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Molecular detection of dermatophytes isolated from tinea corporis infections in the Province of Wasit, Iraq

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***Abstract*---**Background: The detection of fungal components in clinical specimens using direct microscopy in conjunction with culture-based full identification is the basis for dermatophyte identification. Physiological, macro-morphological, and micro-morphological parameters are used to identify colonies phenotypically. In recent years, it has been shown that molecular methods are useful for classifying dermatophyte species. Methods: Fifty samples that were taken from human cases of tinea corporis were examined mycologically. Phenotypic techniques were used to identify isolated dermatophytes. Internal transcribed spacer (ITS) PCR amplification was used for molecular identification. Using a specific primer, PCR was utilized to analyze the *18S rRNA* gene in fungal isolates and the genes for virulence factors. Results: Out of Fifty samples with tinea corporis infections identified. Among them, which 3species were identified distribution to 26 cases Trichophyton mentagrophytes with a percentage of 52%, was the most frequently isolated dermatophyte 12 cases Trichophyton rubrum with a percentage 24% and 12 cases *Microsporum canis* with a percentage 24%. PCR product analysis of the *18S rRNA* gene in dermatophytes isolates showed that all dermatophytes ssp. 100% were positive for *18S rRNA* gene they obtained dermatophyte isolates (n=50) as follows: 26 *Trichophyton*

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mentagrophytes 12 *Trichophyton rubrum*, 12 *Microsporum canis*. PCR analysis of virulence factor; *protease* (*SUB*) gene and *phospholipase* (*tef-1-alpha*) gene showed that 100% of fungi were positive on on all species. Conclusion: In order to identify dermatophytes and determine their virulence factor more quickly and accurately, molecular techniques have been developed; nevertheless, they are too expensive to be employed as a standard procedure.

***Keywords*---**dermatophytes, tinea, fungal infections, trichophyton, ITS, virulence factor.

Introduction

Dermatophytes are keratinophilic fungi that are one of the most adaptable parasite partners of humans that cause dermatophytosis. The three anamorphic genera of dermatophytes are *epidermophyton*, *microsporum*, and *trichophyton* [1]. Traditional markers for identifying dermatophytes include clinical criteria, culture characteristics, microscopic morphology, and physiological test results. However, because to their shared physical characteristics, diversity, and pleomorphism, dermatophyte identification may be challenging or dubious [1,2]. In recent years, it has been demonstrated that molecular methods are useful for addressing species issues in dermatophytes. Genotypic alterations took place. more reliable and accurate than phenotypic characteristics [2]. The effectiveness of identifying various dermatophyte species and strains has been improved by the use of molecular techniques such as PCR, PCR fingerprinting, arbitrarily primed PCR, random amplification of polymorphic DNA, and restriction fragment length polymorphism (RFLP) [3-6]. The current research looked on phenotypic and molecular approaches for identifying dermatophytes and some virulence factor genes.

Materials and Methods

Fifty skin scrapings and hair samples were obtained from patients with tinea corporis who visited Al-Karama Hospital's Dermatology Clinic between December 2021 and April 2022. The Research Ethics Committee approved the study protocol. skin scrapings and hair were taken from each instance in a sterile Petri plate after the lesions were cleaned with 70% ethyl alcohol. KOH (10%) was used for direct microscopic inspection. Skin scrapings and hair samples were inoculated in Sabouraud's Dextrose agar. All inoculation medium were incubated at 28 degrees Celsius for 2 to 3 weeks.

Phenotypic identification was made as follows [7,8]

1. Macro-morphological assessment based on stated rate of growth, uniformity, surface color, and reverse color.
2. Micro-morphological analysis: To check for changes in hyphae, macro-conidia, and micro-conidia, specimens from the growth were placed on a slide with drops of lactophenol cotton blue, coated with a coverslip, and examined under a microscope.

Molecular identification

Molecular identification was used to identify 50 dermatophyte isolates that had previously been recognized using phenotypic techniques.

DNA extraction

According to the manufacturer's instructions, this was done using an extraction kit (the PrestoTM Mini gDNA Yeast Kit was created for quick isolation of genomic DNA from grown yeast and fungus).

