



## Evaluation of the Antioxidant and Melanogenesis Inhibitory Properties of *Pracparatum Mungo* (Lu-Do Huang)

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### ABSTRACT

*Pracparatum mungo* (Lu-Do Huang) is a traditional Chinese functional medicine made from the natural fermentation of mung bean (綠豆 *Lǜ Dòu*) mixed with other Chinese medicines. It has been recognized as having liver protecting and detoxifying effects. As mung beans have been verified to possess anti-inflammatory, antioxidant, antipyretic, and whitening actions, the present research utilized the *in vitro*, *ex vivo*, and *in vivo* experimental models to investigate the antioxidant and melanin inhibiting effects of *P. mungo* on the skin. The *in vitro* experiment revealed that *P. mungo* methanol extract (PMME) and *P. mungo* ethanol extract (PMEE) possess the capacity to clear  $\alpha, \alpha$ -diphenyl-2-picrylhydrazyl (DPPH) radicals and inhibit tyrosinase activity. The *ex vivo* experiment indicated that PMEE can promote the growth of MDCK cells and increase the enzymatic activities of superoxide dismutase (SOD) and catalase in MDCK cells. On the other hand, PMME and PMEE can suppress the proliferation of A375 cells, and PMEE can reduce the enzymatic activities of SOD and catalase in A375 cells. The *in vivo* results showed that *P. mungo* can enhance the enzymatic performance of SOD, Catalase, and glutathione peroxidase (GPx) in the liver. The results also showed that *P. mungo* has antioxidant characteristics and can inhibit tyrosinase activity, thereby promoting the growth of skin tissues and suppressing the proliferation of A375 cells, and thus enhancing the effects that the antioxidant enzymatic performance has on the liver. These results can be applied in the development of tyrosinase inhibitors or antioxidants used for the inhibition of melanin biosynthesis or for auto-oxidation in further industrial applications, particularly those relating to functional food or cosmetic compositions.

**Key words:** Anti-melanogenesis, Antioxidant, *Pracparatum mungo*, Tyrosinase inhibitor

### INTRODUCTION

Melanin production is principally responsible for skin color and plays a significant role in protecting the skin from ultraviolet (UV) light; however, overproduction and accumulation of melanin can result in various dermatological disorders including melisma, freckles, age spots, and sites of actinic damage or other hyperpigmentations.<sup>[1,2]</sup>

Thus, melanogenic inhibitors have become increasingly important ingredients in medication<sup>[3]</sup> and cosmetics<sup>[4]</sup> to prevent hyperpigmentation. Tyrosinase is a copper-containing enzyme catalyzing the oxidation of *o*-diquinones and *o*-diphenols in the first stages of melanin biosynthesis. This enzyme is also responsible for the undesired browning reactions in damaged fruits during post-harvest handling and processing.<sup>[5]</sup> Many tyrosinase inhibitors are topically

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used for treating localized hyperpigmentations such as lentigo, nevus, ephelides, melisma, and post-inflammatory states in humans.<sup>[6,7]</sup>

Previous studies of cultured murine and human skin cells<sup>[8-12]</sup> and human skin<sup>[13-15]</sup> have shown that photodamage involves the generation of reactive oxygen species (ROS) and the depletion of endogenous antioxidant networks. To minimize ROS-induced injuries, skin possesses antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD). Exposure to UV light and oxidative stress caused by excessive ROS are casually linked to skin disorders,<sup>[16]</sup> indicating that antioxidants may benefit skin health. It is also known that ROS play significant roles in the regulation of melanocyte proliferation and melanogenesis, while ROS scavengers and inhibitors may down-regulate hyperpigmentation and UV-induced melanogenesis.<sup>[17]</sup>

Since ancient times, mung beans (綠豆 *Lǜ Dòu*) have been known for their diuretic and detoxifying effects and have been commonly used to relieve summer heat. Previous research findings have shown that mung beans possess anti-inflammatory<sup>[18-20]</sup> and antioxidant activities,<sup>[21-23]</sup> the ability to adapt to warm environments<sup>[24]</sup> and to reduce lipogenesis,<sup>[25]</sup> and whitening effects.<sup>[26]</sup> Sun Si-miao (孫思邈 *Sūn Sī Miǎo*), a famous doctor of the Tang dynasty (唐 *Táng*), known as the “King of Medicine” (藥王 *Yào Wáng*), wrote in his *Supplement to the Formulas of a Thousand Gold Worth* (備急千金藥方 *Bèi Jī Qiān Jīn Yào Fāng*) that mung bean cures chills and fevers (寒熱 *Hán Rè*), heat stroke (熱中 *Rè Zhōng*), ends diarrhea (泄痢 *Xiè Lì*) and sudden afflux dysentery (卒澀 *Cù Pì*), and is good for the fullness and distention of urine (小便脹滿 *Xiǎo Biàn Zhàng Mǎn*). Mung beans can not only be used as a medicine but also as an edible vegetable (sprouts, leguminous crop, or pulse). Li Shi-zhen (李時珍 *Lǐ Shí Zhēn*), a herbalist from the Ming Dynasty (明 *Míng*), praised the mung bean as “an object of real property which, when added to a dish as a vegetable, will bring tranquility throughout the valley.” As food, mung beans can be made into things such as bean porridge, bean rice, bean wine, and bean cakes which, when eaten regularly, will produce results that cannot even be stated. When thought of as a vegetable, people most often use the sprout form of the mung bean. Wang Shi-xiong (王士雄 *Wáng Shì Xióng*), a renowned physician and dietitian of the Qing Dynasty (清 *Qīng*), once reportedly exclaimed, sprouts as vegetables what a refreshingly beautiful taste

