CHAPTER 1

INTRODUCTION

1.1 Introduction

It has been observed by Mahamat et al. (2018), that the consumption of edible mushrooms is rising globally, which has sparked public interest about mushroom dietary and health benefits. The termite mushroom, scientifically known as *Termitomyces*, which is either farmed or gathered in the wild and contains a food bioactive ingredient, is widely taken in Asia and Africa to maintain the health of cancer patients and prevent the progression of chronic diseases (Hsieh et al., 2018). *Termitomyces heimii* and *Termitomyces microcarpus* were utilised in India to cure a cold, fever, and fungal infection (Venkatachalapathi et al., 2016). It was reported by Chandrawati et al., (2014), during wound healing *Termitomyces heimii* acts as blood tonics and blood coagulation while to cure diarrhoea and rheumatic disorder *Termitomyces eurrhizus* is used (Sachan et al., 2013). It was noted in those who had ongoing illnesses, *Termitomyces microcarpus* are used for boosting their immune system and to treat a variety of gastrointestinal issues, including constipation, ulcers, and stomach pain. In Tanzania *T. eurrhizus*, *T. letestui*, *T. aurantiacus,* and *T. titanicus* are used as tonics (Tibuhwa, 2012b).

Termitomyces mushrooms grows effectively in the late rainy seasons from September to December in Malaysia since they are seasonal. It is also locally known as 'cendawan busut' and ''kulat pusu''. However, Ghorai et al., (2009) mentioned that the collected mushrooms are consumed by the collector itself due to their delicacy taste and seasonal factors. After heavy thunderstorm, this mushroom emerges in colony of termite

nest and it will be gathered by the collectors in which only the long branch fruiting body can be consumed. The termite mushroom can sustain for only 4 to 5 days (Santhi et al., 2017). The required symbiotic growth of the mushroom and termites, as described by Aanen et al., (2002) gave rise to the unique name "termite mushroom". In this symbiotic termites-mushroom growth association behaviour, it was noticed that the termites give a nest as a substrate for the mushroom to grow, and in exchange, the mushroom secretes lignocellulosic enzyme that aids the termites' digestion of the substrates they ingest (Wisselink et al., 2020).

In addition to the stated medical benefits of curing diarrhoea, rheumatism, and decreasing blood pressure (Woldegiorgis et al., 2014), *Termitomyces* fruiting bodies contain a considerable quantity of phenolic and polysaccharides compounds that have antioxidant, anticancer, and antibacterial characteristics (Reis et al., 2018). According to Nakalembe et al. (2015), the fruiting bodies of *Termitomyces* mushrooms contain higher levels of ascorbic acid (10–18 mg/g), proteins (27–36%), ergosterol, and vital minerals like phosphorus, copper, iron, potassium, and calcium than those of other edible mushrooms (Malek et al., 2012). Mushrooms lack vitamin D2, and ergosterol may function as a biological precursor to that vitamin. Ergosterol can be transformed into viosterol by ultraviolet light, moisture, and temperature to produce ergocalciferol, a type of vitamin D2 used in food supplements and pharmaceutical applications (Jasinghe et al., 2007).

In this study, a wild termite mushroom was isolated, collected in Negeri Sembilan and later identified using polymerase chain reaction (PCR) molecular sequencing and phylogenetic Molecular Evolutionary Genetic Analysis (MEGA) software in Negeri Sembilan during the monsoon season. Then, after identifying the species submerged liquid fermentation was used to produce the mycelial biomass that is used for extraction

of β-glucan and it was characterised using Fourier-Transform Infrared Spectroscopy (FTIR). Bioactive compound of the fruiting bodies and its cultured mycelium was investigated for its antimicrobial, antifungal and antioxidant. Numerous discoveries should be made that elucidates the nutritional composition and medicinal properties of this mushroom with the advancement of technology, all of which are highly beneficial in maintaining human health and well-being.

1.2 Problem Statement

Termitomyces sp. mushroom is seasonal mushroom in which it only grows during the raining season. Due to the unique taste, it is priced highly compared to other cultivated mushrooms. However, reports on the quad functional including antimicrobial and antioxidant properties of polysaccharide extracted from the *Termitomyces* sp. mycelium has never been reported. Since these mushrooms are seasonal mushroom in which they only grow during the raining season, it is necessary to establish a proper cultivation method using *Termitomyces* sp. mycelium so the valuable bioactive compound from this mushroom can be produced at any time without depending on the seasons. Hence, submerged liquid fermentation was used as it is a promising cultivation method. It is also an alternate method in growing mushroom mycelia and produces the same bioactive compound properties found in the mushroom fruiting bodies. This cultivation techniques definitely help to overcome the difficulties in obtaining bioactive compound from wild fruiting bodies especially from seasonal mushrooms. New findings of medicinal properties of this mushroom are needed to increase the ethnomedicinal knowledge to benefit human health and well-being.

1.3 Research Objectives

The objectives of this study are:

- To isolate and grow the mycelium of wild-termite mushroom (THR2) onto agar medium and submerged liquid fermentation
- To extract the functional bioactive compound from fruiting bodies, mushroom mycelium, and culture broth of THR2 by using SLF method.
- To determine the β-glucan present in various THR2 crude extracts by using FTIR.
- To examine the biological properties (antibacterial, antifungal and antioxidant) of various THR2 crude extract by disk diffusion method and DPPH assay.

1.4 Research Aim

This research aims to cultivate the isolated wild-termite mushrooms onto agar medium and in SLF. The cultivated mycelium was then identified through morphology of the fruiting bodies and molecular sequencing to confirm the species. Batch SLF was carried out to produce mycelium biomass and polysaccharide. Various types of crude polysaccharide were extracted from the fruiting bodies (ENS), mushroom mycelium (IPS) and culture broth (EPS). The extracted polysaccharide was then characterized using FTIR to detect the existing of β-glucan functional group. Finally, the β-glucan polysaccharide was tested on the antibacterial, antifungal and antioxidant test.