

ANTIBIOTIC ACTIVITIES OF CRUDE EXTRACTS FROM
FRENCH LINDA (MICEBIE)

ADIDA ZURADA BINTI MOHAMAD

FAKULTI SAINS DAN TEKNOLOGI
UNIVERSITI MALAYSIA TERENGGANU
2007

C/10: 4586

1100051115

Perpustakaan Sultanah Nur Zahirah (UMT)
Universiti Malaysia Terengganu



LP 2 FST 2 2007



1100051115

Antifungal activities of crude extracts from free-living amoebae
Adida Zuraida Mohamad.

PERPUSTAKAAN
UNIVERSITI MALAYSIA TERENGGANU (UMT)
21030 KUALA TERENGGANU

1100051115		

Lihat sebelah

HAK MILIK
PERPUSTAKAAN UMT

ANTIFUNGAL ACTIVITIES OF CRUDE EXTRACTS FROM FREE-LIVING
AMOEBAE

By

Adida Zuraida Binti Mohamad



Research Report submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Science (Biological Sciences)

Department of Biological Sciences
Faculty of Science and Technology
UNIVERSITI TERENGGANU MALAYSIA
2007

1100051115

This project should be cited as:

Adida, Z. M. 2007. Antifungal activities of crude extracts from free-living amoebae, Bachelor of Science (Biological Sciences), Faculty of Science and Technology, Universiti Malaysia Terengganu. 49p.

No part of this project may be produced by any mechanical, photographic or electronic process, or in the form of phonographic recording, nor may it be stored in retrievals system, transmitted or otherwise copied for public or private use without written permission from the author and the supervisor(s) of the project.



JABATAN SAINS BIOLOGI
FAKULTI SAINS DAN TEKNOLOGI
UNIVERSITI MALAYSIA TERENGGANU

**PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II
RESEARCH REPORT VERIFICATION**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: **ANTIFUNGAL ACTIVITIES OF CRUDE EXTRACTS FROM FREE-LIVING AMOEBAE** oleh **ADIDA ZURAIDA BINTI MOHAMAD**, no. matrik: **UK10078** telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperoleh ijazah Sarjana Muda Sains (Sains Biologi), Fakulti Sains dan Teknologi, Universiti Malaysia Terengganu.

Disahkan oleh: / Verified by:

Penyelia Utama / Main Supervisor

Nama: PROF. MADYA DR. NAKISAH BINTI MAT AMIN

Cop Rasmi: **PROF. MADYA DR. NAKISAH MAT AMIN**
Timbalan Dekan
Pusat Pengajian Siswazah
Universiti Malaysia Terengganu (UMT)
Aras 2, Bangunan Canselori dan Pentadbiran
21030 Kuala Terengganu

Tarikh: 30/4/07

Ketua Jabatan Sains Biologi / Head, Department of Biological Sciences

Nama: DR. AZIZ B. AHMAD

Cop Rasmi: **DR. AZIZ BIN AHMAD**
Ketua
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu

Tarikh: 30/4/07

ACKNOWLEDGEMENTS

Alhamdulillah and thanks to Allah S.W.T for blessing and giving me strength to accomplish the experiment and report writing.

First of all, I want to appreciation to my supervisor, Professor Madya Dr. Nakisah binti Mat Amin and Professor Darah binti Ibrahim from Microbiology Department, Universiti Sains Malaysia, Penang for kindly supply the pathogenic fungal. Their guidance and encouragement for the project was amazing. I have learned so much from them and expand my horizon through the experience that I have gain. I am also wanted to appreciate all lecturers for the comments and advise, master students, Kak Ida, Kak Pae, Kak Kiah, Kak Dah, Kak Shade, Kak Fizah and lab assistants especially Puan Zarina, Puan Ku Naiza, Puan Fatimah and others for their dedication to help me.