Detection of DNA by PCR

Using the primers (internal transcribed spacer – ITS) [9]. In this work, PCR primers were created using the NCBI-Genbank database and primers plus online. Canada's Alpha DNA donated these primers, as shown in the Table 1.

Table 1: Primers with their nucleotide sequence and product size

Fungi	Primer	Forward Reverse	Sequence	Product Size (bp)	Accessions No.
T. mentagro	*18S rRNA*	F	TACCGCCCCATTCTTGTCTACC	238	MG840758.1
		R	GCATTTCGCTGCGTTCTTCATC		
	SUB	F	ACCACCTACCACTATGCCAG	126	MW149246.1
		R	ATCAACGAAGTCCTCATCCC		
	tef-1-alpha	F	TCTCCCTTTCTTCAACCCAC	216	MK467449.1
		R	CCAACTCTTCGGCTTCCTAC		
T. rubrum	*18S rRNA*	F	CCCCCATTCTTGTCTACCTC	235	LC369522.1
		R	TTCGCTGCGTTCTTCATC		
	ALP2	F	GGCAAGTAAAAGGACGACAG	207	AF407183.1
		R	GGTGAAGAGGGAAGAATGAAG		
	phospholipase	F	ACCTCTCTCCTACACAAACC	220	AF407150.1
		R	GACAAGCCAAAGATACCACC		
M. canis	*18S rRNA*	F	CTCAAAACGGGGAAACTCAC	222	LC528690.1
		R	CCAGAACATAGGGCACAGAC		
	protease	F	CAAAGCAAAAGTTCCAACAGTC	230	XM002851066.1
		R	GTTTCCCGTCCACTATTATGTC		
	phospholipase	F	ACAAGCCACGAGGATACGAC	201	XM002850433.1
		R	TATCAGCATATCGGCGGCAC		

The Mixture of PCR Working Solution

The master mix reactions was prepared by (Maxime-PCR-Pre-Mix-kit), and this master mix was completed in accordance with the company's specifications, as shows in the Table 2.

Table 2: Standard PCR master mix protocol

Working Solution	Volume (µL)
Master mix	12.5
Forward and reverse primer	1
Nuclease free water	7.5
Template	3
Total	25

PCR Thermo-cycler Conditions

Utilizing the online tool Optimize Protocol-Writer- Writer™ and a standard PCR thermos-cycler, the PCR thermo-cycler conditions protocol for each gene was calculated, as shown in the Table 3.

Table 3: PCR thermo-cycler conditions protocol

PCR cycle	repeat	Temp.	Time
Initial-denaturation	1	95	5min
Denaturation	34	95	30sec
Annealing		58	30sec
Extension		72	1min
Final extension	1	72	5min
Hold	-	4	2-4min

Results and Discussion

Positively microscopic findings were obtained for 100% of samples on KOH from 50 skin scrapings and hair samples, and dermatophytes were isolated from 50 of samples on SDA agar, as shown in the Table 4.

Table 4: Type and percentage of Dermatophytes spp. according to the gender

Dermatophyte spp.	Gender		Total
	Male	Female	
	NO.(%)	NO.	NO.(%)
T. mentagrophytes	13(26)	13(26)	26(52)
T. rubrum	8(16)	4(8)	12(24)
M. canis	7(14)	5(10)	12(24)
Total	28(56)	22(44)	50(100)

Molecular analysis

Molecular techniques (PCR) used for detection of dermatophytes fungi and their virulence factors. PCR was used to confirm the dermatophytes fungi. In present study PCR was used to detect of dermatophyte spp. A total of T. mentagrophytes (26), T. rubrum (12), and M. cains (12). PCR is a sensitive and accurate technology for molecular identification of M.O, with higher sensitivity, specificity, and quicker turnaround times than traditional methods, including fungus.[11]

Only a few studies confirmed the use of the PCR methodology for genotypic detection and identification of dermatophytes in general, and tinea corpores in particular.[12,13] Molecular diagnostic techniques are becoming more commonly available. Conventional PCR for DNA isolated from clinical specimens is one of the significant molecular methods used in the diagnosis of dermatophytosis.[14]

18S rRNA for Dermatophyte spp

T. mentagrophytes

Diagnosis by PCR recorded *T. mentagrophytes* as 26(100%), as shown in the Figure 1.

