

Detection of the effect of some natural plant products on the most important causes of fungal skin and Cutaneous candidiasis infection

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ABSTRACT

This study was conducted in the Postgraduate Laboratory at the College of Sciences at the University of Kerbala after collect and isolate 115 specimens of dermatophytic patients. The specimens are collected from Imam Al-Hassan -Almujtaba Hospital, in Kerbala Governorate. Where included (100) specimens isolated from (skin scrapings, nail clippings, hair fragments) and 15 specimens isolated from the mouth. After culture in Sabourauds Dextrose Agar, diagnosed microscopically by examining large and small spores, and phenotypically through the color and shape of the colony, period January 2021 to April 2022 for skin fungi, oral sample were collected during the month of May, 2022, and the result of the examination was five types of skin fungi (*Trichophyton mentographites*, *Trichophyton indotinea*, *Trichophyton interdigital*, *Trichophyton quinickeum*, *Microsporum canis*) and *Candida albicans* isolated from mouth. A group of medicinal plants such as Pomegranate, *Oleivera*, *Chamomile*, *Neem*, *Castor* and *Moringa* was collected and a hot water extract was prepared. Then the medium was poisoned by drilling method. This showed that Pomegranate and *Aloe vera* more effective against pathogenic fungi than *Chamomile* and *Castor* which gave little effect on the fungi under study, while *Neem* and *Moringa* did not give any effect against them.

The objective of the study: to detect the ability of some plant extracts to inhibit pathogens of cutaneous fungal diseases and oral candidiasis in Kerbala Governorate.

Keywords; Dermatophytes, medicinal plant, drilling method

INTRODUCTION

Dermatophytes represent the main cause of cutaneous diseases. Dermatophytes attack keratinized tissues, such as hair, nail, and stratum corneum, through its ability to produce keratinolytic enzymes, which leads to dermatophytosis. Medicinal plants have long been used to treat different diseases, and since ancient times, use of plant-based products to treat fungal, bacterial, and parasitic infections. This is because medicinal plants have many features, such as fewer side effects and decreased cost.

Skin diseases are one of the common health problems in developing countries, as fungal infection constitutes a high percentage of the causes of skin infection, affecting all age groups and different parts of the body by three species of filamentous fungi belong to the Eumycetes class *Trichophyton*, *Microsporum* and *Epidermophyton* (1). Transmission of dermatophytes may occur by direct contact with animals or humans or indirectly by contact with contaminated fomites (2). The factors that contribute to a high incidence of superficial mycoses, especially in tropical and subtropical regions are heat and humidity (3) (4). Medicinal plants are used for different purposes, such as for treating infectious diseases and inflammatory processes, as well as to reduce pain and reinforce wound healing. The pharmacological effects of plants often come from the presence of the major constituents, as well as the synergistic action resulting from their many constituents that belong to various chemical classes (flavonoids, phenolic acids, terpenoids, saponins and others). However, most of the plant products used are not only based on the long-term experience of the population, but also showed results of in vitro activity (5). Although few clinical studies have been performed (6) (7).

MATERIALS AND METHODS

A 100 specimens were collected from patients with dermatophytosis in Imam Hassan Al-Mujtaba Hospital in Kerbala, the specimens collected from skin, hair, nail and 15 specimens were collected from the mouth of the children in children's teaching hospital. Dermatophytes isolated diagnosed by direct microscopic examination with 15%-20% KOH and using physiological tests such as vitamin requirement test, growth on rice, hair perforation test and producing pigments in other culture media. Plant extracts were prepared on the basis (8). The selected plants included Pomegranate peel, *Chomomile* flowers, *Alo-vera* leaves and *Castor* leaves. The method included the preparation of 100 gram of powder for each plant in 200 ml of hot distilled water whose temperature ranged from 90-98°C, 500-ml in a flask for 15 minutes. The mixture was mixed by stirring with magnetic electrode, the contents were left for

24 hours then filtered by 2-3 layers of sterilized gauze and the leachate was placed in an open dish to dry in the oven for 2-3 days at a temperature of 40-50 °C after that the precipitate was collected using scraping method and kept in the refrigerator. The aqueous extract was prepared by dissolving (0.5, 0.10, 0.15, 0.20, 0.25, 0.50, 0.75, 1.0)g in 1ml of steril distilled water separately to obtain a concentration of (5%, 10%, 15%, 20%, 25%, 50%, 75%, 100%) respectively to test the anti-efficacy of plant extracts in the growth of pathogenic dermatophytes and candida.

