The present thesis is devoted to the studies of copper containing oxidoreductase enzyme, viz. tyrosinase. It deals with extraction and characterization of tyrosinase from a novel plant source *Amorphophallus companionatus*, its use in the fabrication of a biosensor for the detection of phenolics and the tyrosinase-mediated effects of a folklore medicinal agent, viz. Lawsone, (2-hydroxy-1,4-naphthaquinone), on melanoma *in vitro* as well as *in vivo*. The thesis is divided into four chapters:

**Chapter I** gives a brief account of the tyrosinase enzyme and its significance in food industry, environmental studies and medicinal applications.

**Chapter II** includes the results of our studies on extraction and characterization of tyrosinase from a novel plant source, *Amorphophallus companionatus* (family: Araceae), cultivated in India for edible and medicinal purposes. Tyrosinase appeared to be the major oxidative enzyme in this source. Specificity of the enzyme and *Km* for a number of substrates are described. Tyrosinase and laccase activities were segregated on the basis of substrate specificity and sensitivity to specific inhibitors. On SDS-PAGE for activity staining three tyrosinase isoforms were detected with *Mr* values of ~127, 31 and 27 kDa. From the histochemical studies, stained areas for tyrosinase were located in the intercellular regions of the tissue section suggesting a particulate nature of the enzyme. As far as we are aware of this is the first tyrosinase enzyme obtainable from a plant source which is devoid of any soluble fraction.

In **Chapter III** we have provided an account of immobilization of the enzyme extract of *Amorphophallus companionatus* in a novel composite biopolymer matrix and its subsequent use in the fabrication of amperometric and optic fiber biosensors for detection of phenolic compounds. The composite polymer matrix was prepared from a mixture of biopolymer extracted from tamarind seed kernels and agarose gel. The immobilized enzyme was stable and active with the enhanced shelf life upto four months. The activity of the entrapped enzyme was comparable with the soluble enzyme system.

The amperometric sensor was prepared by casting the biopolymer film directly on the tip of glassy carbon electrode. The response of the modified electrode was measured in terms of cathodic current developed during electrochemical reduction of the *o*-quinone species generated by enzymatic oxidation of *o*-diphenols using L-dopa as the standard substrate. The working time for the enzyme electrode was found to be 8 min with optimum response within 5 min. The response of the enzyme electrode was linear in the substrate concentration range of 10^-2 to 10^-4 mM.

The optic fiber sensor (optrode) was fabricated for one-time use considering constraints of the tyrosinase-catalyzed reaction such as reaction inactivation of the enzyme and practical irreversibility of the reaction. The flow cell for the detection of phenolic compounds was fabricated using the tyrosinase immobilized film and employing the reflectance mode of measurement where the chromogenic nucleophile 3-methyl-2-benzo-thiazohine hydrazone (MBTH) was combined with L-dopa. The optrode working time was also found to be 8 min, however, the optimum response time was 2 min. The linear response of the optrode was obtained in the substrate concentration range of 10^-3 to 10^-5 mM.

**Chapter IV** of the thesis presents an account of our investigations on Lawsone (2-hydroxy-1,4-naphthaquinone), an active constituent of Henna leaves, as an antimelanoma agent with some selectivity in its action. The studies were carried out using B16 mouse melanoma cells *in vitro* and *in vivo*. The molecule caused a dose-dependent inhibition of tyrosinase activity in melanotic cells without manifesting any growth inhibition while in their amelanotic phase it induced re-differentiation where cells lose their ability to proliferate. The observed inhibition of the tyrosinase in the melanotic phase and the activation of the enzyme in the amelanotic phase by lawsone appears to be due to modification of the tyrosinase protein as revealed by activity staining and western blottings.

During *in vivo* studies on C57 BL/6j mice the intraperitoneal administration of lawsone at 70 μmole kg^-1^ body weight was found to arrest the subcutaneous growth of B16 melanoma tumors thereby increasing the life spans of the tumor bearing animals. The increasing number of doses, manifested in stabilization of tumor weight around 3 to 4 g, compared to their dramatic rise up to 12 g in the control group of mice. The effective concentrations of the agent caused necrosis in melanoma cells selectively without exerting toxicities in general and on the selected organs such as liver, spleen, and kidneys.

The results of our studies indicate that the effects produced by lawsone are mediated largely through tyrosinase enzyme. The plausible mechanism of the action involves a two-electron reduction of the lawsone reagent by the bi-cuprous species of the enzyme yielding a cytotoxic compound, viz. 1,2,4-trihydroxynaphthalene. The active constituent of the herbal folklore, thus, turns out to be a novel antimelanoma agent that induces tyrosinase-mediated cytotoxicities in melanotic and amelanotic B16 melanoma cells and hence deserves further investigations.