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Research Article

Effects of some plants extracts in growth of some dermatophytes

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Abstract

Background: This study was conducted to isolate and identify a group of pathogenic fungi from patients with fungi skin infections and detect the active compounds in the aqueous, ethyl alcoholic and acetone extracts of the plants seeds (*Terminalia chebula*, *Pimpinella anisum* and *Artemisia herba alba*) at concentrations (1, 3, 5, 7 ,10 ,15 and 20) mg/ml, against the fungi species that will be isolated from fungi skin infections through the growth retardation zone and determination of the lowest growth minimum inhibitory concentration (MIC). **Objective:** The objective of this study is to provide a safe, effective and available treatment for some fungi infections. **Methods:** 70 pathological isolates were isolated from patients with fungi skin infections and aqueous, alcoholic and acetone extracts were prepare and then the efficiency of these plant extracts against fungi species was evaluated and minimum inhibitory concentration (MIC) was determined. **Results:** 50 fungi isolates were obtained out of 70 samples, 30 isolates of *Trichophyton mentagrophytes* which were isolated, 10 isolates of *Trichophyton rubrum*, 10 isolates of *Microsporum canis* the alcoholic extract of the *Terminalia chebula* plant seeds was the best plant extract in inhibiting the isolated fungi species. **Conclusions**: The Iraqi environment is rich in medicinal plants such as the seeds of *Terminalia chebula*, *Pimpinella anisum* and *Artemisia herba alba*, lt was also found that the alcoholic extracts of the *Terminalia chebula* seeds showed an inhibitory effect on the growth of fungi species, all studied plants contain most of the active compounds, the plants showed no cytotoxicity.

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Introduction

Skin diseases are one of the common health problems in developing countries, as fungal infections constitute a high percentage of the causes of skin infections, as they affect all age groups and different parts of the body by three genera of filamentous fungi belonging to the Euascomycetes class, *Trichophyton*, *Microsporum* and *Epidermophyton* [1].

Dermatophytes infect the outer keratinized layer of the epidermis of the skin and the keratinous tissue of hair and nails, as it can digest keratin through its ability to produce hydrolyzed keratin enzymes, which made it medically important in addition to its ability to transmit from one person to another or from infected animals such as cattle, cats, horses, and birds to humans and may be transmitted from contaminated soil to animals and humans, causing skin diseases known as skin infections and the pathological symptoms range from simple to severe, depending on the immunity of the infected person and the intensity of pathogenicity of the causative fungus. In addition to dermatophytes, many opportunistic fungi such as yeasts and molds exploit weak immunity, such as diabetes, AIDS, chemical drug use, cancerous diseases and other diseases causing skin diseases dermatomycosis [2].

T.mentagrophytes Colonies growing on saproid dextrose agar (SDA) media, where fungal colonies appear with a diameter of 80 mm within 10-14 days, at a temperature of 28 C. The fungal colonies are white, granular in appearance, flat in shape, and in some isolates they were folded from the center and developed into slightly raised bundles. The staining of the base of the colony (Reverse) was reddish-brown microscopic examination shows small, single-celled, thin-walled spores of almost spherical to pear-shaped, either scattered on mycelial hyphae or clustered in clusters, and thin spiral filaments. Distinctive with thin walls, while large spores are rare and if found, they may appear club-shaped and multi-celled with thin walls comprising (2-5) cells with a rounded end, while there were varying numbers of scaly spherical spores [3, 4, 5].

T.rubrum Colonies growing on SDA medium are white, slightly raised and flat, resembling suede. The base of the colony (Reverse) is very light yellow to brownish yellow and some isolates failed to produce the dye, while other isolates produced dark red to red pigments on microscopic examination, we notice small pear-like spores with a wedge or teardrop-like shape while neither large spores nor squamous spores were observed [3, 6].

M.canis Colonies growing on saproid dextrose agar (SDA) medium when incubated at a temperature of 27 °C and after two weeks were white from the center surrounded by a bright yellow color, then gradually became white with age until the colonies turned into a dense cottony appearance and some isolates contain radiating grooves. The base of the colony (Reverse) was golden yellow to orange. With age, the colony turns brown, the fungus grows on the center of the potato dextrose agar (PDA) grows on the center of the potato. The size is spindle-shaped and contains between 5-15 cells. Microconidia are small, pear-shaped, few in number, and single-celled [7].