RESULT & DISCUSSION

The result proved that ringworm of the body (T. corporis) recorded 18 infection with ratio of 16.3% while (T. capitis) was the highest number among clinical cases with 42 out of the total number of sample of 110 sample with ratio of 38.1%, then with ringworm (T. faciei) with 34 infection with ratio 30.9%, and ringworm (T. manum) was number of 4 by 3.6% while nail ringworm was recorded 2 by 1.8% while (Oral candidiasis) was 9%. table 1.

Table (1) distribution of clinical patterns of ringworm and candidiasis according to age and sex. (n=110)

Type Of infection	gender	Age categories			total	%
		10>	11-29	<30		
T.corporis	Male	5	8	3	16	14.5
	female	1	1	0	2	1.8
T.capitis	Male	29	5	2	36	32.7
	female	5	1	0	6	5.4
T.manum	Male	0	1	3	4	3.6
	female	0	0	0	0	0
T.faciei	Male	7	13	2	22	20
	female	3	7	2	12	10.9
T.ungium	Male	0	0	2	2	1.8
	female	0	0	0	0	0
Oral candidiasis	Male	3	1	0	4	3.6
	female	4	2	0	6	5.4
total		57	39	14	110	99.7

The results showed that the most isolated fungal type during the study is *Trichopyton mentagrophytes*. the most five type dermatophytes fungi were isolated and diagnosed are *T. mentagrophytes*, where the number of isolated reached 10 isolated at 23.8% (10/42)

while *T. indotineae*, *T. quinckium*, with 6 isolates for each one at 14.2% (6/42) and *T. interdigital* frequency by 2 isolation by 4.7% while *Microsporum canis* frequency by 8 isolation by 19.4% (8/42) where *albicans* yeast recorded 10 isolates and 23.8% (10/42). table 2.

table(2) frequency of fungal species isolated from the skin and mouth in relation to the causative clinical patterns.

Type of dermatophytes	T.corporis	T.capitis	T.faciei	T.ungium	T.manum	Oral candidiasis	total	%
<i>T.mentagrophytes</i>	6	2	0	0	2	0	10	23.8
<i>T.quinckeanum</i>	3	3	0	0	0	0	6	14.2
<i>T.indotineae</i>	0	2	4	0	0	0	6	14.2
<i>T.interdigital</i>	0	2	0	0	0	0	2	4.7
<i>M.canis</i>	3	4	1	0	0	0	8	19.4
<i>C.albicans</i>	0	0	0	0	0	10	10	23.8
total	12	13	5	0	2	10	42	100

The experiment showed that *Pomegranate* peel extract came first in terms of inhibitory effectiveness, as it excelled in all transactions followed by the extract of the leaves of *Aloe vera* and then the anti fungal (fluconazole) and the inhibition diameters of fungal colonies were high

The spicy aqueous extract of *Pomegranate* plant showed high inhibition effectiveness and that rate of inhibition diameter is directly proportional to the concentration of the extract. five concentration of the hot water extract of *pomegranate* plant were prepared, namely (5,10,15,20,25). the inhibition diameters of *T. mentagrophytes*, *T. benhamea*, *T. interdigital*, *T. quinicium*, *M. canis*, *C. albicans* when the concentration was 10, (2,1,2,0,9,9.5)mm respectively while the inhibition diameters when the concentration was 25, (45,50,37,30,42,43)mm respectively table 3.