Lastly, I would like to convey my special appreciation to my beloved parents, Ariffin bin Ismail and Che Som binti Busu, my beloved fiance, Mohd Ezwan bin Mohd Zin and my friends especially Wana, Lami, Dill, Alan, Tiqah and all my housemates for their supports and encouragement in completing my project. Nevertheless, their passion to help others was the greatest thing somebody can give.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF FIGURES	v
LIST OF TABLES	vi
LIST OF ABBREVIATIONS	vii
LIST OF APPENDICES	viii
ABSTRACT	ix
ABSTRAK	x
CHAPTER 1 INTRODUCTION	
1.1 Introduction	1
1.2 Importance of Study	4
1.3 Objective of Study	4
CHAPTER 2 LITERATURE REVIEW	
2.1 Introduction to <i>Acanthamoeba</i>	5
2.1.1 Pathogenicity of <i>Acanthamoeba</i>	5
2.2 Description of <i>Aspergillus niger</i>	7
2.2.1 Ecology of <i>Aspergillus niger</i>	7
2.2.2 Pathogenicity of <i>Aspergillus niger</i>	8
2.3 The Available Antifungal Agents	9
2.4 Potential of Antifungal from Other Microorganism	9
CHAPTER 3 METHODOLOGY	
3.1 Amoebae	11
3.1.1 Sources of Amoebae	11
3.1.2 Culture Media Preparation and Cultivation of Amoebae	11
3.2 Preparation of Amoebae Extracts	12
3.2.1 Determination of Protein Concentration	13

3.2.2 Determination the Volume of Amoebae Extract With Various Concentration	14
3.3.1 Fungi	15
3.3.1 Sources of Fungus	15
3.3.2 Cultivation of Fungus	15
3.4 Treatment of the Crude Extracts on Fungus	17
3.4.1 Antifungal Disc	17
3.4.2 Screening for Antifungal Activities	17
3.4.2a Streaking Plate Technique	18
3.4.2b Pour Plate Technique	19
CHAPTER 4 RESULTS	
4.1.1 Observation on inhibition zone of <i>Aspergillus niger</i> after treatment with extracts of <i>Acanthamoeba</i> strains AK and AP	20
CHAPTER 5 DISCUSSION	25
CHAPTER 6 CONCLUSION AND RECOMMENDATIONS	28
REFERENCES	29
APPENDICES	35
CURICULUM VITAE	49

LIST OF FIGURE

Figure	Page
3.1 Cultures of AK and AP in cell culture flasks	11
3.2 Original culture of <i>Aspergillus niger</i>	15
3.3 Culture of <i>Aspergillus niger</i> (after subcultivation)	15
3.4 Potato Dextrose Broth	16
3.5 Culture of fungus in Potato Dextrose Broth	16
3.6 Treatment AK extracts on <i>Aspergillus</i> by streak plate technique	18
3.7 Treatment AP extracts on <i>Aspergillus</i> by pour plate technique	18
3.8 Treatment AK and AP extracts on <i>Aspergillus</i> by pour plate technique	19
4.1 Treatment of AK and AP extracts on <i>Aspergillus</i> by streak plate technique with different concentrations	22
4.2 Treatment of AK and AP extracts on <i>Aspergillus</i> by pour plate technique with different concentrations.	23
4.3 Treatment of Tetracycline (Te5) as positive control	24

LIST OF TABLES

Table	Page
4.1 (a) Indication of inhibition zone of <i>Aspergillus niger</i> after treatment with various concentration of amoeba extracts AK and AP using streak plate technique.	20
4.1 (b) Indication of inhibition zone of <i>Aspergillus niger</i> after treatment with various concentration of amoeba extracts AK and AP using pour plate technique.	21

LIST OF ABBREVIATIONS

Abs	absorbance
μm	micrometer
%	percentage
$^{\circ}\text{C}$	Degree of Celcius
g	gram
ml	mililiter
mg	miligram
mg/ml	miligram per mililiter
rpm	rotation per minute
μl	microliter
μm	micrometer
μg	microgram
L	liter
Te5	Tetracycline 5 μg

