

**CULTIVATION AND FUNCTIONAL PROPERTIES  
CHARACTERIZATION OF “CENDAWAN BUSUT”  
(*Termitomyces* sp.) LOCALLY ISOLATED IN NEGERI  
SEMBILAN**

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UNIVERSITI SAINS ISLAM MALAYSIA

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## AUTHOR DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledge.

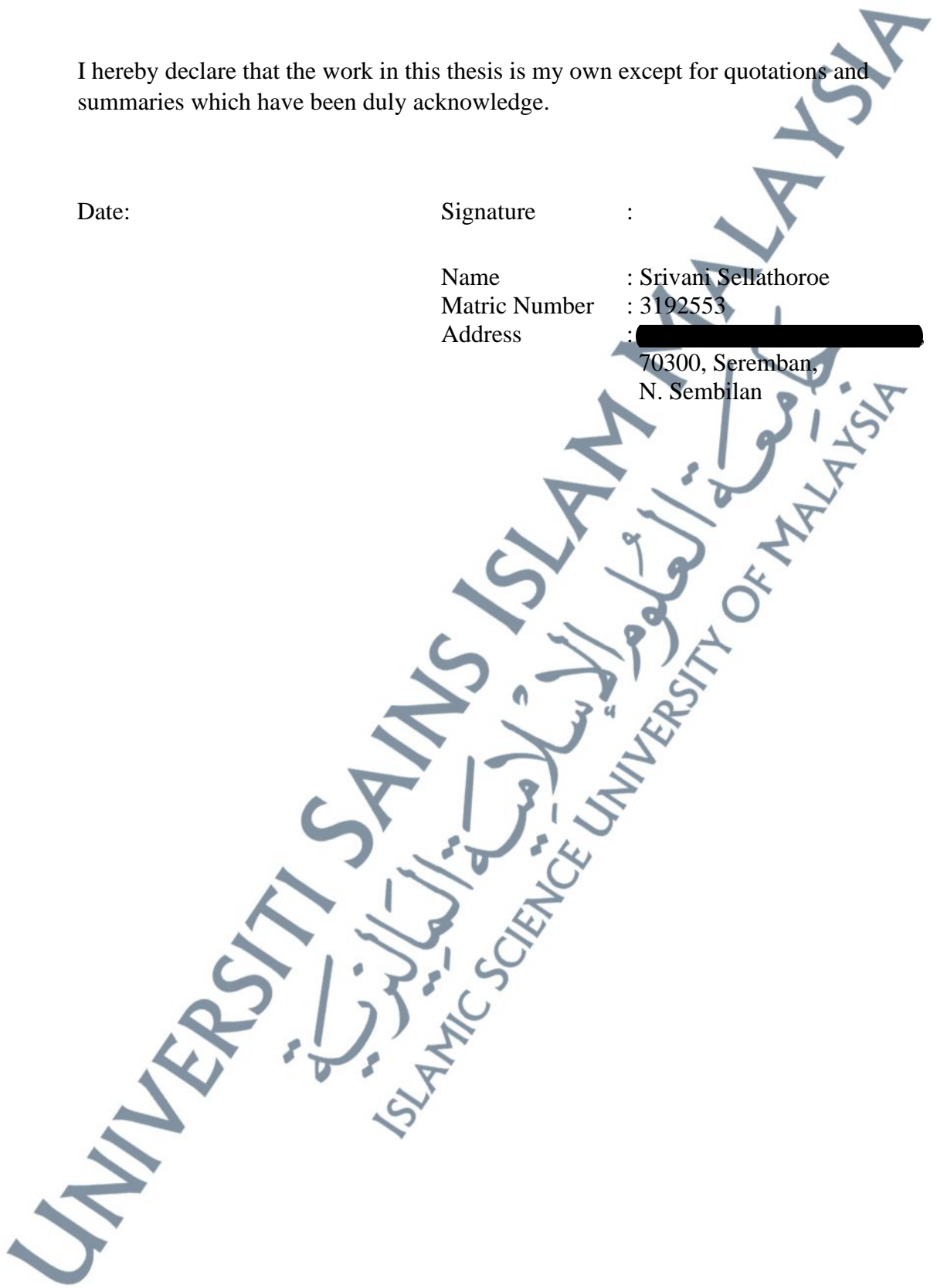
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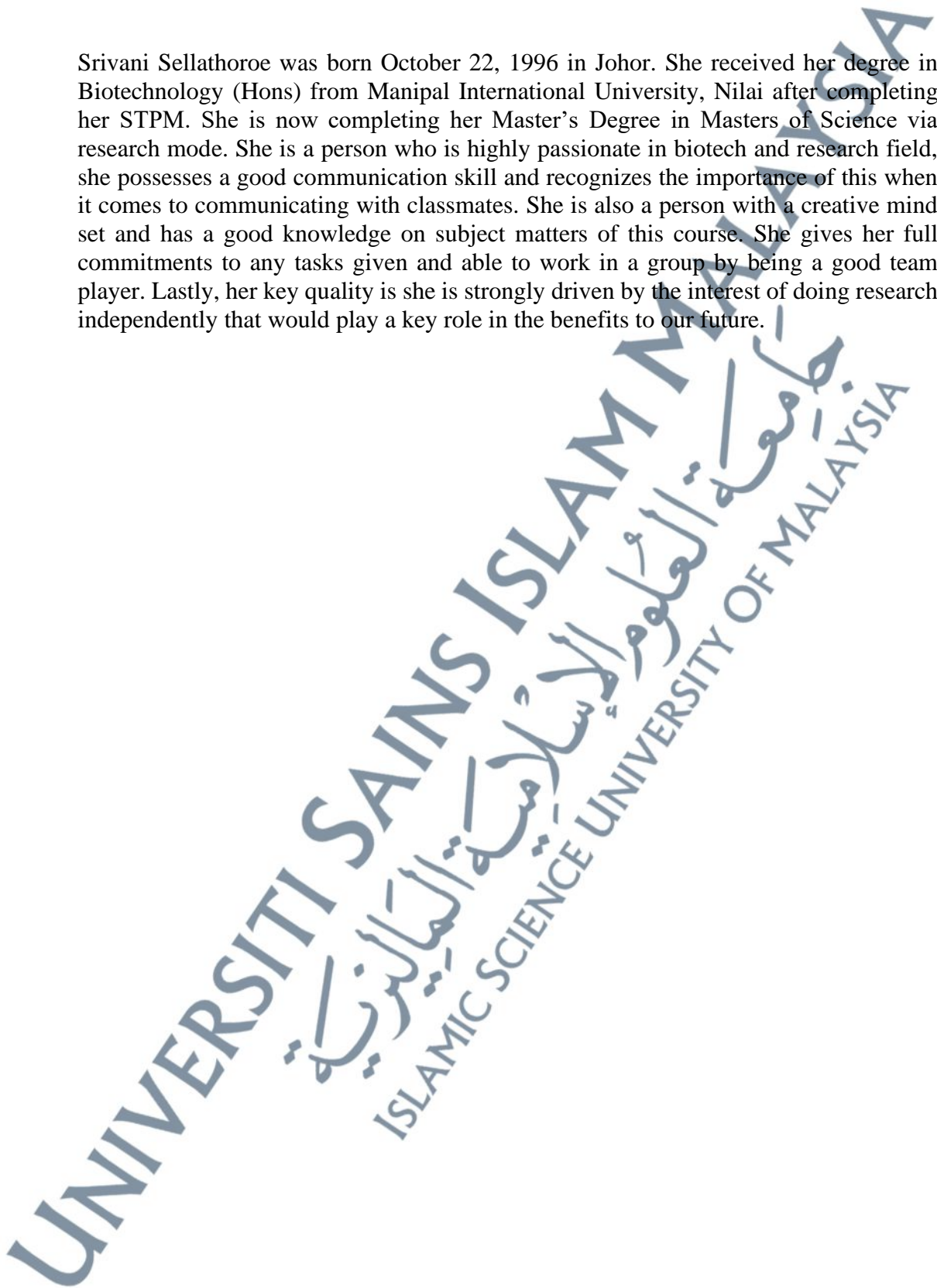
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## BIOGRAPHY

