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SHORT COMMUNICATION

Evaluation of anti-melanogenic activity of *Pterocarpus santalinus* L. using bacterial system

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ABSTRACT
Tyrosinase is a copper-containing enzyme, which is widely distributed in animals, plants and microorganisms. The enzymes showed considerable structural similarity independent to the kingdom they belong. Tyrosinase is a key enzyme in melanin biosynthesis, involved in determining the color of mammalian skin and hair. Increased activity of the enzyme can lead to hyperpigmentation resulting in distressing aesthetic values. The inadequacy of current conventional methods to inhibit tyrosinase activity safely encourages the need to seek new potent tyrosinase inhibitors in cosmetic and therapeutic applications. In the current study we report the effectiveness of hot water extract of *Pterocarpus santalinus* bark against the melanin producing system of *Bacillus cereus.* The extract had shown to inhibit melanin production in bacteria dose dependently. Therefore, our results suggested that *P. santalinus* extract possesses antimelanogenic/antityrosinase activity, which could be utilized as a safe depigmentation agent.

KEY WORDS: *anti-tyrosinase, red sandalwood, melanin, bacteria*

Introduction

The melanin biosynthesis inhibitors have been of great interest all-time due to its esthetic importance (Priestly, 1993). The production of abnormal melanin pigmentation is more prevalent in middle-aged and elderly individuals (Parvez *et al*, 2007). It is cosmetically important in cultures which consider this pigmentation as sign of health or in cultures that are very beauty conscious were the need of depigmenting agents are inevitable (Briganti *et al.*, 2003). Tyrosinase (EC 1.14.18.1) is the key enzyme responsible for this hyperpigmentation. This enzyme plays crucial role in oxidation from L-tyrosine to 3, 4-dihydroxyphenylalanine (L-DOPA) and from DOPA to DOPAquinone, which is the initial step in melanin synthesis (Singh and Pandey, 2016). Increased tyrosinase activity may be considered as the main reason behind hyperpigmentation diseases. Therefore, the need of tyrosinase inhibitors has become increasingly significant in therapeutic as well as cosmetological applications (Roh *et al*., 2004). A number of tyrosinase inhibitors are reported from both natural and synthetic sources, but only a few of them are used as depigmentation agents primarily due to various safety concerns. For example, linoleic acid, hinokitiol, kojic acid, naturally occurring hydroquinones, and catechols were reported to inhibit enzyme activity but possess cytotoxic and mutagenic effects (Seo *et al*., 2003).

This elevates the need of a safe and natural tyrosinase inhibitor. In traditional medicine from time immemorial *Pterocarpus santalinus* (red sandalwood) paste has been used for fair skin complexion (Saikia *et al*., 2006).In a recent report it was found that the active principles in the *P*

santalinus bark extract inhibited melanin production in B16F0 melanoma cells (Hemachandran *et al*., 2016). Many bacteria were known to produce melanin via L-DOPA (Solano, 2014) which had shown similarity with the human melanogenic pathway. The tyrosinase in humans too shares structural similarity with the bacterial counterpart (*Bacillus megaterium*) (Nokinsee *et al*., 2015). So we consider a high melanin producing *Bacillus cereus* could be utilized as a model system in evaluating the anti-melanogenic/anti-tyrosinase activity of *P santalinus* extract, which could substantiate the earlier findings in *in vitro* conditions. To our knowledge this is the first report on anti-melanogenic activity of *P santalinus* against *B. cereus* melanin biosynthesis machinery.

Bark from a home grown *Pterocarpus santalinus* tree of 17 years old and having a diameter of 121 cm was used in the study. Dried bark was pulverized to the fine powder form. This was then subjected to hot water extraction according to Sukhdev *et al*., 2008. Prepared extract is added to the melanin production medium (Yabuuchi and Ohyama, 1972) at a concentration 1- 5 mg/mL. Melanin producing bacteria *Bacillus cereus* strain BTSNGIST5 (unpublished data) was then inoculated to the melanin production medium. After 5 days the produced melanin concentration was determined spectroscopically at 400nm (Turick *et al*., 2002). Melanin produced under different concentration of *P santalinus* extract was compared to the control to determine its anti-melanogenic potential.

The anti-tyrosinase activity of *P santalinus* extract was evaluated against bacterial tyrosinase and it showed profound inhibitory effect to tyrosinase activity in a dose dependent manner which was evident from decrease in melanin production by *B. cereus.* (Fig.1) In control flasks were extract was not added the melanin produced was 120.08 ±1.04µg/mL and was black in color , while addition of 5 mg/mL of extract decreased the melanin production to 35.41±1.14µg/mL. A significant 70% decrease of melanin production was observed here which confirms the anti-tyrosinase activity of *P santalinus* extract against *B. cereus* tyrosinase enzyme.

Due to its significant tyrosinase inhibitory potential, the extract can be utilized to explore into metabolism of melanin in *B. cereus* in place of commonly using inhibitors like Kojic acid (Sajjan *et al*., 2010). Though the common melanin produced in bacteria is via homogenisate i.e. Pyomelanin (Solano, 2014). The melanin inhibitory activity of the extract exhibited in the present study in *B. cereus* revealed the melanin belongs to the subtype, DOPA melanin or eumelanin.

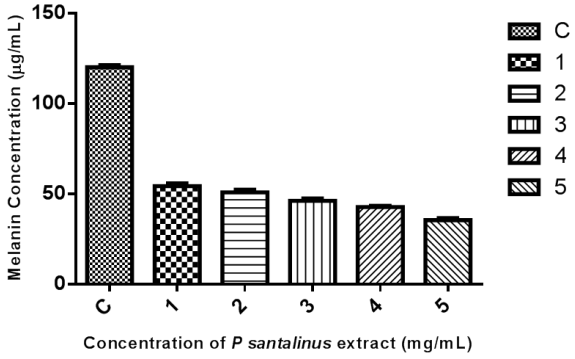

Figure 1: (a) Graph showing inhibition of *B. cereus* melanin production by various concentrations (1-5) of *P santalinus* extracts compared to the control (C)

Cytotoxicity in *in vitro* conditions (Hemachandran *et al*., 2016) and acute and sub-acute toxicity studies in rats (Azamthulla *et al*., 2013) of *P santalinus* extract revealed that the extract is less toxic and can be utilized for cosmetic and therapeutic applications. Thus our investigation concluded that hot water extract of *P santalinus* can be utilized as a depigmentation agent, which could contribute for the replacement of toxic synthetic as well as non-synthetic tyrosinase inhibitors. This research supports the recent findings *in vitro* and affirms that *P. santalinus* extract can be an effective anti-tyrosinase compound irrespective of the enzyme source. We strongly suggest the use of *P santalinus* extract in cosmetics and for therapeutic applications after *in vitro* trails.

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