T.chebula is a common and widespread plant and its fruits are widely used and contain many compounds, including phenols. Phenolic active compounds may play a vital role in the effect of biological activity. *T.chebula* fruit extract possesses various biological properties such as cancer, anti-inflammatory, antioxidant, and protoparasite. It is antimicrobial, has a protective activity for the liver and kidneys and plays a role in the management of metabolic syndrome [8].

As for *P.anisum*, it is a plant that belongs to the Umbelliferae family and is considered one of the oldest medicinal plants. It is also an annual herbaceous plant with a height of 30 - 50 cm. It has white flowers and small green to yellow seeds. It grows in the East, the Mediterranean region, West Asia, the Middle East, Mexico, Egypt and Spain. *P.anisum* seeds also contain essential oil and are used as flavorings [9].

As for the *A.herba alba* plant, it is a plant that belongs to the Asteraceae family and is widely consumed in traditional medicine because it contains many effective compounds. Studies have shown that the

A.herba alba plant contains large amounts of terpenes, flavonoids, coumarins, acetylene and basic sterols, as these compounds have many vital activities [10].

For this, the research aims to provide a safe, effective and available treatment for some fungal infections. In this study, some widely used plants such as *T.chebula* seeds, *P.anisum* and *A.herba alba* were used to investigate their effect on the growth of a group of fungi that cause some pathological infections. Therefore, the research focused on the following:

1. Isolation and identification of pathogenic fungi from pathological samples, preparation of plant extracts (aqueous, alcoholic and acetone), evaluation of the effect of different concentrations of these plant extracts on pathogenic fungi isolated from pathological samples, determination minimum inhibitory concentration (MIC) for alcoholic extracts, and qualitative detection of active compounds in plant extracts and study the toxicity of plant extracts on living cells.

Materials and Methods

Preparation of media for pathogenic fungi

a) Sabouraud Dextrose Agar

It was prepared by dissolving 65 g of the medium in 1 liter of distilled water, then 0.05 mg of chloramphenicol was added to it to inhibit the growth of bacteria, then the medium was sterilized by autoclave at 121°C and pressure of 15 pounds / in 2 for 15-20 minutes.

b) Sabouraud Dextrose broth

It was prepared by dissolving 30 g of the medium in 1 liter of distilled water, then 0.05 mg of chloramphenicol was added to it to inhibit the growth of bacteria, then the medium was sterilized by autoclave at a temperature of 121 ° C and a pressure of 15 pounds / ang 2 for 15-20 minutes, then 15 ml of sterile glycerin was added to it for every 100 ml of the medium at a rate of 15%, this medium was used to preserve fungal isolates.

c) Potato Dextrose Agar

It was prepared by dissolving 39 g of the medium in 1 liter of distilled water, then 0.05 mg of chloramphenicol was added to it to inhibit the growth of bacteria, then the medium was sterilized by autoclave at a temperature of 121 °C and a pressure of 15 pounds / inch2 for 15-20 minutes.

fungal isolates included in the study

Skin scraping were taken from patients attending the consulting clinic for skin diseases at Imam Hussein (Peace on him) Teaching Medical Hospital in the holy city of Karbala, who suffer from fungal skin infections of different ages and of both sexes.

Medicinal plants

Aqueous, alcoholic and acetone extracts were prepared at the following concentration (1, 3, 5, 7, 10, 15 and 20) mg/ml.

Evaluate the inhibitory ability of plant extracts in growth of dermatophytes

The method of [15]. was followed, where by the dried plant extracts were mixed with the melted Sabouraud dextrose agar (SDA) culture medium cooled to 50 °C and with the

28 °C for 2 - 4 weeks, after which the growing fungal colonies were examined outwardly in terms of color, shape, and texture and they were examined microscopically for diagnosis and according to the approved taxonomic keys: [11, 12, 13, 14].

following concentrations (1, 3, 5, 7, 10, 15 and 20) mg/ml, at a rate of 3 replicates for each concentration. After hardening of the culture medium, a disk with a diameter of 6 mm was placed from the fungal colony taken from the growing fungal colony on SDA medium or potato dextrose agar PDA for 7-10 days, where the fungal disk was placed in the center of the plate. Two types of comparison were used, a positive comparison in which the anti-fungal Clotrimazole at a concentration of 2 mg/ml was added to a plate containing SDA solid medium, and a negative comparison that included a plate containing the culture

medium without the addition of any substance, and all plates were planted with the same fungus. The dishes were incubated at a temperature of 25-28 °C for 2-3 weeks, then the diameter of the growing colony was measured (average of two orthogonal diameters) for each colony and each treatment was three replicates for each replicate of one dish.

