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Evaluation of Crude Phenolic Extract of *Alhagi graecorum* Boiss Plant against Some Dermatophytes Isolated from Patients at Al-Yarmouk Teaching Hospital

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Abstract: This study aimed to evaluate the crude phenolic extract from the *Alhagi graecorum* Boiss plant that contains a bioactive compound to treat dermatophytes. Eighty samples were taken from patients who underwent al-Yarmouk teaching hospital. plant extract was performed by GC-Mass technology . Molecular diagnosis of fungal isolation by PCR technique were *Trichophyton rubrum*, *Trichophyton mentographytes*, and *Microsporum canis* . Results show that the fungicidal diameter for *Trichophyton rubrum*, *Trichophyton mentographytes*, and *Microsporum canis* was 20 mm at 3.125 mg/ml phenol. Fungi did not grow at 6.25, 12.5, 25, or 50 mg/ml phenol. Macroconidia and microconidia sporulation concentrations of *Trichophyton rubrum* at 3.125 mg/ml crude phenol were $1x10^4$ and $2x10^4$, respectively. At the same concentration, macroconidia and microconidia counts were zero at $2x10^4$ and $3x10^4$ of *Microsporum canis* macroconidia, respectively. No significant sporulation was reported for macroconidia and microconidia of *Trichophyton rubrum*, *Trichophyton mentographytes*, and *Microsporum canis* at 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, and 50 mg/ml crude phenol concentrations. When the crude phenol content from *Alhagi graecorum* Boiss above 3.125 mg/ml, sporulation halted. In conclusion, the phenol crude isolated from *Alhagi graecorum* Boiss showed strong antifungal activity at concentrations above 3.125 mg/ml by lowering fungus diameter and preventing sporulation.

Keywords: Dermatophytes , PCR technology , Phenol crude extract , *Alhagi graecorum* Boiss plant , GC-MS technology , Antifungal.

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Introduction

A substantial scientific study is devoted to elucidating the reasons for the continued development of plant-based bioactive compounds with diverse health advantages. Plants are considered not only a significant source of human food and animal feed, but also natural therapies for treating several health issues. Plants have a long history of traditional medical usage, which is backed by the fact that they contain a vast diversity of bioactive that are often classified as secondary metabolites, such as phenolics, with varied medicinal qualities and health benefits (1,2).

Nowadays, there is a resurgence of interest in using plants as a source of food and medicine. According to a recent WHO study (2011), around 80% of the world's population depends on

traditional medicines to treat various health issues and illnesses, particularly in poorer nations. The use of phytomedicines for therapeutic purposes is apparent in almost all medical systems (Chinese, Greco-Arab, Ayurveda etc.). Literature describing traditional phytomedicine's therapeutic and pharmacological properties many decipherable studies (3,4).

The principal phenolic compounds in the human diet are plant-based foods such as fruits, vegetables, and drinks (fruit juice, wine, tea, and beer). Polyphenols are a vast, structurally varied category of natural antioxidants whose chemistry, sources, antioxidant activity, and health effects have been widely explored. In recent years, multiple studies have linked polyphenol-rich diets to the protection of cardiovascular illnesses, some forms of cancer, and other oxidative damage-related disorders(5).

Dermatophytes are pathogenic fungi with a strong attraction for keratinized structures in nails, skin, and hair, and they cause surface infections known as dermatophytosis (6). Dermatophyte-caused infections are known by numerous names, including dermatophytosis, ringworm, and tinea. Infections of the skin, hair, and nails produced by colonization of the keratinized tissues of the body (7). India, Iran, and Arabia are all familiar with the *Alhagi graecorum* Boiss (Manna tree). It is used as a general tonic, an anthelmintic, and a treatment for constipation, jaundice, and arthritis, while the roots are employed as an aphrodisiac. In other nations, the plant is recognized as a diuretic, a blood cleanser with antibacterial properties, and a treatment for diarrhea, upper respiratory system issues, wounds, haemorrhoids, and uterine difficulties (8). *A. graecorum* is traditionally used in folk medicine as a treatment for rheumatic aches, bilharziasis, liver problems, and different forms of gastrointestinal distress (9).

The biological processes that natural products engage in are both intriguing and helpful, and they serve a variety of purposes (10). In an effort to discover more effective treatments for cancer, viral, and microbial diseases, researchers are increasingly focusing their attention on medicines derived from natural sources (11).

Gas Chromatography-Mass Spectrometry (GC-MS) is an analytical method that identifies various chemicals contained within a test sample by combining the separation capabilities of gas-liquid chromatography with the detection characteristics of mass spectrometry. This hyphenated method combines the capabilities of gas-liquid chromatography and mass spectrometry.GC separates volatile and thermally stable replacements in a sample, while GC-MS segments the target analyte depending on its mass. GC-MS/MS is the result of adding a mass spectrometer. The single and triple quadrupole modes provide superior performance (12,13).

This study aimed to evaluate the extracted phenolic crud from the *Alhagi graecorum* Boiss plant that contains a bioactive compound to treat the dermatophytes of *Trichophyton rubrum*, *Trichophyton mentographytes*, and *Microsporum canis* .

Materials and methods

Fungal isolation

The cross-sectional research was done between December 2021 and March 2022 at AL- Yarmouk Teaching Hospital. After obtaining agreement from each patient or their family members, 80 patients were recruited in this research. Each patient's medical history was meticulously documented. Additionally, the patient's name, gender, age, affected body area, the existence of an inflammatory margin, symptoms, and sickness duration were documented. After obtaining a thorough history of the patient, a thorough clinical examination was conducted in excellent lighting to determine the location of infection, number of lesion types, presence of an inflammatory margin, etc.

The samples of skin scrapings, nail clippings, and hair have been gathered aseptically; the afflicted region has been cleansed with 70% ethyl alcohol to eliminate dirt and environmental contaminants, such as bacteria, and dried thoroughly. The infection's periphery was scraped using a sterile scalpel to get the skin sample. While a sterile clipper was used to capture the nail sample and sterile scissors were used to acquire the hair sample, the samples were collected. The samples were collected and transferred on folded paper, which prevents the overgrowth of bacterial contaminants by keeping the specimen dry, and then folded and fastened with a paper clip.

Microscopic and macroscopic examination

This investigation identified human pathogenic fungus. This attribution was contingent on the following:

1- Characteristics of the colony (colour, consistency and topography).

2- Colony turns around (colour, significant pigment).

Microconidia and macroconidia: their form, organization, and hyphal structures.

The study consisted of a microscopic inspection of many preparations from various regions of fungal growth, mounted on a clean slide and stained with lactophenol cotton blue to show spores consisting of big septate macroconidia and tiny, single-celled microconidia. The slide was gently heated under a spirit lamp to aid staining and eliminate air bubbles (extra stain was removed using tissue paper) .

Identification of fungal isolates by Polymerase Chain Reaction (PCR):

DNA extraction

General Protocol

1. Transfer 1~ 5 x10 of cultures (fungal/ yeast cells) to a 1.5 ml microcentrifuge tube.
2. Add 1 ml of FA Buffer to the cells and resuspend the cells by pipetting. descend the cells by centrifuging at 5,000 rpm for 2 min and discard the supernatant completely.
3. Resuspend the cells in 550 µl of FB buffer and add 50 µl of lyticase solution, mix well by vortexing. Incubat the sample at 37 °C for 30 min. caution: Lyticase solution and FB Buffer containing 14 mM of ß-mercaptoethanol is hazardous to human health. perform the procedures involving Lyticase solution and FB Buffer in a chemical

fume hood. descend the cells by centrifuging at 5,000 rpm for 10 min. remove the supernatant completely.

