

ABSTRACT

Mushrooms have been important to millions of people not only as food but as traditional medicine. They now serve as new sources for pharmaceuticals and nutriceuticals. For centuries the medicinal or tonic values of certain mushrooms have been known. Through modern research, more and more mushrooms are being identified and tested of their beneficial compounds.

The antifungal activities of the methanolic extracts of 15 selected polypores including *Pycnoporus* sp., *Polyporus* sp., *Microporus* sp., *Albatrellus* sp., *Trametes* sp., *Microporus xanthopus* and *Polypore* sp. against *Saccharomyces pombe*, *Candida albicans*, *Candida parapsilopsis*, *Fusarium oxysporum* f.sp. *cubense* race 1, *Fusarium oxysporum* f.sp. *cubense* race 2, *Fusarium oxysporum* f.sp. *cubense* race 4 and *Ganoderma boninense* were investigated. The strains of polypores were obtained from the Fungal Biotechnology Laboratory, University Malaya. Culture systems (solid agar culture, submerged liquid culture, static liquid culture, effect of culture medium and solid substrate fermentation) in different culture media (potato dextrose, yeast – peptone – glucose, sabouraud dextrose , glucose – yeast – malt – peptone , malt extract and corn meal) were investigated to obtain the bioactive compounds.

Of the 15 strains studied, only *Albatrellus* sp. (KUM 60500) which was cultured on potato dextrose agar displayed high activity against *S. pombe* and *F. oxysporum* in the paper disc assay. The remaining 14 species did not exhibit activity except for extract of *Microporus* sp. (POR 18) inhibited *G. boninense*. The methanolic extracts of *Albatrellus* sp. and POR58 from static culture, using potato dextrose inhibited *F. oxysporum* f.sp. *cubense* race 4 but not *S. pombe*.

The production of oxidases and peroxidases by polypore fungi were examined with plate screening tests for tyrosinase, laccase and lignin peroxidase. Lignin peroxidase was detected in 12 of 15 polypores studied but was only high activity for *Trametes versicolor* (POR 33D), *Polypore* sp. (POR 35), *Pycnoporus* sp. (POR11) and POR 36. The laccase activity was strong for *T. versicolor* (POR 33D) and POR 58. On the other hand, *M. xanthopus* (POR 57) and POR 48 produced the tyrosinase in PDA plate cultures. POR 33D

and POR 35 showed the presence of lignin peroxidase activity of 6.146 U / mL and 8.148 U / mL respectively in submerged cultivation.

ABSTRAK

Cendawan bukan sahaja penting sebagai makanan tetapi juga digunakan sebagai perubatan tradisional. Cendawan merupakan satu sumber baru dalam perkembangan bidang farmaseutikal dan nutriseutikal. Penggunaan perubatan daripada cendawan telah diketahui sejak berabad yang lalu. Melalui penyelidikan terkini, lebih banyak cendawan telah dikenalpasti dan faedahnya telah juga dikaji.

Ekstrak metanolik daripada 15 polypores yang terpilih telah digunakan untuk ujian aktiviti anti-kulat. Polypores yang dipilih termasuk *Pycnoporus* sp., *Polyporus* sp., *Microporus* sp., *Albatrellus* sp., *Trametes* sp., *Microporus xanthopus* dan *Polypore* sp. telah diuji kebolehannya untuk menyekat pertumbuhan kulat seperti *Saccharomyces pombe*, *Candida albicans*, *Candida parapsilopsis*, *Fusarium oxysporum* f.sp. *cubense* race 1, *Fusarium oxysporum* f.sp. *cubense* race 2, *Fusarium oxysporum* f.sp. *cubense* race 4 dan *Ganoderma boninense*. Polypores didapati daripada Makmal Fungal Bioteknologi, Universiti Malaya. Pelbagai jenis sistem kultur ('solid agar culture', 'submerged liquid culture', 'static liquid culture', 'effect of culture medium' dan 'solid substrate fermentation') dalam media yang berlainan (dekstros kentang, yis-peptone-glukosa, 'Sabouraud dextrose', glukosa-yis-malta-pepton, ekstrak malta dan emping jagung) telah dijalankan untuk mendapat bahan bioaktifnya.

Daripada 15 polypores yang diuji, hanya *Albatrellus* sp. (KUM 60500) yang kultur di atas 'potato dextrose agar' dapat mempamerkan aktiviti menyekatan pertumbuhan *S. pombe* dan *F. oxysporum* melalui kaerah difusi cepat. Daripada 14 polypores yang lain, ekstrak POR18 daripada 'potato dextrose agar', juga menyekat pertumbuhan *G. boninense* apabila diuji dengan kaerah difusi cepat. Selain daripada itu, ekstrak methanol daripada kultur *Albatrellus* sp. cecair statik di dalam medium dektros kentang telah menyekat pertumbuhan *F. oxysporum* f.sp. *cubense* race 4 tetapi tiada aktiviti terhadap *S. pombe*. Di samping itu, POR 58 juga menyekat pertumbuhan *F. oxysporum* f.sp. *cubense* race 4.

