POTENTIAL FUNCTIONAL INGREDIENTS FROM TIGER MILK MUSHROOM

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ABSTRACT

Tiger Milk Mushroom (also known as "cendawan susu rimau (harimau)", Lignosus rhinocerotis), hailed as Malaysia's national treasure has been used as a medicinal mushroom since 1664 by local communities to treat asthma, fever, cough, cold, sinusitis, cancer, food poisoning, for wound healing and as a general tonic. In 2002, Tun Dr. Mahathir Mohamad mentioned in his speech at BioMalaysia that his chronic cough was cured by a local mushroom called "cendawan susu rimau (harimau)". Despite its high medicinal values, little attempts had been made to study it, due to the very limited supply as it can only be wild collected from the forest by chance, hence hindering the discovery of its full potential for health and economical benefits. We have now successfully cultivated the mushroom in a controlled environment producing high yield. Our success in inducing the formation of sclerotia which eventually led to development of stem and formation of the mushroom cup is no small feat, This paper reports the research and development journey of the Lignosus rhinocerotis cultivar development for the past 10 years, its safety and efficacy investigations as part of attempt to validate the medicinal benefits and ethno-botanical claims of Tiger Milk Mushroom.

INTRODUCTION

Some 400 hundred years ago, an important medicinal product was given to an European government agent who sailed to this region, The Diary of John Evelyn (publication dated 22 June 1664) recorded the name of the medicinal product as "*Lac tygridis*" (meaning tiger's milk). It was described as a fungus used by local communities to treat diseases that European doctors could find no cure to. This fungus was also clearly described by Sir Henry Nicholas Ridley, "the father of Malaya's rubber industry" (Ridley, 1890), as an important medicinal mushroom used by the local communities. In the year 1890, Cooke pioneered the scientific documentation of this fungus and named it as *Fomes rhinocerotis* using a specimen obtained from Penang. The scientific synonym commonly used currently is *Lignosus rhinocerus/rhinocerotis.*

The Tiger Milk Mushroom (*Lignosus rhinocerus*) is an important medicinal mushroom in Southeast Asia and is one of the most popular medicinal mushroom used by indigenous communities of Peninsular Malaysia (Lee et al., 2009). The Tiger Milk Mushroom, "Cendawan susu rimau (harimau)" has traditionally been used by the Malays, Chinese and indigenous communities in Malaysia for treatment of cough, fever, chronic

hepatitis, gastric ulcer, liver and breast cancer food poisoning and as a general tonic (Chang and Lee, 2004; Wong et al., 2009; Lee et al., 2009). Even our former prime minister, Tun Dr. Mahathir, during his opening speech at Biomalaysia, 2002 mentioned that his chronic cough had been cured by this medicinal mushroom. In China, *L. rhinocerus* has also been used in the traditional Chinese medicine to treat liver cancer, chronic hepatitis and gastric ulcer (Huang, 1999). Research findings have shown that the sclerotial polysaccharides from the mushroom demonstrates very high anti-inflammatory, antioxidant, anti-proliferative (Lai et. al. 2008) and immuno-modulating effects (Wong et al., 2009; Wong et al., 2010; Guo et al., 2011).

L. rhinocerus is structurally characterised to have a central stipitate pilei arising from distinct sclerotia. The sclerotium of *L. rhinocerus* is the part with medicinal value. Its geographical distribution is only in the tropical rainforest in the region of Thailand, Malaysia, Indonesia, Philippines, and Papua New Guinea. The existence of this mushroom in the jungle is always solitary and this makes the collection of its sclerotia a difficult task. As a result, its supply is limited and the sclerotium is costly. Recently, we reported a breakthrough in the cultivation of the *Lignosus rhinocerus* strain, LiGNO™ TM02 on agar, solid and spawn medium with high production yield (Tan et al., 2009), thus overcoming the supply problem.

