



Review Article

Morphological, Molecular, and Therapeutic Perspectives on Malaysia's Tiger Milk Mushrooms -An Appraisal

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Abstract

Medicinal mushrooms have been utilized by ancient civilizations for both nutritional and therapeutic purposes, reflecting their historical significance in ethnopharmacology. Due to their health-promoting properties, the value of mushrooms has appreciated, and this has revitalized scientific interest, with modern research validating their bioactive properties and highlighting their potential applications in food and biopharmaceutical industries. An example of such a medicinal mushroom is the Tiger Milk Mushroom (TMM), locally referred to as *cendawan susu harimau* among indigenous communities in Southeast Asia. Traditionally, TMM has been in use for the treatment of respiratory disorders and inflammatory conditions. It is one of the highly valued mushrooms known for its role in promoting general health and well-being. Tiger milk mushroom belongs to the genus *Lignosus* Lloyd ex Torrend of the family *Polyporaceae* (*Polyporales*). To date, three distinct species of this genus have been discovered in the tropical rainforests of Malaysia: *L. rhinocerus* (Cooke) Ryvarden, *L. tigris* Chon S. Tan, and *L. cameronensis* Chon S. Tan, identified using both morphological and specific genetic DNA sequences. Among the three, *L. rhinocerus* is the highly studied, followed by *L. tigris* and *L. cameronensis*. Comprehensive genomic, transcriptomic, and proteomic analyses have been carried out for *L. rhinocerus* and *L. tigris*, yielding valuable insights into their biology and medicinal potential (antioxidant, antitumor, anti-inflammatory, antimicrobial). In contrast, *L. cameronensis*, despite its discovery in 2013, remains largely unexplored at the molecular level, with limited research focusing on its nutritional value and safety assessment. This review highlights the key research findings on the three *Lignosus* species, with particular emphasis on their medicinal properties and recent advancements in omics-based molecular investigations.

Keywords: Mushrooms, Tiger milk mushroom, Malaysia, Medicinal, Omics.

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1. Introduction

Mushrooms belonging to the Kingdom *Fungi* are the Earth's most diverse and ecologically crucial organisms, important for both ecosystem replenishment and human welfare (Atri et al., 2017). The therapeutic role of mushrooms dates back to the Neolithic period, reflecting their long-standing role in traditional medicine (Wasser, 2014). Globally, over 14,000 mushroom species have been discovered, with 10% being classified as macro-fungi (Devi et al., 2024). Based upon their nutritional roles, mushrooms are categorised as symbiotic, saprophytic and parasitic (Katoch et al., 2023). Naked-eye visible edible mushrooms are widely preferred for culinary and medicinal purposes, as they are rich in flavor and health-promoting properties.

Mushrooms are classified as edible when it is free from toxic compounds and exhibit organoleptic properties such as acceptable aroma, taste, and texture, which are suitable for human consumption (Kong et al., 2025). The fruiting bodies of around 700 edible species are classified as safe for the human diet and are associated with various health benefits (Gupta et al., 2018). Being rich in polysaccharides, dietary fibre, proteo-glucans, and essential vitamins (El-Ramady et al., 2022), medicinal mushrooms not only serve as a valuable dietary food, but also in the development of dietary supplements, nutraceuticals, and mycotherapy products (Venturella et al., 2021). The food and biopharmaceutical industries have demonstrated increasing interest in mushroom-based products due to their notable nutritional and medicinal properties (Rathore et al., 2017). Extensive research over the past decades has demonstrated their bioactive properties, including antioxidant, antitumor, anti-inflammatory, and antimicrobial activities (Contato and Conte-Junior, 2025; Effiong et al., 2024; Jakopovic et al., 2024; Mizuno & Minato, 2024; Trivedi et al., 2024).

In recent years, the growing interest in ethnomycological knowledge has highlighted *Lignosus* Lloyd ex Torrend species as a rare and valued species among polyporoid mushrooms due to their medicinal properties (Fig.1). Natural occurrence of this genus is limited to specific geographic regions of South China, Thailand, Malaysia, Indonesia, the Philippines, Papua New Guinea, New Zealand, and Australia (Lai et al., 2011). As many as six species of genus *Lignosus*, namely *L. dimiticus* Ryvarden, *L. ekombitii* Douanla-Meli and Langer, *L. goetzii* (Hennings) Ryvarden, *L. rhinocerus* (Cooke) Ryvarden, *L. sacer* (Fries) Ryvarden, and *L. hainanensis* B.K. Cui are documented by Ryvarden & Johansen, (1980). Malaysia, recognized as one of the world's 17 mega-diverse countries, boasts about the occurrence of variety of mushrooms, including both edible and non-edible species (Kong et al., 2025). Out of these in Malaysia, *L. rhinocerus* is hailed as the most recognized and significant medicinal mushroom by the Semai aborigines (Lee et al., 2009).

The collection of wild *L. rhinocerus* is challenging as it is both time-consuming and labour-intensive because it grows solitarily in the wild (Abdullah et al., 2013). Being challenged by the cost and supply issues, the large-scale cultivation of *L. rhinocerus* in a controlled environment was a success in Malaysia since the 2000s (Lau et al., 2011; Lau et al., 2013), thereby providing an opportunity for potential pharmacological and nutraceutical investigation, especially in developing dietary supplements and functional foods (Nallathamby et al., 2018).

1.1 Historical Overview

Mordecai Cubitt Cooke (1879) was the first to scientifically document the TMM in 1879 on Penang Island, Malaysia, and named it *Polyporus rhinocerus*. The Diary of John Evelyn (1664) recorded TMM's as *Lac tygridis* and their use by the local communities beyond the reach of Western medicine to treat illnesses. Tiger milk mushroom was known by various local common names (cendawan susu harimau, betes kismas, hurulingzhi, hijiritake) and formerly classified under different synonyms (*Polyporus sacer* var. *rhinocerus* and *Microporus rhinocerus*), but the current accepted name is *Lignosus rhinocerus* (Fung & Tan, 2019). The prominence of TMM can be traced to the maritime trade era of the Malay Peninsula. Tiger milk mushrooms are among the important medicinal mushrooms carried by Chinese traders for their long and arduous journeys. This is possibly due to its resemblance to Lingzhi, a well-known medicinal fungus in China (Fung & Tan, 2019).