Polypores juga telah diuji kebolehannya dalam penghasilan enzim seperti tirosinase, lakkase dan lignin peroksidase melalui ujian penyaringan plat. 12 daripada 15 polypores telah menghasilkan enzim lignin peroksidase tetapi hanya *Trametes versicolor* (POR 33D),

Polypore sp. (POR 35), *Pycnoporus* sp. (POR11) dan POR 36 menunjukkan aktiviti yang tinggi. Selain itu, *T. versicolor* (POR 33D) dan POR 58 juga menunjukkan aktiviti yang tinggi terhadap penghasilan enzim lakkase. Untuk penghasilan enzim tirosinase, *M. xanthopus* (POR 57) dan POR 48 telah memberikan keputusan yang baik. Ekstrak daripada kultur cecair ‘potato dextrose’ POR 33D dan POR 35 telah diuji untuk aktiviti lignin peroksidase, didapati nilai lignin peroksidase yang dihasilkan oleh POR 33D dan POR 35 adalah 6.146 U / mL dan 8.148 U / mL masing-masing.

ACKNOWLEDGEMENTS

I would like to express my gratitude to my supervisor; Prof. Dr. S. Vikineswary for her guidance, constructive in guiding me throughout the project is deeply appreciated.

I also would like to express my sincere appreciation to my friends in Mycology Laboratory, especially Agne, Wong Kah Hui and Ng Ching Ching. Thank for teaching me the methods and giving me many helpful comments and suggestion that have improved my project greatly.

Many thanks to my wife, Chok Sook Fan for your continuous supports, patience and assistance throughout my project and will always cherish the memories. Lastly, I would like to share my greatest pleasure in completing this thesis with my parents. Thanks for your encouragement and having faith in me.

TABLE OF CONTENTS

CONTENTS	PAGE
Abstract	II
Abstrak	IV
Acknowledgements	VI
Table of Contents	VII
List of Figures	X
List of Plates	XI
List of Tables	XII
List of Symbols and Abbreviations	XIII
CHAPTER ONE: Introduction	1
CHAPTER TWO: Literature Review	5
2.1 Medicinal Mushrooms	5
2.2 Introduction to the Fungi Kingdom	6
2.2.1 History and Discovery	6
2.2.2 Importance of Fungi	7
2.3 Fungal Systematics	8
2.3.1 <i>Aphyllophorales order</i>	9
2.3.2 <i>Polyporaceae Family</i>	10
2.4 Basidiomycetes Systematics	12
2.5 Importance of Basidiomycetes	13
2.5.1 Distribution of Basidiomycetes in Malaysia	14
2.6 Alternative Medicine	16
2.6.1 Antifungal Agents	18
2.6.2 Antitumor Agents	21
2.6.3 Enzymes Activity	23
2.7 Culture Media for Fungi	24
2.7.1 Aqueous Culture System	24
2.7.2 Surface Culture System	25
2.7.3 Agitated Culture System	25

2.7.4 Solid Substrate Fermentation	26
CHAPTER THREE: Materials and Methods	28
3.1 Selected Polypores Cultures	28
3.2 Test Fungi Cultures	30
3.3 Inoculum Preparation	31
3.4 Culture Systems for Cultivation of Selected Polyporales.	31
3.4.1 Solid Culture System	31
3.4.2 Static and Submerged Agitated Culture System	31
3.4.3 Solid Substrate Culture System	32
3.4.4 Submerged and Agitated Culture System for Enzymatic Activity Test	32
3.5 Extraction of Bioactive Components	32
3.5.1 Extraction from Solid Culture	32
3.5.2 Extraction from Static and Submerged Agitated Culture	33
3.5.3 Extraction from Solid Substrate Culture	33
3.6 Plate Assay for Antagonistic Activity	33
3.7 Paper Disc Diffusion Test to Study Antifungal Activities against <i>Candida</i> spp. and <i>Saccharomyces pombe</i> .	35
3.8 Paper Disc Diffusion Test to Study Antifungal Activities against <i>Ganoderma</i> sp. and <i>Fusarium</i> sp.	36
3.9 Agar Spot Test to Investigate Enzymatic Activity	37
3.10 Lignin Peroxidase (LiP) Enzyme Activity	38
CHAPTER FOUR: Results, Discussion and Conclusion	39
4.1 Plate Assay for Antagonistic Activity	39
4.2 Paper Disc Diffusion with Polypores Extracts from Agar Plate Culture Against Test Fungi	42
4.3 Paper Disc Diffusion with Polypores Extracts from Static Liquid Culture Against Test Fungi.	48
4.4 Effect of Submerged Agitated Culture Medium of <i>Albatrellus</i> sp. against Test Fungi.	51
4.5 Effect of Extracts from Solid Substrate Fermentation Against <i>Saccharomyces pombe</i> .	51
4.6 Agar Spot Test for Enzymatic Activity	54

4.7	Lignin Peroxidase (LiP) Enzyme Activity	56
4.8	Conclusion	58
	References	61
	Appendix A: Media and reagent	73
	Appendix B: Preparation for Lignin Peroxidase enzymatic activity	74