The nutrition industry is ever growing, despite poor economic outlook. The market for functional foods remains bullish. In this modern society, functional food demandis driven by the desire for products that help build and maintain optimum health. Functional foods contain special ingredients with unique beneficial effects from cardiovascular to mental health function, immunity. The list of contemporary health issue of public concern is growing rapidly. The continuous demand for nutraceuticals creates new opportunities and commercial pressures. Hence, the nutrition industry is always looking for potential functional ingredients. The cultivated *L. rhinocerus* has a huge potential to be exploited in this arena.

ISOLATION AND CULTIVATION OF TIGER MILK MUSHROOM

Although *L. rhinocerus* is recognized as a rare species, it still can be found in the jungle. The study started with *L. rhinocerus* specimens collected from the forest in Cameron Highland, Sungai Perak, Gerik, Hulu Langat, and Raub. The spore and the tissue from its sclerotium, stem and pileus were grown in a special formulated culture media. The mycelium growth was then subjected to many cycles of sub-culturing in order to obtain a clean, pure culture. Genetic marker was developed in order to authenticate the specimen collected (Tan et al., 2010). The *L. rhinocerus* culture growth conditions in liquid and solid media were optimised. Since the sclerotia of *L. rhinocerus* is the part that contained medicinal properties, cultivation factors and conditions for the formation of sclerotia were also determined and optimised. The cultivation process takes approximately 6 months (for the sclerotia formation) to up to 2-3 years (for stem and pileus formation).

At present, we are able to produce the sclerotia of *L. rhinocerus* at commercial scale in an environmentally controlled culture room. The production is about 100 kg per month and can easily be expanded by increasing the parameters of its culture room.

SAFETY ASSESSMENT

In the development of any functional ingredient, the safety has to be established based on its historical use, its intrinsic nature, usage or based on information generally known and accepted by qualified scientific experts.

Although history reports that *L. rhinocerus* has been extensively used safely for over hundreds of years, a scientific assessment remains essential. Preliminary toxicity study showed that oral administration of the cultivated sclerotial powder at daily dose of up to 10% of the experimental animals' body weight continuously for three months did not show any adverse effects. Various toxicity studies in compliance with the OECD guidelines were subsequently conducted.

Acute toxicity study showed that there were no treatment-related acute toxicities in rats following oral administration at a high dose of up to 2000 mg/kg. 28 days sub-acute toxicity study (Lee et. al., 2011) showed that oral administration of the sclerotial powder at daily dose of up to 1000 mg/kg had no adverse effects on the growth rate, hematological and clinical biochemical parameters. Histological studies showed that the treatments did not induce any pathological changes in the liver, kidney, heart, spleen and lung of the animals. As the highest tested dose of 1000 mg/kg was not associated with any toxicity concerns, the NOAEL dose is concluded to be higher than 1000 mg/kg.

The cultivated *L. rhinocerus* was also subjected to corticosteroid screening by Toxicology Laboratory, National Poison Centre. The results confirmed the absence of corticosteroid as part of its ingredient.

Currently, there are few other ongoing toxicity studies such as chronic toxicity, genotoxicity, mutagenicity and reproductive toxicity studies.

EFFICACY ASSESSMENT

Continuous research on the bioactive properties of *L. rhinocerus* is essential to generate proof to substantiate the ethno-botanical claims of *L. rhinocerus* and to support its contentst with evidence-based proofs.

Anti-inflammatory

The present study investigates the anti-inflammatory activity of the sclerotia of the cultivated *L. rhinocerus* using carrageenan induced anti-inflammatory model (rat paw edema). The cold water extract has shown significant anti-inflammatory effects comparable to standard drug, indomethacin (unpublished data).

Immuno-modulating

Sclerotial polysaccharides of *L. rhinocerus* have been associated in animal studies with multiple bioactive properties. Extracts of *L. rhinocerus*, and particularly the sclerotial polysaccharide constituents have shown stimulatory effects on human innate immune cells. The constituents have also been associated with immune modulation in preclinical study, and are hypothesised to exert anti-tumor effects as a result of these immune properties (Wong et. al., 2009; Wong et. al., 2010; Guo et. al., 2011).

