The Inhibition Effect of Paliurus Spina-Christi Methanolic Extract on Trichophyton Mentagrophytes Growth and Tri M4 Gene

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Abstract

Back ground: Paliurus spina-christi methanolic extract is rich of phenolic compounds as well as other biological active compounds (sugars ,esters, fatty acids and alkaloids) .
Aim:To asses that Paliurus spina-christi a good anti dermatophytic activity (Trichophyton mentagrophytes) .
Materials and methods: five different concentrations of methanolic extract of P. spina-christi against T. mentagrophytes followed by the RNA extraction of Ttichophyton mentagrophytes grown in media containing P. spina-christi methanolic extract followed by complementary DNA synthesis to examine the expression of Tri m4 gene .
Results: Statistical analysis revealed that at concentrations(25 and 50)µg/ml the differences between means of the growth diameters are non-significant ($P \geq 0.05$) as compared with the control while at concentrations (75,100,125)µg/ml the effect of methanolic extract on diameter growth was statistically significant ($P \leq 0.05$). Maximum growth inhibition was at 125µg/ml while minimum inhibition was at concentration 25 µg/ml .The percentage of inhibition was statistically significant especially at concentration 125 µg/ml it recorded 82%. The expression of Tri m4 gene was identified in presence of extract .
Conclusion :Methanolic extract of P. spina-christi didn't affect the expression of Tri m4 gene.
Key words: Paliurus spina-christi, active compounds, Trichophyton mentagrophytes, Tri m4 gene.

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Introduction

Paliurus spina Christi belong to Rhamnaceae family, its shrub-1-2m in long, *Paliurus spina-christi*. is a traditional Mediterranean and Asiatic medicinal plant [1]. Widely distributed in dry and rocky places in the Mediterranean region and Asia [2]. *Paliurus spina-christi*, commonly known as Jerusalem Thorn, Garland Thorn, Christ's Thorn, or Crown of Thorns[3].

Chemical investigation of *Paliurus spina-christi* fruits and leaves indicated the

presence of the flavonoides isoquercitin, rutin, hyperoside [4][5]. Sterols [6].

Paliurus spina-christi commonly used as a diuretic and against diarrhea and rheumatism [7]. A remarkable antibacterial activity, mainly on Gram-positive bacteria, of the ethanolic extracts from different plant parts was found ,*Paliurus spina-christi* demonstrate a clear inhibition zone against gram positive and negative bacteria (*Staph aureus, Streptococcus faecalis, Micrococcus luteus, Shigella sonnei, Proteus mirabilis and E.coli*) [8]. It is also used against rheumatism in ethnomedicine [9].Antifungal activity of *P.spina-christi* refers to phenolic compounds [10] In host pathogen interactions the gene expression of the pathogen is modulated by signals from the host and understanding the expression patterns may provide in sight into the mechanisms of disease .Little or no information is available on the transcription patterns of dermatophytes .In contrast to other fungal pathogens such as *Aspergillus fumagatus*, *Cryptococcous neoformance* and other pathogens of plant. [11][12].[13] characterized the expression of an aminopeptidase gene, *Trichophyton mentagrophytes* homolog of *T. rubrum* ,Tri r4 gene ,*T. mentagrophytes* Tri m4 is closely related to Tri r4 .Trim4 resemble other protease encoding genes though to be virulence factors .

The aim of present study was undertaken to investigate the *Paliurus spina-christi* as antidermatophytic activity.

Materials and Method

Extraction of *P. spina-christi* dried stem was done according to (Arokiyaraj *elal*.,2007). Detection of active compounds performed by HPLC type Ajilent 1100/1200 and GC/MS Ajilent type of Varian 450.

Invitro antifungal evaluation plant extracts were added to modified sabouraud dextrose agar containing cephalexin and cycloheximide at the ratio 1.5:1.5ml. All petridishes were inoculated with spore and incubated at 30°C for 5-7 days [15]. The diameter of fungal colonies was determined after the period of incubation.

DNA Extraction:

The genomic DNA was extracted according to the method of intron biotechnology company kit (korea). Mycelial samples of *T. mentagrophytes* were grounded in liquid N_2 and transferred into eppendorf tubes to start the extraction steps.

Spectrophotometric analysis and electrophoresis:

The extracted DNA was quantified using spectrophotometer .According to method of (Hube *etal.*,1994).

PCR of Template DNA

PCR was performed according to method of (Shmitt *etal*.,1990).Ready mix Taq PCR was carried out in reaction.

Table(1): Sequence of Primers used for molecular identification of *T.mentagrophytes*.

