CHAPTER 2

LITREATURE REVIEW

2.1 Medicinal Mushroom

The use of fungi has long been a significant component of both conventional and scientific medicine. For a long time, the widespread use of medicinal mushrooms was prohibited in Asian nations, but recently, the use of medicinal mushrooms has increased in Western and other nations (Guedes et al., 2008). Higher basidiomycetes, often known as therapeutic mushrooms, are microscopic fungus that are utilised for nutrition, illness prevention, and treatment in the form of extracts or powder (Barros et al., 2007).

In contrast to their texture and exquisite flavour, medicinal mushrooms are prized for their chemical and nutritional properties; they contain a family of polysaccharides known as β-glucan (Manzi et al., 1999). In the study made by Ubaidillah et al., (2014) it claims that β-glucan stimulates complement receptors, which in turn increases antitumor immunity associated to antibody-Fc interactions. The most frequently recommended natural anticancer medicines are β-glucan, which operate synergistically with therapeutic antibodies like "trastuzumab" or "rituximab" as shown in mouse models (Ubaidillah et al., 2014).

Due to their rareness, difficulty in cultivation, and insufficient evidence there are few species of mushroom that have become highly valued partially which includes *Mirchella esculenta* (morel), *Ganoderma lucidum* (Lingzhi), *Grifola frondosa* (maitake), *Pleurotus eryngi* (king oyster mushroom) and *Termitomyces* sp. (termite mushrooms) also known as “cendawan busut” (Tsai et al., 2006). Malek et al., (2012) state that attempts to develop and cultivate *termitomyces* sp. in laboratories have been

unsuccessful due to their rareness and difficulty in cultivation. *Termitomyces* species have been shown to be highly nutritious edible therapeutic mushrooms in the wild. *T. heimii* is used as blood tonics and for blood coagulation in India to treat wounds (Chandrawathi et al., 2014). Moreover, *T. heimii* have chemo preventative properties of specific human diseases (Abd Malek et al., 2012). However, given these are seasonal mushrooms, there are little publications on the chemical components of *Termitomyces* sp. due to the challenges in acquiring the wild fruiting bodies (Malek et al., 2012). In Malaysia, *Termitomyces heimii* is one of the commonly encountered wild mushroom and is considered as a delicacy.

2.2 Diversity of *Termitomyces* sp. Medicinal Properties

Health promotion and treatment are achieved through using several species of *Termitomyces* ethnomedicinally. In China, *Termitomyces albuminosus* (*T. albuminosus*) are used to improve brain and stomach functions or for curing haemorrhoids (Hsieh et al., 2018). In India *T.heimii* and *T. microcarpus* are used to treat fungal infection, fever and cold (Venkatachalpathi et al., 2016). Rheumatic disorder, diarrhea, and high blood pressure can be cured with *Termitomyces eurrhizus* (Sachan et al., 2013).

In Nigeria, a preparation using *T. microcarpus* aids in the treatment of gonorrhoea (Oyetayo, 2011). *T. titanicus*, *T. letestui*, *T. eurrhizus*, and *T. aurantiacus* are used as tonics in Tanzania for a variety of gastrointestinal issues, such as abdominal pain, constipation, stomach-ache, and ulcers. *T. microcarpus* can enhance the immune system of persons with protracted illnesses (Tibuhwa, 2012a). *Termitomyces* sp. bioactive components have been shown to contain phenolic compounds (Woldegiorgis et al., 2014) and β-carotene (Puttaraju et al., 2006) that exhibit antioxidant action.