Determination of the minimal inhibitory concentration (MIC) of plant extracts on the growth of dermatophytes and their associated

The aforementioned method was followed in testing the antagonistic efficacy of plant extracts on the growth of the studied fungi by mixing the sterile water, alcohol, or acetone extract of each plant separately with the medium and at the following concentrations (2, 4, 6, 8, 9, 11, 12, 13, 14, 16, 17, 18 and 19) mg/ml, at a rate of three replicates for each concentration, and the results were recorded based on the presence of growth (+) or the absence of growth (-), as the lowest concentration of the extract did not show fungal growth is the lowest inhibitory concentration.

Cellular toxicity test

Hemolysis was performed as described by [16] and as follows:

30 µL of an alcoholic plant extract solution was mixed with the seeds at a concentration of (2,4,6,13 and 14) mg/ml. It was gently mixed with 0.2 mL of blood (healthy, non-smoking) for 5 minutes, 20 mL of normal saline was added to prevent any decomposition and then centrifuged at 3000 rpm for 10 minutes.

30 µL of DMSO was prepared with normal saline and blood in the same proportion used as positive control while 100% hemolysis was determined by diluting the blood used with a 100-fold greater volume of distilled water rather than normal saline. The absorption was measured at 540 nm and the hemolysis was evaluated by the following equation:

Hemolysis%= (AT-AN)/(A 100% H-AN) x 100%

AT: Absorbance of test solution

AN: Absorbance of normal saline

A100% H: Absorbance of 100% hemolysis

Ethical approval

The research proposal was fully discussed and approved by the scientific and ethical committee in Kerbala university\ college of science\ department of Biology\ Iraq. The agreement of Al-Hussein Medical City\ Karbala city was taken before the start of data collection in number (73) in date (9\1\2021). A verbal consent was taken from all included patients after a full explanation of the aim of study and insurance of confidentiality of the collected data which will not be used but for research purposes.

Statistical analyses

A complete random design was used and the data were analyzed statistically using the computer, and (L.S.D) values were used to compare the averages of the treatments at a probability level (0.05) in all experiments, and the rate of negative comparisons (aqueous, alcoholic and acetone) was extracted according to [17]. for a negative comparison (-) Cont. and entered the statistical analysis program.

Results

Fungal general isolated from skin infections

The results of the current study showed that 50 samples out of a total of 70 samples, with a rate of 71.4%, were positive for culture, as they showed the growth of skin fungi belonging to Dermatophytes as fungi that cause infections with ringworm (Tinea), table (1).

Table (1): cutaneous fungal species isolated from 70 patients infected with dermatophytes

Sample type	Number of samples	Number of males(%)	Number of females(%)
Skin scrapping	30	15(50%)	15(50%)
Hair fragments	25	13(52%)	12(48%)
Nail clipping	15	6(40%)	9(60%)
Total	70	34 (49%)	36(51%)

Three types of dermatophytes were isolated, namely *T.mentographytes* with a number of 30 isolation with a rate of (50%), *T.rubrum* with a number of 10 isolation with a rate of (20%) and *M.canis* with a number of 10 isolation with a rate of (20%), as in table (2).

Table (2): Fungal species isolated from skin infections and the percentage of their recurrence

Type of fungal isolation	The number	Bercent(%)
T.mentagrophytes	30	(60%)
T.rubrum	10	(20%)
M.canis	10	(20%)
Total	50	(100%)

The fungus *T.mentographytes* (60%) was the most common because it is resistant to unfavorable environmental conditions, as well as the most common fungus that causes zoonophilic skin diseases and is often isolated from dogs, cats, rabbits and other rodents [18].

Table (3): Distribution of patients with fungal skin diseases according to the affected sex

Type of fungal isolation	The number (%)	The number of males(%)	The number of females(%)
T.mentagrophytes	30(50%)	14(46.7%)	16(53.3%)
T.rubrum	10(20%)	5(50%)	5(50%)
M.canis	10(20%)	4(40%)	6(60%)
Total	50(100%)	23(46%)	27(54%)

Tinea capitis, especially in the ages that ranged between 41-50 years, ranked first, followed by Tinea corpus, in the second rank, Tinea unguium, in the third rank, followed by Tinea pedis, and Tinea cruris in the fourth rank, especially in the ages that ranged between 21-30 years, followed by, Tinea cruris of the face, Tinea faciei, in the fifth rank in table (4).

