample, 54 sample were positive to fungal isolation with ratio 67.5%, male 72.0% and female 62.1% the results show that's highest infection case was Tinea Capitis with ratio 26.2% and lowest was Tinea cruris with ratio 2.5% , according to culture and PCR results 38.8% of isolated type belong to *Trichophyton mentagrophytes* and 3.7% of Epidermophyton floccosum .

Trichophyton mentagrophytes resistant to Nystatin and Fluconazole while sensitive to Griesofulvin, Clotrimazole and Flucytosin. MIC of Ago and Zno nanoparticle against *Trichophyton mentagrophytes* were 250 and 275 µg /ml while MFC were 275 and 300 µg /ml respectively. Results of RAPD PCR showed that both Ago and Zno nanoparticle effect in genetic material which showed as appear or disappear in thickness of bands of *Trichophyton mentagrophytes* .

Introduction

Skin is first line define against infection, it protects the body from external physical, chemical and biological effects, as well as it has physiological functions such as balancing the body temperature, its complex system formed about 15% from total body weight [1].

Dermatophytes belong to deuteromycota fungi, it consist from three genus which are: Microsporum, Trichophyton and Epidermophyton. Its classified according to sources of infection in to: Anthropophilic species, Zoophilic species and Geophilic species [2]. Dermatophytes is most common types of skin infection, these group of fungi called keratophilic, it have Keratinase which help them in use of keratin as source of protein [3].

Clinical of Dermatomycosis infection can be classified according anatomical location of infection in: Tinea capitis, Tinea ungium, Tinea corporis, Tinea manum, Tinea barbae, Tinea pedis, Tinea faieci and Tinea cruris [4].

Similarities between fungi cell and host cell and development of fungi resistant against antifungal drugs lead to difficulty in treatment of mycotic infection [5].

Nanoparticles is one alternatives treatment to antifungal, which is a particles in size 10-100 nanometer, Manufactured by Down-Up Fabbrication or Up- Down Fabbrication, have different physical and chemical properties in compare with its origin, act as antifungal and a microbial agents [6].

Material and methods

Sample collection and fungal diagnosis: (80) pathological skin samples were collected from outpatient clinics Under the supervision of dermatologists. The samples direct cultivation in Sabouraud dextrose agar and incubated at 25-30 ° C for 7days. After colony development, one colony selected for phenotypic examination and Microscopic examination applied (according to Atlas,2005) and a group of biochemical tests were applied (Hair penetration test, Urease enzyme test and Protease production test) according to [8].

PCR test for confirmation diagnosis of *Trichophyton mentagrophytes*

A- DNA extraction : DNA was extracted according to [7].

B- DNA amplification mixture: as in Table (1)

C- Thermocycler program: as in table (2) according to [9].

Table 1: Compounds used in preparation of Reaction Mixture

Compounds used in preparation of Reaction Mixture	Reference	Amount
Taq PCR Master Mix KIT (Qiagen, Germany) Which contain Taq DNA Polymerase (2.5 Unit), PCR Buffer with 3mM MgCL2, 200µMdNTP	3	25
Panderm_F(5'GAAGAAGATTGTCGTTTGCATCGTCTC3	7 Out product size 336bp	0.3 from 100pM Solution.
Panderm_R(5'CTCGAGGTCAAAAGCACGCCAGAG3'		0.3 from 100pM Solution.
DNA Template	Samples	3
DNA free water (Qiagen, Germany)	8	21.4
Total		50

Table 2: showed thermocycler program

Stage	Temperature	Time	No. of cycles
First Denaturation step	95c°	3 mints	1cycle
Denaturation step	94c°	45sec.	35 cycles
Primer-annealing step	56 c°	45sec.	
DNA extension step	72 c°	1mint	
Final DNA extension	72 c°	10 mints	--------
End Temperature	4 c°	---------	----------

- Antifungal sensitivity test: applied by disc methods against Nystatin, Griesofulvin , Fluconazole, Clotrimazole , Flucytosin and according to [10].
- Minimum inhibitory concentration (MIC)and Minimum fungicidal concentration (MFC) of nanoparticles (Ago and Zno) : applied by tube methods according to [11]. with final nanoparticles concentration (100,200, 225, 250, 275, 300, 325, 350 µg /ml).
- Study effect of nanoparticles on genetic materials of *Trichophyton mentagrophytes* by RAPD PCR:

A- DNA was extracted according to[12].