4. Add 350 µl TG1 Buffer and mix well by pipetting. transfer the sample mixture to a bead tube. mix well by plus vortexing for 5 minutes.
5. Add 20 µl of Proteinase K (10 mg/ml) and mix well by vortexing. Incubate at 55 °C for 15 min, vortex 30 seconds for every 5 minutes incubation.
6. Descend the cells by centrifuging at 5,000 rpm for 1 min and transfer 200 µl of supernatant to a new 1.5 ml microcentrifuge tube.
7. Add 200 µl of TG2 Buffer and mix well by pipetting.
8. Add 200 µl of ethanol (96-100%) and mix well by pulse-vortexing for 10 seconds.
9. Place a TG (Tiagen) mini column in collection tube. transfer the sample mixture (including any precipitate) carefully to TG mini column. centrifuge at 14,000 rpm for 30 second then place the TG mini column to a new collection tube.
10. Add 400 µl of W1 (Wash 1) Buffer to the TG mini column. centrifuge at 14,000 rpm for 30 seconds and discard the flow-through. place the TG mini column back to the collection tube.
11. Add 750 µl of Wash Buffer to the TG mini column. centrifuge at 14,000 rpm for 30 seconds and discard the flow-through. place the TG mini column back to the collection tube .
12. Centrifuge at full speed 14,000 rpm for an additional 3 min to dry the column.
13. Place the TG mini column to a elution tube.
14. Add 50 ~100 µl of elution buffer or ddH2O (deionized distil water) to the membrane center of the TG mini column. stand TG mini column for 3 min.
15. Centrifuge at full speed 14,000 rpm for 1 min to elute total DNA. 16. Store total DNA at 4°C or -20°C.
16. Agarose gel electrophoresis of DNA:
    a. An agarose solution was prepared by dissolve 1g of agarose powder in 100 ml of 1x TBE (Tris Borate EDTA) in the (100) ml flask, agarose was melted in hot block until the solution became clear.2- The agarose solution was made cool to about (50- 55 ℃) , swirling the flask occasionally to cool evenly.
    b. Red stain (3 µl) was added to the warm gel then sealed the ends of the casting tray with two layer of tape.
    c. The combs were placed in the gel-casting tray
    d. Melted agarose solution was poured into the casting tray.
    e. The agarose was allowed to solidify at room temperature, the comb pulled out carefully and the tape was removed. The gel was placed onto the electrophoresis chamber that was filled with TBE (1x) buffer.
    f. DNA samples (5µl) were mixed with (3µl) DNA loading stain and loaded in agarose gel wells.
    g. The agarose gel electrophoresis was completed at 70V, 65Amp for 1hour.The DNA was observed by viewed under UVtrans illuminator.

The primers preparation

The primers were lyophilized and dissolved in free ddH_2O (deionized distil water) to give a final concentration of 100 pmol/l as a stock solution, and the stock was kept at -20

℃. To prepare 10 pmol/l concentration as work primer, 10 ml of the stock solution was suspended in 90 ml of free ddH_2O to reach a final volume of 100 µl.

Primers used in the interaction

Table (1): The sequence of primers used in the interaction

Pimer	Sequence	Primer sequence	Tm (°C)	GC %	Size of Product (bp)
ITS	F	5′- TCCGTAGGTGAACCTGCGG -3′	60.3	50 %	550-650
ITS	F	5′- TCCGTAGGTGAACCTGCGG -3′	60.3	50 %	
	R	5′ TCCTCCGCTTATTGATATGC-3′	57.8	41 %	

Master Mix or GoTaq® Green Master Mix was used for PCR amplification The components of kit are listed in table (2).

Table (2): The components of kit

Materials	Volume
Taq DNA Polymerase	2.5 U
DNTPs	2.5 Mm
Reaction buffer	1X
Gel Loading buffer	1X

The PCR amplification was performed in a total volume of 25µl containing 1.5µl DNA, 5 µl Taq PCR PreMix , 1µl of each primer (10 pmol) then distilled water was added into tube to a total volume of 25µl as in table (3).

Table (3): PCR components

Component	25 µl (Final volume)
Taq PCR PreMix	5µl
Forward primer	(1 µl) 10 picomols/µl
Reverse primer	(1 µl) 10 picomols/µl
DNA	1.5µl
Distill water	16.5 µl

Amplification of ITS region:

The universal primers (ITS-1 ITS-2) were used for the amplification of rRNA gene and ITS regions which is found in all eukaryote as a conserved regions (14). The thermal cycling conditions were done as follows: Initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 45s, 52°C for 1 min and 72 °C for 1 min with extension 2 at 72 °C for 5 min using a thermal cycled as in table (4):

Table (4) : The optimum condition of detection

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	5 min.	1 cycle
2-	Denaturation	95°C	45 sec.	35 cycle
3-	Annealing	52°C	1 min.	
4-	Extension	72°C	1 min.	
5-	Extension	72°C	5 min.	1 cycle

The PCR products were separated by 1.5% agarose gel electrophoresis and visualized by exposure to ultraviolet light after staining by safe red stain.

Sequencing and sequence alignment of fungi

After confirming the amplification of gene by conventional PCR, 20µl from PCR reaction with 50µl of forward primer for this genes were send to Macrogene company to determine the DNA sequencing in these genes. Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov), and BioEdit program . both of which are accessible from the National Center for Biotechnology Information (NCBI). The findings were compared to data provided from Gene Bank's published ExPASY software, which is accessible online via the NCBI.

Collection of plant sample

Plants sample, including the roots of *Alhagi graecorum* Boiss was collected from Al-Anbar province orchards during november and december 2021. after collection, the sample was cleaned, by washing with tap water then dried at room temperature, and then stored at 25 °C in dark and clean containers until use.

Crude phenol extract

According to Harborne (1984) (15), the Crude phenols were isolated . 200 g of plant powder was divided into two equal portions, 300 ml of 1% hydrochloric acid was added to one portion, and 300 ml of D.W. was added to the other portion. The two portions were placed in the electric blender for five minutes. The two mixtures were then placed in a boiled water bath for 30 to 40 minutes, cooled, filtered through muslin cloth, then centrifuged for 10 minutes at a speed of 3000 revolutions per 10 minute. Both supernatants were mixed . equal quantity of n-propanol was added to the mixture and sodium chloride was added until the solution was separating into two layers. The lower layer was extracted in separating funnel with Ethyl acetate and the solvent layer was collected and evaporated in rotary evaporated at 40°C for (1-2) hours . The upper layer was evaporated in rotary evaporated at 40°C for (1-2) hours . the dried material of both layers were mixed and dissolved in 5ml of 96% ethanol then transferred to oven 37 °C . then the extract was kept in dark containers in refrigerator until use.

Extracts indicators

Lead acetate reagent

Lead acetate reagent is a 1% solution of lead acetate in alcohol ($Pb(C_2H_3O_2)_2.3H_2O$). after adding the reagent to an equivalent volume of alcohol extract, a white precipitate formed, indicating the presence of phenols (15).

Ferric chloride solution (1%)

Three ml of plant extract was added to 2 ml of 1% Ferric chloride ($FeCl_3$). The appearance of blue green color refers to phenolic compounds existence(16).

Detection of saponins

The extract 5 ml was added to 3 ml of mercuric chloride solution ($HgCl_3$). The appearance of a white precipitate indicates the presence of saponins and also the appearance of big foam for a long time as a result of stirring the aqueous solution of extract powder in test tube indicates to saponins existence(17).

Detection of resins

Abortion of 50 ml from 95% ethanol alcohol was added to 5 g extract powder and put in water boiling bath for 2 min. After cooling the filtered mixture, 10ml of distilled water containing 4% hydrochloric acid (HCL) was added to the filtered solution, thereafter turbidity appearance refers to resins existence(17).

Detection of glycoside

Few drops of 10% hydrochloric acid (HCL) were added to 5 ml of extract, and then left in a boiling water path for 2 min. Then 2 ml of benedict reagent was added and left in boiling water bath for 5 min. The appearance of red precipitate refers to a positive result (18).

Biochemical analysis of plants extract Gas chromatography-mass spectrometry (GC-Mass) analysis :

In labs of the Ministry of Industry and Minerals. GC-MS analysis was carried out on a GC - mass 5977A Series Agilent system auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused silica capillary column HP-5MS (30 mm×0.25 mm I.D) operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 uL was employed (split ratio of10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 60°C (isothermal for 2 min), with an increase of 10 °C/min, to 270°C, then 5°C/min to 290°C, ending with a 9 min isothermal at 310°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time is 60 min (19).

Determination of minimal inhibitory concentration (MIC) of *Alhagi graecorum* Boiss extract on isolated dermatophytes

1. Various amounts of phenol extract was made, and each volume was separately combined with 100 ml of SDA (Sabouraud Dextrose Agar) to achieve the desired concentrations (3.125 , 6.25 ,12.5 ,25 ,50 mg/ml).Three replicates were made for each concentration . The mixture of extract and SDA is put onto petri plates and allowed to harden under sterile conditions.
2. Five millimeters of mycelial growth from a 7 day fungal culture was placed in the middle of each plate at 28°C, the inoculated plates were incubated.
3. After seven days, the diameters of fungal growth were measured and the antifungal activity of each concentration was determined by measuring the growth inhibition(20).