Table (4): Distribution of patients with fungal skin diseases according to age and type of skin infection

Clinical type	Age (year)				
	10-20	21-30	31-40	41-50	Total
Tinea capitis	2	2	5	6	15
Tinea corpois	1	5	3	3	12
Tinea unguium	2	4	3	1	10
Tinea cruris	1	3	1	1	6
Tinea faciei	0	2	2	0	4
Tinea pedis	0	0	0	1	1
Tinea barbae	0	0	0	1	1
Tinea manum	0	0	0	1	1
Total					50

Identification of fungal species

Five pathogenic fungal species (*T.mentographytes*, *T.rubrum*, and *M.canis*) were diagnosed based on the agronomic characteristics of the colonies growing on the nutrient media, as well as on the microscopic characteristics such as spores and filaments and on the approved taxonomic keys: [11, 12, 13, 14].

The inhibitory effect of different plant extracts in the growth of pathogenic fungi *in vitro*:

The inhibitory effect of aqueous, alcoholic and acetone extracts of the *T.chebula* seeds in the growth of pathogenic fungi under study.

The results showed that the effect depended on the type of extract and its concentration as well as on the type of fungal isolate. The alcoholic extract showed a high inhibitory effectiveness and came in the first place, followed by the acetone extract and then the aqueous extract. In the alcoholic extract, the diameters of the fungal colonies were In the alcoholic extract, the diameters of the fungal colonies were *T.mentographytes*, *T.rubrum* and *M.canis* (40, 33 and 25) mm, respectively, at a concentration of 1 mg / ml, in the average of the colonies with fungal growths were (0, 0 and 0) mm respectively at a concentration of 7 mg/ml, while the rates of fungal colonies diameter were zero with an inhibition rate of 100% at a concentration of 20 mg/ml.

As for the acetone extract, it also showed a high inhibitory activity against fungal species, as the diameters of the fungal colonies were (45, 38 and 31) mm, respectively, at a concentration of 1 mg/ml, while they were (10, 0 and 0) mm, respectively, at a concentration of 7 mg/ml, while it was zero with a 100% inhibition rate at a concentration of 20 mg/ml.

As for the aqueous extract, it also showed a high inhibitory activity against fungal species, as the average diameters of the fungal colonies were (45, 42, and 35) mm, respectively, at a concentration of 1 mg/ml, while they were (21, 14, and 0) mm, respectively, at a concentration of 7 mg/ml, while it was zero with a 100% inhibition rate at a concentration of 20 mg / ml as in the table (5).

Also, fungal isolates showed significant differences towards plant extracts, as *M.canis* showed the highest sensitivity to plant extracts, followed by *T.rubrum*, then *T.mentagropiytes*. When a statistical comparison was made between aqueous,

alcoholic and acetone extracts between them and the anti-fungal Clotrimazole (2mg/ml), the alcoholic extract showed an equal effect of the antifungal against *M.canis* at a concentration of 3 mg/ml, and against *T.rubrum* at a concentration of 5 mg/ml. and *T.mentagrophytes* at a concentration of 7 mg/ml.

As for the acetone extract, it showed an equal effect of the antifungal against *M.canis* at a concentration of 3 mg/ml and against *T.rubrum* at a concentration of 7 mg/ml, while it showed an equal effect of the antifungal against *T.mentagrophytes* at a concentration of 10 mg/ml.

As for the aqueous extract, it showed an equal effect of the antifungal against *M.canis* at a concentration of 7 mg/ml, *T.rubrum* at a concentration of 10 mg/ml, and *T.mentagrophytes* at a concentration of 15 mg/ml.

Table (5): The inhibitory effect of aqueous, alcoholic and acetone extracts of *T.chebula* seeds in the radial growth (mm) of the pathogenic fungi under study in SDA medium at a temperature of 25-28°C.

