



ChemTech

International Journal of ChemTech Research

CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.9, pp 1156-1167, 2017

Isolation and Characterisation of Mushroom Tyrosinase and Screening of Herbal Extracts for Anti Tyrosinase Activity

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Abstract : Objective : The present study focus on synthesizing and characterizing tyrosinase from Indian edible mushroom *Agaricus bisporus* and analysis for its inhibition by selected herbal plants.

Methods : Crude extract were prepared by homogenization in 100mM cold phosphate buffer (pH 5.8). After centrifugation, the supernatant was applied to ammonium sulphate precipitation. Different precipitation steps were carried out for tyrosinase enzyme precipitation(45%-80%) and precipitate were collected. The precipitate was dialyzed against 100mM potassium phosphate buffer (pH 7.0). The dialyzed fractionate was used for tyrosinase activity and protein content. The dialyzed ammonium sulfate fraction was applied to a sephadex G-100 column that was obtained after ammonium sulphate precipitation and sephadex G-100 column was subjected to ion exchange chromatography using DEAE cellulose column. The dialyzed enzyme preparation was loaded on DEAE-cellulose column which was preequilibrated with potassium phosphate buffer (100mM pH7.0).The fractions were collected and assayed for tyrosinase activity and those showing high activity were pooled and used for protein concentration. Protein concentration was determined by the method of Bradford (1976) using bovine serum albumin(BSA) standard. After protein estimation the purified mushroom sample is subjected to SDS-PAGE analysis. Powdered samples were extracted with 500 ml of methanol each using Soxhlet apparatus for ten hours of time. The extracts were condensed using rotary evaporator.

Result : The Methanolic extracts of *Glycyrrhiza glabra*, *Vetiveria zizanioides*, *Rosa indica* exhibited mushroom tyrosinase inhibition. The maximal toxic free concentration of plant extract was evaluated on vero cell line and found to be non toxic at the concentrations tested.

Conclusion : Tyrosinase inhibition is one major strategy used to treat hyperpigmentation. The results obtained from biological assays showed that *Glycyrrhiza glabra*, *Vetiveria zizanioides*, *Rosa indica* possessed anti-tyrosinase properties, which exhibited potential for application in medical cosmetology.

Soundhari. C *et al* /International Journal of ChemTech Research, 2017,10(9):1156-1167.