Spores production rate (sporulation):

A plug mycelium (5mm) of selected dermatophytes was cut from treat colony with extract and transferred to test tubes containing 10 ml distilled water. Then dilutions were made and the numbers of spores were counted in a haemocytometer (21).

Statistical analysis

The Statistical Analysis System-SAS (2018) (22) program was used to detect the effect of different factors on study parameters. Least significant difference – LSD test (Analysis of Variation-ANOVA) was used to

significant compare between means in this study.

Resultss

Fungal isolation

The results of fungal isolation from patients showed that the types were *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Microsporum cains*

The morphological description of *Trichophyton rubrum*: The colonies' surfaces are granular or fluffy, white to buff. Their reverse is deep red or purplish; occasionally, it is brown, yellow-orange, or even colourless.

The morphology of the *Trichophyton mentographytes* colonies was greatly varied; their surface was buff and powdery or white and downy. At the same time, others become pinkish or yellowish. Their reverse is usually brownish tan but may be colourless, yellow, or red, as shown in figure (1).

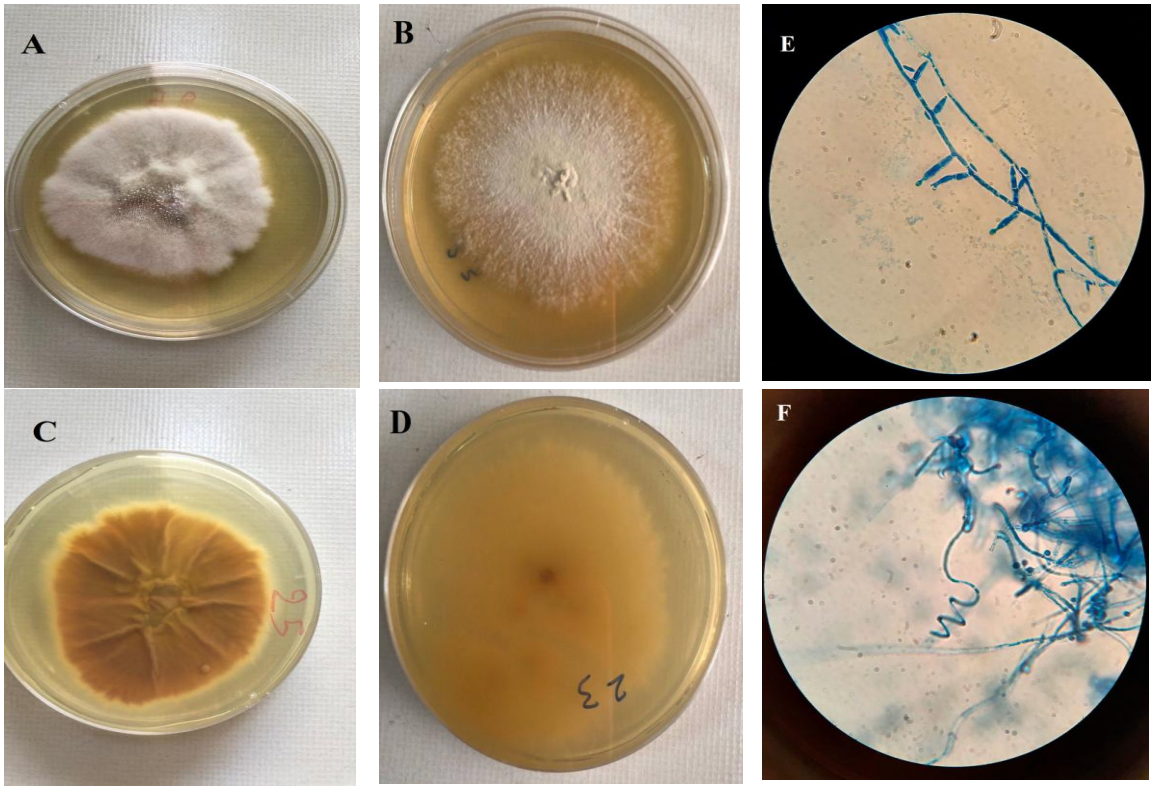

Figure (1): *Trichophyton* species grown on SDA after ten days of incubation at 28±2C: (A)Top view: *Trichophyton rubrum* (B) Top view: *Trichophyton mentographytes* (C) Reverse view: *Trichophyton rubrum* (D) Reverse view: *Trichophyton mentographytes*. (E) Microscopic features of *Trichophyton rubrum*. (F) Microscopic features of *Trichophytons mentographytes.*

The structure of the colonies of the fungus *Microsporum canis* surface was white, coarsely fluffy, hairy to silky or fur-like, with yellow pigment at the periphery and closely spaced radial grooves. Surface had a texture similar to fur, as shown in figure (2), their undersides have a dark yellow color that fades to a brownish yellow with time.

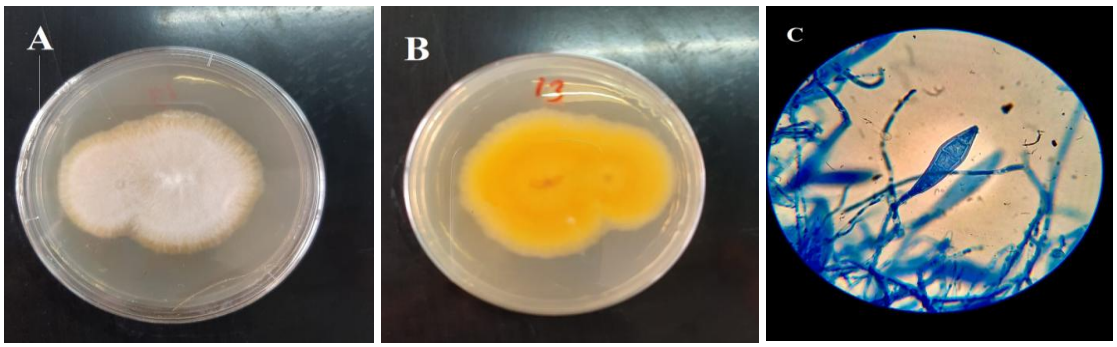

Figure (2): *Microsporum canis* grown on SDA after ten days of incubation at 28±2C(A) Top view (B) Reverse view. (C) Microscopic feature of *Microsporum canis*.

Molecular identification of dermatophytes by Polymerase Chain Reactrion (PCR)

To confirm the identification of some dermatophytes species, three isolates were identified by PCR and sent for gene sequence, these isolates showed the formation of concentric rings that are typical of dermatophytes species which is consistent with the characteristics previously described for this fungus. However, although the colony morphology serves to identify fungi of this genus, it is insufficient to distinguish the species, which makes it necessary to confirm the species through molecular methods, one of these methods is sequencing assay (23). The extraction of genomic DNA was done efficiently using a Mini Kit for Favorep Fungi/ Yeast Genomic DNA extraction (Ca5t. No.: FAFYG 001) . After finishing the DNA extraction, the concentration and purity of DNA were measured by nanodrop, the result showed a concentration between (37.1 to 72.5 $\mu g\ ml^{-1}$) and purity (1.4-1.8). Then the purity of DNA was confirmed by agarose gel electrophoresis (Figure 3) . The molecular characterization was based on the ITS region where the nuclear rDNA region spanning the ITS1, ITS2 and 5.8S rRNA gene was amplified for each of the some dermatophytes isolates. The inter genic spacer region was successfully amplified from all isolates tested, and a distinct product size was consistently obtained for all isolates of a given species and all isolates yielded a unique product size of approximately ~ 550-650 bp , The amplicon obtained of this region for each of the isolates was in the size range of (~565 bp), (Figure 4).

Figure (3): Agarose gel electrophoresis of the total genomic DNA for some dermatophytes species isolates. Fragments were fractionated by electrophoresis on 1.5 % agarose gel visualized under U.V. light after staining with red safe stain lan (1-3).