Extract type	Conc. mg/ml	Avarge diameters of fungal colonies (mm)			extract effect
		T.m.	*T.r.*	*M.c.*	
Aqueous	1	45	42	35	30.79
	3	40	37	27	
	5	32	30	23	
	7	21	14	0	
	10	17	0	0	
	15	0	0	0	
	20	0	0	0	
	Cont. (-)	80	65	60	
	Clot. 2mg/ml	0	0	0	
Ethanol	1	40	33	25	23.73
	3	32	19	0	
	5	22	0	0	
	7	0	0	0	
	10	0	0	0	
	15	0	0	0	
	20	0	0	0	
	Cont. (-)	80	65	60	
	Clot. 2mg/ml	0	0	0	
Acetone	1	45	38	31	26.80
	3	35	25	0	
	5	27	15	0	
	7	10	0	0	
	10	0	0	0	
	15	0	0	0	
	20	0	0	0	
	Cont. (-)	80	65	60	
	Clot. 2mg/ml	0	0	0	
Fungi		31.07	13.84	7.04	

Conc.	1	3	5	7	10	15	20
	33.73	38.33	37.67	30.18	10.20	15.20	19.93

L.S.D.0.05	Extracts	Fungi	Conc.	Extracts* fungi *Conc.
	0.48	0.62	0.82	3.24

- The results in the above table represent the average of three replications.

M.c = Microsporum canis.
T.m =Trichophyton mentagrophytes.
T.r = Trichophyton rubrum.
Clot = Clotrimazole.
Cont = Control.(-) A negative comparison represents the rate of aqueous, alcoholic and acetone comparisons.

The inhibitory effect of aqueous, alcoholic, and acetone extracts of the *P.anisum* seeds in the growth of pathogenic fungi under study.

The results showed that the effect depended on the type of extract and its concentration as well as on the type of fungal isolate. The alcoholic extract showed a high inhibitory effectiveness and came in the first place, followed by the acetone extract and then the aqueous extract. In the alcoholic extract, the average diameters of the colonies of *T.mentagrophytes*, *T.rubrum* and *M.canis* were (48, 40 and 37) mm, respectively, at a concentration of 1 mg / ml, in the average of the colonies with fungal growths were (0, 0 and 0) mm on tulle at a concentration of 7 mg/ml, while the average diameters of fungal colonies were (0, 0 and 0) mm at a concentration of 20 mg/ml.

As for the acetone extract, it also showed a high inhibitory activity against fungal species, as the average diameters of the fungal colonies were (50, 45 and 40) mm, respectively, at a concentration of 1 mg/ml, while they were (31, 15 and 0) mm, respectively, at a concentration of 7 mg/ml, while it was (0, 0 and 0) mm at a concentration of 20 mg/ml.

As for the aqueous extract, it also showed a high inhibitory activity against fungal species, as the average diameters of the fungal colonies were (55, 50 and 45) mm, respectively, at a concentration of 1 mg/ml, while they were (33, 22 and 0) mm, respectively, at a concentration of 7 mg/ml, while it was (0, 0 and 0) mm at a concentration of 20 mg/ml, as in table (6).

Also, fungal isolates showed significant differences towards plant extracts, as *M.canis* showed the highest sensitivity to plant extracts, followed by *T.rubrum*, then *T.mentagropiytes*.

When a statistical comparison was made between aqueous, alcoholic and acetone extracts between them and the anti-fungal Clotrimazole (2mg/ml), the alcoholic extract showed an equal effect of the antifungal against *M.canis* at a concentration of 5 mg/ml, and for *T.mentagrophytes* and *T.rubrum* at a concentration of 7 mg/ml.

As for the acetone extract, it showed an equal effect of the antifungal against *M.canis* at a concentration of 7 mg/ml and against two fungi *T.rubrum* and *T.mentagrophytes* at a concentration of 10 mg/ml.

As for the aqueous extract, it showed an equal effect of the antifungal against *M.canis* at a concentration of 7 mg/ml, *T.rubrum* at a concentration of 10 mg/ml and *T.mentagrophytes* at a concentration of 15 mg/ml.

Table (6): The inhibitory effect of aqueous, alcoholic and acetone extracts of *P.anisum* seeds in the radial growth (mm) of the pathogenic fungi under study in SDA medium at a temperature of 25-28°C.

