CONFIRMATION OF *Trichophyton rubrum* BY MICROMORPHOLOGICAL
AND MOLECULAR TECHNIQUES AND *IN-VITRO* ANTIFUNGAL
ACTIVITIES OF ALLICIN AND GARLIC EXTRACTS

BY

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Dermatophytosis caused by a group of pathogenic fungi namely, dermatophytes, is among the most prevalent infectious diseases worldwide. One of the most frequently isolated pathogenic dermatophytes is *Trichophyton rubrum*. Chemical drugs are widely used in the treatment of dermatomycosis, but can cause various side effects to the patients and drug resistance to the pathogens. Hence, alternative natural compounds should be assessed to solve this problem. Garlic can be considered as a good replacement due to its high level of sulfur compounds (e.g., allicin). Therefore, the main objective of this study was to evaluate allicin and garlic extract against various isolates of *T. rubrum*. These studies were divided into three parts. The first was to confirm ten different isolates of *T. rubrum* which were obtained from the Laboratory of Medical Mycology Department in
Tehran University of Medical Sciences, Iran by using both conventional and molecular methods. The second part was to evaluate the in vitro antifungal activity of allicin, aqueous garlic extract, ketoconazole and fluconazole and the combination of these azoles drugs with allicin and aqueous garlic extract against these ten isolate of T. rubrum. The third objective was to see the effect of allicin and garlic extract to the ultrastructure of T. rubrum using electron microscope. In this study, colony characterizations of all isolates of T. rubrum are varied and inconclusive. However the molecular study based on Internal Transcribed Spacer, 1 and 4 (ITS1 and 4) revealed that all the different isolates tested with reference from data base in Genbank (BLAST search) showing more than 95% similarity. In vitro antifungal study on the effects of allicin and aqueous garlic extract singly and in combination with ketoconazole and fluconazole against ten clinical isolate of T. rubrum were effective in inhibiting the fungal growth. The results showed that MICs for allicin ranged from 0.78 – 12.5 µg/ml, ketoconazole 0.25 – 8.0 µg/ml and fluconazole 1.0 - 32.0 µg/ml respectively. Combination of allicin or garlic extract with ketoconazole or fluconazole caused the synergistic or additive effect on dermatophytes, which may increase fungicidal effects, decrease toxicity, side effects and drug resistance. Besides, in vitro studies revealed that allicin and aqueous garlic extract alone and in combination with these two azoles drugs, has a good potential as antifungal drug based on the results of MICs (Minimal Inhibitory Concentration) and FICIs (Fractional Inhibitory Concentration Index). The SEM (Scanning Electron Microscopy) micrographs showed that allicin and garlic extract caused shrinkage, rough
and irregular-shaped hypha with expanded hyphal tip of *T. rubrum*. The TEM (Transmission Electron Microscopy) micrographs revealed that allicin and garlic extract caused cell wall thickening, disorganization of cytoplasmic contents and breaking down of cell membrane and cell wall of *T. rubrum*. SEM and TEM studies proved that allicin and garlic extract possessed antifungal activity by inhibiting the growth of *T. rubrum*, and can be considered to treat fungal infections. This study showed that although different isolates exhibited different morphological characteristics but molecular analysis proved that they belong to the *T. rubrum* species. Allicin and garlic extracts were effective in inhibiting dermatophytes growth. In addition, SEM and TEM studies demonstrated that allicin and garlic extract possessed antifungal activity which inhibits the hyphal growth of *T. rubrum*. 
isolat klinikal *T. rubrum* yang diperolehi dari Jabatan Perubatan Mikologi, Universiti Perubatan Tehran, Iran dengan menggunakan kaedah Konvesional dan molekular. Bahagian kedua adalah untuk menentukan kombinasi yang terbaik antara kumpulan azole dengan allicin (Alexis– Biochemicals Co, USA), dan Ekstrak Akueus bawang putih sebagai bahan antikulat. Bahagian ketiga untuk melihat kesan allicin dan Ekstrak Akueus bawang putih ke atas struktur ultra *T. rubrum* menggunakan mikroskop electron. Kajian ini mendapati kaedah konvensional untuk pengesahan spesies dermatofit bergantung kepada perbezaan fenotip adalah tidak konklusif. Pendekatan kaedah molekular berdasarkan “Internal Transcribed Spacer” 1 dan 4 (ITS1 dan 4) menunjukkan keseluruhan isolat berbeza yang diuji dan dibandingkan dengan isolat rujukan dari pengkalan data “Genbank” (carian BLAST) menunjukkan persamaan melebihi 95%. Kajian antikulat secara *in vitro* kesan allicin dan Ekstrak Akueus bawang putih secara bersendirian atau digabungkan dengan kptonazole dan fluconazole terhadap 10 isolat klinikal *T. rubrum* adalah berkesan menghambat pertumbuhan kulat tersebut. Keputusan kajian mendapati MICs untuk allicin masing masing berjulat antara 0.78-12.5 µg/ml, ketoconazole 0.25-8.0 µg/ml dan fluconazole 1.0-32.0 µg/ml. Kombinasi allicin atau Ekstrak Akueus bawang putih dengan ketoconazole atau fluconazole menghasilkan kesan synergistik atau kesan penambahan terhadap dermatofit, seterusnya berkemungkin meningkatkan kesan antikulat, mengurangkan toksisiti, kesan sampingan dan kesan kerintangan terhadap bahan antikulat. Kajian *in vitro* juga menunjukkan allicin dan Ekstrak Akueus bawang putih secara bersendirian atau
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I certify that a Thesis Examination Committee has met on 22 February 2011 to conduct the final examination of Farzad Aala on his Doctor of Philosophy thesis entitled "In vitro Antifungal Activities of Allicin and Garlic Extracts, and Molecular and Micro Morphological Identification of *Trichophyton rubrum*, in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, or is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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Date: 22 February 2011
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>viii</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>ix</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xix</td>
</tr>
</tbody>
</table>