By boosting the expression of alcohol metabolism, *T. albuminosus* polysaccharides protected mice livers from damage brought on by alcohol (Zhao et al., 2015). From *Termitomyces* fruiting bodies, several polysaccharides have been extracted for their potential as immunomodulators, antioxidants, and structure elucidation with implications (Mondal et al., 2006). Immunomodulators are substances which help to regulate and balance the immune system, while antioxidants are compounds that helps to protect cells from damage caused by harmful molecules called free radicals. The impact of immunomodulators can be quite significant as they enhance the immune response, making it more effective in fighting off infections and diseases. They can also help to reduce inflammation, which is a common immune response that can sometimes become excessive and lead to various health issues. As for antioxidants, their impact is equally important. Antioxidants can help prevent oxidative stress and damage to cells which is associated with aging and the development of chronic diseases by neutralizing free radicals.

2.3 *Termitomyces* sp. Mushroom

An edible mushroom of the genus *Termitomyces* is a member of the *Lyophyllaceae* family, the Basidiomycota phylum, and the Fungi Kingdom. The classification of *Termitomyces* according to the Integrated Taxonomic Information System and species (2000) is displayed in Table 2.1. According to Hsieh et al. (2017), this mushroom has been found in forests, gardens, and pastures where *Odontotermes* termites are present. Typically, there are more than 300 specimens of this mushroom rooted in a single termite nest, which has room for up to 40 or more mushroom chambers associated with termites. Due to the *Termitomyces* sp. mushroom's symbiotic growth

with termites in the hills, which allows it to only survive and produce offspring while in this stage, it is also known as the "termite mushroom" (Abd Malek et al., 2012).

According to Abd Malek et al., (2012) T*ermitomyces* sp., associated with macro termite mushrooms it is a group of agarics mutualistically unlike other mushrooms which grow in usual situation on sand. A ‘fungus garden’ will be constructed by these termites within their nests and cultivate a basidiomycete fungus belonging to the genus *Termitomyces* shown in Figure 2.1. Numerous pearly white nodules will be produced by the fungus gardens known as primordial (Rahmad et al., 2014) *Termitomyces* sp. mushroom helps the termites to digest substrates called as lignocellulosic by producing various enzymes. It was reported that the mycelia can grow on agar media but the fruiting bodies of *Termitomyces* sp. has not been successful yet under artificial cultivation (Hsieh et al., 2017).

The estimated number of *Termitomyces* species worldwide is thirty, and twenty or more of these species are particularly regarded for their delicate flavour and fruiting body texture. Many species of *Termitomyces* are used by various ethnic groups who are knowledgeable about ethnomedicine (Ghorai et al., 2009). In regions where people consume foods with low nutritional qualities, *Termitomyces* sp. mushrooms become a significant source of nutrients as well as micronutrients like vitamins and minerals (Nakalembe et al., 2015; Sargunam et al., 2012). Eight *Termitomyces* species, including *T. clypeatus*, *T. entolomoides*, and *T. heimii*, have been found in Peninsular Malaysia. *T. radicatus*, *T. aurantiacus*, *T. auhrizus*, *T. microcarpus*, and *T. striatus*. There hasn't been much investigation into the lipid composition of *T. heimii* in Malaysia (Abd Malek et al., 2012). Hence, research was done by Sathiya et al., (2020) to describe another new species as an addition to the genus *Termitomyces* found within Sabah, Malaysia but it

is often misidentified based on morphology by the local communities especially in Malaysia for *Chlorophyllum molybdites* which is a highly poisonous mushroom.

Table 2.1: Scientific classification of *Termitomyces* sp. mushroom

Kingdom	Fungi
Phylum	Basidiomycota
Class	Agaricomycetes
Order	Agaricales
Family	*Lyophyllaceae*
Genus	*Termitomyces*

Source: Rahmad et al., 2014

Figure 2.1: Primordia growing on termite nest

2.4 *Termitomyces heimii*

In Malaysia, the most common wild-termite mushroom found is *T. heimii,* belonging to the family *Lyophyllaceae.* It is highly sought edible mushroom, grows

seasonally in a symbiotic environment with termites. Several research has been carried out on *T. heimii* to study the locality, nutrient composition as well as the medicinal properties (Abd malek et al., 2012). But the study has been very limited due to the difficulties in obtaining fresh fruiting bodies since it's a seasonal mushroom thus, the mycelium cultivation is also difficult to carry out (Hsieh et al., 2017). It develops periodically and together with termites. One of the *Termitomyces* species that is frequently found in the wild and is regarded as a delicacy in Malaysia is *T. heimii*.