B- Preparation of RAPD-PCR reactions according to [11], were performed using the (GoTaq® G2 Green master mix.

C- Thermocycler program: as in Table 3.

Table 3: Thermocycler program

Step	Temperature (C°)	Time	No. of cycles
Initial denaturation	95	4min	1
Denaturation	92	30sec.	40
Annealing	36	45sec.	
Extension	72	45sec.	
Final extension	72	5min	1

D- Primers used in RAPD PCR as in table (4)

Table 4: Primers used in RAPD PCR

No	Primer code	Nucleotide sequence 5 to 3
1	OP V-20	CAGCATGGTC
2	OP Q-02	TCTGTCGGTC
3	OP G-05	CTGAGACGGA
4	OP P-04	GTGTCTCAGG
5	OP U-12	TCACCAGCCA

Results

A- Result of fungal isolation:

Out of 80 sample, 54 sample were positive to fungal isolation with ratio 67.5%. Table 5

Table 5: Number and ratio of fungal isolation

Gander of patient	Number of sample	Number of positive case	Ratio
Male	43	31	72.0%
Female	37	23	62.1%
Total	80	54	67.5%

B- from table (6) show that's highest infection case was Tinea capitis and lowest was Tinea cruris

Table 6: type of tinea and ratio of fungal isolation

Type of tinea	Clinical case		Isolation	
	No	%	No	%
Tinea capitis	21	26.2%	16	76.1%
Tinea corpis	19	23.7%	13	68.4%
Tinea manum	14	17.5%	8	57.1%
Tinea pedis	11	13.7%	7	63.6%
Tinea faciei	8	10%	5	62.5%
Tinea ungium	5	6.25%	4	80.0%
Tinea cruris	2	2.5%	1	50.0%
Total	80	100%	54	67.5%

C- Genus and Species of isolated fungi: from table (7) showed that highest isolated species was *Trichophyton mentagrophytes* (Figure 1 and Figure 2) and lowest isolated species was *Epidermophyton floccosum*

Table 7: Genus and Species of isolated fungi

Dermatophyte species	Number	Ratio
Trichophyton mentagrophytes	21	%38.8
Trichophyton tonsurans	15	%27.7
Trichophyton verrucosum	3	%5.5
Trichophyton rubrum	3	5.5%
Microsporum canis	6	%11.1
Microsporum ferrugineum	4	%7.4
Epidermophyton floccosum	2	%3.7
TOTAL	54	%100

Fig. 1: *Trichophyton mentagrophytes* colony on SDA

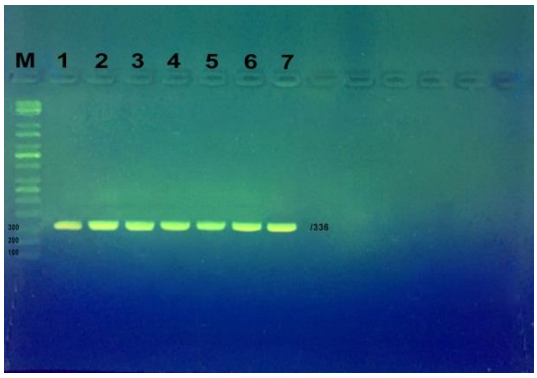

Fig. 2: Agarose gel electrophoresis of PCR products. M: 100 bp DNA ladder, lines (1-7) positive result at 336bp for *Trichophyton mentagrophytes*

D- Antifungal sensitivity test: as in table (8).