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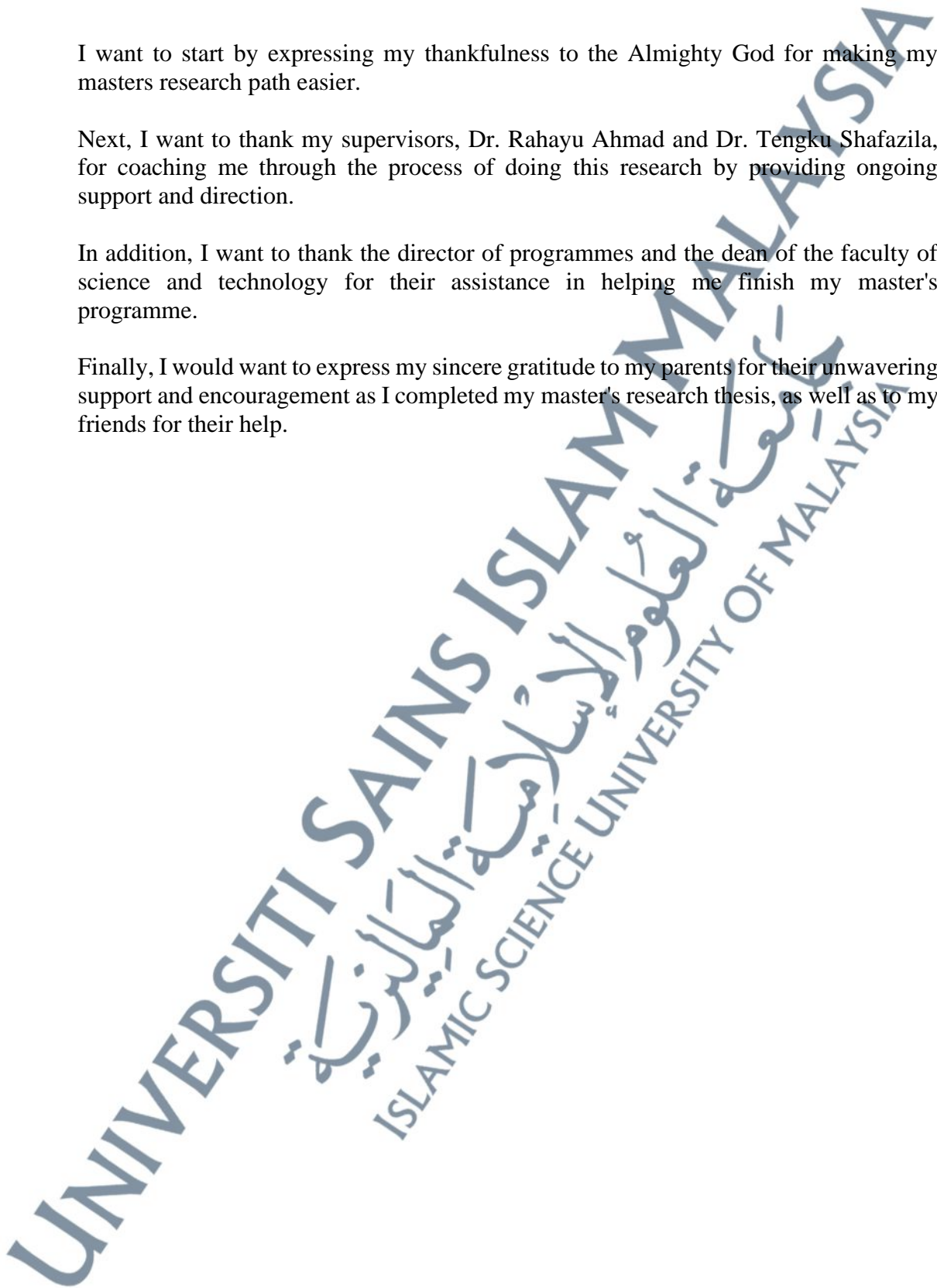
## ACKNOWLEDGEMENTS