2.4.1 Appearance of *T. heimii*

The observable characteristics of *T. heimii* is the stipe which appear to look cylindrical, creamy and stuffed with thick annulus (Figure 2.2). The stipes length usually said to be between 10-30 cm and the colour of *T. heimii* fruiting bodies found to be cream in colour (Srivastava et al., 2011).

Source: Rahmad et al., 2014

Figure 2.2: Fully grown *T. heimii* fruiting bodies on top of termite nest,

2.4.2 Cultivation of *T. heimii*

There are different types of cultivation method to grow or propagate the mushroom. The growing of the fruiting bodies by germination of spores where the fruiting bodies was gathered from the natural habitat and place on the fresh substrate causing the spores to germinate and colonise the substrate. Using the artificial solid media is another traditional method to culture the mycelium of the mushroom in which for the first fruiting body to appear it takes up to 3 months (Manan et al., 2018). The fruiting bodies were removed from their normal environment and placed in an area with "fresh" substrates, where they caused the spores to germinate and colonise the substrate. For the formation of fruiting bodies and polysaccharides, artificial solid media namely potato dextrose agar, PDA) have traditionally been employed to cultivate mushrooms, however this method requires a lengthy growing period (Manan et al., 2018).

Submerged liquid fermentation, SLF offers a lot in terms of the growing method, allowing for shorter cultivation times, smaller spaces, and a lower risk of contamination. According to Ahmad et al., (2014) a wider production of bioactive compounds from basidiomycetes are able to be produced by manipulating and improving the culture conditions through SLF.

2.4.2.1 Polysaccharides and β-glucan

Mushroom polysaccharides are a type of carbohydrate polymer formed from a mushroom's cell wall, and β-glucan is one of these polysaccharides that may be found in the fruiting bodies of mushrooms (Wan-Mohtar et al., 2016a). Previous studies reported that polysaccharides extracted from various mushroom were one of the obvious reasons to carry out investigations related to therapeutic uses (Chandrawathi et al.,

2014). Fungal polysaccharide has been reported to exhibit beneficial effects to our body including hormonal, nervous, and immune systems (Hsieh & Ju, 2018).

The polysaccharide contain in the fruiting bodies and mycelium of mushroom was known as intracellular (IPS) or endopolysaccharide (ENS) (Wan-Mohtar et al., 2016b). Exopolysaccharide (EPS), on the other hand, is the term for a polysaccharide that has spread throughout the liquid culture broth during the fermentation process. Due to their constant excretion into the medium, EPS look unattached, renewable, free, and simple to extract through fermentation. Due to the host's immune system being activated, mushroom β-glucan has potent antibacterial, antiviral, and anticancer effects (Villares et al., 2012). Many pharmacological studies using polysaccharide isolated from some mushrooms are proved to have antiviral properties antibiotic activity, antitumor, immune-stimulating properties, serum lipids and sugar level reduction (Song et al., 2010). Due to the wide range of health advantages that mushrooms offer, β-glucans could be used as functional foods, nutraceuticals, or dietary supplements (Giavasis, 2014).

2.5 Extraction Process of the Bioactive Compounds

The extraction of mushroom can be carried out in numerous methods in which the main target is to obtain the extracts with higher yield and lower cost. Non-conventional techniques are more environmentally friendly because of diminished utilization of manufactured and natural chemicals, better yield, and decreased operational time and increase quality of extract (Azmir et al., 2013).

