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Edited by J.J.P. Baars & A.S.M. Sonnenberg

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The Medicinal Properties and Efficacy of Cultivated Tiger Milk Mushroom (*Lignosus rhinocerotis* TM02)

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ABSTRACT

Lignosus rhinocerotis, commonly known as Tiger Milk Mushroom, has been used for more than 400 years by South East Asian communities for its healing properties on more than 15 types of medical ailments including asthma, fever, cough, cold, sinusitis, cancer, food poisoning, joint pain, and wound healing. It has also been consumed as general health tonic for improving vitality. However, research and development of this rare mushroom was hindered by its limited supply from the wild. Recent successful commercial cultivation and mass production of *L. rhinocerotis* TM02 has enabled numerous scientific studies to be conducted to verify claims of its bioactivity and, the efficacy and safety profile of *L. rhinocerotis* TM02. Scientific investigations revealed that *L. rhinocerotis* TM02 contains various active components such as the polysaccharides-protein complexes, β -glucan, protein, immune-modulatory proteins, all of which are biologically active in rendering significant medicinal properties such as anti-inflammatory, immuno-modulatory, anti-proliferative, antioxidative, neurite outgrowth stimulation etc. Genomic study has shown the presence of a novel fungal immuno-modulatory protein (FIP) in this mushroom. The gene sequence and three dimensional model of this novel fungal immuno-modulatory protein have been recently elucidated. Pre-clinical toxicology evaluations showed that *L.rhinocerotis* TM02 did not induce any adverse effect on fertility, teratogenicity and genotoxicity. The no-observed-adverse-effect level (NOAEL) dose of the *L.rhinocerotis* TM02 in a 180-day chronic toxicity study is more than 1000 mg/kg which is equivalent to 162 mg/kg human dose whilst the anticipated dose for a 60 kg adult is only ~10g. The effective dosage of *L.rhinocerotis* TM02 is 0.5g per day for an adult, therefore there is a wide safety margin for consumption. Based on users' experiences and feedback, *L.rhinocerotis* TM02 has been shown to be effective in a broad spectrum of respiratory health and immune health conditions, maintaining the health of joints, improving vitality and stamina, and improving the quality of life of cancer patients.

Keywords: anti-inflammatory, immuno-modulatory, anti-proliferative, antioxidative.

INTRODUCTION

Lignosus rhinocerotis (Cooke) Ryvardeen, locally referred to as Tiger Milk Mushroom, is one of the most popular medicinal mushrooms consumed by the various communities in South East Asia from time immemorial. It belongs to the family of *Polyporaceae* (Basidiomycota) and consists of sclerotium, fruiting body with pileus and stipe (Cooke, 1879). The sclerotium is the part containing valuable medicinal value (Chang and Lee, 2004). Its history of usage can be traced back to 1664 when it was obtained from the local indigenous people and was described as “*Lac tygridis*” (in Latin) and was recorded to be used for treating diseases (Everlyn, 1664). Both Ridley (1899) and Burkill (1966) described a fungi species, *Polyporus rhinocerus* (synonym of *Lignosus rhinocerotis*) (in Malay ‘*susu rimau*’ translating to “tiger’s milk”) and recorded its use to treat congestion and cough. A survey by Lee *et al* (2009) showed that the *Lignosus* species (‘*susu rimau*’) is the most common macrofungi used by majority of the indigenous communities in Peninsular Malaysia. These indigenous communities usually use it to treat cough, asthma, fever, cancer, food poisoning, wound healing, and as a general tonic. For postpartum, the traditional way of preparation is to boil sliced sclerotium with other herbs such as “tongkat ali” (*Eurycoma longifolia*) or with *Polyalthia bullata* (black tongkat). For other purposes, it is orally consumed as a decoction (Chang and Lee, 2004; Lee *et al*, 2009). In China, *Polyporus rhinocerus*, or ‘*hurulingzhi*’ in Chinese, is a common traditional medicine to treat liver cancer, chronic hepatitis and gastric ulcer (Huang, 1999). Despite its various traditional claims, there are limited scientific studies of the mushroom mainly due to its scarcity and difficulty in cultivation. It was not until 2009 that the cultivation of *L. rhinocerotis* TM02 was successful and subsequently commercialized. The unlimited supply from its successful cultivation enabled biopharmacological studies, and made therapeutic use possible (Tan, 2009).

