Reactive oxygen species such as $\text{O}_2^-$, $\cdot\text{OH}$ and $^1\text{O}_2$, play an important role in the cytotoxicity and photodynamic action of various chemotherapeutic agents. In the present thesis, an attempt is made to study the cytotoxic action of various naturally occurring quinones and to investigate the formation of ROS from these quinones. The three fourth of the thesis deals with the studies on these quinones extracted from three different ethanomedicinal plants such as *Thespesia populena*, *Ervatamia coroneria* and *Cassia tora*. These studies include the cytotoxicity of these quinones against human breast adenocarcinoma cell lines (MCF-7), the ability of these quinones to generate $\text{O}_2^-$ and $\text{H}_2\text{O}_2$ upon enzymatic reduction and EPR spin trapping investigations of $\text{O}_2^-$. Photogeneration of ROS by these quinones is also studied by using optical and spin trapping methods. The last quarter of the thesis concerns with the study of comparative basis towards the generation of ROS particularly $\text{O}_2^-$ and $^1\text{O}_2$ (Type I and Type II) among the natural emodin-quinone (PS1) and anthraquinone (PS2), a synthetic analogue of podophyllotoxin, an etoposide (PS3) and a synthetic analogue of alkaloids, MPT quinoline (PS4). With these PS’s, a study looking for the plausible phototoxicity mechanism over mamma carcinoma cell lines (MCF-7) has been envisaged by taking into the account of all *in situ* cellular (biochemicals) experiments which are inevitable and conducive to any living cell system.
On understanding the MPT quinoline as the efficient cytotoxic candidature from the results of photocytocidal activities of the all four PS’s with MCF-7 cell lines, the reason for effective photokilling has been analysed systematically. This study is followed to understand various mode of free radical generation with an aid of EPR spin trapping techniques. Converting singlet oxygen and superoxide anion to an EPR observable nitroxide radical signal and DMPO-O$_2$• adduct signal respectively for the photogenerated species, $^1$O$_2$ and O$_2$• from synthetic three ketocoumarins have been formed the subject of study in the last part of thesis.

EPR spin trapping techniques, UV-visible spectra technique, Oxymetry and Cyclic Voltammetry have been used in these studies. A brief account of the various chapters, given in this thesis, is outlined below:

Chapter 1 gives an introduction to biological importance of reactive oxygen species (ROS) and their determination using various analytical techniques. The chemical, radiolytic, photochemical and enzymatic methods of generating ROS, the normal pathways of their formation in vivo are discussed. An extensive discussion is presented on the mechanisms of action of clinically useful anticancer quinones in which oxygen free radicals have been implicated for their cytotoxic and photodynamic action. Mechanism of PDT, photosensitization, photosensitizer, ALA-PDT, non-porphyrin compounds, anti-cancer agents from plants and role of quinone and coumarins in ROS generation. A brief account of the methods used to detect these ROS, is presented. An outline of EPR spin trapping experiment and the nature of various spin adducts are also presented. Scope and objectives of this study is also outlined.

Chapter 2 gives a description of the experimental methods used to detect the reactive oxygen species. The analytical instruments used in this
investigation are indicated with appropriate explanation. Description of phytochemical isolation of compounds from natural plant (like emodin and anthraquinone from leaves and root of *Cassia tora*), synthesis of compounds such as etoposide and MPT quinoline is presented. Furthermore, the compounds prior to use in this study are characterized and identified for authenticity, using spectral data from Mass, IR and NMR spectroscopy as well as their melting points.

Chapter 3 describes the study of cytotoxic action of four naturally occurring quinones, *viz.*, mansonone-D (MDQ), mansonone-H (MHQ), thespone (TPQ) and thesposone (TPEQ), upon aerobic incubation with human breast adenocarcinoma (MCF-7) cell lines. These quinones are extracted from *Thespesia populnea*. Cytotoxicity of the quinones followed the order MDQ > TPQ > MHQ ≃ TPEQ. EPR spectrometric and Clark electrode oxymetric studies indicate that redox cycling of these quinones produce superoxide anion radical (\(O_2^-\)) and \(H_2O_2\) on aerobic incubation with NADH: cytochrome c reductase. Generation of superoxide radical during enzymatic reduction of quinones is confirmed by EPR spin trapping experiment using 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as a spin trap. Cyclic voltammetric studies show reversible redox couples for MDQ and TPQ whereas TPEQ and MHQ show irreversible redox couple. The electrochemical results indicate that MHQ and TPEQ are more difficult to reduce than TPQ and MDQ.

