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4. Name of Topic: Design and development of stable Polyphenol Oxidase for biomedical application
5. Keywords: Bioseparation, Immobilization, Biosensor, Magnetic cross-linked enzyme aggregates, Biofilm

FINDINGS OF THESIS

The key findings of the thesis may be summarised as under:

Polyphenol oxidases (PPO) (EC 1.14.18.1) are metalloenzymes containing copper which catalyzes the oxidation of mono-, di-, and polyhydric phenols to quinones, hence, they are bi-functional in nature. The product o-quinones formed gets polymerized further which results in the formation of black, brown, or red pigments such as melanin. These enzymes are responsible for the browning of cut fruits, and vegetables and are found in fruits, vegetables, humans, and microorganisms. PPO has a broad application in the bioremediation of phenolic effluents, designing of biosensors for the detection of phenols, food additives during food processing, immunoassays, and biosynthesis of pharmaceutical drugs like L-dopa/levodopa (l-3,4-dihydroxy phenylalanine). As this enzyme has diverse allocations with a wide range of applications in different fields, hence, necessitates the need for their purification and stabilization. Therefore, the enzyme was immobilized as it is one such approach that can make the system cost-effective and enhance productivity as well as stability.

PPO was purified from bio-waste using an economical, cost-effective, non-chromatographic and phase separation method- three-phase partitioning with high purification fold and immobilized using different techniques. Adsorption of PPO was done to fabricate low-cost, economical, portable, rapid, and sensitive colorimetric paper-based biosensor for on-site monitoring of phenolic contaminants. The biosensor was validated and applied using environmental water (collected from Yamuna River) and an artificial urine sample to show its reliability and sensitivity. Further, PPO was cross-linked on iron oxide nanoparticles using the bio-imprinting method to develop nano-biocatalyst (bio-imprinted magnetic cross-linked enzyme aggregates) and used in the production of L-3,4-dihydroxyphenylalanine (L-dopa, which is used to treat Parkinson's disease) with enhanced productivity and validated using HPLC. As biofilms are a big menace to public health, their eradication is necessary. This nano-formulation was used as an anti-biofilm agent against pathogens including multi-drug resistant strains and validated through in vitro, microscopic, and in silico approaches. Since enzymes are proteinaceous in nature, they get degraded in the presence of proteases, so we evaluated different nanoparticles for oxidase-like activity and applied them for L-dopa production. These nanozymes are gaining momentum as a next-generation artificial enzyme.