Primer name	Sequences (5' to 3')	Target gene	PCR product/bp
T.mentagrophytes	F)5'-CGA GCG TGG CTA CAG CTT CT-3' R)5'-CTCCTTGAT ACG GAC GAT-3'		<120
M4#804	f)5'-CAG GAC TTC AAC GGA ACC TTC T-3'	Tri m4	<120
M4#1859	R)5'-CAA TCC CAG CGG TCA TAG TTC T-3'	Tri m4	<120
#219	F)5'-CGA GCG TGG CTA CAG CTT CT-3'	Actin	<120
#279	R)5'-CTC CTT GAT GTC ACG-3'	Actin	<120

Total RNA was extracted from mycelia growth of *T. mentagrophytes* according to the method of (Sharma *etal*.,2011).to prepare cDNA .cDNA synthesis was according to the kit protocol of Intron Biotechnology and as following:

One micro liter of Oligo (dt)15 or 1µl of random primer was added to RNA sample and heated at 75 °C for 5 min. after that the tubes were spined briefly in order to collect the solution at the bottom of the tube. Then the plates were placed on ice for at least 1 min..The reagents were added in the order and mixed gently they are (1µl) (RNase inhibitor, (4µl) 5Xrt buffer, 2µl dntP, 2µl DTT and 0.5µl AMV RT. followed by incubation at 42°C for 60 min. and heating to 70°C.Finally the above reactant diluted by adding 50-80 µl sterile water .The resulted product electrophoresis and examined under U.V.