Chapter 4 presents the study of photogeneration of superoxide anion radical (\(O_2^-\)) and singlet oxygen (\(^1O_2\)) from TPQ, TPEQ, MHQ and MDQ. Photolysis of these quinones in DMSO, in the presence of DMPO generates twelve line EPR spectrum with hyperfine coupling constants as \(a_N = 1.2\) mT; \(a_H^\beta = 1.03\) mT; \(a_H^\gamma = 0.1\) mT. The generation of \(O_2^-\) radical is
effectively enhanced by the presence of electron donor such as EDTA and DETAPAC. The results indicate that both TPQ and MDQ possess high ability to photogenerate the reactive oxygen species.

Chapter 5 explores the study of two naturally occurring anthraquinones namely, erwatamia-I (EQ-I) and erwatamia-II (EQ-II), extracted from *Ervatamia coroneria* for the their cytotoxic action during aerobic incubation with human breast adenocarcinoma (MCF7) cells. Cytotoxicity of EQ-I and EQ-II, measured as their LD$_{50}$ (50% inhibition of colony formation) values shows EQ-II to be more effective than EQ-I. EPR studies confirm that EQ-II is reductively activated by NADH: cytochrome c reductase top superoxide anion radical ($O_2^{-*}$). Cyclic voltammetric studies show one quasi-reversible redox couple for both EQ-I and EQ-II. In comparison to the reduction potential of TPQ and MDQ (around 0.60 V), the reduction of EQ-II (-1.24) is low, suggesting EQ-II to be less efficient than TPQ and MDQ. Also, aerobic solutions of both EQ-I and EQ-II, on visible light illumination generate reactive oxygen species (ROS).

Chapter 6 presents a comparative study for the generation of both $^1O_2$ and $O_2^{-*}$ during photosensitization has been carried out among the naturally occurring quinones such as emodin-quinone (PS1), anthraquinone (PS2), synthetic derivatives such as an etoposide VP-16, 1 (PS3, an analogue of natural podophyllotoxin) and MPTQ quinoline (PS4, an analogue of natural alkaloid ellipticine). The phototoxic species, $^1O_2$ and superoxide generation during photosensitization are confirmed from the results of RNO bleaching, SOD inhibitable cytochrome c reduction and EPR spin trapping experiments. Among the compounds studied, MPTQ (PS4) exhibits efficient generation of ROS.
Chapter 7 describes the mechanism involving in photocytotoxicity of MCF-7 cell lines with all the vital parameters that are essential for the survival of living cells. Studies pertaining to staining ability or dye exclusion ability and MTT assays of MCF-7 cell lines sensitized by four sensitizers are manifesting that the PS4 has the most damaging effect to membranes. The PDA of sensitizer is in the order of PS4 > PS3 > PS1 > PS2. Photosensitized cells have shown to consume O$_2$ to lesser extent, which virtually disturb mitochondrial metabolism resulting to dysfunctioning. The rate of O$_2$ consumption follows the order PS2 > PS1 > PS3 > PS4. Significant reduction in glucose, glycolytic enzymes intracellular ATP content, glutathione, enhanced degradation rate of cysteine, histidine and tryptophan and the enhancement of MDQ (products of LPO) are some of the cellular factors in sensitized cell lines associated with phototoxicity and photokilling.

Chapter 8 elucidates the vital role of light in the molecular mode mechanism of free radical generation from MPTQ. Taking together all the chemical processes cumulatively, the efficient cause of cell death (MCF-7 cell lines) ultimately with MPTQ is resulted due to the involvement of simultaneous generation of various mode and differential quantum of OFR. As a result of facile photosensitized production of semiquinoline radical, Q$^*$ and NAD$^*$ radical, efficient generation of O$_2$$^*$ is observed. Because the O$_2$$^*$ mediate formation of H$_2$O$_2$, and reaction concerted with Fenton reaction yields highly reactive hydroxyl radicals (OH) which are very likely to behave powerful photokilling radical and attributes to the deleterious effect towards cell system via bioreductivity of drug quinoline moiety and characteristic subsequent re-oxidation, (redox cycling) property.

Chapter 9 enumerates the study of synthesis, characterization and identification of three ketocoumarins viz., 3-benzoyl-7-methoxycoumarin
(BMC), 5,7-dimethoxy-3-(1-naphthoyl) coumarin (DMNC) and 7-diethylamino-3- (2-thenol) coumarin (DETC). The photodynamic activity of all the three ketocoumarins through Type I and Type II mechanism has been studied by optical method and EPR technique. The cytotoxicity of all the quinones have been measured as their Dq and LD$_{50}$ values. The cytotoxic efficiency of all the three quinones follows the order BMC $>$ DMNC $>$ DETC. Also the photosensitization in BMC is found to be more efficient in the participation of Type I and Type II pathways.