PCR has been effective for the detection of a great variety of microorganisms and it may be useful tool in diagnosis process. This result is near to those obtained in previous studies on dermatophytes identification by sequencing analysis of the ITS region is a faster, precise, and more dependable diagnosis at species and subspecies levels than the conventional laboratory methods (24) . Use of the ITS region is first step tower spices typing method for dermatophytes species identification; thesome dermatophytes species specific PCR primers, ITS1 and ITS2, provide a fast and accurate tool for identification and characterization of dermatophytes species.

Figure (4): Agarose gel electrophoresis. of the total genomic DNA for fungal isolates. Fragments were fractionated by electrophoresis on 1.5% agarose gel visualized under U.V. light after staining with red safe stain . M : 100bp ladder marker , lane *T. mentographytes* , lane 2 : *M. canis* , lane 3 : *T. rubrum.*

The three PCR product samples were sent for sequence analysis; of some dermatophytes species and 50 µl (10 pmol) from the forward primer.for this gene were submitted to the Macrogene business to evaluate the DNA sequencing in these genes. The result of the sequence analysis was analysed by blast search in the National Centre Biotechnology Information (NCBI) online at (http:// www.ncbi.nlm.nih.gov) and BioEdit program to detect polymorphism. After sequencing analysis on blast website, acquired results allowed to determine some dermatophytes at species level. Result indicated the presence of three different species which is *Trichophyton mentographytes*, *Microsporum canis* and *Trichophyton rubrum*. The three species alignments with universal isolate recorded on BLAST program showed 100% identification and 0% gaps (Figure 4, 5, 6).

Score	Expect	Identities	Gaps	Strand
1097 bits(1216)	0.0	608/608(100%)	0/608(0%)	Plus/Plus

```
Query  1
ACGATAGGGCCAAACGTCCGTCAGGGGTGAGCAGATGTGCGCCGGCCGTACCGCCCCATT  60

Sbjct  163
.............................................................  222


Query  61
CTTGTCTACCTTACTCGGTTGCCTCGGCGGGCCGCGCTCTTCCAGGAGAGCCGTTCGGCG  120

Sbjct  223
.............................................................  282


Query  121
AGCCTCTCTTTAGTGGCTAAACGCTGGACCGCGCCCGCCGGAGGACAGACGCaaaaaaaT  180

Sbjct  283
.............................................................  342


Query  181
TCTTTCAGAAGAGCTGTCAGTCTGAGCGTTAGCAAGCAAAAATCAGTTAAAACTTTCAAC  240

Sbjct  343
.............................................................  402


Query  241
AACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGA  300

Sbjct  403
.............................................................  462


Query  301
ATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGCATTCCG  360

Sbjct  463
.............................................................  522


Query  361
GGGGGCATGCCTGTTCGAGCGTCATTTCAGCCCCTCAAGCCCGGCTTGTGTGATGGACGA  420

Sbjct  523
.............................................................  582


Query  421
CCGTCCGGCGCCCCCGTTTTTGGGGGTGCGGGACGCGCCCGAAAAGCAGTGGCCAGGCCG  480

Sbjct  583
.............................................................  642


Query  481
CGATTCCGGCTTCCTAGGCGAATGGGCAACAAACCAGCGCCTCCAGGACCGGCCGCCCTG  540

Sbjct  643
.............................................................  702
```

Figure (4): *Trichophyton mentagrophytes* strain 600313/19 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MT371235.1Length: 990Number of Matches: 1
Range 1: 163 to 770GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
1216 bits(1348)	0.0	674/674(100%)	0/674(0%)	Plus/Plus

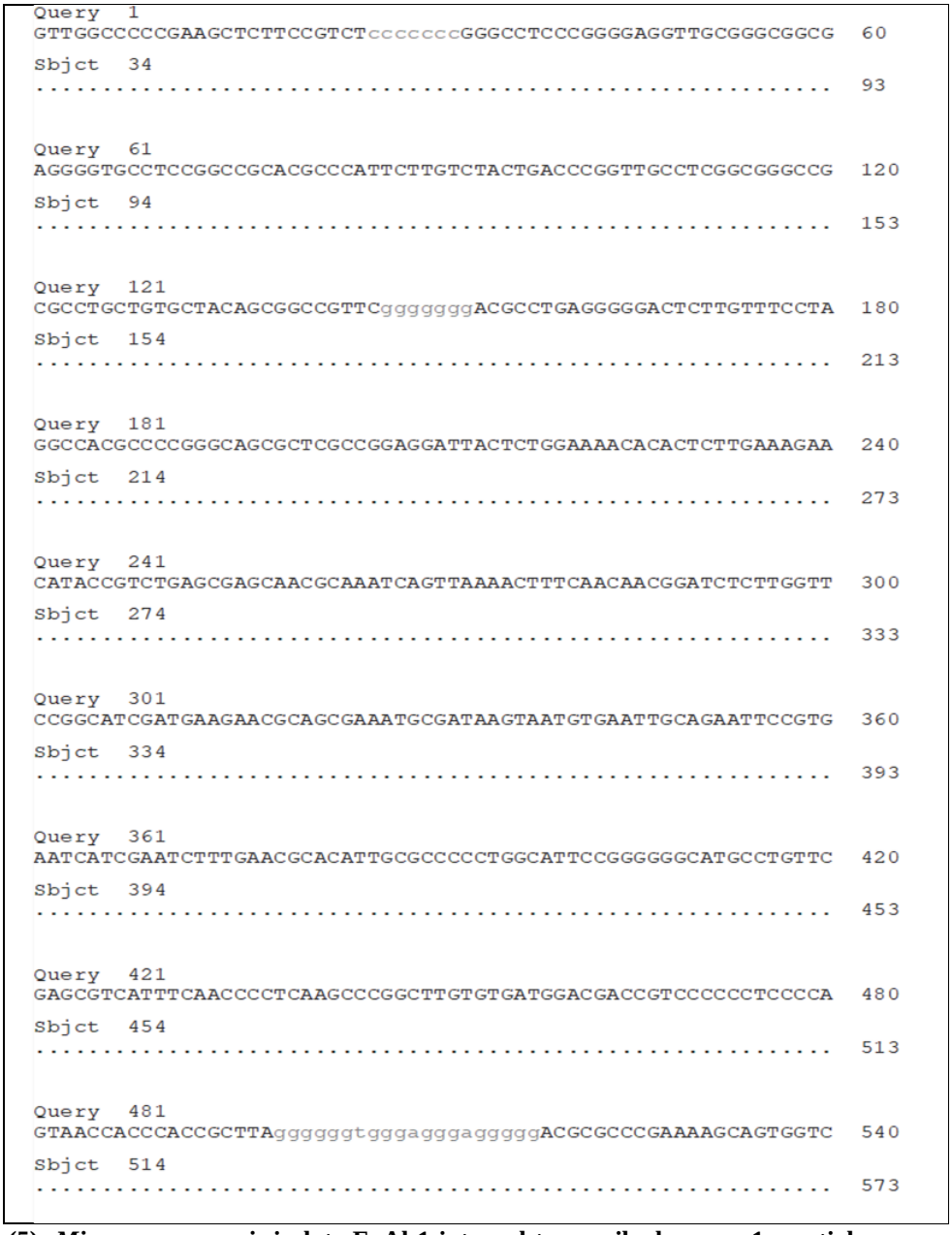

Figure (5): *Microsporum canis* isolate FpAl-1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MT441906.1Length: 516Number of Matches:
Range 1: 15 to 510GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
895 bits(992)	0.0	496/496(100%)	0/496(0%)	Plus/Plus