Extract type	Conc. mg/ml	Avarge diameters of fungal colonies (mm)					extract effect
		T.m.		*T.r.*		*M.c.*	
Aqueous	1	55		50		45	34.16
	3	48		43		37	
	5	42		35		30	
	7	33		22		0	
	10	25		0		0	
	15	0		0		0	
	20	0		0		0	
	(-) Cont.	80		65		60	
	Clot. 2mg/ml	0		0		0	
Ethanol	1	48		40		37	27.98
	3	40		35		25	
	5	32		27		0	
	7	0		0		0	
	10	0		0		0	
	15	0		0		0	
	20	0		0		0	
	(-) Cont.	80		65		60	
	Clot. 2mg/ml	0		0		0	
Acetone	1	50		45		40	31.13
	3	43		37		32	
	5	37		28		23	
	7	31		15		0	
	10	0		0		0	
	15	0		0		0	
	20	0		0		0	
	(-) Cont.	80		65		60	
	Clot. 2mg/ml	0		0		0	
Fungi		19.15		4.33		6.37	
Conc.	1	3	5	7	10	15	20
	37.07	36.27	38.53	40.00	18.80	16.13	18.40
L.S.D.0.05	Extracts		Fungi		Conc.		Extracts* fungi *Conc.
	0.50		0.64		0.86		3.34

- The results in the above table represent the average of three replications.

M.c= Microsporum canis.
T.m =Trichophyton mentagrophytes.
T.r = Trichophyton rubrum.
Clot = Clotrimazole.
Cont = Control.(-) A negative comparison represents the rate of aqueous, alcoholic and acetone comparisons.

The inhibitory effect of aqueous, alcoholic, and acetone extracts of the *A.herba alba* seeds in the growth of pathogenic fungi under study.

The results showed that the effect depended on the type of extract and its concentration, as well as on the type of fungal isolate. The alcoholic extract showed a high inhibitory effectiveness and came in the first place, followed by the acetone extract, then the aqueous extract. In the alcoholic extract, the average diameter of the colonies of *T.mentagrophytes*, *T.rubrum* and *M.canis* (50, 40 and 20) mm, respectively, at a concentration of 1 mg/ml. Fungal growths (0, 0 and 0) mm were obtained at a concentration of 7 mg/ml, while the fungal colony diameters were zero with an inhibition rate of 100% at a concentration of 20 mg/ml.

As for the acetone extract, it also showed a high inhibitory activity against fungal species, as the diameters of the fungal colonies reached (57, 42 and 30) mm, respectively, at a concentration of 1 mg/ml, while they were (22, 0 and 0) mm, respectively, at a concentration of 7 mg/ml, while it was (0, 0 and 0) mm at a concentration of 20 mg/ml.

As for the aqueous extract, it also showed a high inhibitory activity against fungal species, as the average diameters of the fungal colonies were (60, 52 and 42) mm, respectively, at a concentration of 1 mg/ml, while they were (37, 30 and 23) mm, respectively, at a concentration of 7 mg/ml, while it was (0, 0 and 0) mm at a concentration of 20 mg/ml, as shown in table (7).

Also, fungal isolates showed significant differences towards plant extracts, as *M.canis* showed the highest sensitivity to plant extracts, followed by *T.rubrum*, then *T.mentagropiytes*. When a statistical comparison was made between aqueous, alcoholic and acetone extracts between it and the anti-fungal Clotrimazole (2mg/ml), the alcoholic extract showed an equal effect of the antifungal against *M.canis* at a concentration of 3 mg/ml, and *T.rubrum* and *T.mentagrophytes* at a concentration of 5 mg/ml.

Table (7): The inhibitory effect of aqueous, alcoholic and acetone extracts of *A.herba alba* seeds in the radial growth (mm) of the pathogenic fungi under study in SDA medium at a temperature of 25-28°C.

Extract type	Conc. mg/ml	Avarge diameters of fungal colonies (mm)					extract effect
		T.m.		*T.r.*		*M.c.*	
Aqueous	1	60		52		42	36.20
	3	52		45		35	
	5	47		37		29	
	7	37		30		23	
	10	17		0		0	
	15	0		0		0	
	20	0		0		0	
	(-) Cont.	80		65		60	
	Clot. 2mg/ml	0		0		0	
Ethanol	1	50		40		20	22.64
	3	33		27		0	
	5	0		0		0	
	7	0		0		0	
	10	0		0		0	
	15	0		0		0	
	20	0		0		0	
	(-) Cont.	80		65		60	
	Clot. 2mg/ml	0		0		0	
Acetone	1	37		35		22	30.64
	3	30		25		13	
	5	22		0		0	
	7	0		0		0	
	10	0		0		0	
	15	0		0		0	
	20	0		0		0	
	(-) Cont.	80		65		60	
	Clot. 2mg/ml	0		0		0	
Fungi		18.44		4.56		6.85	
Conc.	1	3	5	7	10	15	20
	36.60	40.33	40.47	36.40	13.33	16.40	20.00
L.S.D.0.05	Extracts		Fungi		Conc.		Extracts* fungi *Conc.
	0.49		0.64		0.85		3.30

- The results in the above table represent the average of three replications.