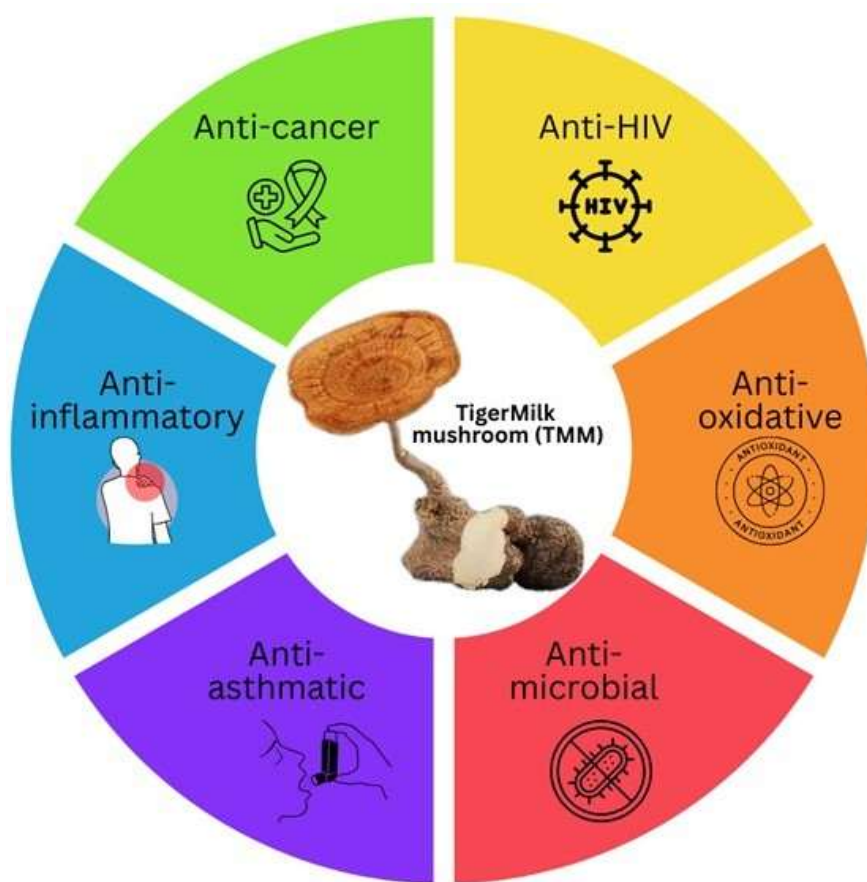


Fig. 1. Medicinal properties of Tiger milk mushroom.



As a result, from this trade and integration of Chinese and Malay Peninsula traders, TMM became well known among the Peranakan communities for its extraordinary medicinal value. Nowadays, the traditional use of TMM has also been reported from Singapore, China, Indonesia, Thailand, Philippines, and Papua New Guinea. Tiger milk mushroom is categorized as a mushroom with large economic significance in 'A Dictionary of the Economic Products of the Malay Peninsula (Burkill, 1966; Fung & Tan, 2019).

Tiger milk mushroom is extensively utilized as a traditional remedy by indigenous communities across Southeast Asia and China. Tiger milk mushroom has long been valued for its therapeutic properties, particularly in the treatment of a broad range of ailments such as asthma, cough, fever, and cancer. Cultural stories of Malaysian folklore believe that TMMs sprout in spots where prowling tigress milk dropped to the ground while nursing their cubs (Ng et al., 2024). In the tropical forest, TMM is rare and scarce, as not more than one is found within a radius of 5 km (Fung & Tan, 2019).

2. *Lignosus* species in Malaysia

The genus *Lignosus* belongs to the group of medicinal polypore fungi of Southeast Asia. To date, a total of eight species of this polyporaceous macrofungus have been recognized and documented from different parts of the world (Fig.2), three of which, *L. rhinocerus*, *L. tigris*, *L. cameronensis* have been identified in Malaysia (Fig.3) (Ng et al., 2023; Yap et al., 2022). In Malaysia, *L. rhinocerus* was first discovered on Penang Island (Cooke, 1879), followed by two more additional species (*L. tigris* and *L. cameronensis*) in Pahang's tropical forest back in 2013. These species were formerly misclassified as *L. rhinocerus* due to their incomplete morphological features. The pore size and basidiospore dimension in these species are quite variable based on which these can be clearly differentiated from one another (*L. rhinocerus*: 7-8 /mm, *L. tigris*: 1-2 /mm, *L. cameronensis*: 2-4/mm) and dimensions of basidiospores (*L. rhinocerus*: 3-3.5 × 2.5-3 µm, *L. tigris*: 2.5-5.5 × 1.8-3.6 µm, *L. cameronensis*: 2.4-4.8 × 1.9-3.2 µm) (Tan et al., 2013). *L. rhinocerus* (Cooke) Ryvarden (MycoBank MB 316915) was the first species to be described as *Polyporus rhinoceros* by Cooke in 1879 in Penang, Malaysia (Cooke, 1879). Later, Ridley (1890) documented its medicinal value by comparing *Pleurotus tuber-regium* and *Wolfiporia cocos*. This species was then reclassified in different genera (*Fomes*, *Scindalma*, *Polystictus*, *Microporulus*) and named as *L. rhinocerotis* (Cooke) Ryvarden (*L. rhinocerus*) (Index Fungorum 2014). In Malaysia, *L. rhinocerus* have been found in a few places, including Penang, Cameron Highlands, Hulu Langat, and Gerik (Cunningham, 1965; Tan et al., 2009), and occasionally from remote regions of Pahang and Perak. Development of molecular tools such as DNA barcode markers solved taxonomic ambiguity as the *L. rhinocerus* has often been mistaken for *Pleurotus tuber-regium* or *Lentinus tuber-regium* (Cui et al., 2011; Sotome et al., 2008). It differs from other *Lignosus* species by its characteristics, such as pale-brown pore surface, ellipsoid-subglobose basidiospores, and long pseudorrhiza that anchors the basidiocarp deep into the soil. The name *L. rhinocerus* originates from a rhinoceros horn, which was sent together with a fungus-like mushroom 'Lac tigridis' as gifts from Jesuits for their medicinal values (Fung & Tan, 2019). *Lignosus tigris* (MycoBank MB 800843) was the second species to be discovered in Malaysia, distinguished from *L. sacer* by its larger pores (1–2 pores per mm) and smaller basidiospores (2.5–