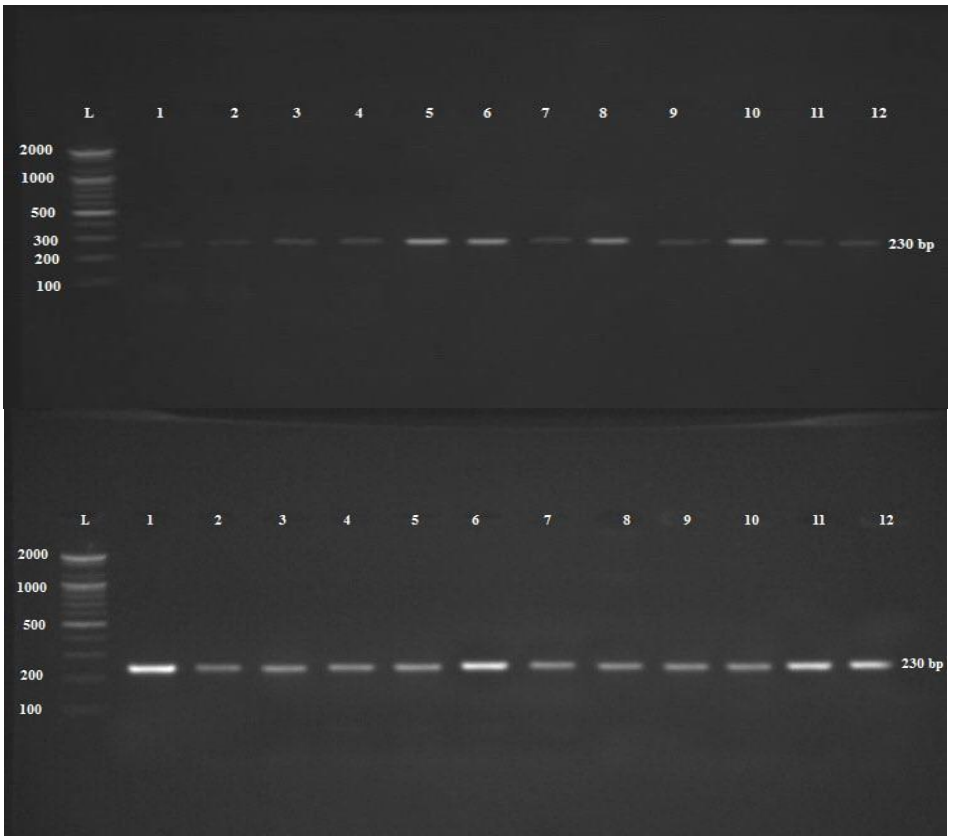

Figure 1: Agarose-gel-electrophoresis for *T. mentagrophytes*. Whereas the Lane (L): DNA marker ladder (100-2000bp) and the Lane (1-12) displayed positive PCR amplification of the ITS1 gene at 230bp PCR product size in *T. mentagrophytes*.

T. rubrum

Diagnosis by PCR recorded *T. rubrum* as12(100%), as shown in the Figure 2.

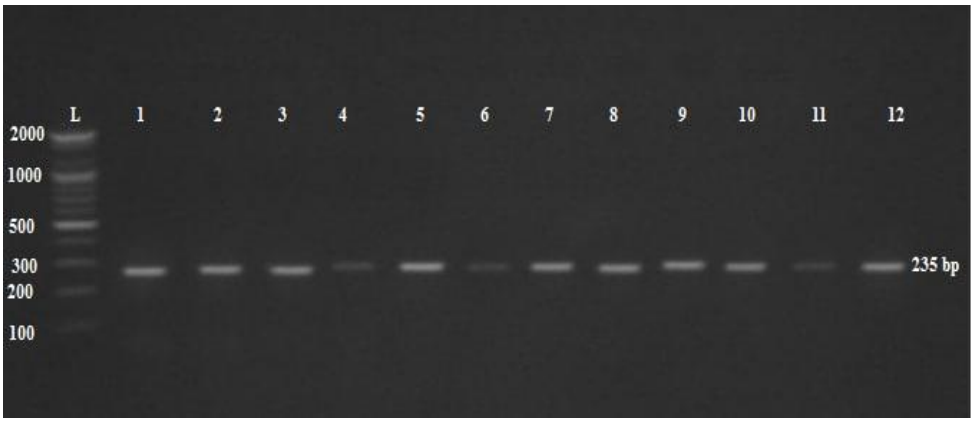

Figure 2: Agarose-gel-electrophoresis for *T. rubrum*. Whereas the Lane (L): DNA marker ladder (100-2000bp) and the Lane (1-12) displayed positive PCR amplification of the ITS1 gene at 235bp PCR product size in *T. rubrum*.