For thousands of years, *Pracparatum mungo*, a derivative of mung beans, has been known as a “sacred detoxification medicine” that is widely used for its liver detoxifying properties. *P. mungo* is a natural food product refined from *Phaseolus mungo* beans of the plants in the Leguminosae family. The refinement process involves natural fermentation and soaking in a complex traditional Chinese medicine preparation. There are a variety of purposes for *P. mungo* as a traditional Chinese medicine, such as removing impurities, adjusting smells, reducing or eliminating toxicities or side effects, easing excessive medicinal properties, whitening skin, and inducting medicines into affected channels to enhance their efficacy. The traditional manufacturing process of *P. mungo* is quite unique because the concepts of yin and yang (陰陽 *Yīn Yáng*) and the Five Elements (Wu Xing) (五行 *Wǔ Xíng*) have been incorporated. The overall process can roughly be divided into four stages. In the first stage, Mountains, the process begins in

the White Dew period in early autumn. Chinese herbal medicine is added to freshly selected mung beans and the mixture is placed within a living 3-5-year-old, high mountain green bamboo stem for approximately 120 days through various solar periods such as the White Dew, Autumnal Equinox, Cold Dew, Frost’s Descent, Beginning of Winter, Slight Snow, Great Snow, and Lesser Cold periods. In the Rivers stage, during the Lesser Cold period, the entire section of living green bamboo containing the mung beans is excised, placed in boys’ urine (specifically urine from prepubescent boys because the mung bean possesses cold characteristics that require the yang energy of boys to neutralize it and moderate the medicinal property of the *P. mungo*) and covered. The beans are then allowed to ferment naturally for approximately 120 days. By the beginning of summer, during the Grain Full period, the beans are rinsed in a stream of water deep in the mountains for approximately 120 days. The third stage, or Day stage, occurs by the beginning of autumn during the White Dew and Autumnal Equinox periods. During this stage, the bamboo section is split open and the fully fermented mung beans that have a complete bean shape and beautiful appearance and color are removed. The beans are then soaked in Chinese herbal soup once every 5-7 days and dried in the morning, with the herb species being changed in accordance with the 24 solar terms. In the fourth stage, or Night stage, the mung beans are dried at dawn but exposed to dew water at night for several months until the Grain Rain period. Each day/night alternating cycle of the preparation lasts for approximately 120 days and for a total of approximately 480 days.

*P. mungo* (also known as Lu-Do Huang) is a traditional and functional food that is made from mung beans (*Ph. mungo L.*). Previous studies have shown that mung beans contain natural antioxidants and can offer protection against injuries due to heat stress.<sup>[24,27]</sup> While there is one study discussing the modern applications of *P. mungo* for its hepatoprotective effects associated with increase in SOD and glutathione (GSH) levels, there is no scientific evidence showing the ability of *P. mungo* to protect skin. In this study, we used *in vitro*, *ex vivo*, and *in vivo* examinations to investigate the inhibition of melanogenesis and antioxidant properties of *P. mungo* extracts. These results can be used to develop tyrosinase inhibitors or antioxidants to inhibit melanin biosynthesis and auto-oxidation, and could have further industrial applications, especially in functional food or cosmetic compositions.

## MATERIALS AND METHODS

### Preparation of *P. mungo* methanol (PMME) and ethanol (PMEE) extracts

*P. mungo* (Lu-Do Huang) was purchased from a local company (Eight Princes Biotechnology Co. Ltd., Cha-yi, Taiwan) and then powdered. The powder (100 g) was extracted with ethanol (90% ethanol, 1000 ml, two extractions) and methanol (100% methanol, 1000 ml, two extractions) for 24 h at 4°C and then centrifuged at 500 ×g for 20 min. The extract was filtered and then evaporated to dryness under reduced pressure in a rotary evaporator; the eventual yield was more than 10 g of methanol extract (PMME) and ethanol extract (PMEE). The PMME and PMEE were then lyophilized (EYMA Freeze Dryer, FDU-540).

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