1. Results & Discussion

1.1. Identification and Authentication

In Malaysia, three species of *Lignosus* were discovered. Besides the commonly known *Lignosus rhinocerotis*, two other species, *L. cameronensis* and *L. tigris* were identified (Tan *et al*, 2013). They are similar in morphology, and their identification and differentiation is largely based on pileus pore size. However this identification criteria can be imprecise and unreliable. Identification by genetic markers is more precise and hence, genetic markers were developed in 2010 to allow identification of *L.rhinocerotis* in mycelia culture and the sclerotia where pileuses are absent (Tan *et al*, 2010). The nucleotides identity in internal transcribed spacer (ITS) gene region of rRNA of known *Lignosus* sp are sequenced, compared and subsequently, a specific primer pair based on the DNA sequences within the ITS region is designed as the specific genetic marker for *L.rhinocerotis* (Tan *et al*, 2010).

1.2. Healthful composition

This list below are the content of certain important healthful composition found in *L. rhinocerotis* TM02 : β -1,3/1,6-glucan = 286.8 mg/g, α -glucan = 29.91 mg/g, Adenosine (mw 264.24) = 0.282 mg/g, Cordycepin (mw 251.24) = 0.873 mg/g, Adenine (mw 135.13) = 0.024

mg/g, Hydroxyethyl-adenosine (mw 311.12) = 0.268 mg/g, Ethyl-Adenosine (mw 295.12) = 0.399 mg/g, Glycoprotein = 186.1 mg/g, and Total Polysaccharides = 401.4 mg/g.

1.3. Safety Evaluation

As *L. rhinocerotis* became increasingly popular in recent years as a nutraceutical, safety evaluation of the sclerotial powder likewise, has become an important prerequisite. The *Lignosus rhinocerotis* TM02 cultivar were selected for production into sclerotial powder, and the recommended dosage is 5–10 mg/kg/day. Pre-clinical studies (using *Sprague Dawley* rats) were carried out to ascertain its safety profile. Acute (7 days), subacute (28 days) and chronic (180 days) toxicity study revealed no adverse effect with doses up to 2000mg/kg/day (for acute), 1000mg/kg/day (subacute and chronic) respectively (Lee *et al*, 2013; Lee *et al*, 2011). There were no adverse change or toxic effect on body weight, haematological and clinical biochemistry parameters and histological examination . The toxicity studies' dosage is equivalent to 162 mg/kg human dose and the anticipated dose for a 60 kg adult is only ~10g which translates to 0.5g per day for an adult, thus there is wide safety margin. In terms of impact on fertility and teratogenicity, oral administration of 100mg/kg/day sclerotial powder for 7-8 weeks did not cause any adverse effect in rats. *In vitro* assessment of genotoxicity was conducted using Ames test and the results revealed no gene mutations. In conclusion, the no-observed-adverse-effect level (NOAEL) dose for fertility, teratogenicity and genotoxicity studies was determined to be higher than 1000mg/kg (Lee *et al*, 2013). The establishment of safety profile of *L.rhinocerotis* TM02 ensures safe consumption of the mushroom as a nutraceutical to promote general well-being and a multitude of medicinal purposes.

1.4 Biopharmacological Efficacy Assessment Based on Scientific Studies

Research on the pharmaco-properties of *L.rhinocerotis* is essential to generate evidence-based proofs to substantiate the ethno-botanical claims of *L.rhinocerotis*.