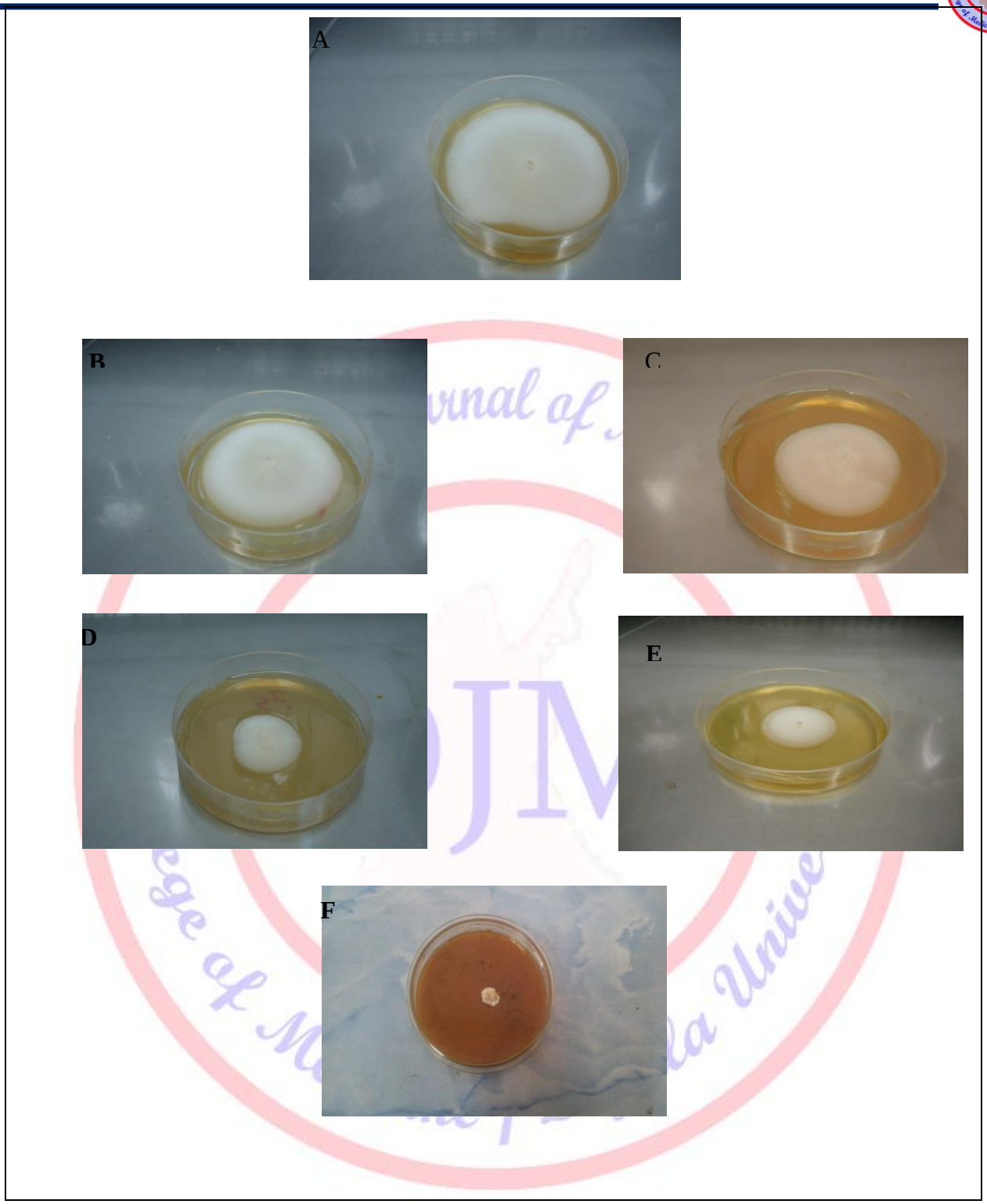
Results

The results of present study revealed that maximum growth inhibition of *P. spina Christi* was observed at 125µg/ml while minimum growth inhibition observed at concentration 25µg/ml as shown in Table(1).The means of *T. mentagrophytes* growth diameter with percentage of inhibition ,the differences between means of growth diameter statistically non significant (P≥0.05) for the concentrations (25and 50)µg/ml while the differences between means of growth diameter statistically significant (P≤0.05) for the concentrations (75,100and 125)µg/ml differences between percentage of inhibition is statistically significant (P≤0.05) for all concentrations of *P.spina-christi* extract. At the concentration 25µg/ml of methanolic plant extract *T. mentagrophytes* growth diameter after period of incubation was(6.90) and the percentage of inhibition was (16%) .Using concentration 50 µg/ml of *P. spina- christi* methanolic extract the *T. mentagrophytes* growth diameter after incubation for seven days was (6.50)cm as demonstrated in Table (2).The inhibition percentage at this concentration was (21%) . By increasing the concentration to 75 µg/ml of plant extract, the mean diameter of *T. mentagrophytes* after seven days of incubation was (4.56)cm this indicated the ability of this concentration in inhibit the growth of *T. mentagrophytes* .The inhibition percentage was (45%) .

The *T. mentagrophytes* maximum growth diameter was (2.73) cm after incubation for seven days in media treated with 100 µg/ml of plant extract concentration. The inhibition percentage was statistically significant(67%). While at the 125 µg/ml of plant methanolic extract concentration *T. mentagrophytes* growth diameter after incubation for seven days was (1.50) cm. This effect was statistically non significant P≥0.05 but the inhibition concentration (82%) was statistically significant.

Table 2: The antifungal activity of Methanolic extract (diameter of the Growth, cm) of *paliurus spina-christi* against *Trichophyton mentagrophytes*.

Concentration (µgl/ml)	**Average of colonial diameter (cm) ± SE**	**Percentage of inhibition (%)**
0	8.30 ± 0.87	0 ± 0.0
25	6.90 ± 0.62	16 ± 0.94a
50	6.50 ± 0.49	21 ± 1.02a
75	4.56 ± 0.31	45 ± 2.37b
100	2.73 ± 0.25	67 ± 2.93c
125	1.50 ± 0.03	82 ± 3.77d
LSD value	2.508 *	9.366 *
* (P<0.05)		



Figure(1). Antifungal activity of methanolic extract of *P. spina-christi* different concentrations against *T.mentagrophytes*. A: control, B: 25µg/ml, C: 50µg/ml, D: 75µg/ml, E, 100µg/ml, F: 125 µg/ml .

The molecular identification of Tri m 4 gene characterized by PCR analysis of gene product from *T.mentagrophytes* species cultured on media without plant extract and *T.mentagrophytes* grown on media treated with 125µg/ml of plant extract ,in addition to Actin gene which acted as reference gene. The gene doesn't express in both non treated and treated *T.mentagrophytes* as shown in figure (3).

Discussion

A numbers of plants extract were tested for their antifungal activity against dermatophytes species *T.mentagrophytes* or indirectly treated dermatophytes caused tinea (Tinea corporis ,Tinea cruris and Tinea facie) [19].This inhibition may be attributed to different compounds detected in methanolic extract such as flavonoids, phenols (including tannins, gallic acid and other) .

These ingredients were known to have antimicrobial activity and were found in many plant species and genera.

Aloe vera gel acts as anti-inflammatory drug reducing the pruritus and the scales of the lesion caused by Tinea corporis and tinea cruris [20]*Aloe vera* is known to contain flavonoids,glycosides [21].while the methanolic extract of *Lemon grass* ,*lanta* and *nerium* followed by their ethyl acetate extracts showed the highest activities against *T.mentagroph- ytes* ,extract of lemon were the most effective followed by lanta ,nerium and basil showed moderate activities this affectivity may be due to free and bound flavonoid fractions [21]. In addition the presence of phenolic compounds which can be hold a good promise as a natural fungicide against common pathogens of crops [10]. A wide variety of flavonoids, sesquiterpenoid alcohols, triterpenoids and quinic acid caffeates product from plants may also be useful as antimicrobials [22]. The activity is probably due to their ability to form a complex with extracellular and soluble proteins, which binds to bacterial cell wall. More lipophilic flavonoids may also disrupt microbial membranes [23].