Table (3) the effect of hot water extract of *Pomegranate* plant on growth rate of diameter in (mm) fungi under study.

Fungi Conc- entration	<i>T. Mentag rophytes</i>	<i>T. quinic um</i>	<i>T. indotin eae</i>	<i>T. interdigi tal</i>	<i>M. Canis</i>	<i>C. albicans</i>	average	LSD P value
5	0	0	0	0	0	2	0.33 e	4.63: 0.0054
10	2	0	1	2	9	9.5	3.92 d	
15	9	5	17	16	17	18.5	13.75 c	
20	25	15	25	21	25	27	23.00 b	
25	45	30	50	37	42	43	41.17 a	
Flu	0	0	0	20	30	32	13.67 c	
average	13.5 cd	8.33 d	15.5 c	16 c	20.5 b	22 a		
LSD P value	3.692 0.0247 ^S							

S: significant difference at 0.05 level (p value < 0.05)
 HS: high significant difference at 0.01 level (p value < 0.01)
 . Different letters are significantly difference between groups

Also, the extract of the *Aloe vera* plant showed high inhibitory effectiveness against pathogenic fungi, so that the rate of inhibition diameters is directly proportional to that concentration of the extract the prepared five concentration of the hot water extract of the *Aloe vera* plant (5,10,15,20,25) each of *T. mentagrophytes*, *T. benhamea*, *T. interdigital*, *T. quinicium*, *M. canis*, *C. albicans*. the inhibition diameters when the concentration was 10, (1,1.5,1.5,2,14,16)mm in row while the inhibition diameters when the concentration was 25 (37,35,27,15,63,62)mm respectively.

Table (4) the effect of hot water extract of *Aloe vera* plant on growth rate of diameter in (mm) fungi under study

fungi conc- entration	<i>T. Mentag rophytes</i>	<i>T. quinic um</i>	<i>T. indotin eae</i>	<i>T. interdigi tal</i>	<i>M. Canis</i>	<i>C. albicans</i>	average	ANO (LS P va
5	0	0.5	0	0	0	5	0.92 e	3.1: 0.00:
10	1	2	1.5	1.5	14	16	6.00 d	
15	7	4	5.5	8	21	27	12.08 c	
20	20	9	21	19	32	39	23.33 b	
25	37	15	35	27	63	62	39.83 a	
Flu	0	0	0	20	30	32	13.67 c	
average	10.83 b	5.08 c	10.50 b	12.58 b	26.67 a	30.17 a		
LSD P value	2.292 0.0428 ^S							

S: significant difference at 0.05 level (0.01 < p value < 0.05)
 HS: high significant difference at 0.01 level (p value < 0.01)
 . Different letters are significantly difference between groups (Duncan Test)

there was no effect of hot water extract of *chamomil* and *castor* plants even at concentration 100 except for *M.canis* fungus it gives a inhibition of 3mm at concentration 100% to *chamomil* plant .and inhibition of 5mm at a concentration of 100% for *castor* plant for the fungus *T. mentagrophytes*.

Table (5)the effect of hot water extract of *Matricaria Chamomile* plant on growth rate of diameter in(mm)fungi under study

fungi Concentration	<i>T.Mentagrophytes</i>	<i>T.quinckanum</i>	<i>T.indotinea</i>	<i>T.interdigital</i>	<i>M.Canis</i>	<i>C.albican</i>	average	LSD P value
25	0	0	0	0	0	0	0 d	3.223 0.0429 ^S
50	0	0	0	0	0	0	0 c	
100	0	0	0	0	3	0	0.50 a	
Flu	0	0	0	20	30	32	13.67 b	
average	0 c	0 c	0 c	5 b	8.25 a	8 a		
LSD P value	3.113 0.0071 ^{HS}							

S: significant difference at 0.05 level (p value <0.05)
 HS: high significant difference at 0.01 level (p value < 0.01)
 . Different letters are significantly difference between groups