Table 8: antifungal sensitivity test

Inhibition diameter Zone (mm) / Isolates	Antifungal agents				
	Nystatin	Griesofulvin	Fluconazole	Clotrimazole	Flucytosin
Trichophyton mentagrophytes	—	16	—	14	16
Trichophyton rubrum	—	12	4	12	8
Trichophyton.tonsurans	—	12	—	10	—
Trichophyton verrucosum	—	14	—	12	—
Microsporum canis	—	18	8	16	16
Microsporum ferrugineum	—	20	6	18	14
Epidermophyton floccosum	—	16	—	12	8

E- Antifungal activity of nanoparticle(Ago and Zno) against *Trichophyton mentagrophytes*: from table (9) showed that MIC of Ago and Zno nanoparticle against *Trichophyton mentagrophytes* were 250 and 275 µg /ml while MFC of Ago and Zno nanoparticle against *Trichophyton mentagrophytes* were 275 and 300 µg /ml respectively.

Table 9: MIC and MFC of Ago and Zno nanoparticle against *Trichophyton mentagrophytes*

Concentration of nanoparticles (µg /ml)	Type of nanoparticles	
	Ago	Zno
100	Turbidity/ growth	Turbidity/ growth
200	Turbidity/ growth	Turbidity/ growth
225	Turbidity/ growth	Turbidity/ growth
250	Clear / growth	Turbidity / growth
257	Clear / sterile	Clear / growth
300	Clear / sterile	Clear / sterile
325	Clear / sterile	Clear / sterile
350	Clear / sterile	Clear / sterile

F- Result of RAPD PCR test: from figure 3,4,5 showed that both Ago and Zno nanoparticles were effected in genetic materials which showed as appear or disappear and increase or decrease in thickness of bands

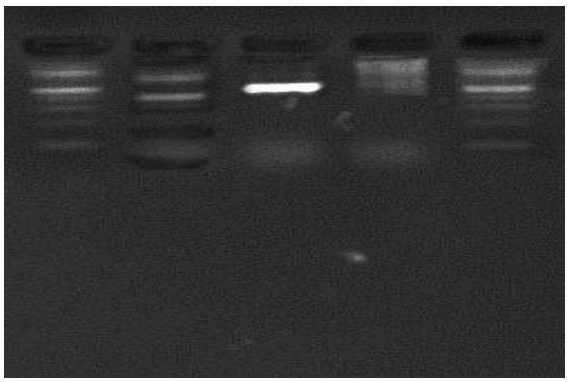

Fig. 3: Agarose gel electrophoresis of RAPD- PCR products. lines (1-5) positive result of *Trichophyton mentagrophytes* with5 different primers, before treatment with Nanoparticle

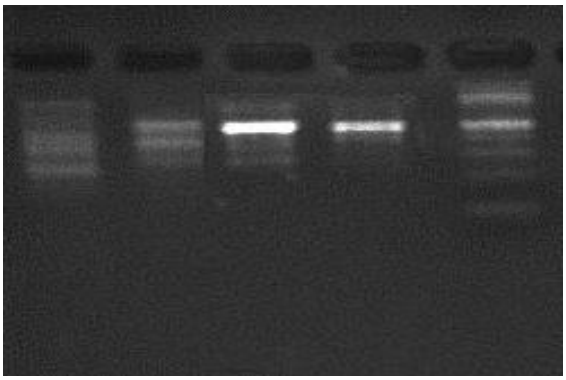

Fig. 4: Agarose gel electrophoresis of RAPD- PCR products. lines (1-5) positive result of *Trichophyton mentagrophytes*, after treatment with Ago Nanoparticle

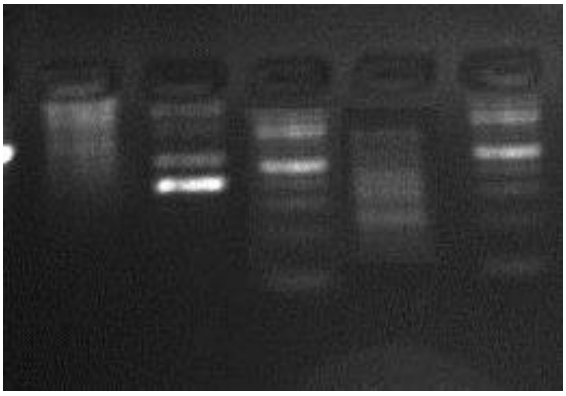

Fig. 5: Agarose gel electrophoresis of RAPD- PCR products. lines (1-5) positive result of *Trichophyton mentagrophytes* with5different primers, after treatment with Zno Nanoparticle

Discussion

The common type of skin diseases were Superficial fungal infections which affecting millions of healthy and immunocompromised people worldwide [13].