Tri m4 gene expressed in both non treated and treated *T.mentagrophytes* culture

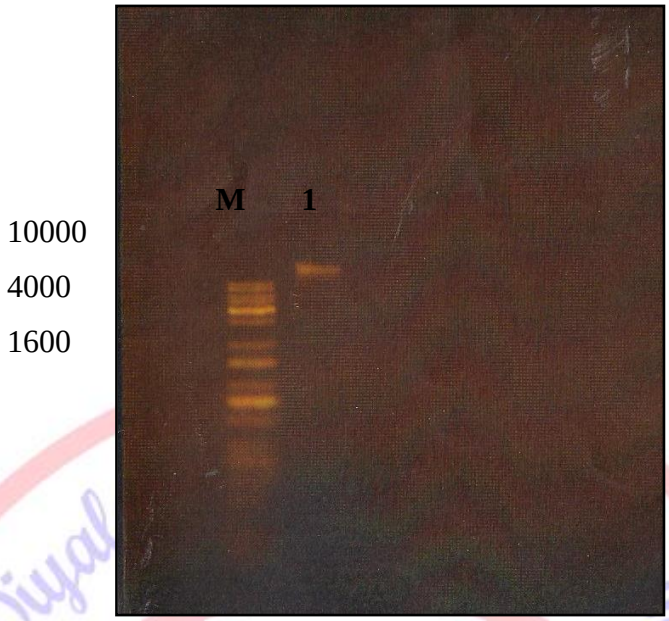
this may be refered to several factors :-

1-This gene expressed in media supplemented with salts, keratin and elastin as a sole source of carbon and nitrogen [24].

2-The primers were designed to detect the transcripts of several other candidates genes ,including the protease Tri m2 and Alp genes, metalloprotease Mep2 and Mep3 genes.

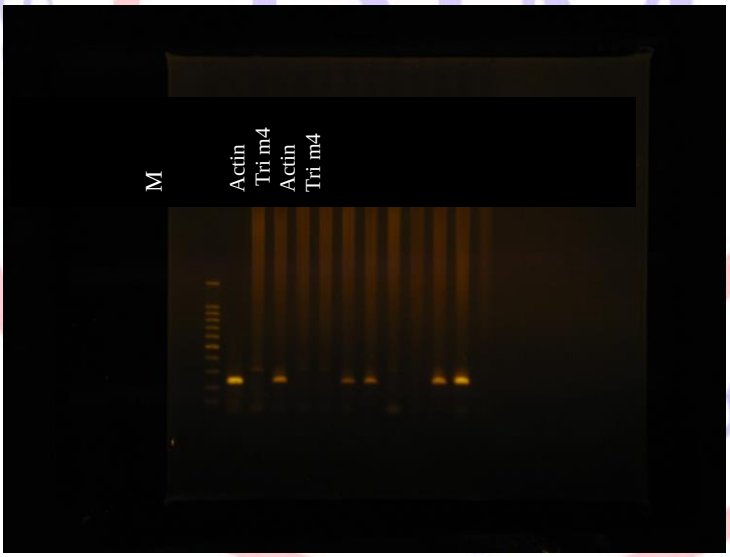
3-Tri m4 gave a strong signal which detected by RT-PCR from RNA isolated from cultures grown on blood plasma [26].

The expression of the Tri m4 gene in presence of blood plasma with plant extract was confused they produce the same bands after 40 cycle of PCR. The combination of additives (glucose ,keratin and elastin) with plant extract doesn't recommend due to the effect of inhibition will be of little chance [27].There must be an alternative way to regulate the expression of this gene without additives only the natural components of the fungal media e.g.(malt extract ,glucose ,peptone) then identified the effect of plant extract on the expression of this gene wither does it inhibit its expression or not.



Figure(2): gel electrophoresis of *T. mentagrophytes* DNA Extract.
Lanes: M, molecular weight marker (kappa ,universal ladder), size range, (100-10000) Bp.
1: DNA Extract Of *T. mentagrophyte*(less than 10,000bp)

[Without plant extract] [with plant extract]



Figure(3):PCR Product of actin and Tri m4 gene of *T.mentagrophyte* species.

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