5.5 × 1.8–3.6 µm). It was discovered by Chon S. Tan during an expedition to the tropical rainforests of Lata Iskandar, Pahang. The species named *tigris* derived from local folklore, which tells of the mushroom arising from the milk that dropped onto the forest floor while a tigress was nursing her cubs (Tan et al., 2013). *Lignosus cameronensis* (MycoBank MB 800844) was discovered by Chon S. Tan along with *L. tigris* in Lata Iskandar, Pahang. It was distinguished from *L. ekombitii* by its smaller basidiospores (2.4–4.8 × 1.9–3.2 µm). The name *cameronensis* refers to the Cameron Highlands, the locality where the type specimen was collected. However, initially both *L. tigris* and *L. cameronensis* were interchangeably referred to as *L. rhinocerus* (Tan et al., 2013).

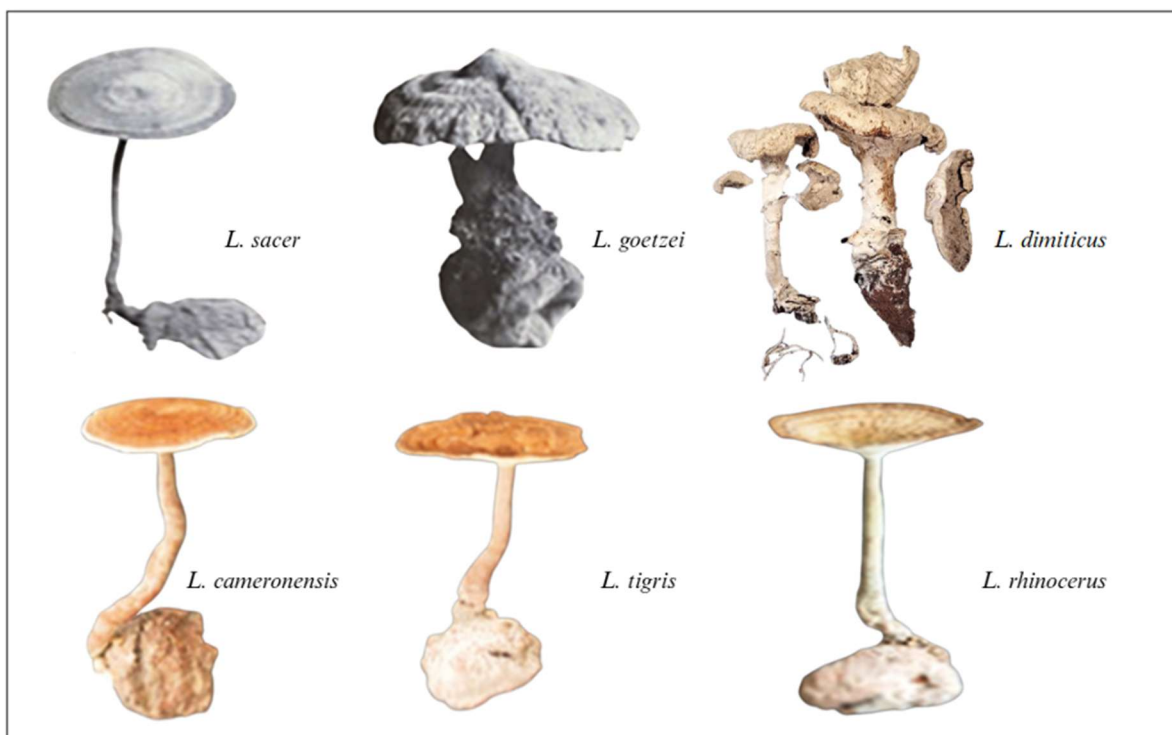


Fig.2. *Lignosus* species. *L. sacer*^{*}, *L. goetzei*^{*}, *L. dimiticus*[#], *L. cameronensis*, *L. tigris* and *L. rhinocerus*

^{*}Picture from Index of Mycological Writings Volume III, Lloyd, C.G. 1909–1912

[#] Adapted from Yayaro et al., (2025).

3. Morphological comparison of *L. rhinocerus*, *L. tigris*, and *L. cameronensis*

Lignosus species feature three key structures: pileus (cap), stipe (stem), and sclerotium (tuber). It contains a unique polypore feature as the fruiting body (cap and stem) emerges from the underground tuber instead of a woody substrate. The pileus and stipe are woody, meanwhile the sclerotium is denser with a food-reserve-rich mass of mycelium. Mycelium is whitish in colour; meanwhile, the sclerotium exudes milk-like solution, which has a milk taste and is hard to harvest (Tan et al., 2009).

From a morphological point of view, *L. rhinocerus*, *L. tigris*, and *L. cameronensis* have centrally stipitate basidiocarps emerging from underground sclerotia, a trimitic hyphal system– generative hyphae with abundant clamp connections and thin-walled. They show differences in terms of pileus characteristics, stipe dimensions, sclerotium size, and pore density. *L. tigris* has the longest stipe, *L. cameronensis* has the widest pileus, while *L. rhinocerus* exhibits an intermediate morphology with the largest sclerotium. The pore size varies among the three species; *L. tigris* features the largest pores (0.5-1 pores/mm), followed by *L. cameronensis* (2-3 pores/mm), and *L. rhinocerus* (6-8 pores/mm) (Fig.3).