Table (6)the effect of hot water extract of *Ricinus communis* on growth rate of diameter in (mm) fungi under study

fungi concentration	<i>T.mentagrophytes</i>	<i>T.qunickanum</i>	<i>T.indotinea</i>	<i>T.interdigital</i>	<i>M.canis</i>	<i>C.albican</i>	average	LSI P va
25	0	0	0	0	0	0	0.00 b	5.21 0.002
50	0	0	0	0	0	0	0.00 b	
100	5	0	0	0	0	0	0.83 b	
Flu	0	0	0	20	30	32	14.5 a	
average	1.25 c	0 d	0 d	5 b	7.5 a	8 a		
LSD P value	4.733 0.0071 ^{HS}							

S: significant difference at 0.05 level (p value <0.05)
 HS: high significant difference at 0.01 level (p value < 0.01)
 . Different letters are significantly difference between groups

The results of the evaluation of the opposite effectiveness showed that fluconazol ranked third in terms of contrast effectiveness , as the result showed that this anti against had high effectiveness against Candidasis yeast with inhibition diameters (32)mm while no efficacy against *T. mentagrophytes*, *T.qunickanum*, *T.indotinea* was shown, , which indicated that this antigen has effectiveness against skin fungi , and that the aqueous extract of *Pomegranate* peels was highly effective and consistent with result of(9) the anti-efficacy of *Pomrgranate* extract comes because it contains phenolic ingredients and biologically effective tannins against many microbiology .these results are consistent with findings of(10)(11)(12)(13).Although the *Aleo vera* plant has a role in inhibiting the growth and progression of pathogenic fungi , including *Candida* yeast ,it was agreed with(14) that the *Aleo vera* plant leads to the formation of an inhibitory area around the colony against *C.albicans* and inhibition increases with increased concentration .and because of *Aloe vera* contains various that have shown antifungal activity such as anthraquinones ,saponins ,phenolic compounds these compounds may extra their antifungal effects through different mechanisms, including inhibition of fungal enzyme, inter ference with fungal cell signaling pathways or

modulation of the host immune respons . saponins can reduce surface tension resulting in increased permeability or cell leakage and cause intercellular compoundsto come out .(15)

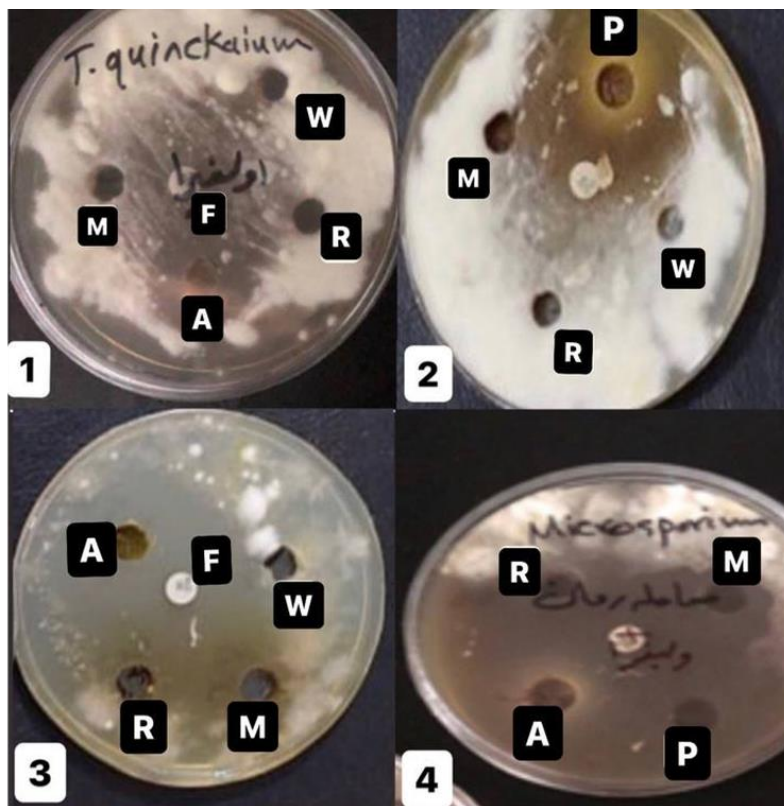


Figure (5)Inhibitory efficacy of plant extracts against pathogenic fungi

1&2 *T.quinckaium* ((M: *Matricaria Chamomile* ,P: *Pomegranate*,A:*Aleo vera* ,R:*Ricinus communis* W:hot water ,F:fluconozol 10mg)