M.c = Microsporum canis.
T.m =Trichophyton mentagrophytes.
T.r = Trichophyton rubrum.
Clot = Clotrimazole.
Cont = Control.(-) A negative comparison represents the rate of aqueous, alcoholic and acetone comparisons.

Determine of the minimum inhibitory concentration (MIC) of alcoholic extracts of pathogenic fungi under study.

The results in determining the value of the minimum nhibitory concentration (MIC) when using the method of mixing the crude extract with the nutritional medium showed that the value of the minimum inhibitory concentration differed according to the fungal isolate as well as the type of extract, where the decrease in the values of the minimum inhibitory concentration (MIC) of the alcoholic extract was clear for one fungal isolate in all the plants included in the study, the alcoholic extract of the *T.chebula* seeds plant gave the highest values of inhibition as it reached the minimum inhibitory concentration (MIC) where the fungus was. The fungal isolates also showed significant differences towards the plant extracts, as the fungus *M.canis* showed the highest sensitivity to the plant extracts, followed by the fungus *T.rubrum* and then *T.mentagropiytes* in table (8).

Table (8): The minimum inhibitory concentration (MIC) of the alcoholic extracts of the *T.chebula* seeds plant in pathogenic fungi under study

Conc. mg/ml	*T.m.*	*T.r.*	*M.c.*
2	+	+	-
4	+	-	-
6	-	-	-
8	-	-	-
9	-	-	-
11	-	-	-
12	-	-	-
13	-	-	-
14	-	-	-
16	-	-	-
17	-	-	-
18	-	-	-
19	-	-	-

presence of growth = (+)
no growth = (-)

Preliminary chemical detection of the active ingredients in the plants

In light of the results of the current study on the inhibitory effect of the aqueous, alcoholic and acetone extracts of the plants included in the study, their content of active compounds was investigated using some chemical reagents in table (9).

Table (9): Preliminary chemical detection results for the active ingredients in the plants

No	Qualitative disclosures	*T.chebula*	*P.anisum*	*A.herba alba*
1	detection of carbohydrates			
a	Detection of phenol with concentrated sulfuric acid H_2SO_4	+	+	+
b	Muelch revealed	+	+	+
2	detection of alkaloids			
a	Dargendorf detector	+	+	+
b	Mark detector	+	+	+
c	Meyer detector	+	+	+
d	Wakener detector	+	+	+
3	detection of tannins			
a	Lead acetate detection	+	+	+
b	Ferric chloride detection	+	+	+
4	detection of phenols			
a	Follen revealed	+	+	+
b	Ferric Chloride Detection 1%	+	+	+
5	detection of flavonoids			
a	Detection of alcoholic potassium hydroxide	+	+	+
b	Detection of flavonoids and flavonols	+	+	+
6	Detection of phytochemicals	+	+	+
7	detection of saponins	+	+	+
8	Detection of glycosides	+	+	+
9	Detection of resins	+	+	+
10	Turbines detection	+	+	+

The presence of the active chemical compound = (+)
The absence of the active chemical compound = (-)

The percentage of cytotoxicity of the alcoholic extracts of the plants used under study

Table (10) shows the percentage of cytotoxicity present in the alcoholic extracts of the plants used under study, as it reached 0.096% in the alcoholic extract of the *T.chebula* plant, while in the alcoholic extract of the *P.anisum* plant, it reached 0.016%, and in the alcoholic extract of the *A.herba alba* plant, as it reached 0.01%, and the percentages were very small and lowest. The normal limit of 0.13%, according to [16], shows that the use of plant extracts as alternatives to treatment does not hurt the body of the organism compared to antibiotics.

