## Inhibitory Effect of *Pinus rigida* × *Pinus taeda* on Melanogenesis in B16 F10 Cells

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The cone of *Pinus rigida*  $\times$  *Pinus taeda* (PRT), a plant in the Pinaceae family, has long been used in traditional medicine to treat hemostasis, bruises, and burns. Previous research has shown that regulating oxidation-reduction reactions in reactive oxygen species can help inhibit melanogenesis, the process of melanin synthesis, which is a common target for addressing hyperpigmentation. Inhibiting tyrosinase is also known to be effective in this regard. Based on these findings, we conducted an investigation into the inhibitory effect of the ethyl acetate fraction of PRT (ERT) on melanogenesis in B16 F10 cells. We know that the expression levels of melanin biosynthesis-related proteins, including tyrosinase, TRP-1, and TRP-2, are regulated by MITF (microphthalmia-associated transcription factor) and cAMP, with cAMP affecting the activity of protein kinase A (PKA). PKA can reduce melanogenesis, and CREB reduces the phosphorylation of melanin-producing enzymes. In addition, the MAPK signaling pathway, composed of ERK, JNK, p38, and other factors, is also known to play a role in the inhibition of melanogenesis in melanocytes. Our immunoblotting results showed that ERT inhibited the expression of melanin production-related proteins (tyrosinase, TRP-1, TRP-2, and MITF) that were significantly increased by a-MSH treatment to promote melanin production. Furthermore, the phosphorylation levels of factors related to cAMP/PKA/CREB and MAPK signaling pathways were significantly reduced without affecting the total form. In conclusion, we believe that treatment with ERT can inhibit melanin synthesis by modulating the phosphorylation of cAMP/PKA/CREB and MAPK signaling pathways at the cellular level. These findings suggest the potential of ERT as a raw material for functional cosmetics and pharmaceuticals, thanks to its antioxidant activity and ability to inhibit melanogenesis. We thought that these findings of ERT as a natural plant resource will inspire further research and development in this area.

## Key words: α-MSH, Melanogenesis, Pinus rigida × Pinus taeda, Tyrosinase

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