3&4 *M.canis*(M: *Matricaria Chamomile* ,P: *Pomegranate*,A:*Aleo vera* ,R:*Ricinus communis* W:hot water ,F:fluconzole 10mg).

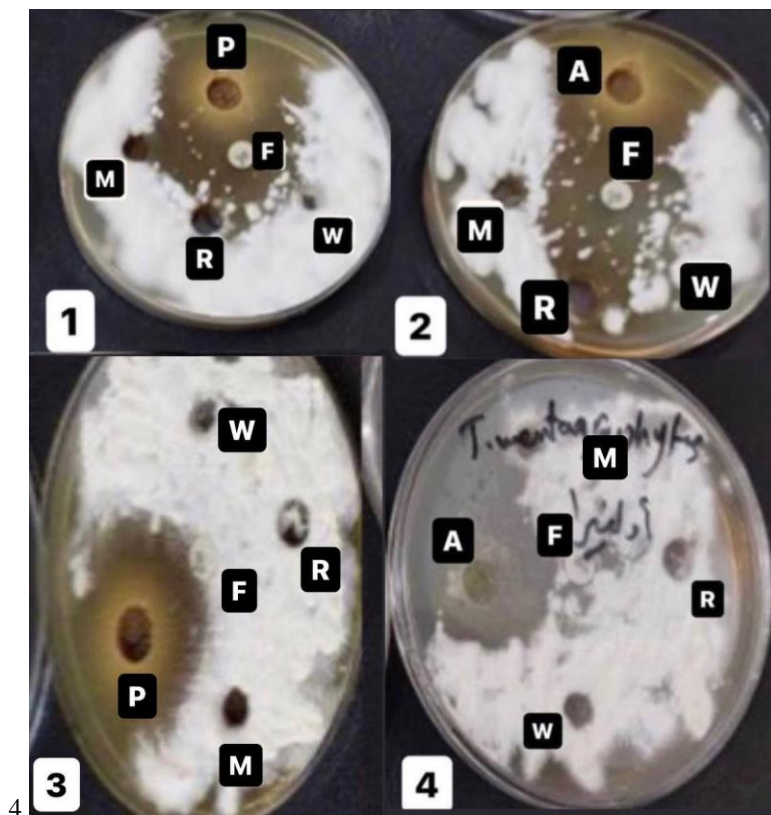


Figure (6) Inhibitory efficacy of plant extracts against pathogenic fungi

1&2 *T.interdigital* (M: *Matricaria Chamomile* ,P: *Pomegranate* ,A: *Aleo vera* ,R: *Ricinus communis* W: hot water ,F: fluconazol 10mg)

3&4 *T.mentagrophytes* (M: *Matricaria Chamomile* ,P: *pomegranate* ,A: *Aleo vera* ,R: *Ricinus communis* , W: hot water ,F: fluconazol 10mg)(