An effective mushroom extraction procedure is essential for successfully recovering specified bioactive components. Hot water extraction has the capability of extracting different classes of compounds which mainly depends on the temperature

and time. A substance extracted from a mixture via hot water (80°C) using a hot water bath is considered as hot water extraction. Majority of the active elements that found in the mushroom can be extracted using this method and the efficacious values also will be retained. By using this method there are several advantages such as; its low cost, simplicity, cleanness and its possibility of automation. According to a study by Abdullah et al. (2017), different mushroom hot water extracts have variable glucan concentrations and induce diverse immune-stimulatory effects.

A substance extracted from a mixture via warm room temperature water is considered as cold water extraction. It is a type of fractional crystallization (Azmir et al., 2013). The advantages of cold water extraction include the potential to retain bioactive compounds. This was deduced from Chen et al., (2016) study results, which showed that a low extraction temperature was chosen for the extraction of *P. citrinopileatus* because a high extraction temperature could destroy bioactive compounds.

Hot and cold water extractions are commonly used for extracting, mushroom because the main active ingredient in most medicinal mushrooms, β-glucans are water-soluble which means they are able to dissolve in water. Hence, in this research hot and cold water extraction method was used to extract the components from both *Termitomyces* fruiting bodies and the mycelium biomass.

2.6 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis scans test and examines chemical characteristics of materials using infrared light. A sample is passed through an FTIR device using infrared light that ranges in wavelength from 10,000 to 100 cm^{-1} (Villares et al., 2012). The range of wavelengths absorbed by a substance in the infrared region will be measured. This is

accomplished by exposing samples of a substance to infrared light (IR). The ability of the sample to absorb the energy of the infrared light at different wavelengths is examined to ascertain the chemical composition and structure of the substance. The resultant signal at the detector typically exhibits a spectrum between 4000 cm^{-1} and 400 cm^{-1}. The production of a distinctive spectrum by each molecule or chemical structure makes FTIR analysis a fantastic tool for chemical identification. The spectrum shows many absorption peaks to analyse the existence of specific functional groups like β-glucans and laminarin in this study.

Laminarin is the standard polysaccharide used in this research to compare with the existing sample (HW-ENS, CW-ENS, EPS, IPS) by using FTIR. It is commonly known as brown algae starch, is an active component of kelp that exhibits a variety of properties, including hypoglycemic, anticancer, immunomodulatory, antibacterial, antiviral, blood lipid-regulating, anti-oxidation, and anticlotting properties (Chen et al., 2013).

2.7 Functional Properties of *T. heimii*

2.7.1 Antimicrobial and Antifungal Activity

Chemical substances known as antimicrobial agents either stop or eliminate bacteria development. β-glucan presence in mushroom possess a strong antibacterial, antiviral, and antitumor properties action due to the activation of the host's immune system (Villares et al., 2012). Numerous species of *Termitomyces* have also been shown to have therapeutic characteristics in addition to their nutritional value and industrial use (Mahamat et al., 2018). For instance, prior studies have concentrated on the antimicrobial and antifungal effects of *Termitomyces* sp. on nosocomial agents like

Escherichia coli, *Pseudomonas aeruginosa*, *Streptococcus* spp., *Candida albicans*, and *Enterobacter cloacae*, all of which have been identified as the most frequently causing bacteraemia and fungemia (Paul, 2017). The effect of the antimicrobial activities from other *Termitomyces* sp. have been reported previously, for example, the aqueous extracts (hot water extraction process) from *Termitomyces clypeatus* fruiting bodies was reported to highly inhibited the growth of several bacteria such as *E. coli*, *S. aureus*, *Salmonella typhi* and *E. aerogenes* in the range of 2.6 – 10.55 mm zone of inhibition (Mahamat et al., 2018). Apart from water extracts, *T. hemii* acetone extract had demonstrated antibacterial effectiveness against *Staphylococcus aureus* and *Klebsiella pneumoniae* (Singha et al., 2017).

Antifungal agents are chemical compound that inhibits fungal growth. Mushrooms must produce and secrete antifungal compounds of survive in the wild against competing or pathogenic organism since the mushrooms always grow in those places where the possibilities of finding the microbes is severe. Hence mushrooms must have strong antifungal properties to fight such fungus microbes to survive (Romi, 2017).