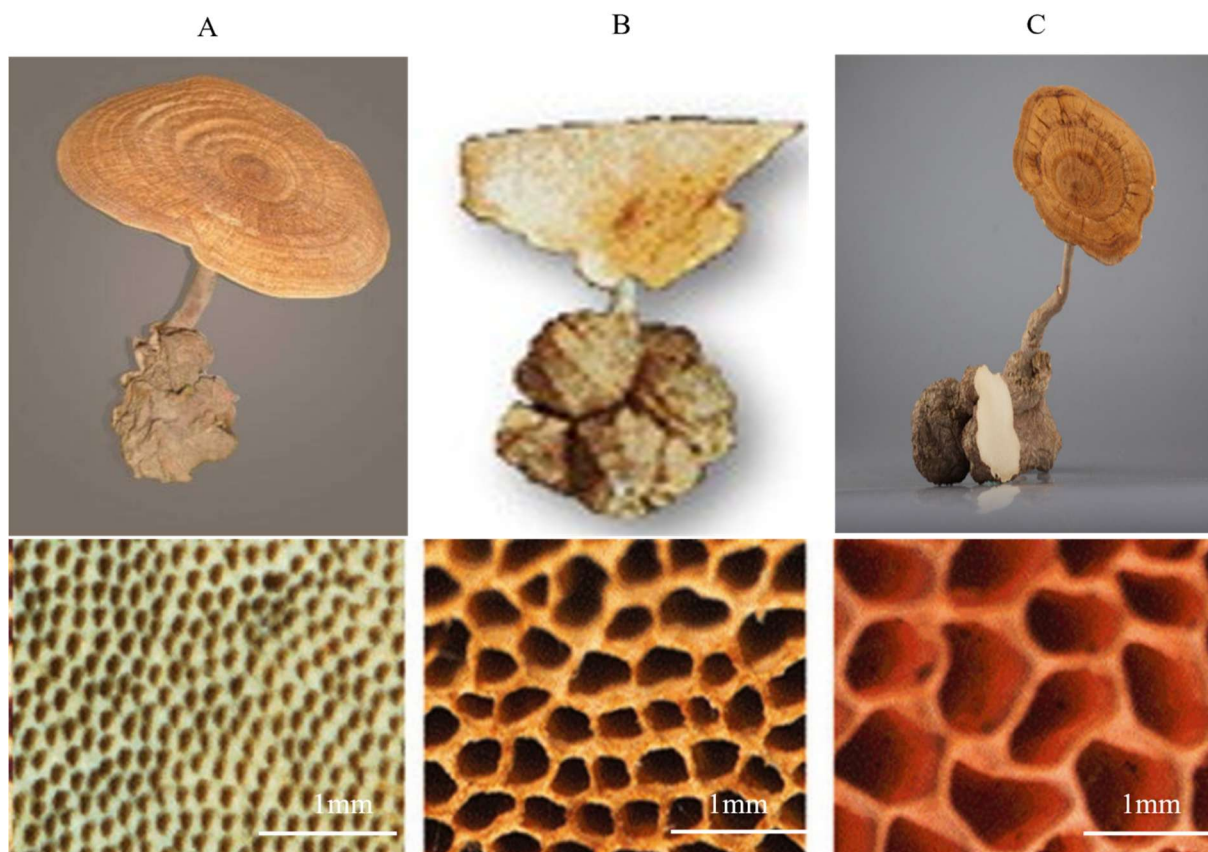


Fig.3. Morphology and pore sizes of three *Lignosus* species in Malaysia. (A)**L. rhinocerus* (6-8 pores/mm) (B)[#] *L. cameronensis* (2-3 pores/mm) (C)⁺*L. tigris* (0.5-1 pores/mm)

*downloaded from <https://www.ligno.com.my/>, [#]Adapted from Yap et al., (2024),

⁺downloaded from <https://drinkmycotea.com/lignosus-tigris/>

4. Multi-omics Studies of *Lignosus* in Malaysia

4.1 Genomic Studies

The 34.3 Mb genome of *L. rhinocerus* and the 45.9 Mb draft genome of *L. tigris* comprises approximately 10,742 and 12,800 predicted protein-coding genes, respectively (Ng et al., 2023;



Yap et al., 2014b). Comparative analysis with *L. rhinocerus* revealed significant divergence, where 50.51% of *L. tigris* genes were unmatched with *L. rhinocerus*, indicating strong divergence of these two sister species of *Lignosus* spp. A total of 4,399 genes of *L. tigris* were unique compared to *L. rhinocerus*, signifying the potential to encode for novel proteins (Ng et al., 2023).

The *L. rhinocerus* genome encodes for the complete biosynthetic pathways of both 1,3- and 1,6- β -glucans, supporting its immunomodulatory properties (Xu et al., 2011; Yap et al., 2014b). Meanwhile, *L. tigris* genome encodes for the full biosynthetic pathways of vitamins B6 and D2, crucial in treating dermatitis and anaemia, suggesting a unique nutritional advantage not reported in *L. rhinocerus* (Ng et al., 2023; Parra et al., 2018). Two fungal immunomodulatory proteins (FIPs) (GME7566_g and GME10641_g) showing 64% sequence identity to the well-studied LZ-8 protein from *Ganoderma lucidum*, possessing anti-cancer and immunomodulatory properties, were also identified in *L. rhinocerus* (Kino et al., 1989; Wu et al., 2011; Yap et al., 2014b). Similarly, *L. tigris* encodes for two putative FIPs (LTGME11499_g and LTGME87_g) containing the Fve domain associated with immunomodulatory and anticancer activities, showing up to 79.4% sequence identity to FIP-Lrh from *L. rhinocerus* (Ng et al., 2023; Pushparajah et al., 2016). *L. tigris* was reported to have a larger lectin gene repertoire (66 genes) containing ricin B, jacalin-like, and C-type lectin families, compared to only nine lectins in *L. rhinocerus* (Ng et al., 2023; Yap et al., 2014b). *L. rhinocerus* comprises of sesquiterpenoid biosynthesis genes, with 12 sesquiterpene synthase genes, uncommon among Basidiomycota, and a total of 136 cytochrome P450 enzymes (CYP5144 and CYP5150 families) involved in detoxification and secondary metabolism (Črešnar & Petrič, 2011). Likewise, *L. tigris* encodes for 14 terpene biosynthetic gene clusters involved in antimicrobial, anti-inflammatory, antifungal and anticancer properties (Ng et al., 2023; Rabi & Bishayee, 2009; Theis & Lerda, 2003) as well as β -lactone biosynthetic cluster with potential anti-obesity and anticancer relevance (Ng et al., 2023; Robinson et al., 2019). *L. rhinocerus* genome also encodes 332 carbohydrate-active enzymes (CAZymes) signifying strong polysaccharide degradation capabilities, 535 enzymes unique among Basidiomycetes, genes responsible for both catabolism and secondary metabolites biosynthesis such as limonene and pinene degradation, indole alkaloid, β -lactam, and gingerol biosynthesis, highlighting its diverse bioactivities and ethnomedicinal use (Yap et al., 2014b). In contrast, *L. tigris* enriched in chromatin-associated proteins, such as HP1-like and chromodomain proteins, indicating a more complex regulatory capacity and greater potential for epigenetic control and environmental adaptability (Ng et al., 2023).