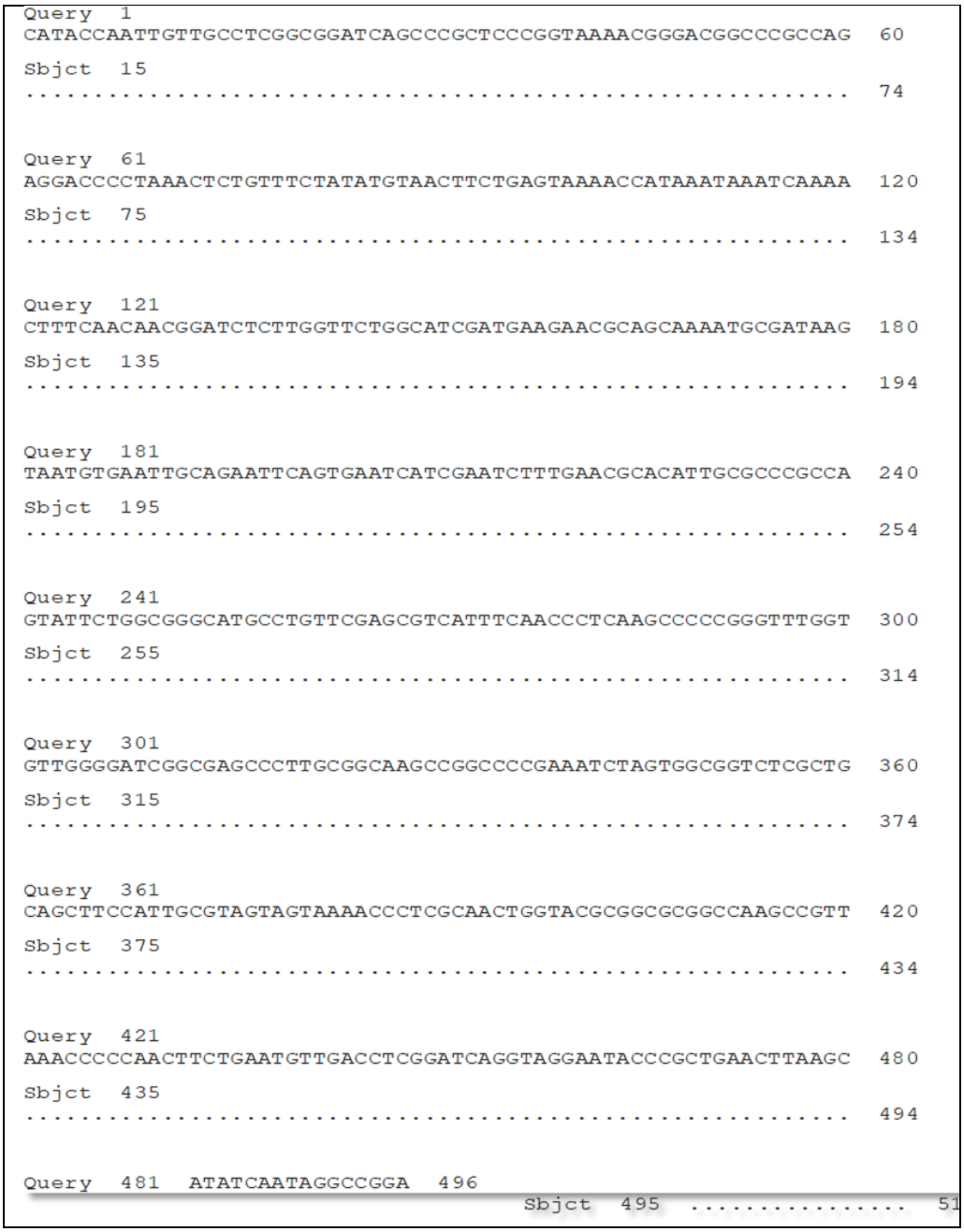

Figure (6): ***Trichophyton rubrum*** **isolate FpAl-1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence**

3Sequence ID: KF437402.1 Length: 516Number of Matches: 1
Range 1: 15 to 510GenBankGraphicsNext MatchPrevious Match

GC-Mass technology for detect of active compounds

The results of detecting many active compounds included phenolic compounds of *Alhagi graecorum* Boiss plant performed by the GC-Mass technology shown in figure (7).

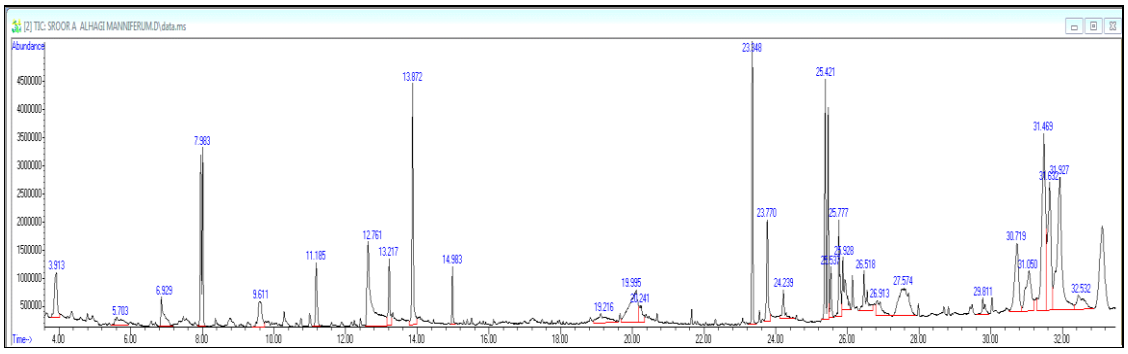

Figure (7): The detection of the active compounds by the GC-Mass technology.

The GC-MS of crude phenolic extract of *Alhagi graecorum* Boiss plant as shown in table (5) indicated the presence high content of Tetracosane (10.56 %) are similar to the result of (25) mentioned that the organic compound Tetracosane (0.81%) produced in *Punica granatum* fruit peel extract act as antimicrobial and antifungal .

Table (5): The component of *Alhagi graecorum* Boiss plant.

No.	Constituents	Retention Time	Peak Area%
1	3,5-dimethylpyrazole 3-Furaldehyde Furfural	3.916	2.09
2	Hyrazinecarboxamide 1,2- Hydrazinedicarboxylic acid , di ethyl ester 1,4,6- Trimethyl-2-azafluorenone	5.699	0.97
3	2-Furancarboxaldehyde	6.928	1.44
4	2,4-Pentanediol Dimethylmalonic , hexyl 3-phenylpropyl ester Pyrimido [4,5-b] cyclopentathiophen-4(3H)-one , 4,5,6,7-tetrahydro-2-methyl-3-phenyl	7.984	5.51
5	2,5-Furandicarboxaldehyde Orcinol	9.611	1.77
6	1-(2-Thienyl)-1-propane Thiophene , 2-methyl-5-propyl-1-(2-Thienyl)-1-propyl-	12.761	1.61
7	5-Hydroxymethylfufural Thiophene,2-propyl	12.761	6.05
8	1,2-Cyclohexanedicarboxylic acid , isohexyl 4-octyl ester 1,2-Cyclohexanedicarboxy5lic acid , isohexyl 4-isoproyloxyphenyl diester 2,6-Difluoro-3-methylbenzoic acid , 2-methylpentyl ester	13.220	1.67
9	2-Fluorobenzoic acid undecyl este 3,4-Difluorobenzoic acid , 3-dodecyl ester 2,5-Difluorobenzoic acid , 3-dodecyl ester	13.869	4.84
10	Eugenol 3-Allyl-6-methoxyphenol	14.986	0.95