M. canis

Diagnosis by PCR recorded *M. canis* as 12(100%), as shown in the Figure 3.

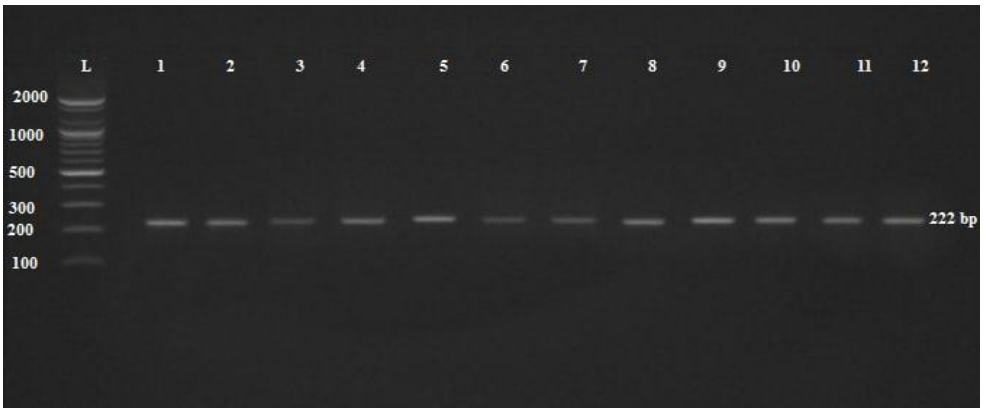

Figure 3: Agarose-gel-electrophoresis for *M. canis*. Whereas the Lane (L): DNA marker ladder (100-2000bp) and the Lane (1-12) displayed positive PCR amplification of the ITS1 gene at 222bp PCR product size in *M. canis*.

Detection of virulence factors

In the present study were design to detection of virulence factors (*Protease* and *Phospholipase*), for each *T. mentagrophytes*, *T. rubrum*, and *M. cains*.

***T. mentagrophytes* virulence factors**

The results in the present study were showed that the presence of subtilisin-like protease (SUB) gene in T. mentagrophytes isolates by PCR, as shown in the Figure 4