Anti-Oxidant

The findings from a recent *in vitro* study (unpublished data) suggest that the antioxidant capacity of the mushroom sclerotium is comparable to many other medicinal mushrooms, which is generally moderately low. The water extracts, however, exhibited strong superoxide anion scavenging activity, indicating that the extract might be helpful in preventing certain type of oxidative stress. Taken as a whole, the *L. rhinocerus* sclerotial powder offer a promising source of functional ingredient potentially attributed to its antioxidant capacity, specifically the superoxide anion scavenging activity.

Anti-proliferative

Lai et al. (2008) was the first to investigate the anti-proliferative effects of the sclerotial polysaccharides of the mushroom. They reported that the hot water extract of *P. rhinocerus* (synonym of *L. rhinocerus*) exhibited anti-proliferative activity against different kinds of leukemic cells.

Recently, Lee et. al. (2012, *in press*) reported that the cold water extract of the sclerotia of the cultivated *L. rhinocerus* exhibited significant anti-proliferative activity against the breast cancer cell MCF-7 and lung cancer cell A549. Their results also showed that the cold water extract was essentially not cytotoxic against the normal breast and lung cells. Its cytotoxicity action is due to a high molecular weight fraction isolated from the cold water extract, and that the cytotoxic action is mediated via apoptosis. The anti-proliferative action against MCF-7 cells provides a plausible scientific basis for the traditional use of *L. rhinocerus* sclerotia in breast cancer treatment by the Malaysian indigenous communities.

REGULATORY AND PRODUCT REGISTRATION

Traditional medicine is a system of medicine based on cultural beliefs and practices handed down from generations to generations. The practice continues until today and the World Health Organization (WHO) estimates that 65-80% of the world's population rely on traditional medicine for their primary health care needs.

Regulation of traditional medicine in Malaysia began in 1992 and applications for product registration have to be submitted to the National Pharmaceutical Control Bureau (NPCB). Only ingredients listed in the NPCB Traditional Medicine Active Ingredients List are eligible to be used in any registered products. Unfortunately *L. rhinocerus* was not in the list prior to September 2010. In order to qualify as functional ingredient, we have submitted to NPCB the supporting documents on the safety, history of use and bioactivities of *L. rhinocerus*. NPCB had accepted *L. rhinocerus* to be listed in the traditional medicine active ingredient list in September 2010. This enables *L. rhinocerus* to be exploited commercially as a functional ingredient.

POTENTIAL FUNCTIONAL INGREDIENT

Functional foods have entered the global market at full force in the past decade and have rapidly gained market share as a value added product. This category is generally thought to include products that influence specific functions in the body and thereby offers benefit for health, well-being or performance, beyond their regular nutritional value. As natural

substances are clearly preferred over chemical ones, functional botanical ingredients are more popular than ever in the functional food market. Thus, *L. rhinocerus* may be proposed as a new potential functional ingredient due to its high anti-inflammatory, immuno-modulating and antioxidant activity. Its bioactivities render it a valuable item to be incorporated in functional foods, beverages and nutraceuticals.

CONCLUSION

The full potential of *L. rhinocerus* high in medicinal and nutritional values has never been realised due to its limited supply. Now with the successful cultivation technology at commercial scale, the supply problem is a thing of the past. In order to further qualify as a functional ingredient, evidencebased safety and efficacy assessment is crucial and essential.

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REFERENCES

Chang, Y.S. and Lee, S.S. (2004). Utilisation of macrofungi species in Malaysia. Fungal Diversity. 15, 15-22.

Cooke (1879). 'XV. Enumeration of Polyporus'. *Trans. & Proc. Bot. Soc. Edinb.* 13(1): 131-159.