Table (10): Results of the cytotoxicity test of the plants used under study (Cellular toxicity test)

Plant name	The percentage cytotoxicity of the alcoholic plant extracts under study
T.chebula	0.096%
P.anisum	0.016%
A.herba alba	0.01%

Discussion

Three types of dermatophytes were obtained, namely: *T.mentagrophytes*, *T.rubrum*, *M.canis*. The high infection rate in females is due to the constant wearing of shoes by females, which provides the appropriate environment for the growth of moisture-loving skin fungi, as well as the women's dealing with kitchen floors and polluted places, which leads to fungal spores from reaching the foot and providing the appropriate environment, which helps in the occurrence of infection and this the finding is consistent with several studies, including the study [19,20].

The reason for the efficiency of the alcoholic extract of *T.chebula* seeds may be attributed to the nature of the active compounds in it, especially the glycosides, saponins, resins, phenols, tannins (tanners) and others table (9), In a study conducted by [21]. to evaluate the efficiency of the aqueous extract of the seeds of the *T.chebula* plant against various dermatophytes, yeast *Floccosum*, *Epidermophyton*, *T.rubrum*, *M.gypseum* and *C.albicans*, the aqueous extract of the seeds of the *T.chebula* plant showed antifungal activity against dermatophytes. It also agrees with the study conducted by [22]. to evaluate the efficacy of alcoholic extract of *T.chebula* seeds against several types of pathogenic fungi and yeasts *A.flavus*, *A.niger*, *A.fumigatus*, *T.mentagrophytes*, *T.rubrum*, *M.gypsum* and *C.albicans*, *C.tropicalis*, *C.glabrata*, *C.krusei* and *C.parapsilosis*, as the alcoholic extract of the seeds of the *T.chebula* plant showed high effectiveness in inhibiting various dermatophytes and yeasts.

The reason for the efficiency of the alcoholic extract of the *P.anisum* seeds plant may be attributed to the nature of the active compounds in it, especially phenols, flavonoids, essential oils and resins, which do not dissolve except in polar solvents table (9). These results also agreed with the results of the study conducted by [23]. conducted on antifungal activities *in vitro* on the use of crude extracts of *P.anisum* which is 50% methanol and aqueous extracts against two types of fungi (*A.fiumigatus*, *A.niger*). The results showed that *P.anisum* extracts have antifungal activity and the results of this study indicated that the alcoholic extracts are more active than the aqueous extracts and that *P.anisum* can be used as a natural antimicrobial agenst.

As for the acetone extract, it showed an equal effect of the antifungal against *M.canis* and *T.rubrum* at a concentration of 7 mg/ml and against *T.mentagrophytes* at a concentration of 10 mg/ml.

As for the aqueous extract, it showed an equal effect of the antifungal against *M.canis* and *T.rubrum* at a concentration of 10 mg/ml and against *T.mentagrophytes* at a concentration

of 15 mg/ml. The reason for the efficiency of the alcoholic extract of *A.herba alba* seeds may be attributed to the nature of the active compounds in it, especially saponins, flavonoids, alkaloids and others, table (9), *A.herba alba* contains large amounts of active compounds such as terpenes, flavonoids, coumarins, acetylene and essential sterols [10]. The results are consistent with what was mentioned by [24]. using it as an ointment against skin diseases.

As for the minimum inhibitory concentration (MIC), or perhaps the reason for the preference of alcoholic extracts is due to the ability of alcohol to dissolve some active substances that do not dissolve in acetone and distilled water as a result of the difference in the polarity of this solvent, as the extraction conditions are the same, as the polarity of the solvent used in preparing the extract plays an important role in determining the inhibitory potency of the extract, hence the difference in the inhibitory potency of fungal growth between aqueous, alcoholic and acetone extracts [25] indicated the solubility of some active compounds in one of the extracts without their solubility in the other extracts, which may affect the effectiveness of the extract against microorganisms.

It was also found that the seeds of *T.chebula*, *P.anisum* and *A.herba alba*, used in the study possess many active compounds, such as the glycosides, saponins, resins, phenols, tannins (tanners) and others.

As it was found through conducting a toxicological test for plant extracts that the plants did not contain cytotoxic effects when comparing the results with the limit allowed according to [16].

Conclusions

The environment is rich in medicinal plants such as the seeds of *T.chebula*, *P.anisum* and *A.herba alba*, lt was also found that the alcoholic extract of the seeds of the *T.chebula* plant is sufficient in inhibiting the growth of the fungus under study , all studied plants contain most of the active compounds, the plants showed no cytotoxicity.

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