LIST OF FIGURES

Figures	Page
3.1 Plate screening for antagonistic activity of <i>Ganoderma boninense</i> and <i>Fusarium oxysporum</i> (PDA plate; 24 to 48 hours of incubation at $27 \pm 2^{\circ}\text{C}$.)	34
3.2 Plate screening for antagonistic activity of <i>C. albicans</i> , <i>C. parapsilosis</i> and <i>S. pombe</i> (SDA plate for <i>Candida sp</i> and YPG agar plate for <i>S. pombe</i> ; 24 to 48 hours of incubation at $27 \pm 2^{\circ}\text{C}$ for <i>Candida sp</i> and $37 \pm 2^{\circ}\text{C}$ for <i>S. pombe</i>).	34
3.3 Paper disc diffusion test to <i>C. albicans</i> , <i>C. parapsilosis</i> and <i>S. pombe</i> (SDA plate for <i>Candida sp</i> and YPG plate for <i>S. pombe</i> ; 24 to 48 hours of incubation at $27 \pm 2^{\circ}\text{C}$ for <i>Candida sp</i> and $37 \pm 2^{\circ}\text{C}$ for <i>S. pombe</i>).	35
3.4 Paper disc diffusion test to <i>F. oxysporum</i> and <i>G. boninense</i> (PDA plate; 24 to 48 hours of incubation at $27 \pm 2^{\circ}\text{C}$).	37

LIST OF PLATES

Plates	Page
3.1 Fruiting body structure of polypores selected for this study. a) <i>Albatrellus</i> sp. (KUM 60500); b) <i>Polypore</i> sp. (POR 35); c) <i>Polyporus</i> sp. (POR 26); d) <i>Trametes</i> sp. (POR 33); e) <i>Microporus</i> sp. (POR 34); f) <i>Microporus</i> sp.(POR 18); g) Unidentified 1 (POR 36) and h) Unidentified 2 (POR 38).	29
4.1 Inhibition zones of <i>Microporus</i> sp. (POR34) and <i>Albatrellus</i> sp. (KUM 60500) against <i>F. oxysporum</i> in antagonistic activity in plate assay (PDA; 72 hours at $27 \pm 2^{\circ}\text{C}$).	40
4.2 Inhibition zones produced by methanolic extracts of <i>Albatrellus</i> sp. and nystatin as control against <i>S. pombe</i> after 48 hours at $27 \pm 2^{\circ}\text{C}$ in paper disc diffusion test.	43
4.3 The growth morphology of ten days old culture of <i>Albatrellus</i> sp. (KUM 60500) grown on different agar media.	44
4.4 Inhibition zones of paper disc diffusion for methanolic extracts from static liquid culture (PDA plate; 72 hours at $27 \pm 2^{\circ}\text{C}$.).	50

LIST OF TABLES

Tables	Page
3.1 Code, identity and collection site of selected polypores for this study.	28
4.1 Inhibition zones for antagonistic activity plate assay produced by selected polypores (PDA; 72 hours at $27 \pm 2^{\circ}\text{C}$)	39
4.2 Inhibition zones (mm) produced by methanolic extracts <i>Albatrellus</i> sp. and <i>Microporus</i> sp. from PDA plate culture (incubated at $27 \pm 2^{\circ}\text{C}$ for 48 hours) at a concentration of 200 mg / ml against test fungi.	43
4.3 Inhibition zones (mm) produced by methanolic extracts (200 mg/ml) of <i>Albatrellus</i> sp. (KUM 60500) from different media agar plate against <i>S. pombe</i> , which incubated at $27 \pm 2^{\circ}\text{C}$ for 48 hours in discs diffusion test.	45
4.4 Inhibition zones (mm) produced by <i>Albatrellus</i> sp. and POR 58 methanolic extracts from static liquid culture at a concentration of 200 mg/ml against test fungi.	49
4.5 Laccase, tyrosinase and lignin peroxidase activities of polyporales on agar spot test (Polyporales were cultured on PDA plate; then incubated at $27 \pm 2^{\circ}\text{C}$ for 7 – 8 days).	55

LIST OF SYMBOLS AND ABBREVIATIONS

%	Percentage
$^{\circ}\text{C}$	Degree Celsius
Abs	Absorbance
DMSO	Dimethylsulphoxide
DPPH	1, 1-Diphenyl-2-Picrylhydrazyl
EC_{50}	Effective concentration that produces 50% of the maximal possible effect.
e.g.	Example
<i>et al.</i>	and others
etc	and others
g	gram
GYMP	Glucose-Yeast-Malt-Peptone
KUM	Kulat Universiti Malaya
Lac	laccase
LiP	lignin peroxidase
μg	microgram
ME	malt extract
mg	milligram
ml	mililiter
mm	milimeter
MMN	modified Melin Norkrans medium
MnP	Manganese-dependent lignin peroxidase
PDA	potato dextrose agar
SDA	sabouraud dextrose agar
sp.	specie
spp.	Species
SSF	Solid substrate fermentation
VP	versatile peroxidase
w/w	weight/weight
YPG	yeast – peptone – glucose