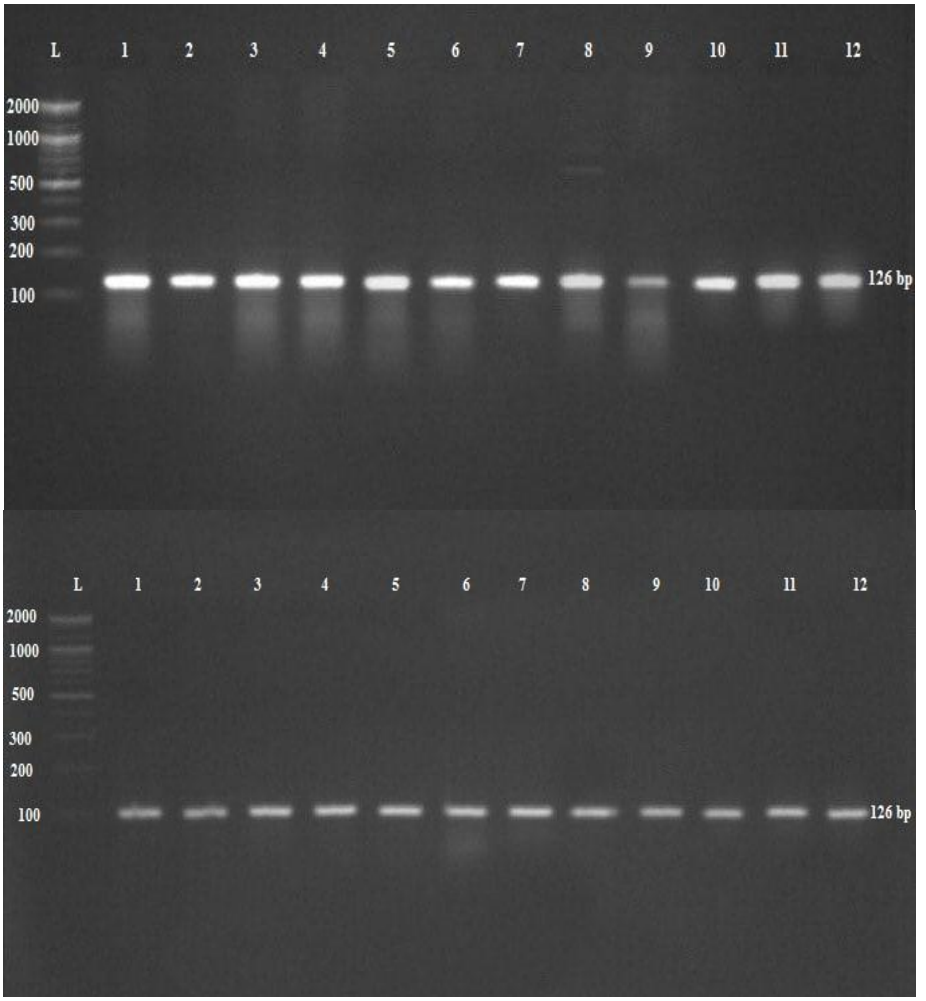

Figure 4: Agarose-gel electrophoresis for subtilisin-like protease *SUB* gene in *T. mentagrophytes*. Marker ladder, lane (M) (100-2000bp), lane (1-12): showed positive *SUB* gene at 126bp PCR product size.

The results in this study show presence of translation elongation factor *1-alpha* (*tef-1-alpha*) gene in *T. mentagrophytes* isolates by PCR, as shown in the Figure 5.

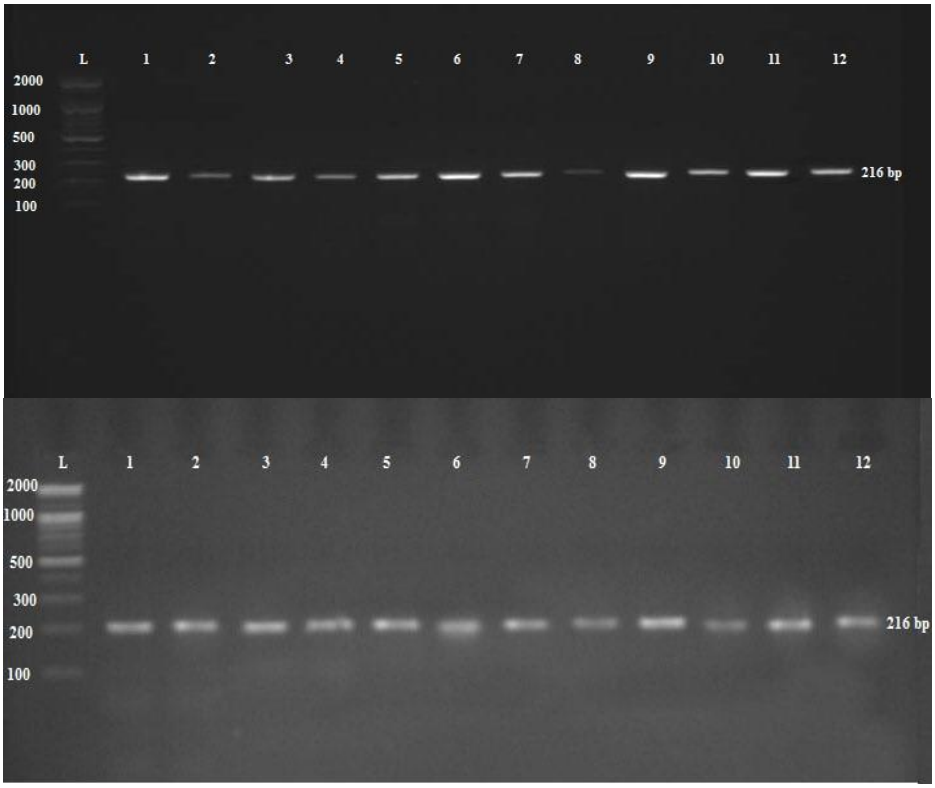

Figure 5: Agarose gel electrophoresis for 1-alpha (*tef-1-alpha*) gene in *T. mentagrophytes*. Marker ladder, lane (M) (100-2000bp), lane (1-12):showed positive *tef-1-alpha* at 216bp PCR product size.