2.7.1.1 Disk Diffusion Method

In this study, the antibacterial and antifungal activity is tested using the disk diffusion method. When an antibiotic-impregnated disc is placed on agar that has already been inoculated with the test bacteria or fungus, the antibiotic picks up moisture and diffuses radially outward through the agar medium, creating an antibiotic concentration gradient. The antibiotic is most concentrated at the disk's edge, and as one moves away from it, its concentration steadily decreases until it no longer inhibits the organism's growth, at which time it becomes non-inhibitory. If the substance prevents

bacterial growth after incubation, a clear zone forms around an antibiotic disc (Mahamat et al., 2018).

2.7.2 Antioxidant Activity

Antioxidant is a bioactive compound that is needed by humans in order to prevent oxidation of other molecules that is found in our body. This is because the oxidation of the molecule could result in free radicals which is an unstable atom that scavenge the body to seek electrons and become a pair. This eventually will damage the body cells and leads to various disease (Mitra et al., 2016). Antioxidant is available either as natural or synthetic. However, the synthetic antioxidant is no longer considered to be safe to consume as it was recently reported that it has many health problems (Mitra et al., 2016). Their toxic and carcinogenic properties lead to liver damage and other harmful effects (Mitra et al., 2016). Thus, the natural source of antioxidant is more preferred.

Mushrooms are a source of powerful bioactive chemicals as well as various nutritional and nutraceutical benefits, according to research done in recent decades. As stated in stated in Gebreyohannes et al., (2019) all extracts from *Termitomyces* sp. mushrooms have revealed potent antioxidant activities. At times when our body immune system becomes insufficient to fight against excessive oxidative damage, antioxidants from external sources becomes necessary, which may be in the form of food supplements, where *Termitomyces* sp. mushrooms have striking significance (Mitra et al., 2016).

2.7.2.1 Antioxidant Compound

A different amount of antioxidant content is found in different types of mushrooms. According to Yang et al., (2002) antioxidant activity is often correlated to their molecular structure known as phenols. The molecular structure of a phenolic compound consists of a hydroxyl group tie to an aromatic hydrocarbon group since flavonoids often make up a large collection of phenolic compounds it is categorized under phenolic group (Yang et al., 2002). Flavonoids or bioflavonoid is the word from the Latin word flavus which means yellow, their shading in nature and it is a class of plant and growth auxiliary metabolites. Flavonoids have been appeared to have antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, and hostile to thrombotic, and vasodilatory movement (Woldegiorgis et al., 2014). Mycochemical antioxidant investigations, according to Njouonkou et al., (2020), identified polyphenols as the primary antioxidant molecules, followed by flavonoids and thiols.

2.7.2.2 DPPH (2, 2-diphenyl-1-picrylhydrazyl) Assay

In order to analyse the antioxidant activity of the determined phytochemicals, the antioxidant assay need to be carried out. DPPH provides a faster and simple way to evaluate potential antioxidant based on the concept of transferring an electron. DPPH is basically an organic chemical compound that is in the form of dark coloured crystalline powder form. This powder is composed of stable free radical molecules. This synthetic chemical was discovered to be stable at room temperature and will not break down when exposed to water, methanol, or ethanol. Based on the compounds' ability to shed their hydrogen and the components' confirmed structural integrity, the free radical scavenging activity functions. The strong ability of DPPH to receive an electron or a

hydrogen atom and create a diamagnetic molecule is demonstrated by its 517 nm wavelength. Once in contact with the antioxidant molecule, DPPH is subsequently decreased. A colourless ethanol solution is the end result, and a spectrophotometer is used to measure the antioxidant activity. The discolouration is a result of the low levels of DPPH radicals in the environment and it shows the extract's capacity to scavenge radicals (Mitra et al., 2016).