4.2 Transcriptomic Studies

Genes encoding Jacalin-like lectins, cysteine-rich proteins (CPs), hydrophobins, and glucan biosynthesis enzymes were reported to be highly expressed in *L. rhinocerus* (Yap et al., 2015a). Among them, the most abundant transcripts with significant high expression levels (RPKM: 1,198.14-5,842.62) and protein abundance up to 14.96% were lectin genes (GME275_g, GME272_g, and GME273_g) which are close homologs of Jacalin-type lectins from *Grifola frondosa*, displaying antiproliferative potential (Hassan et al., 2015; Kawagishi et al., 1990; Yap et al., 2015a). In comparison, *L. tigris* revealed 68.06% of actively expressed predicted genes in the sclerotium, with 80.38% of them being coding transcripts. Several highly expressed genes encode proteins with known or putative medicinal properties, reinforcing its therapeutic potential.



For instance, putative hydrophobin and serine proteases exhibited remarkably high expression levels with FPKM value of 16742.98 and 7356.68, respectively, suggesting its role in drug delivery (Cai et al., 2021; Ng et al., 2024)), protein degradation, immune modulation, and apoptosis induction in cancer cells (Tagirasa & Yoo, 2022). Similarly, transcripts encoding deoxyribonucleases (FPKM = 3777.98), jacalin-like lectins (FPKM=3690.87), and fungal immunomodulatory proteins (FIPs; FPKM=2337.84) were abundantly expressed (Ng et al., 2024). These proteins have been widely reported in other medicinal fungi for their anticancer, antiviral, and immune-enhancing properties (Chettri et al., 2021; Lannoo & Van Damme, 2014). Additionally, genes responsible for the biosynthesis of pharmacologically relevant β -glucans (1,3- β - and 1,6- β -glucans) were also highly expressed (RPKM 22.52–910.75) in *L. rhinocerus* highlighting its potential in the production of bioactive polysaccharides with immunomodulatory and antitumor properties (Ooi & Liu, 1999; Wasser, 2002; Yap et al., 2015a). Likewise, in *L. tigris* antioxidant defence genes such as superoxide dismutase (SOD; FPKM=1161.69) and catalase (FPKM=1905.83) involved in detoxification of reactive oxygen species (ROS) and oxidative stress mitigation, critical for maintaining cellular redox balance and preventing oxidative damage-related diseases (e.g. cancer and neurodegenerative disorders) were highly expressed (Anwar et al., 2024; Chidambaram et al., 2024; Ng et al., 2024; Zheng et al., 2023). Moreover, the transcriptome of *L. rhinocerus* also revealed the active expression of genes linked to the biosynthesis of secondary metabolite, particularly terpene pathways, contributing to its ethnopharmacological potential (Oliveira et al., 2021; Yap et al., 2015a). Collectively, these transcriptomic findings provide meaningful insights on the molecular basis of *Lignosus*'s bioactive properties and underscore their importance as a medicinal macro fungus.

4.3 Comparative Transcriptomics Overview

The transcriptome analysis of *L. tigris* reveals distinct expression patterns compared to *L. rhinocerus*, underscoring species-specific functional adaptations. Notably, *L. tigris* exhibited a higher number of highly expressed genes within the COG categories “translation, ribosomal structure and biogenesis” (14 vs. 4 in *L. rhinocerus*) and “lipid transport and metabolism” (5 vs. none in *L. rhinocerus*), suggesting an elevated rate of protein synthesis and lipid-associated activity. In contrast, *L. rhinocerus* showed slightly high expression in categories such as “energy production and conversion,” “secondary metabolite biosynthesis,” and “defence mechanisms,” highlighting differential stress or metabolic responses (Ng et al., 2024; Yap et al., 2015a). Furthermore, *L. tigris* expressed a broader repertoire of abundant transcripts, including serine proteases, heat shock proteins (e.g., HSP70), deoxyribonuclease, phosphatidylserine decarboxylase, RNA-binding proteins, and fungal immunomodulatory proteins (FIPs), many of which were absent or not highly expressed in *L. rhinocerus*. This suggests their role in protecting the cells of organisms from harmful effect of chemical toxins such as UV and oxidative agents. (Anwar et al., 2024; Chidambaram et al., 2024; Hu et al., 2022; Singh et al., 2024; Tagirasa & Yoo, 2022), These transcriptional differences observed despite similarity in cultured environment and nutritional conditions, emphasizes the intrinsic species-specific regulatory mechanisms and divergent ecological or physiological roles.