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## ABSTRAK

Cendawan anai-anai liar *Termitomyces heimii* RFES 230622 (THR2) dikumpul dari Negeri Sembilan semasa musim monsun dijujukan dan didapati 99% sama dengan *Termitomyces heimii* spesis yang mana jarak evolusi ( $K_{nuc}$ ) turut mengesahkannya. Ciri-ciri morfologi THR2 yang boleh diperhatikan ialah batang stipe yang kelihatan seperti silinder, warna putih kekuningan dipenuhi dengan anulus tebal dan tumbuh dengan anai-anai dalam sikat sarang anai-anai. Cendawan yang dikumpul digunakan dalam kultur tisu untuk pertumbuhan miselium pada medium agar yang telah dimanipulasi, dan diperhatikan bahawa gabungan PDA + ME + YE menunjukkan pertumbuhan miselium yang penuh selepas 14 hari pengkulturan. Ekstrak THR2 telah dihasilkan daripada jasad buah, biojisim miselial dan kaldu kultur THR2 untuk analisis antimikrob, antioksidan, antikulat dan penentuan polisakarida  $\beta$ -glukan. Miselium THR2 mampu membesar dan menghasilkan polisakarida dalam fermentasi kultur tenggelam (SLF) dengan biojisim miselia sebanyak 8.55 g/L selama 7 hari fermentasi. Pelbagai ekstrak disediakan daripada jasad buah (ENS), miselium cendawan (IPS) dan kaldu kultur (EPS) melalui keadah pengekstrakan air panas, air sejuk dan pengampaian dalam etanol. Pengekstrakan air panas dan air sejuk jasad buah THR2 menghasilkan HW-ENS dan CW-ENS dengan masing-masing 3.20 g/L dan 1.36 g/L polisakarida. Manakala, pengekstrakan air panas miselium menghasilkan IPS sebanyak 0.80 g/L dan polisakarida ekstraselular (EPS) yang diperolehi ialah 1.44 g/L. Polisakarida telah disahkan sebagai  $\beta$ -glukan oleh analisis spektrum FTIR.  $\beta$ -glukan ini mempamerkan sifat antimikrob dengan tindak balas tertinggi diperhatikan dalam  $\beta$ -glukan yang diekstrak daripada jasad buah (HW-ENS) berbanding dengan  $\beta$ -glukan yang diekstrak daripada miselium.  $\beta$ -glukan tidak menunjukkan sebarang sifat antikulat. Kehadiran fitokimia seperti flavonoid, saponin dan terpenoid dikesan dalam semua ekstrak THR2. Jumlah kandungan flavonoid (TFC) dikira menggunakan Quercetin sebagai standard dan kandungannya berada dalam julat 2.16 – 2.86 mg/g dalam semua ekstrak THR2. Walau bagaimanapun, kandungan flavonoid paling tinggi didapati dalam HW-ENS iaitu  $2.86 \pm 0.15$ . Aktiviti antioksidan ekstrak THR2 diukur menggunakan ujian DPPH dan nilai  $IC_{50}$  ditentukan. Nilai  $IC_{50}$  semua ekstrak terletak di antara 4.44 mg/ml hingga 6.71 mg/ml. Sampel HW-ENS ekstrak THR2 menunjukkan nilai  $IC_{50}$  paling sedikit iaitu 4.44 mg/ml manakala EPS menunjukkan nilai  $IC_{50}$  tertinggi iaitu 6.71 mg/ml. Daripada empat ekstrak THR2, HW-ENS ini boleh dianggap sebagai ekstrak yang berpotensi tinggi untuk aktiviti antioksidan kerana nilai  $IC_{50}$  paling rendah dengan peratusan perencatan yang tinggi pada 10 mg/ml iaitu 90.83% berbanding dengan ekstrak lain.

