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Objectives of the project:

Preparation of Biogenic Selenium Nano conjugate emulsion from *Lentinula edodes* extract to treat skin hyperpigmentation.

Skin hyperpigmentation is a common and distressing dermatologic condition. Since tyrosinase enzyme plays a vital role in melanogenesis, its inhibition is considered a logical approach along with other therapeutic methods to prevent the accumulation of melanin in the skin. Major challenges experienced in using TYR inhibitors such as arbutin, kojic acid, deoxyarbutin, and hydroquinone are the concern about safety, toxicity and instability, especially when used for a long time and at a high dose[1]. Therefore, Nano medicine has been recently proposed in the treatment of hyperpigmentation in addition to the traditional approaches. Since oxidative stress is a contributing variable that appears to be a large player facilitating melanogenesis, antioxidants seems to be the potent inhibitors of tyrosinase activity and melanin production[2]. While Selenium is an essential trace element involved in numerous vital biological processes its toxicity is a major concern.

Nevertheless, Selenium nanoparticles (SeNPs), have exceptional in vivo bioavailability, reduced toxicity and higher antioxidant potential in comparison to its organic and inorganic analogs[3]. *Lentinula edodes*, an edible mushroom enriched with several bioactive agents exhibit multiple pharmacological activities including significant antioxidant activity[4].

Here is an attempt to synthesize Selenium Nano conjugate emulsion from *Lentinula edodes* extract to treat skin hyperpigmentation. Since emulsions maintain water-lipid balance of epidermis and demonstrate an ability to transport active ingredients (polar and nonpolar alike) into deeper layers of the skin; this effect increases the efficacy of biogenic Selenium Nano conjugate

Methodology:

1. **Preparation of Lentinula edodes** aqueous extract: 5g of dried mushrooms material shall be added to 50 mL of distilled water and the mixture is sonicated at room temperature for 24hrs. The solution is centrifuged and filtered. The filtrate is used for the biogenic synthesis of Selenium nanoparticle.
2. **Biogenic synthesis of Selenium Nanoconjugate (SeNc):** 10 mL of L. edodes aqueous extract is drop-wise admixed with 200ml of 350 mM selenious acid solution using magnetic stirrer. Further 2 mL of 400 mM ascorbic acid is added to the mixture and stirred for 48 h at 37°C. The reaction mixture is centrifuged for 20 min at 3,500 rpm and the pellet is washed three times with deionized water dissolved in phosphate buffer (pH 7.4) using an ultra-sonicator and stored at 5°C for further analysis. The SeNc is analysed using UV–Vis spectrophotometer and transmission electron microscope.
3. **Tyrosinase inhibition assay:** Tyrosinase inhibition potential of the SeNc is performed using L-DOPA as substrate. The reaction mixture with 685 µL of 0.05 M phosphate buffer (pH 6.5), 15 µL of tyrosinase enzyme, 100 µL of 5 mM L-DOPA. is included with different aliquots of SeNc. The absorbance is immediately monitored at 492 nm for Dopachrome formation in the reaction mixture. Kojic acid and the reaction mixture without SeNc are used as a positive and negative control respectively.
4. **Preparation of Selenium Nano conjugate emulsion:** The emulsion is prepared using a Homogenizer. The PUFA from mushrooms and sterile distilled water are individually heated in water baths maintained at 70–75 °C and mixed in 2:5 ratio with continuous stirring. Further SeNc is added and stirred constantly until a homogenous composition is achieved. Ascorbic acid is added in 0.5 ratio as preservative and pH is adjusted to 6.

5. References:

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3. Ullah A, Mu J, Wang F, Chan MWH, Yin X, Liao Y, Mirani ZA, Sebt-E-Hassan S, Aslam S, Naveed M, Khan MN, Khatoon Z, Kazmi MR. Biogenic Selenium Nanoparticles and Their Anticancer Effects Pertaining to Probiotic Bacteria-A Review. Antioxidants (Basel). 2022 Sep 27;11(10):1916. doi: 10.3390/antiox11101916. PMID: 36290639; PMCID: PMC9598137.

4. Ibrahima Diallo, Frédéric Boudard, Sylvie Morel, Manon Vitou, Caroline Guzman, et al. Antioxidant and Anti-inflammatory Potential of Shiitake Culinary-Medicinal Mushroom, Lentinus edodes (Agaricomycetes), Sporophores from Various Culture Conditions. International Journal of Medicinal Mushrooms, 2020, 22 (6), pp.535-546. ff10.1615/IntJMedMushrooms.2020034864ff. f

Expected Outcome of the project:

Current study is an attempt to synthesize Selenium nanoparticle from *Lentinula edodes* aqueous extract to target skin hyperpigmentation. The resulting Nano conjugate is used for preparation of emulsion which is expected to have effective dermal penetration to inhibit Tyrosinase enzyme thereby reducing the melanin production in the melanocytes