4.4 Proteomic Studies

Proteomic profiling of *L. rhinocerus* confirmed the presence of key proteins translated from highly expressed genes thus validating their functional roles (Yap et al., 2015b). However, the initial identification of most protein spots using public fungal databases us MALDI-MS is a failure. This highlights the structural divergence of *L. rhinocerus* proteins from other known fungal proteins. However, subsequent LC-MS analysis successfully identified all 40 protein spots, aligned with the species-specific genome. Jacalin-like lectins were among the most abundant proteins (39.13%), aligning with transcriptomic data, and showed multiple isoforms with post-translational modifications such as phosphorylation and glycosylation. These features suggest diverse functional roles beyond gene-level prediction. Other detected proteins include serine proteases (11.08%), glycoside hydrolases (1.90%), and antioxidant enzymes (e.g., manganese superoxide dismutases (0.91%), catalases (0.38%), glutathione transferase (0.54%)), responsible for active defence, metabolism, and stress response mechanisms (Anwar et al., 2024; Chidambaram et al., 2024; Islam et al., 2022; Zheng et al., 2023). 14-3-3 domain proteins (0.07%) and cerato-platanins (12.10%) were also included in the proteome indicating roles in signalling and fungal-host interaction (Yap et al., 2015b). These findings complement transcriptomic data by confirming the actual protein machinery at work, reinforcing the biochemical complexity and medicinal relevance of this macro fungus.

5. Phylogenetic Relationships among *L. rhinocerus*, *L. tigris*, and *L. cameronensis*

Based on the phylogenetics analysis done on ITS sequences of *Lignosus* species, *L. rhinocerus* strongly delineates from both *L. tigris* and *L. cameronensis*. It formed a distinct and well-supported clade with high confidence values (Clade A: Maximum Likelihood = 100%, Maximum Parsimony = 100%, Bayesian Inference = 1.00), highlighting its divergence from other *Lignosus* species (Tan et al., 2013). Meanwhile both *L. cameronensis* and *L. tigris* are placed within Clade B, forming two separate subclades B1 and B2, respectively, together with other closely related species (*L. sacer* and *L. ekombitii*). *L. rhinocerus*, as a sister group to Clade B reveals a deeper evolutionary split, supporting its distinct and unique taxonomy within the genus (Tan et al., 2013). Evidence from this molecular phylogeny aligns with distinct morphological differences, including basidiocarp size, sclerotia structure, and spore characteristics observed earlier.

6. Cultivation of Tiger Milk Mushroom

The traditional usage of TMM has reduced over time because of its rarity and sporadic natural occurrence. Tiger milk mushroom only emerges under optimal environmental conditions, sprouting from its underground sclerotium. Locating wild TMMs is challenging, and labour-intensive as typically only single fruiting body can be found within a radius of approximately 5 km (Fung & Tan, 2019). Environmental destruction such as deforestation and urban development has further threatened the natural growth of TMMs in the wild (Vikineswary & Chang, 2013). As response, many efforts have been undertaken for large scale cultivation of TMM. Initial cultivation using sawdust-based substrate (80% sawdust, 18% wheat bran, 1% sugarcane and 1% CaCO₃) successfully achieved sclerotium formation after approximately 18 months (Huang, 1999). Further advancement in cultivation techniques, via the submerged culture technique using



Mushroom Complete Medium (MCM) has achieved mycelia growth (Rahman et al., 2012). Subsequently, solid-state culture conditions along with the optimization of medium composition and culture conditions were reported to significantly enhance both mycelia biomass and exopolysaccharides production (Lai et al., 2014).

Abdullah et al. (2013) reported the enhancement of optimal mycelia growth using the substrate mixture of sawdust, paddy straw, and spent yeast (7.9:1:1) which facilitated sclerotium formation within 3–4 weeks post-burial. Besides, Lau et al. (2011) and Chen et al. (2013) further advanced cultivation methods by employing submerged fermentation under both static and shaking conditions, including usage of bioreactor systems for large-scale applications. In 2009, a significant milestone was achieved by LiGNO™ Biotech Sdn. Bhd., via the development of a proprietary cultivation protocol for *L. rhinocerus*. This protocol incorporated formulated rice-based medium within environmentally controlled chambers enabling large-scale sclerotium production within 6 months. The resulting strain, designated as TM02®, was subsequently used in pre-commercial safety and efficacy studies, culminating in the official registration of *L. rhinocerus* as a dietary supplement in Malaysia (MAL13025025TC). Following the success, *L. tigris* has been successfully cultivated and the resulting strain is designated as TG-K. Both TM02® and TG-K represent important advancements in the cultivation of TMM, by LiGNO™ Biotech Sdn. Bhd. marking a pivotal step in transitioning these once rare ethnomedicinal fungi into widely accessible and scientifically supported health products (Fung & Tan, 2019).

7. Nutritional Content

The cultivated strain of *L. rhinocerus* (TM02®) and *L. tigris* (TG-K) were reported to possess high nutritional content compared to their respective wild strain thus showing promising potential to be developed into functional foods and nutraceuticals (Yap et al. 2013, 2014a). Besides, sclerotia of *L. rhinocerus*, *L. tigris* and *L. cameronensis* possesses carbohydrates including both monosaccharides and disaccharides with moderate protein content and the fat content being >1% (Fung et al., 2019; Nallathamby et al., 2018; Yap et al., 2014a).

According to Lau et al. (2013), in *L. rhinocerus*, potassium was the predominant mineral found in fruiting body, mycelium and sclerotium, with the mycelium containing the highest concentration (~1.4 g/100 g DW), followed by sclerotium and fruiting body. The mycelium contained the greatest amounts of magnesium, phosphorus, riboflavin, niacin and sodium. Minerals such as iron and manganese were concentrated in the fruit body. Zinc and copper were most abundant in the mycelium. Selenium was present in traces (~0.01 mg/100 g DW) in all three parts of *L. rhinocerus*.

Lignosus cameronensis was reported to have high amount of total carbohydrate, calcium, and energy among the *Lignosus* species. Major amino acids include glutamic acid, aspartic acid, and leucine, with essential amino acids comprising ~40% of the total, slightly higher than *L. tigris*. The potassium content is lower than other *Lignosus* species, but significantly higher than *Pleurotus tuber-regium* (sclerotium-forming mushroom), with a favourable Na:K ratio (<0.6), supporting potential benefits in blood pressure regulation (Fung et al., 2019).