Anti-inflammatory: The present study investigates the anti-inflammatory activity of the sclerotia of the *L.rhinocerotis* TM02 using carrageenan induced anti-inflammatory model (rat paw edema). The anti-inflammatory effect of methanol extract, cold water extract and hot water extract was measured via carrageenan-induced paw oedema test in *Sprague Dawley* rat. All three extracts exhibited significant anti-inflammatory effect *in vivo*, with highest potency in cold water extract (Lee *et al*, 2011). There was an unpublished *in vivo* study on ovalbumin-sensitized asthmatic *Sprague Dawley* rats treated with *L.rhinocerotis*. The study showed the ability of *L.rhinocerotis* treatment to reduce total immunoglobulin E in serum, cytokines e.g. IL-4, IL-5, and IL-13 in bronchoalveolar lavage fluid, and also reducing lung eosinophil infiltration

Immuno-modulating: Extracts of *L.rhinocerotis*, and particularly the sclerotial polysaccharide constituents exhibit 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*, 2010; Guo *et al*, 2011). Genomic and proteomic studies showed that *L.rhinocerotis* contains fungal immunomodulating protein (FIP) that may be attributed to this activity (Yap *et al*, 2014).

Anti-Oxidant : *L.rhinocerotis* TM02 exhibited ORAC 6.0 value of 1,480 umol TE/g. A recent *in vitro* study (Yap *et al*, 2013) suggested 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.rhinocerotis* sclerotial powder offers a promising source of functional ingredient . Its efficacy in this function is 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. rhinocerotis* exhibited anti-proliferative activity against different kinds of leukemic cells. Recently, Lee *et al* (2012) reported that the cold water extract of *L.rhinocerotis* TM02 exhibited direct specific cytotoxicity on human breast carcinoma (MCF-7) and human lung carcinoma (A549) cell lines with IC50 of 96.7µg/ml and 466.7µg/ml. Their results also showed that the cold water extract was essentially not cytotoxic against the normal breast and lung cells. Its cytotoxic action is attributed 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.rhinocerotis* sclerotia in breast cancer treatment by indigenous communities in Malaysia.

1.5. Efficacy Assessment Based on Functional Observation

A survey was conducted among the volunteers who had taken Tiger Milk Mushroom for various health concerns. The volunteers were given 0.5g of Tiger Milk Mushroom per day continuously for 1-2 weeks and response were collected after the completion of treatment. The volunteers' testimonials can be categorized as follows:

Relief of respiratory-related illness: Tiger Milk Mushroom improves breathing in patients with respiratory health concerns. It also enables them to expel phlegm with ease, especially for volunteers who smoke.

Relief of asthmatic symptoms: One of the most prominent benefits of Tiger Milk Mushroom is its ability to relieve asthmatic symptoms. It improves the patients' breathing and also reduces the frequency of inhaler usage. For those suffering from asthmatic attacks, consumption of Tiger Milk Mushroom was able to shorten the recovery period and decreases the recurrence of subsequent asthmatic attacks.

Treatment of chronic cough: Tiger Milk Mushroom had successfully cured many chronic cough cases, and the testimony of Malaysia's former Prime Minister, Tun Dr. Mahathir (who is a medical doctor by training) was one of the most prominent report.

Relief of allergy: Tiger Milk Mushroom helps to relief various allergy symptoms including respiratory allergy such as nasal and sinus symptoms. In cases of skin allergy such as eczema and allergy to food or chemicals, it has been reported that rashes subsided within few days of tropical application.

Treatment of joint pains: Tiger Milk Mushroom was found to be effective in the treatment of joint pains (ie as a result of dengue fever), one week after consumption. It is also effective

in relieving joint pains in the elderly, rheumatoid arthritis and osteoarthritis patients. This may be attributed to its anti-inflammatory activity.

Improvement of stamina: Tiger Milk Mushroom was also found to improve stamina and alertness in healthy volunteers. It also significantly prolongs the stamina of athletes (unpublished survey).

Anti-tumor: Traditionally, tiger milk mushroom was used to heal cancer, especially breast cancer. Cancer patients who consumed Tiger Milk Mushroom for more than a week showed greatly improved the quality of life and were more energetic.

REFERENCES

All papers cited are list in <http://www.ligno.com.my> under the section publications.