LIST OF APPENDICES

Appendix	Page
A	<ul style="list-style-type: none"> a. Determination of protein concentration of amoebae extracts; <i>Acanthamoeba</i> strain (AK) and <i>Acanthamoeba polyphaga</i> strain (AP) from absorbance values of protein. 35 b. Calculation of protein concentration from absorbance value as a stock. 35 c. Determination for the volume of protein amoeba extracts using the formula. 36
B	<ul style="list-style-type: none"> Table 4.1 (a) Indication of inhibition zone of <i>Aspergillus niger</i> after treatment with various concentration of amoeba extracts AK and AP using streak plate technique. 38 Table 4.1 (b) Indication of inhibition zone of <i>Aspergillus niger</i> after treatment with various concentration of amoeba extracts AK and AP using pour plate technique. 40
C	<ul style="list-style-type: none"> Figure 1 <i>Acanthamoeba</i> keratitis in cell culture flask 42 Figure 2 <i>Acanthamoeba polyphaga</i> in cell culture flask 42 Figure 3 Protease Peptone D-glucose media 43 Figure 4 Phosphate Buffer Saline 43 Figure 5 Incubation of amoebae 44 Figure 6 Biohazard Laminar Flow 44 Figure 7 Biohazard Laminar Flow 45 Figure 8 Vertical Laminar Flow 45 Figure 9 Harrier 15/8 Centrifuge 46 Figure 10 Spectrofotometer (Eppendorf BioPhotometer) 46 Figure 11 Eppendorf Centrifuge 5417R 47 Figure 12 Harrier 15/8 Centrifuge 47 Figure 13 Sonicator Microson™ ULTRASONIC CELL DISRUPTOR 48 Figure 14 Rotating Shaker 48

ABSTRACT

In this study, extracts of two free-living amoebae, *Acanthamoeba* strain (AK) and *Acanthamoeba polyphaga* strain (AP) were tested on pathogenic fungus *Aspergillus niger*. Both strains of *Acanthamoeba* were axenically cultured in Protease Peptone D-glucose (PPG) media at Biotechnology 3 Laboratory, INOS, Universiti Malaysia Terengganu. The amoebae extracts of AK and AP were prepared in various concentrations (0.5, 1, 2, 4, 6, 8, 10 and 12 mg/ml) and were tested on *Aspergillus niger*. Thirty μ l of each extract concentration were dropped on plain discs. The disc was left for a few minutes before placed onto treatment plate. For the screening process, two techniques (streaking plate technique and pour plate technique) were done to see if there are any differences in results obtained between the two techniques employed. The observation was done after 24 and 72 hours. *Aspergillus* spp. are well-known filamentous fungi to play a role in three different clinical settings in man: (i) opportunistic infections; (ii) allergic states; and (iii) toxicoses. Results obtained from this study show that both extracts at various concentrations which had been used in treatment on this fungus have no antifungal potential against *Aspergillus niger*. There was no inhibition zone seen at all concentrations of the extracts used, indicating that the amoebae extracts did not have antifungal activities.

AKTIVITI ANTIKULAT DARIPADA EKSTRAK MENTAH AMEBA

ABSTRAK

Dua ekstrak daripada ameba iaitu *Acanthamoeba* strain (AK) dan *Acanthamoeba polyphaga* strain (AP) telah dirawat ke atas kulat patogenik, *Aspergillus niger* dalam kajian ini. Kedua-dua strain ameba dibiakkan di dalam media Peptone D-glucose (PPG) di Makmal Bioteknologi 3, INOS, Universiti Malaysia Terengganu. Kedua-dua ekstrak ameba iaitu AK dan AP disediakan pada kepekatan yang berbeza (0.5, 1, 2, 4, 6, 8, 10 dan 12 mg/ml) dan diuji ke atas *Aspergillus niger*. Sebanyak 30 µl ekstrak bagi semua kepekatan dititikkan ke atas disk kosong. Disk dibiarkan kering selama beberapa minit sebelum diletakkan ke atas plat rawatan. Bagi proses saringan, dua teknik (teknik plat coretan dan teknik plat tuangan) diaplikasikan untuk melihat sama ada terdapat perbezaan dalam keputusan yang diperolehi bagi kedua-dua teknik tersebut. Pemerhatian dilakukan selepas 24 dan 72 jam. Kulat *Aspergillus* diketahui umum memainkan peranan dalam tiga keadaan yang berbeza dalam tubuh manusia: (i) jangkitan bersifat oportunistik; (ii) menunjukkan alergi; dan (iii) ketoksikan. Keputusan dari kajian ini menunjukkan kesemua ekstrak yang digunakan tidak berpotensi melawan *Aspergillus niger*. Pada semua kepekatan ekstrak yang digunakan, didapati tiada zon perencatan yang menunjukkan ekstrak ameba tidak mempunyai aktiviti antikulat .