T. rubrum **virulence factors**

The results in the present study were showed that the presence the presence of other two genes (*protease* and *phospholipase*) were detected in *T. rubrum* isolates by PCR, as shown in the Figure 6 – 7.

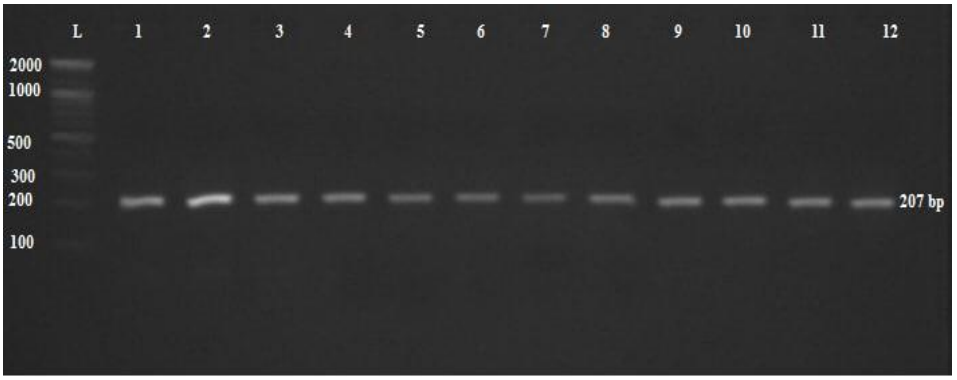

Figure 6: Agarose gel electrophoresis for *protease* gene in *T. rubrum*. Marker ladder, lane (M) (100-2000bp), lane (1-12): showed positive *protease* gene at 207bp PCR product size.

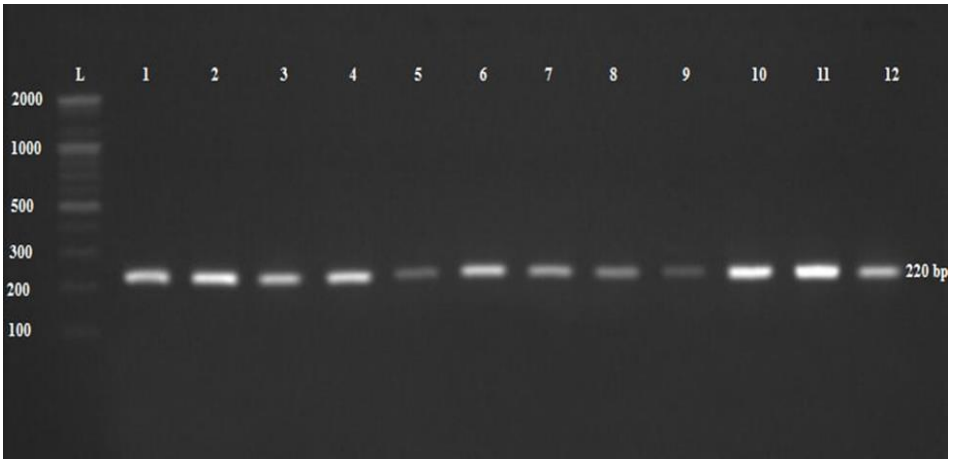

Figure 7: Agarose gel electrophoresis for *phospholipase* gene in *T. rubrum*. Marker ladder, lane (M) (100-2000bp), lane (1-12): showed positive *phospholipase* gene at 220bp PCR product size.

***M. cains* virulence factors**

The results in the present study were showed that the presence the presence of other two genes (*protease* and *phospholipase*) were detected in *M. canis* isolates by PCR, as shown in the Figure 8 – 9.