Guo, C., Wong, K.H, Cheung, P.C.K. (2011). Hot water extract of the sclerotium of *Polyporus rhinocerus* Cooke enhances the immune functions of murine macrophages. *Int J MMushrooms.* 13(3):237-44.

Huang N L (1999). Identification of the scientific name of Hurulingzhi. Acta Edulis Fungi, 6(1), 30-32.

John Evelyn; William Bray; Georges W.E. Russell. The Diary of John Evelyn. / Volume 1. London; New York : J.M. Dent & Sons : E.P. Dutton, 1907. Pg. 373-374.

Lai, C.K.M., Wong, K.H. and Cheung, P.C.K. (2008). Antiproliferative effects of sclerotial polysaccharides from *Polyporus rhinocerus Cooke* (*Aphyllophoromycetidead*) on different kinds of leukemia cells," *Int. J. Med. Mushroom, vol.* 10, no.3, pp. 255-264.

Lee, M.L., Lee, S.S., Tan, N.H., Fung, S.Y., Tan, C.S. and Ng, S.T. (2010). Investigation on the anticancer properties and subacute toxicity of cultivated tiger milk mushroom (*Lignosus rhinocerus).* FEBS Journal. 277 (Suppl 1), 62.

Lee, M.L., Tan, N.H., Fung, S.Y., Tan, C.S. and Ng, S.T. (2012, In press). The anti-proliferative activity of sclerotia of *Lignosus rhinocerus* (tiger milk mushroom). *Evidence-Based Complementary and Alternative Medicine. In Press.*

Lee, S.S., Chang, Y.S., Noraswati, M.N.R. (2009). Utilization of macrofungi by some indigenous communities for food and medicine in Peninsular Malaysia. Forest Ecology and Management. 257, 2062–2065.

Lee, S.S., Tan, N.H., Fung, S.Y., Pailoor, J., Sim, S.M. (2011). Evaluation of the sub-acute toxicity of the sclerotium of Lignosus rhinocerus (Cooke), the Tiger Milk mushroom. *J Ethnopharmacol.* 2011 Oct. 31;138(1):192-200. Epub 2011 Sep 10.

Ridley, H.N. (1890). On the so-called Tiger's Milk, "Susu rimau" of the Malays. Journal of the Straits Branch of the Royal Asiatic Society. Pg. 341-344.

Ryvarden, L. and Johansen, I. (1980). A preliminary polypore flora of East Africa. Fungiflora, p636. (Olso, Norway).

Tan, C.S., Ng, S.T., Vikineswary, S., and Tee, C.S. (2009). Development of Lignosus rhinocerus (Cendawan Susu Rimau) Cultivar - Bring to Life to a Valuable Malaysian Medicinal Mushroom. Paper presented at International Congress of Malaysian Society for Microbiology (ICMSM 2009), 1-4 December, Penang, Malaysia.

Tan, C.S., Ng, S.T., Vikineswary, S., Lo, F.P. and Tee, C.S. (2010). Genetic markers for identification of a Malaysian medicinal mushroom *Lignosus rhinocerus* (Cendawan susu rimau). Acta Hort. (ISHS). 859, 161-167.

Wong, K.H. and Cheung, P.C.K. (2008). Sclerotium of culinary-medicinal king tuber oyster mushroom, pleurotus tuber-regium (Fr.) Singer (Agaricomyccidcae) : its cultivation, bio-chemical composition, and biopharmacological effects (review). Int J Med Mushr. 10 : 303-13.

Wong, K.H., Lai, C.K.M., Cheung, P.C.K. (2009). Stimulation of human innate immune cells by mushroom sclerotial polysaccahrides. *International Journal of Medicinal Mushrooms,* 11, 215-223.

Wong, K.H., Lai, C.K.M., Cheung, P.C.K. (2010). Immunomodulatory activities of mushroom sclerotial polysaccharides. *Food Hydrocolloids*, 25, 150-158.