No.	Constituents	Retention Time	Peak Area%
11	Allantoin Trimethylsilyl 20-acetoxy-3,6,9,15,18-hexaxoxaicosan-1-oate n,N-Dimethylformamide trimethylene acetal	19.218	1.36
12	4-O-Methylmannose 2-[2-(2-Butoxyethoxy) ethoxy] ethoxy]etyl acetate Methyl (methyl 4-O-methyl-.alpha.-d-mannopyranoside) urinate	19.997	4.24
13	Hydroperoxide , 1,4-dioxan-2-yl 3-Methylmannoside Trimethylsilyl 20-acetoxy-3,6,9.1518-hexaoxaicosan-1-oate	20.240	1.03
14	Hexadecanoic acid , methyl ester	23.347	4.21
15	n-Hexadecanoic acid Pentadecanoic acid	24.238	0.97
16	Octatriaconty trifluoroacetate 1-Decanol , 2-hexyl - Pentafluoropropionate	24.238	0.97
17	7-Oxabicyclo[4.1.0] heptane , 1-methyl-4-(2-metyloxiranyl)- 9,12,15-Octadecatrienoic acid , methyl ester , (z,z,z) - Methyl 5 , 12-octadienoate	25.424	6.97
18	11-octadecenoic acid , methyl ester 9-Octadecenoic acid , methyl ester (E)- cis 13-Octadecenoic acid , methyl ester	25.537	1.05
19	Heptadecanoic acid , 16-methyl-, methyl ester Methyl stearate	25.779	2.34
20	9-Octadecenoic acid , (E)- Oleic Acid	25.779	2.36
21	Tetrapentacontane , 1,54-dibromo-Oxirane , tridecyl- Nonahexcontanoic acid	26.1515	1.78
22	Octadecane , 1-chloro- Sulfurous acid , octadecyl 2- propyl ester Octadecane , 1-chloro-	26.913	1.36
23	Stigmasterol	27.571	4.57
24	Cyclopropa[5,6]-33-norgorgostan-3-ol,3,6-dihydro-, (3.beta., 5.beta., alpha.,22.xi.,23.xi.)-24-Methyl-5,28-stigmastadien-3.beta.ol Ergost-25-ene-3,5,6,12-tetrol , (3.beta., 5.alpha., 6.beta ., 12.beta)-	29.812	0.74
25	.gamma.-Sitosterol .beta.-Sitosterol	30.721	4.34
26	1,13-Tetradecadiene Bicyclo[5.3.0] decane (R) – (-)-14-Methyl-8-hexadecyn-1-ol	31.050	3.66
27	Lupan-3-one Phosphine, dimenthoxy-menthyl- Humulane-1,6-dien-3-ol	31.466	10.34
28	.beta.-Amyrin Olean-12-ene 4,4,6a,6b,8a, 11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	31.630	7.10
29	Tetracosane Nonadecane,9-methyl- Octacosane	31.924	10.56
30	9,19-Cycloergost-24(28)-en-3-ol,4,14-dimethyl-,acetate,(3.beta.,4.alpha.,5.alpha.)- 9,19-Cyclolanostan-3-ol, 24,24-epoxymethano-,acetate 1H-Indene , 5-butyl -6-hexyloctahydro-	32,530	1.75

Plants extracts constituents

In this work, root parts of *Alhagi graecorum* Boiss plant, was extracted for detection of the phenolic extracts. chemical detection of the alcoholic extracts of these samples was summarized in table (6). The results showed the phenols indicated the positive result of the crude phenolic while the saponins, resins, alkaloids, glycosides, and were absent from this extract. This result agrees with phenolic compounds derived from natural sources(26).

Table (6): Chemicals test of crude phenolic extract the test was based on color and appearance

Test Name	*Alhagi graecorum*
Phenols detection	+Ve
Saponins detection	-Ve
Resins detection	-Ve
Glycoside detection	-Ve
Alkaloids detection	-Ve

The symbol (+ve) refers to presence of the compound that appearance in white color in lead acetate reagent and green color in ferric chloride reagent but the symbol (-ve) refers to the absence of the compound.

The effect of *Alhagi graecorum* Boiss phenolic extraction on the fungal growth

The effect of the phenolic extraction of *Alhagi graecorum* Boiss on the three types of fungal is shown in table (7). The phenolic effect is calculated by measuring the diameter of the fungal. The analysis shows that at the 3.125 mg/ml of the phenol concentration, the fungicidal diameter for *T. rubrum* and *T.mentographytes* and *M. canis* were increased to 20 mm, 19 mm, and 20 mm, respectively, as shown in figure (8). In contrast, when the concentrations of phenol increased to 6.25 mg/ml, 12.5 mg/ml, 25, 50 mg/ml, no growth were found in those fungi. that means when the concentration of the phenol from *Alhagi graecorum* Boiss increase, this leads to an inhibition of the fungal activity, as shown in figures (9), (10), (11), and (12), respectively. The effect of crude phenol extracted from *Alhagi graecorum* Boiss on fungal growth shows that *Alhagi graecorum* Boiss extraction had a significant effective result against dermatophytes for treating *T. rubrum*, *T. mentographytes*, and *M. canis* fungi.

Table (7): Effect of *Alhagi graecorum* Boiss crude phenolic extract at different concentrations on fungal growth or colony on SDA at 28±2 °C

Concentration (mg/ml)	Mean ±SE		
	T.rubrum	***T.mentographytes***	***M.canis***
3.125	20 ±0.86 b	19 ±0.71 b	20 ±0.86 b
6.25	0 ±0 c	0 ±0 c	0 ±0 c
12.5	0 ±0 c	0 ±0 c	0 ±0 c
25	0 ±0 c	0 ±0 c	0 ±0 c
50	0 ±0 c	0 ±0 c	0 ±0 c
Griseo.(2mg /ml)	0 ±0 c	0 ±0 c	0 ±0 c
Cont.(-)	72 ±3.47 a	54 ±2.39 a	65 ±2.86 a
LSD value	7.489 *	6.031 *	6.495 *
Means having with the different letters in same column differed significantly. * (P≤0.05).			

T.r = *Trichophyton rubrum* , **T.m** = *Trichophyton mentographytes* , **M.c** = *Microsporum canis* , **Griseo.** = Griseofulvin , **Cont.** = Control.

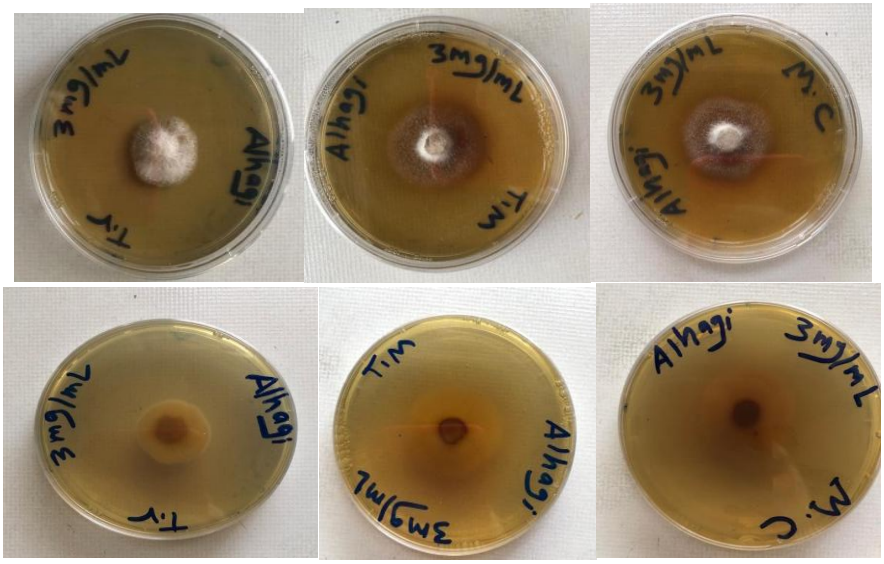

Figure (8): The effect of crude phenol extract for *Alhagi graecorum* Boiss plant with 3.125 mg/ml concentration on fungal growth.

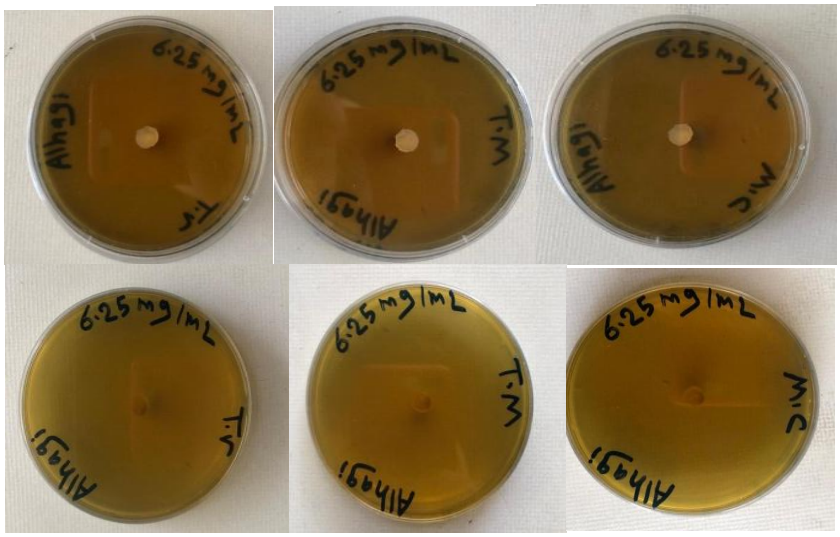

Figure (9): The effect of crude phenol extract for *Alhagi graecorum* Boiss plant with 6.25 mg/ml concentration on fungal growth.