8. Ethnomedicinal Uses of TMM

Tiger milk mushrooms have been traditionally used by various Malaysian aborigines (Semai, Temuan, and Jakun) each with their own folk practices and belief systems due to their significant cultural and medicinal value. According to Burkill (1966), sclerotium of TMM was used in the treatment of respiratory ailments such as coughs and lung congestion. Despite limited formal studies on macrofungal utilization in Malaysia, Chang & Lee (2004) reported the wide usage of this mushroom by Malays, Chinese, and indigenous groups.

Furthermore, indigenous beliefs emphasize TMM as a crucial tonic used in enhancing mental clarity and energy during jungle expeditions. Native forest trekkers are reported to navigate safely back to the base by chewing the sclerotium during their rest periods. Besides, TMMs are used in reduction of swelling; particularly breast swelling in women who have ceased breastfeeding. Villagers in Cameron Highlands, Pahang also reported the wound healing properties of TMM. These properties (swelling reduction and wound healing) of TMM suggest that this mushroom possesses anti-inflammatory and possibly antiproliferative properties, which may suggest its traditional use in reducing tumor-like swellings. Additionally, sclerotiums of TMM have also been used by the Chinese communities for lung and respiratory problems. Above ethnomycological observations highlights the medicinal importance of TMM (Fung & Tan, 2019).

9. The health enhancing effects of TMM

9.1. Immunomodulatory

Lignosus rhinocerus TM02® was reported for its anti-inflammatory properties by strong inhibitory effects on TNF- α and eosinophil infiltration in animal models highlighting its role in treating acute asthma and airway inflammation (Johnathan et al., 2016; Lee et al., 2014). It demonstrates bronchorelaxation, alleviates airway inflammations and asthma symptoms suggesting its role in managing overall lung function (Kong et al., 2025). *L. rhinocerus* has a semi-crystalline structured polysaccharide composed of $\rightarrow 4$)- α -D-Glcp-(1 \rightarrow and $\rightarrow 3$)- β -D-Glcp-(1 \rightarrow linkages which significantly reduce the eosinophil counts, Th2 cytokines, IgE levels, epithelial damage, and airway smooth muscle thickness in an ovalbumin induced asthma mouse model. It also downregulated iNOS gene expression responsible for airway contraction and mucus secretion (Bushra et al., 2025; Salyaev et al., 2007).

TNF- α plays a dual role in cancer, promoting either apoptosis or inflammation-driven tumor growth. *L. rhinocerus* was reported to have selective cytotoxicity against oral cancer cells via TNF signalling pathway modulation with minimal effects on normal fibroblasts. *L. rhinocerus* TM02® induced apoptosis, leading to G0/G1 cell cycle arrest, and inhibition of TNF-related immunomodulators (MIP-2 and COX-2) (Sum et al., 2020; Yap et al., 2022). This finding suggests that the extract modulates TNF signalling via suppression of tumor-promoting inflammation while enhancing apoptotic activity, supporting its potential as a natural anti-cancer agent.



9.2. Anti-inflammatory and Wound healing

Lignosus rhinocerus has been traditionally used by Aborigines as a valuable medicine in wound healing. The combination of *L. rhinocerus* powder and Chinese rice wine has been applied for the treatment of lumps, sores and boils (Wong, 2011). Wound-healing properties, such as improved cell migration and protection against ulcers, also highlights its role in supporting skin health and injury recovery. *L. rhinocerus* shows promising skincare potential due to its ability to inhibit melanin, boost collagen synthesis, and protect against UV-induced damage (Kong et al., 2025). *Lignosus rhinocerus* have been reported to modulate immune response by the increment of cytokines such as IL-5, IL-6, and MIP-2 in RAW 264.7 cells, likely through activation of the NF- κ B and MAPK signalling pathways (Sum et al., 2020). These pathways are reported to play key roles in corneal epithelial repair, scratch-induced injury and cutaneous wound healing (Heo et al., 2011; Thuraisingam et al., 2010). MIP-2 responsible for the early inflammatory phase by guiding granulocyte and stem cell migration. Meanwhile, IL-5 and IL-6 promote inflammation by recruiting immune cells to the wound site and stimulating growth factors involved in angiogenesis and collagen production (Hayta et al., 2018). Thus, the upregulation of IL-5, IL-6, and MIP-2 by *L. rhinocerus* may accelerate the inflammatory phase, enhancing early immune cell infiltration and clearance of neutrophils and macrophages.

9.3. Antioxidative

Antioxidants prevent oxidative damage and inhibit lipid oxidation through free radical scavenging (Abdullah et al., 2012). The antioxidant richness of TMM extract unveils significant scavenging activities against free radicals, which might be useful in the mitigation of oxidative stress related damage (Kong et al., 2025). Their antioxidant potential was demonstrated by DPPH scavenging activity with IC₅₀ values comparable or lower than most medicinal mushrooms (Yap et al., 2014a). Superoxide radicals produced from metabolism can trigger ROS formation, causing oxidative damage in DNA, lipids and proteins thus contributing degenerative diseases (Fang et al., 2002; Valko et al., 2007). Interestingly, both *L. rhinocerus* (TM02) and *L. tigris* (TG-K) were reported to show strong superoxide anion scavenging activity which could be attributed to the presence of soluble polysaccharides suggesting their role in the prevention of oxidative stress (Kong et al., 2016b; Yap et al., 2013). Additionally, β -glucan extracted from *L. rhinocerus* mycelium demonstrated notable antioxidant activity, with DPPH and ABTS radical scavenging rates of 53.31% and 47.31%, respectively that were comparable to, or even exceeded, those of the sclerotium (Lertworapreecha et al., 2025). Comparative analysis performed on CWE of *L. cameronensis* (LC-CWE) exhibited moderate antioxidant activity, with notable Trolox equivalent antioxidant capacity and high phenolic content (Yap et al., 2024).