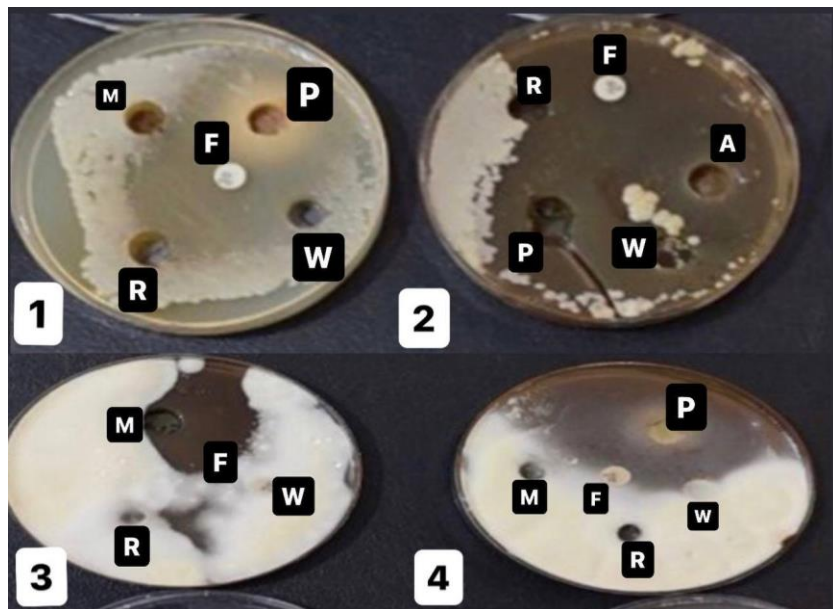


Figure (7) Inhibitory efficacy of plant extracts against pathogenic fungi

1&2 *C.albicans* (M: *Matricaria Chamomile*, P: *Pomegranate*, A: *Aleo vera*, R: *Ricinus communis*, W: hot water, F: fluconozol 10mg)

3&4 *T.indotinia* (M: *Matricaria Chamomile*, P: *Pomegranate*, A: *Aleo vera*, R: *Ricinus communis*, W: hot water, F: fluconozol 10mg).

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الخلاصة

أجريت هذه الدراسة في مختبر الدراسات العليا في كلية العلوم جامعة كربلاء حيث تم جمع وعزل 115 عينة من المرضى المصابين بالأمراض الفطرية والتي تم جمعها من مستشفى الامام الحسن المجتبي عليه السلام ومستشفى الأطفال التعليمي في محافظة كربلاء. حيث شملت 100 عينة من مرضى الإصابات الجلدية مثل (قشور جلدية, عينات من الاظافر وعينات من الشعر) و 15 عينة عزلت من الفم. وبعد زراعة الفطريات المرضية على وسط السابرويد دكستروز اكار وتشخيصها مجهريا من خلال فحص الابواغ الكبيرة والصغيرة وفحصها مظهريا عن طريق لون وشكل المستعمرة للفترة من كانون الثاني 2021 لغاية نيسان 2022 بالنسبة للفطريات الجلدية وعينات الفم التي عزلت خلال شهر أيار لسنة 2022 وكانت نتيجة

الفحص خمس أنواع من الفطريات الجلدية (*Trichophyton mentoglyphytes* , *Trichophyton indotimea*)

Trichophyton interdigetal, *Trichophyton qunickeueam*, *Microsporum canis*

وخميرة *Candida albicans* المعزولة من الفم. ايضا تم اخذ مجموعة من النباتات الطبية وذلك لاختبار كفاءتها في تثبيط هذه الفطريات المرضية حيث تم عمل مستخلصات الماء الحار لكل من (قشور الرمان واوراق الاوليفيرا, البابونج, الخروع, النيم والمورينجا) واختبار تأثيرها في تثبيط الفطريات المرضية من خلال تسميم الوسط بطريقة الحفر. وقد اعطى نبات الرمان والاوليفيرا نتائج عالية في تثبيط الفطريات المرضية اما نباتي البابونج والخروع فقد اعطيا نتائج تثبيط ضعيفة بينما لم يعطي نباتي النيم والمورينجا أي تثبيط ضد الفطريات المرضية.

الهدف من الدراسة :اختبار كفاءة المستخلصات النباتية في تثبيط الفطريات الجلدية المرضية والفطريات المعزولة من الفم في محافظة كربلاء

الكلمات المفتاحية :الفطريات الجلدية,النباتات الطبية ,طريقة الحفر