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NATURAL MODULATION OF MELANIN PRODUCTION: TYROSINASE INHIBITION BY *Stachys byzantina* K. KOCH

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Stachys byzantina is a plant species of medicinal interest due to its high content of phenols and remarkable antioxidant and anti-inflammatory activities. For this reason, *S. byzantina* presents potential applications in skin care, particularly for conditions associated with oxidative stress, inflammation, skin aging, and pigmentation diseases, including melasma and solar lentigines, which are particularly linked to increased tyrosinase activity. Previous studies from our group reported that the dichloromethane fraction (DF) obtained from the ethanolic extract of *S. byzantina* presents relevant tyrosinase inhibitory action. The present study aimed to fractionate DF in order to identify at least one compound responsible for this inhibition. *S. byzantina* aerial parts were collected at UFJF Faculty of Pharmacy Garden (SisGen A3DD429). A voucher was deposited in CESJF herbarium (no. 46598). The ethanolic extract was obtained by ultrasound-assisted extraction and subsequently fractionated (30 g) by liquid partition using organic solvents of increasing polarity, yielding DF (6.75 g). DF was further fractionated on a silica gel column, resulting in 29 fractions. Tyrosinase inhibitory activity was evaluated according to Khatib et al. (2005). Each fraction was incubated at 62.5 µg/mL with tyrosinase (250 U/mL) and L-tyrosine (2 nM), and the absorbance was monitored at 450 nm for 30 minutes. Fraction F12.2 was further analyzed by HPLC-UV due to its highest inhibitory activity (81.33 ± 2.62 %), using a C18 column. The mobile phase was 0-5 min: 5-15% methanol (95-85% water); 5.01-30 min: gradient 45-100% methanol (55-0% water). The injection volume was 20 uL, and the wavelength was set to 330 nm. The major peak was identified as apigenin (RT = 16.11 min) by comparing the retention time and co-elution with the standard (0.025 mg/mL). A standard curve was constructed (R^2 = 0.9998), and apigenin was quantified at 32.03 ± 4.17 mg/g. Apigenin is a flavonoid widely recognized for its tyrosinase inhibitory activity, as well as its antioxidant and anti-inflammatory properties, which may collectively contribute to reducing hyperpigmentation. These findings highlight the potential of this plant as a source of natural cosmetic products for modulating melanin production, and further encourage studies to identify additional bioactive compounds.

Keywords: *Tyrosinase inhibition, Hyperpigmentation, Stachys byzantina, Apigenin, Dermatological applications.*

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