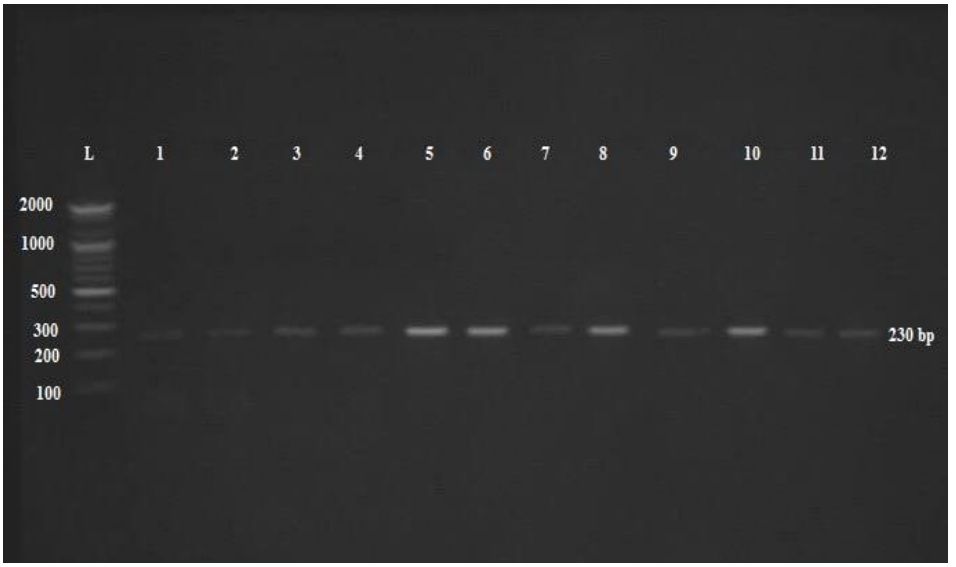

Figure 8: Agarose gel electrophoresis for *protease* gene in *M. canis*. Marker ladder, lane (M) (100-2000bp), lane (1-12): showed positive *protease* gene at 230bp PCR product size.

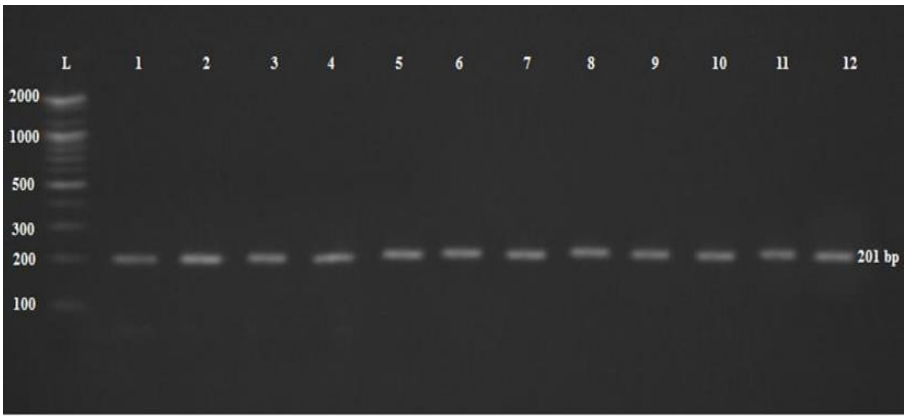

Figure 9: Agarose gel electrophoresis for *phospholipase* gene in *M. canis*. Marker ladder, lane (M) (100-2000bp), lane (1-12): showed positive *phospholipase* gene at 201bp PCR product size.

Keratinase, which is released by *Trichophyton* ssp., is a major virulence factor. The MEP and SUB gene families in *T. mentagrophytes* encode proteins involved in the synthesis and release of keratinase [15]. A crucial element in the invasion, use, and subsequent diffusion across the host's stratum corneum is the release of proteolytic enzymes by dermatophytes [16]. Enzymes including phospholipases and proteases have been found to be particular virulence factors linked to the pathogenicity of *T. rubrum* [17]. The expansion of MEP genes in dermatophytes finally led to species divergence, according to a phylogenetic analysis of genomic and protein sequences, proving that these genes play a crucial and conserved function in dermatophytes [18].

Conclusion

Although the molecular approach is quick and represents a technical leap in dermatophyte identification, it is too costly to be used routinely and requires more work. In the absence of qualified mycologists or in unusual and variant dermatophytes, we propose its usage.

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