**Kata-kunci:** *Termitomyces heimii*, fermentasi kultur tenggelam, polisakarida, antimikrob, antioksidan

## ABSTRACT

*Termitomyces heimii* mushroom is seasonal in which they only grow during the rainy season, it is necessary to establish a proper cultivation method so that the valuable bioactive compound can be produced at any time. *Termitomyces heimii* RFES 230622 (THR2), a wild-termite mushroom that was isolated from Negeri Sembilan during the monsoon, was sequenced and found to be 99% identical to the *Termitomyces heimii* strain, which was further supported by the mushroom's evolutionary distance (Knuc). The stipe, which seems to be cylindrical, creamy in colour, stuffed with thick annulus, and growing in the comb of termite nest, is the observable morphological trait of THR2. The isolated mushrooms were employed in tissue culture to grow mycelium on modified agar medium, and it was found that the PDA + ME + YE combination produced thick, full-plate mycelium growth after 14 days of incubation. Fruiting bodies, mycelial biomass, and cultured broth of THR2 were used to make extracts for antibacterial, antioxidant, antifungal analysis and determination of  $\beta$ -glucan polysaccharides. After 7 days of incubation, the THR2 mycelium was able to grow and generate polysaccharide in submerged liquid fermentation (SLF), with mycelial biomass of 8.55 g/L. Various extracts from fruiting bodies (ENS), mushroom mycelium (IPS), and culture broth were made (EPS). The THR2 fruiting bodies yield HW-ENS and CW-ENS, with corresponding polysaccharide contents of 3.20 g/L and 1.36 g/L, after hot-water and cold water extraction. Meanwhile, the hot-water extraction of mycelium produces IPS with 0.80 g/L and the extracellular polysaccharide (EPS) obtained was 1.44 g/L. The polysaccharides were verified as  $\beta$ -glucan by FTIR spectra analysis. These  $\beta$ -glucan exhibits antimicrobial properties with highest response observed in the  $\beta$ -glucan extracted from fruiting bodies (HW-ENS) compared to the  $\beta$ -glucan extracted from the mycelium whereas there was no zone of inhibition against *Aspergillus niger* which means it does not exhibit antifungal properties. All of the THR2 extract was found to contain phytochemicals like flavonoids, saponins, and terpenoids. Total flavonoid content (TFC), which was determined using quercetin as the standard, ranged from 2.16 to 2.86 mg/g in each THR2 extract. However, the highest concentration of flavonoid content was found in HW-ENS which is  $2.86 \pm 0.15$ . Using the DPPH assay, the antioxidant activity of THR2 extracts was evaluated, and the  $IC_{50}$  value was calculated. All extracts had  $IC_{50}$  values that range from 4.44 mg/ml to 6.71 mg/ml. HW-ENS sample of THR2 extract shows the least  $IC_{50}$  value which is 4.44 mg/ml whereas EPS showed the highest  $IC_{50}$  value which is 6.71 mg/ml. Out of this four THR2 extracts HW-ENS can be considered as extract with high potential of antioxidant activity as the  $IC_{50}$  value was the lowest with high percentage of inhibition at 10 mg/ml which was 90.83% compared to the other extracts.

**Keywords:** *Termitomyces heimii*, submerged liquid fermentation, polysaccharide, antimicrobial, antioxidant

## AL-MULAKHKHAS

تم ترتيب تسلسل فطر النمل الأبيض البري *Termitomyces heimii* RFES 230622 (THR2) الذي تم جمعه من Negeri Sembilan خلال موسم الرياح الموسمية ووجد أنه مطابق بنسبة 99% للأصناف *Termitomyces heimii* التي أكدتها المسافة التطورية (Knuc) أيضاً. السمة المورفولوجية لـ THR2 التي يمكن ملاحظتها هي شكل أسطواناني الشكل ، ذو لون أبيض مائل للصفرة ملىء بحلقة سميكة وينمو مع النمل الأبيض في أمشاط عش النمل الأبيض. تم استخدام الفطر الذي تم جمعه في زراعة الأنسجة لنمو الفطريات على وسط أجار معالج ، وقد لوحظ أن مزيج PDA + ME + YE أظهر نمواً كاملاً للفطر السميك بعد 14 يوماً من الاستنبات. تم إنتاج مستخلصات THR2 من الأجسام المثمرة والكتلة الحيوية الفطرية ومرق ثقافة THR2 لتحليل مضادات الميكروبات ومضادات الأكسدة ومضادات الفطريات وتحديد عديد السكاريد  $\beta$ -glucan. THR2 mycelium قادر على النمو وإنتاج السكريات في التخمر المغمور (SLF) مع الكتلة الحيوية الفطرية البالغة 8.55 جم / لتر لمدة 7 أيام من التخمر. تم تحضير مستخلصات مختلفة من أجسام الفاكهة (ENS) وفطر الفطر (IPS) ومرق الاستزراع (EPS) من خلال الاستخلاص بالماء الساخن والاستخلاص بالماء البارد والمعلق في الإيثانول. أنتج استخراج الماء الساخن والماء البارد من أجسام الفاكهة THR2 HW-ENS و CW-ENS مع 3.20 جم / لتر و 1.36 جم / لتر من السكريات على التوالي. وفي الوقت نفسه ، أنتج استخراج الماء الساخن من mycelium IPS مع 0.80 جم / لتر وعديد السكاريد خارج الخلية (EPS) الذي تم الحصول عليه كان 1.44 جم / لتر. تم تأكيد عديد السكاريد على أنه  $\beta$ -glucan بواسطة تحليل طيف FTIR. أظهر هذا  $\beta$ -glucan خصائص مضادة للميكروبات مع أعلى استجابة لوحظت في  $\beta$ -glucan المستخرج من الجسم الثمر (HW-ENS) مقارنة بـ  $\beta$ -glucan المستخرج من الفطريات بينما لم يظهر أي خصائص مضادة للفطريات. تم الكشف عن وجود مواد كيميائية نباتية مثل الفلافونويد والسابونين والتريبتويدات في جميع مستخلصات THR2. تم حساب محتوى الفلافونويد الكلي (TFC) باستخدام Quercetin كمعيار وكان المحتوى في حدود 2.16 - 2.86 مجم / جم في جميع مستخلصات THR2. ومع ذلك ، تم العثور على أعلى محتوى من الفلافونويد في HW-ENS والذي كان  $0.15 \pm 2.86$ . تم قياس النشاط المضاد للأكسدة لمستخلص THR2 باستخدام اختبار DPPH وتم تحديد قيمة IC50. تراوحت قيم IC50 لجميع المستخلصات من 4.44 مجم / مل إلى 6.71 مجم / مل. أظهرت عينة HW-ENS لمستخلص THR2 أقل قيمة IC50 قدرها 4.44 مجم / مل بينما أظهرت EPS أعلى