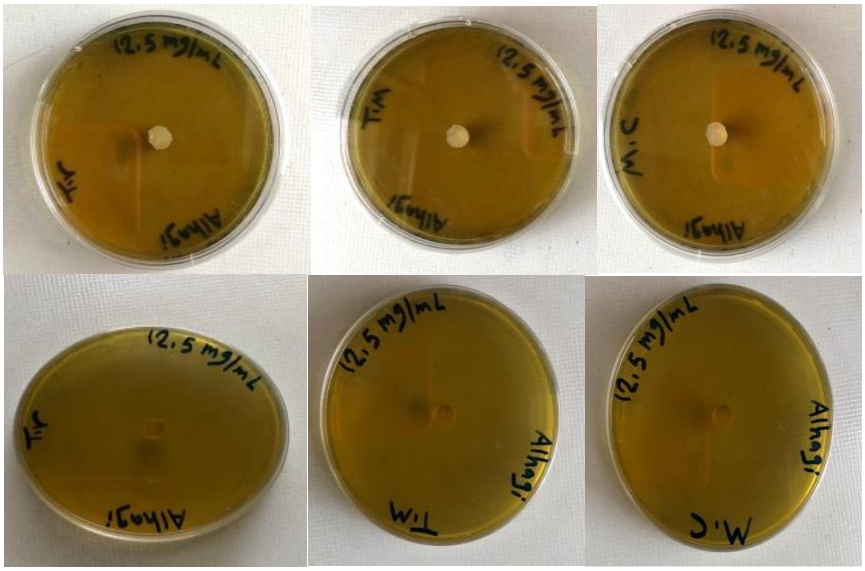

Figure (10): The effect of crude phenol extract for *Alhagi graecorum* Boiss plant with 12.5 mg/ml concentration on fungal growth.

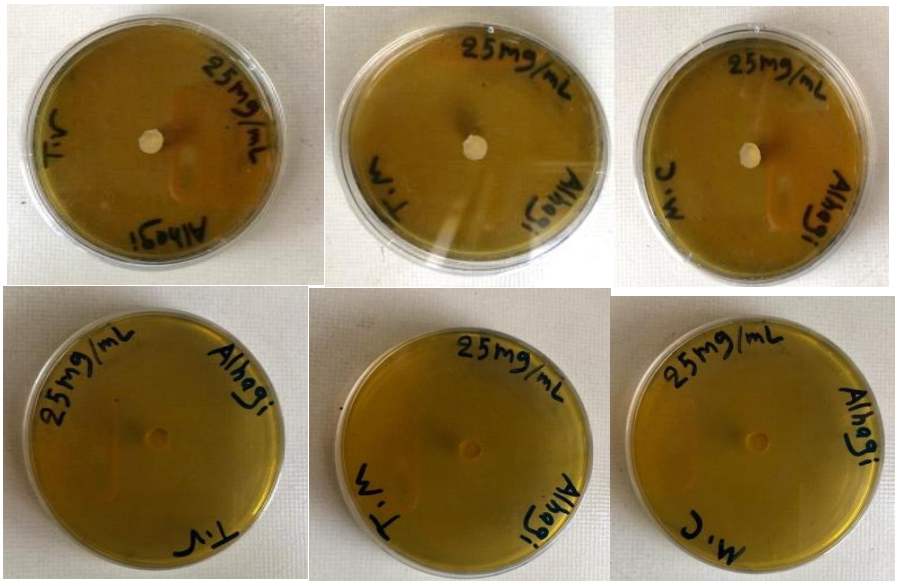

Figure (11): The effect of crude phenol extract for *Alhagi graecorum* Boiss plant with 25 mg/ml concentration on fungal growth.

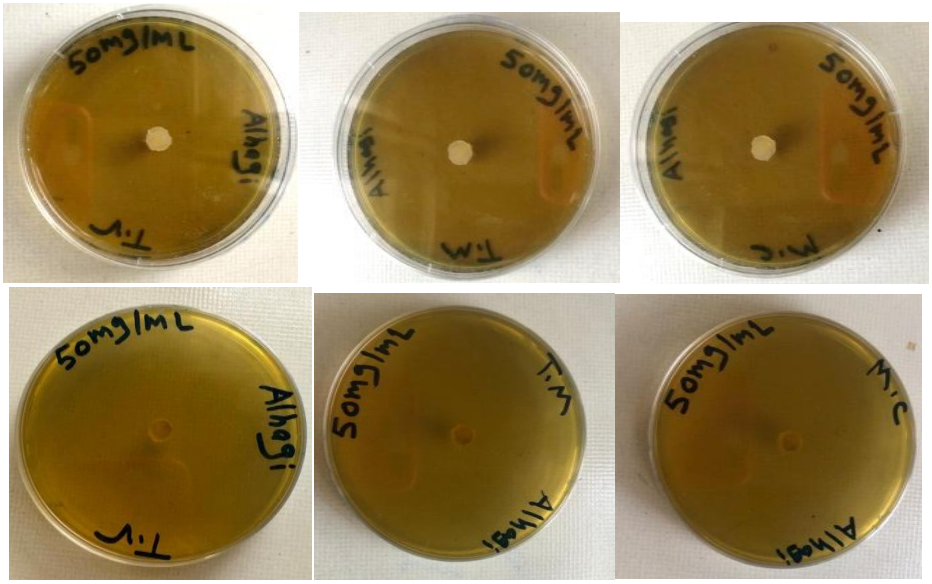

Figure (12): The effect of crude phenol extract for *Alhagi graecorum* Boiss plant with 50 mg/ml concentration on fungal growth.

The effect of griseofulvin medicine on the fungal growth

The antifungal griseofulvin used for fungal infections of the scalp, fingernails, and toenails, as well as skin infections, are all treated with griseofulvin. The side effects of griseofulvin are fever, sore throat, skin rash, and mouth soreness or irritation (27). In this study, griseofulvin used to compare the medical drugs used as antifungal for the skin and the extraction of phenolic extracts by studying the efficiency and side effects of these plants and drugs. For this purpose, a (2%) concentration of Griseofulvin was used with the three types of fungal: *T. rubrum*, *T. mentographytes*, and *M. canis* fungi. Two samples were prepared and used for each type of the three fungi. They were divided into two groups : The first groups was treated with a meshed griseofulvin drug for each type. The results show that the grisefulvin kills the three types of fungi (inhibited to 100%) , as shown in figure (13) . the second groups is the control of the three types of fungi without any treatments, as shown in figure (14) .

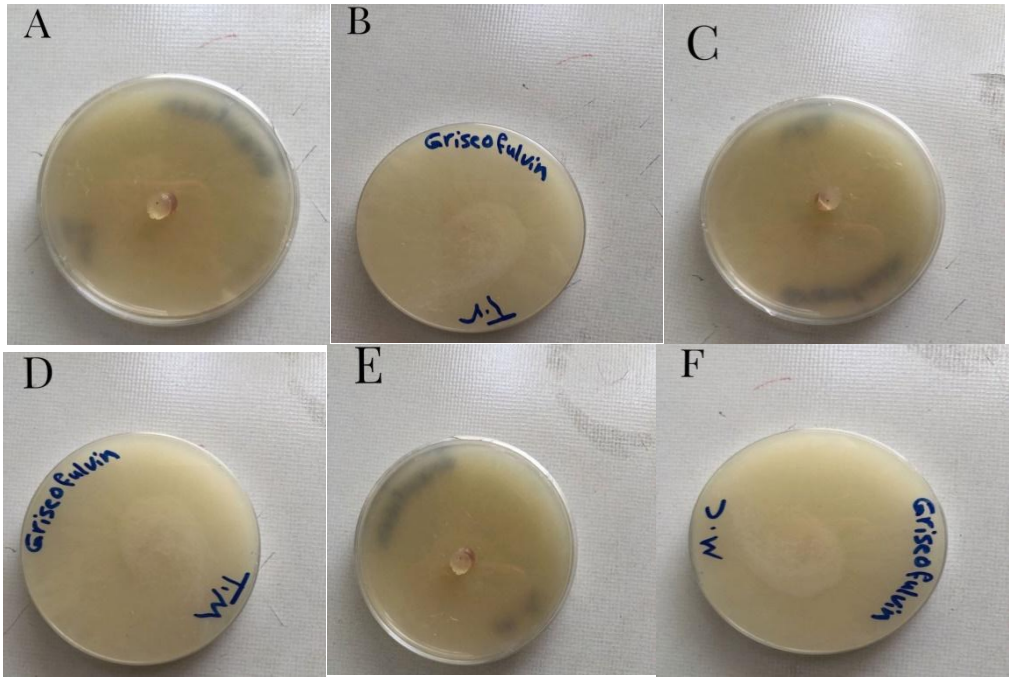

Figure (13): The effect of 2 % concentration of griseofulvin on fungal growth (A) Top view : *Trichophyton rubrum* (B) Reverse view : *Trichophyton rubrum* (C) Top view: *Trichophyton mentographytes* (D) Reverse view: *Trichophyton mentographytes* (E) Top view: *Microsporum canis* (F) *Microsporum canis*.