In the current study showed presence of 32.5% of clinical infected cases were negative to culture that's may be due to take of patient to antifungal drugs or error in transport of sample or culture technique, or due to contamination of sample with other organism or maybe the infected not fungal origin [14]. In present study showed that main type of infection were Tinea capitis that's perhaps due to factors related with the community culture, such as Clean the barbers, Free barber tools from the causes of disease.

In current study showed that the dominants fungal isolated type was *Trichophyton mentagrophytes* that's agreement with [15] and disagreement with [16]. The dominance of fungal type in compare with other type depend on geographic location, type of samples, season and Community habits.

In this study show high effect of Ago nanoparticles as antifungal and according to RAPD PCR showed effect in genetic materials, that's agreement with [17]. Ago nanoparticles have high specific surface area and high fraction of surface atoms in compare silver metal, that's may be gives him the antifungal characteristic [18]. The antifungal activity of AgNPs due to their ability to accumulation on fungal membrane of microorganisms, formation of pores which lead to change in permeability of cell wall. Also AgNPs can cause inhibit cellular respiration, DNA replication, and cell division, which result in the loss of cell viability, and lead to cell death [19].

In the current showed obvious effect of Zno nanoparticles on *Trichophyton mentagrophytes* and according to RAPD PCR showed effect in genetic materials. This agreement with [20, 21, 22]. These activity may be due to electrostatic interaction between negative charge of cell membrane and positive charge of nanoparticles [23]. Zn o have ability to cause damage to the cell membrane directly leads to the leakage of minerals, al may be act as antifungal due to toxic properties of metal oxide NPs, also have ability to bind with thethiol(-SH)groups of protein present in the cell wall. This interaction decreases the cell permeability which leads to cell lyses [24, 25, 26].

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عزل وتشخيص بعض الفطريات الجلدية ودراسة حساسيتها للمضادات الفطرية والدقائق النانوية للخارصين والفضة

هبه يونس خلف

كلية الطب البيطري ، جامعة تكريت ، تكريت ، العراق

الملخص

هدفت هذه الدراسة إلى التعرف على الأنواع الرئيسية للفطريات الجلدية التي تسبب إصابة الجلد وتأثير مضادات الفطريات و الجسيمات النانوية Ago و Zno معهم. أظهرت نتيجة هذه الدراسة أنه من أصل 80 عينة ، كان هناك 54 عينة ايجابية فطرية بنسبة 67.5٪ معزولة من الذكور بنسبة 72.0% والاناث بنسبة 62.1%. واظهرت النتائج ان اعلى حالة اصابة كانت سعفة الرأس بنسبة 26.2% واقلها كانت سعفة الفخذ بنسبة 2.5 % . وتبعا للوسط الزرعي ونتائج ال PCR 38.8٪ من النوع المعزول تنتمي إلى Trichophyton mentagrophytes و 3.7% تنتمي الى Epidermophyton floccosum .

نبتة الشعراويةTrichophyton mentagrophytes المقاومة للنيستاتين والفلوكونازول حساسة للكريزوفولفين ، كلوتريمازول وفلوسيتوزين. MIC من الجسيمات النانوية Ago و Zno ضد Trichophyton mentagrophytes 250 و 275 ميكروغرام / مل بينما MFC كانت 275 و 300 ميكروغرام / مل على التوالي. نتائج RAPD PCR أظهرت أن كلا من الجسيمات النانوية Ago و Zno لها تأثير في المادة الوراثية بأن تظهر او تختفي في سمك شريط Trichophyton mentagrophytes.