CHAPTER

1. GENERAL INTRODUCTION                                          1

2. LITERATURE REVIEW
   2.1 Dermatophytes
      2.1.1 *Trichophyton rubrum*  7
      2.1.2 Pathophysiology  8
      2.1.3 Clinical manifestations  10
      2.1.4 Epidemiology  13
      2.1.5 Treatment  14
   2.2 Identification of dermatophytes  15
      2.2.1 Conventional method  15
      2.2.2 *Trichophyton* species  17
      2.2.3 Molecular methods  19
   2.3 Treatment of dermatophytosis and drugs  25
      2.3.1 The azoles group  26
      2.3.2 Classification of azoles  27
      2.3.3 Mechanism of action  27
      2.3.4 Ketoconazole  28
      2.3.5 Fluconazole  30
      2.3.6 The development of azole-resistance  31
      2.3.7 Dermatomycosis and azoles group  32
   2.4 Plant extracts
      2.4.1 Garlic (*Allium sativum*)  35
      2.4.2 Allicin  39
   2.5 Antifungal susceptibility testing  43
   2.6 Electron microscopy  46

3. CONVENTIONAL AND MOLECULAR CHARACTERIZATION OF *Trichophyton rubrum*  50
   3.1 Introduction  50
   3.2 Materials and methods  51
3.2.1 Conventional method 52
3.2.2 Molecular method 52
3.2.3 DNA extraction 53
3.2.4 PCR Amplification 54
3.2.5 PCR purification 55
3.3 Results 55
  3.3.1 Morphological characteristics of colonies 55
    *T. rubrum*
  3.3.2 Isolation, identification and molecular characterization of ITS1 of *T. rubrum*
3.4 Discussion 60
3.5 Conclusion 62

4 \textit{IN VITRO} ANTIFUNGAL ACTIVITIES OF ALLICIN AND AQUEOUS GARLIC EXTRACTS AGAINST DERMATOPHYTIC FUNGI 63
4.1 Introduction 63
4.2 Materials and methods 65
  4.2.1 Experimental design 65
  4.2.2 Isolates 66
  4.2.3 Media 66
  4.2.4 Preparation of aqueous garlic extract 67
  4.2.5 Inoculum preparation 67
  4.2.6 Antifungal compounds 69
  4.2.7 Broth microdilution method (NCCLS M38-A protocol) for determination of MIC 70
  4.2.8 Incubation time and temperature 71
  4.2.9 Evaluation of the MIC, MFC and FICI 71
  4.2.10 Time-kill studies 73
4.3 Results 74
  4.3.1 Data analysis 78
4.4 Discussion 86
4.5 Conclusion 91

5 SCANNING AND TRANSMISSION ELECTRON MICROSCOPY FOR EVALUATION OF THE EFFECTS OF ALLICIN AND GARLIC EXTRACT ON *Trichophyton rubrum* 92
5.1 Introduction 92
5.2 Materials and methods 94
  5.2.1 Preparation of antifungal agents and fungus 94
  5.2.2 Culture conditions for the microscopic observation 94
  5.2.3 Specimen preparation for SEM and TEM 95
5.3 Results 97
5.4 Discussion 128