9.4. Antitumor and Cytotoxic Components

Lignosus tigris TG-K was reported to inhibit the growth of MCF-7 (breast), A549 (lung), PC3 cells (prostate) (IC₅₀: 28.9 – 95.0 μ g/mL). Besides, *L. tigris* (TG-K) selectively induced apoptosis in MCF-7 cells via cell death proteins activation (caspase 8, 9 and Bax) and Bcl-2 suppression. Other major cytotoxic components such as lectins, serine proteases and DNase were also identified via proteomic analysis (Yap et al., 2018). Similarly, a serine protease (termed F5) isolated from *L. rhinocerus* TM02 has been reported to exhibit strong cytotoxicity toward MCF-7



cells by apoptosis induction (Yap et al., 2018). Interestingly, in *L. tigris*, 74.6% serine proteases were produced by the gene responsible for F5 serine protease production (Kong et al., 2020). Selective cytotoxicity against MCF-7 breast cancer cells ($IC_{50}=215 \mu\text{g/mL}$) and anti-inflammatory effects with an ED_{50} of 60 mg/kg in a carrageenan-induced rat paw oedema model were also expressed in *L. cameronensis* (Yap et al., 2024). These findings highlight the role of TMMs as anti-cancer agent in the traditional use against breast cancer treatment.

TM02® was demonstrated to be well tolerated in patients with localized breast cancer post-chemotherapy, exhibiting minimal mild side effects and no changes in vital signs or biochemical parameters. This supplementation demonstrated a positive trend toward improved physical functioning and reduced fatigue, aligning with prior findings on mitochondrial function (Eng et al., 2025). Cold water extract (CWE) of *L. rhinocerus* TM02®(xLr®) exhibited selective antiproliferative effects against MCF-7 and A549 cancer cells, with no significant toxicity against normal cell lines. Its anti-proliferative activity was linked to apoptosis and attributed to a high-molecular-weight fraction rich in carbohydrates and low in protein, suggesting the role of a protein-carbohydrate complex in cytotoxicity (Lee et al., 2012; Yap et al., 2013). *L. rhinocerus* sclerotia has also been reported to exhibit antiproliferative effects against HCT 116 (colorectal cancer cells) with no toxicity against normal cells via apoptosis induction and cell cycle arrest. However, the strain of *L. rhinocerus* used in this study was not specified (Zaila et al., 2013).

Angiogenesis refers to formation of blood vessels from pre-existing blood vessels surrounding tumor cells that provides required nutrients and oxygens. This helps the tumor cells to sustain their growth, survival and metastasis (Jiang et al., 2020). Hot-water extracts (HWE) of *L. rhinocerus* sclerotium showed anti-angiogenic and anti-tumor effects without toxicity against normal cells, reducing tumor size and blood vessel formation in HCT116 xenografted embryos (Yong et al., 2024). These findings highlighted the possible mechanisms of TMMs anti-cancer effect apart from the cytotoxicity and apoptotic induction.

9.5 Anti-HIV

Lignosus rhinocerus cold water extract (xLr®) significantly inhibited the activities of HIV-1 protease (PR) and reverse transcriptase (RT) by 25–55%, while the hexane extract (LRH) selectively and strongly inhibited HIV-1 PR activity by 88.97%. LRW also markedly reduced HIV-1-induced syncytial formation and p24 antigen production in infected MOLT-4 cells at a concentration of 0.5 mg/ml. Chemical analysis revealed that the major constituents in the extracts were peptides, terpenoids and fatty acids. In silico docking studies reported strong binding affinities of helianthriol F and 6 α -fluoroprogesterone for HIV-1 PR and RT, respectively. These findings highlight the preventive and curing potential of *L. rhinocerus* as a source of bioactive compounds in developing novel inhibitors targeting HIV-1 PR and RT (Sillapachaiyaporn & Chuchawankul, 2020; Wong et al., 2024).

10. Toxicological Studies

No signs of dermal toxicity were observed in rats provided with cultivated *L. rhinocerus* sclerotial powder TM02® at 2000 mg/kg, accompanied by normal behaviour, body weight, organ function,



and histology (Yap et al., 2025). Besides, sub-acute oral toxicity studies conducted on *L. rhinocerus* (TM02®), *L. tigris* (TG-K) and *L. cameronensis* confirmed that TMMs' sclerotial powder is safe for oral consumption at doses up to 1000 mg kg⁻¹, with no adverse effects observed on body weight, behaviour, food and water intake, haematological or biochemical parameters, organ weights, or histopathology of major organs (spleen, heart, kidney, liver, lung). Additionally, *L. rhinocerus* TM02® showed no adverse effects on fertility, teratogenicity, or genotoxicity in extended evaluations (Kong et al 2016a, b; Lee et al., 2013; Lee et al., 2017). These findings collectively established a no-observed-adverse-effect level (NOAEL) above 1000 mg/kg for both species, thus highlighting their safe use for medicinal, nutraceutical, pharmaceutical and cosmetic applications.

Future Studies

To date, research on *L. cameronensis* has been limited to preliminary studies of its nutritional composition, medicinal potential, and toxicity assessments. LC-CWE displayed distinct bioactive properties, highlighting its therapeutic potential and warranting further mechanistic investigation compared to other *Lignosus* species. Despite its promising bioactive profile, *L. cameronensis* remains largely underexplored with no published omics data to date. Omics-based studies such as genomics, transcriptomics, proteomics are crucial to be carried out to uncover the molecular basis of its bioactive mechanisms. This can help in uncovering its complete bioactive potential, clarifying the mechanisms underlying its medicinal properties, and enabling valuable comparisons among other *Lignosus* species. Omics study will also support and pave the way for further pharmacological research, cancer-focused investigations, mechanism-based assays and bioactive compound isolation essential for validating its therapeutic potential.

Conclusion

The Tiger milk mushroom (TMM) possesses significant ethnomedicinal value and has been traditionally used in treating wide range of ailments. This review highlights several potential therapeutic effects of TMM that are beneficial to human health such as antioxidant, anti-inflammatory, immunomodulatory and anticancer effects. Among the three local species studied from the genus *Lignosus* (*L. rhinocerus*, *L. tigris*, *L. cameronensis*), most pharmacological investigations have focussed on *L. rhinocerus*. However, *L. cameronensis* remains underexplored despite its promising bioactive compounds and biomedicinal potential. Future research should focus on elucidating the molecular mechanisms and bioactive compounds of *L. cameronensis* to better understand its medicinal properties and to support the development of evidence-based therapeutic application.

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