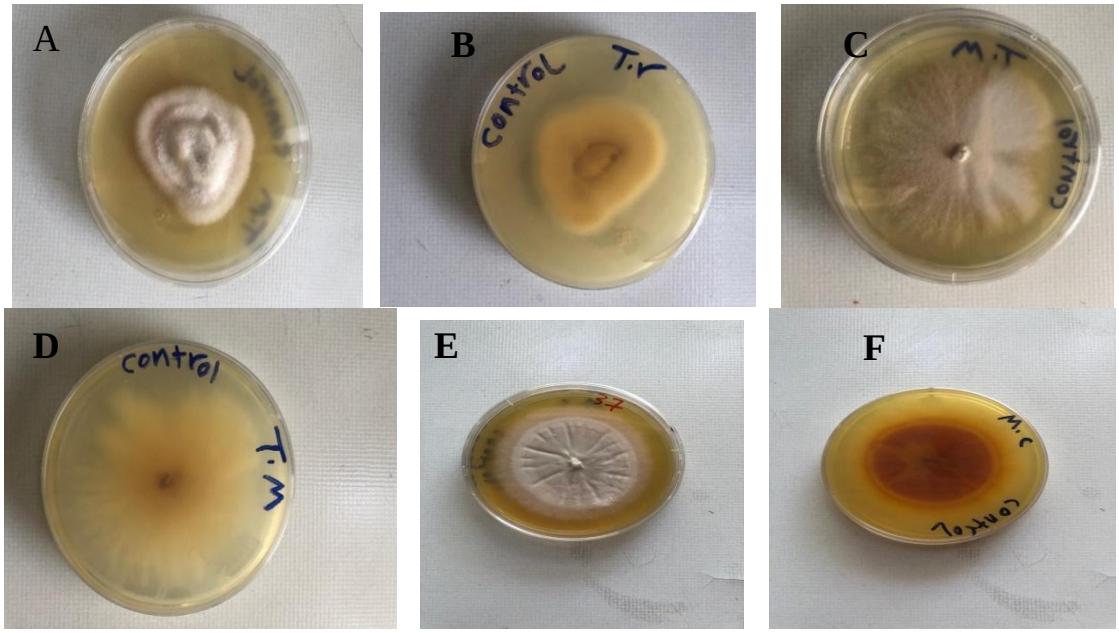

Figure (14): The control group of the three types of fungi: (A) Top view: *Trichophyton rubrum* (B): reverse view: *Trichophyton rubrum* (C) Top view: *Trichophyton mrntographytes* (D) reverse view: *Trichophyton mentographytes* (E) Top view: *Microsporum canis* (F) reverse view: *Microsporum canis*

The effect of *Alhagi graecorum* Boiss extraction on the fungal growth (sporulation)

The results of sporulation test number for *Trichophyton rubrum and Trichophyton mentographytes*, *Microsporum canis*, are shown in Table(8).

Table (8): The sporulation numbers of some dermatophytes isolated

Conc. (mg/ml)	Mean ±SE x 10^4					
	T.rubrum		*T.mentographytes*		*M.canis*	
	Macro conidial	Micro conidial	Macro conidial	Micro conidial	Macro conidial	Micro conidial
3.125	1 ±0.02 b	2 ±0.05 b	0 ±0 b	1 ±0.02 b	2 ±0.05 b	3 ±0.08 b
6.25	0 ±0 c	0 ±0 c	0 ±0 b	0 ±0 c	0 ±0 c	0 ±0 c
12.5	0 ±0 c	0 ±0 c	0 ±0 b	0 ±0 c	0 ±0 c	0 ±0 c
25	0 ±0 c	0 ±0 c	0 ±0 b	0 ±0 c	0 ±0 c	0 ±0 c
50	0 ±0 c	0 ±0 c	0 ±0 b	0 ±0 c	0 ±0 c	0 ±0 c
Griseo. (2 mg/ml	0 ±0 c	0 ±0 c	0 ±0 b	0 ±0 c	0 ±0 c	0 ±0 c
Control	3 ±0.08 a	22 ±0.73 a	1 ±0.02 a	20 ±0.57 a	6 ±0.32 a	25 ±0.87 a
LSD value	0.347 *	0.794 *	0.281 *	0.655 *	0.502 *	0.903 *
Means having with the different letters in same column differed significantly. * (P≤0.05).						

The sporulation concentrations in this study of macroconidia and microconidia of *Trichophyton rubrum* at a crude phenol concentration of 3.125 mg/ml were found to be $1x10^4$ and $2x10^4$, respectively. The macroconidia and microconidia counts of *Trichophyton mentographytes* at the same concentration were zero. *Microsporum canis* macroconidia and microconidia were, at the same concentration, $2x10^4$ and $3x10^4$, respectively.

No significant sporulation number was found for macroconidia and microconidia of *Trichophyton rubrum*, *Trichophyton mentographytes*, and *Microsporum canis* with crude phenol concentrations of 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, and 50 mg/ml). These findings show that when the crude phenol extracted from *Alhagi graecorum* Boiss concentration increased above 3.125 mg/ml, the sporulation of the fungi stopped.

Discussion

Alhagi graecorum Boiss plant contains one of the important natural compounds that possess a broad range of biological activities, including antifungal activity (28).

To find more powerful and safer antifungal compounds and the well-known therapeutic potential of plant-based compounds and dectecting of the active compounds of *Alhagi graecorum* Boiss plant by Gas Chromatography-Mass Spectrometry (GC-MS) technique.

The fungicidal diameter of *Trichophyton rubrum*, *Trichophyton mentographytes*, and *Microsporum canis* was 20 mm, 19 mm, and 20 mm, respectively, at a phenol concentration of 3.125 mg/ml. more over phenol concentrations were raised to 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, and 50 mg/ml, the fungi did not proliferate. This indicates that , an increase the

concentration of phenol content of *Alhagi graecorum* Boiss inhibited the fungal growth . In this regard, Boeck *et al*. (29) demonstrated that all clinical isolates of *T. rubrum* obtained from cutaneous infections in immunocompromised individuals with MIC90 values ranging from 12.5 to 25 g/mL were eliminated by the chalcone. The filamentous fungi *Aspergillus Niger*, *A. fumigates*, and *A. flavus* were not affected by any of the studied chalcones.

In this study, when the crude phenol extracted from *Alhagi graecorum* Boiss concentration increased above 3.125 mg/ml, the sporulation of the fungi stopped. No significant sporulation number was found for macroconidia and microconidia of *Trichophyton rubrum* or *Microsporum canis*.

An agreement was found with Perrins *et al*., 2005 (30), who tested miconazole, chlorhexidine, and combination of both medicines against *Trichophyton mentagrophytes*, *Trichophyton echinacea*, and *Microsporum versicolor* isolates using agar dilution (31). The majority (15 of 23) of isolates examined in vitro support the use of miconazole and chlorhexidine in supplementary topical treatment of *T. mentagrophytes*, *T. erinacei*, and *M. persicolor* dermatophytosis. Chlorhexidine MICs did not vary among the three tested dermatophyte species.

In this study, the *Alhagi graecorum* Boiss plant proved its efficiency in growth limited of one of the most popular fungi: the *Trichophyton rubrum*, *Trichophyton mentographytes*, and *Microsporum canis* . According to our knowledge, no study has previously been performed on such findings.

The griseofulvin drug also stop the fungal growth but the *Alhagi graecorum* Boiss plant is more safer , causes no side effects and more cheap.

Conclusion

We conclude that the phenol crude extracted from *Alhagi graecorum* Boiss exhibited significant antifungal activity at concentrations higher than 3.125 mg/ml by decreasing in diameter of fungi and inhibiting the sporulation process.

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