قيمة IC50 قدرها 6.71 مجم / مل. من بين مستخلصات THR2 الأربعة ، يمكن اعتبار هذا HW-ENS كمستخلص ذو قدرة عالية على النشاط المضاد للأوكسدة بسبب أقل قيمة IC50 مع نسبة تثبيط عالية عند 10 مجم / مل وهي 90.83٪ مقارنة بالمستخلصات الأخرى.

الكلمات المفتاحية: *Termitomyces heimii* ، التخمير المغمور ، السكريات ، مضادات

الميكروبات ، مضادات الأوكسدة

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## LIST OF UNIT AND MEASUREMENTS

Centimetre	cm
Evolutionary distance	K <sub>nuc</sub>
Gram per litre	g/L
Grams	g
Microgram	µg
Microlitre	µL
Milligram per gram	mg/g
Milligram per Millilitre	mg/ml
Millilitre	ml
Millimetre	mm
Molar	M
Nanometre	nm
Pounds per Square Inch	psi
Revolutions per minute	rpm
Wavelength per distance	cm <sup>-1</sup>

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## LIST OF SYMBOLS

$\beta$   
 $^{\circ}\text{C}$   
 $\pm$   
 $\%$

Beta  
Degree Celsius  
Plus- Minus sign  
Percentage



## LIST OF ABBREVIATIONS

<i>A. niger</i>	<i>Aspergillus niger</i>
bp	Base pairs
CWE	Cold water extraction
CW-ENS	Cold water endo- polysaccharide
DPPH	2, 2-diphenyl-1-picrylhydrazyl
<i>E. coli</i>	<i>Escherichia coli</i>
ENS	Endo-polysaccharides
EPS	Extracellular polysaccharide
FTIR	Fourier transform infrared spectroscopy
gDNA	Genomic deoxyribonucleic acid
HWE	Hot water extraction
HW-ENS	Hot water endo- polysaccharide
IC <sub>50</sub>	Half maximal inhibitory concentration
IPS	Intracellular polysaccharide
ME	Malt extract
min	Minutes
NA	Nutrient agar
NB	Nutrient broth
OD	Optical density
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
Pen- Strep	Penicillin- streptomycin
Pen- Strep- Amp B	Penicillin- streptomycin- amphotericin B
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SD	Standard deviation
SDA	Sabouraud dextrose agar
SDB	Sabouraud dextrose broth
secs	Seconds
SLF	Submerged liquid fermentation
<i>T. albuminosus</i>	<i>Termitomyces albuminosus</i>
<i>T. aurantiacus</i>	<i>Termitomyces aurantiacus</i>
<i>T. eurrhizus</i>	<i>Termitomyces eurrhizus</i>
<i>T. letestui</i>	<i>Termitomyces letestui</i>
<i>T. microcarpus</i>	<i>Termitomyces microcarpus</i>
<i>T. titanicus</i>	<i>Termitomyces titanicus</i>
<i>T.heimii</i>	<i>Termitomyces heimii</i>
TBE	Tris-borate-EDTA
TFC	Total flavonoid content
Vol	Volume
YE	Yeast extract