CHAPTER –4

OBJECTIVES OF THE STUDY
Bacterial pathogens such as *Pseudomonas aeruginosa* and many others (Clark. V. L. and P. M. Bavoil, 1994; Salyers. A. et al., 1994) produce a range of virulence factors that allow the bacteria to escape host defense and cause disease. Some of these virulence factors induce apoptosis in phagocytic cells such as macrophages to subvert the host defense (Monack. D. M. et al., 1997; Zychlinsky. A. et al., 1997). Very little information is available, however, about the role of any purified bacterial virulence factor in triggering apoptosis in mammalian cells. An important inducer of mammalian cell apoptosis is the tumor suppressor protein p53 (Agarwal. M. L. et al., 1998; Schuler. M. and D. R. Green, 2001; Vogelstein. B. et al., 2000). A model for p53-induced apoptosis predicted three critical steps:

(i) the transcriptional induction of redox related genes
(ii) the formation of reactive oxygen species (ROS) and
(iii) the oxidative degradation of mitochondrial components, culminating in cell death (Polyak. K. et al., 1997).

Microbial based therapy of cancer is one of the emerging cancer treatment modalities. Over the past few years, important advances have been made to study and develop live bacteria, with or without additional cloned genes that encode toxins targeted specifically to cancer cells, or bacterial products with cancer killing ability. Various types of obligate anaerobic and facultative anaerobic bacteria can preferentially target cancer cells for growth in the hypoxic regions of the solid tumour. Attention has also recently been directed not only to the use of live bacteria, but bacterial proteins that can enter preferentially into cancer cells and disrupt their growth or kill them by multiple mechanisms [Bernardes. N. et al., 2010].

Azurin, a potent anticancer redox protein secreted by *Pseudomonas aeruginosa* (*P. aeruginosa*) species has been reported to have activity against breast cancer cell lines; this had prompted researchers to search for novel methods to enhance this protein’s production. Researchers previously have reported on the synthesis of blue copper protein azurin from different microbial sources specifically from *P. aeruginosa*. Our investigation used customized methods to focus on synthesizing azurin from Clinical isolates of *P. aeruginosa* and ATCC strain 2453 which was used for optimal Azurin production by Sankar Ramachandran et al., 2011. We screened the growth of different *P.
Pseudomonas aeruginosa strains for the synthesis of azurin and followed standardized methods for extraction and purification of azurin.

A low molecular weight redox protein elaborated from the pathogenic bacteria Pseudomonas aeruginosa, azurin is one of the representative bacterial products used in the treatment of tumours.

Pseudomonas aeruginosa secretes a variety of pigments including pyocyanin, pyoverdine and pyorubin. Previous researchers (Goto. M. et al., 2003) adopted genetic engineering techniques and other bacterial species for purification of azurin. Sankar Ramachandran et al., 2012 studied on enhancing azurin synthesis from different strains of P. aeruginosa MTCC strains 1934, 741, 2453, and 1942. The growth of these strains for the synthesis of azurin were scrutinized for enhanced azurin synthesis. High yield was reported in P. aeruginosa 2453 strain than other strains. (Sankar Ramachandran et al., 2012). Classical microbiological techniques were used to isolate 95 Pseudomonas aeruginosa from inpatients and outpatients attended the clinics at Mansoura University Hospitals from January 2010 to July 2012. All isolates were identified using manual biochemical tests and confirmed by the Microscan Walk away 90 systems. The gene encodes for azurin in the local P. aeruginosa isolates was detected using specific oligonucleotide primers in a PCR, amplifying a single 545bp DNA fragment characteristic of azurin gene. Column chromatography (superdex 75) followed by dialysis was used to purify the ammonium sulphate precipitated azurin to near homogeneity. The crude and partially purified azurins killed the breast cancer cell line, MCF-7 cells, with an estimated IC50= 37.6 μg/ml (pure) for the ATCC 15442 strain and IC50= 3 μg/ml for purified azurin from local isolate II. (Yehia A. Osman et al., 2013).

So far in Indian scenario the reports for anti-cancerous activity of bacterial azurin are very limited. People have done extensive research of different activities by using various cell lines. Every year the prevalence of breast cancer as well as Colon cancer deaths were increased tremendously all over the globe. Earlier reports demonstrates that the bacterial secondary metabolite azurin acts like a promising anti cancerous agent by arresting cell proliferation process. Based on earlier reports we want to validate the effect azurin isolated from the blood of Immuno compromised patients in
Hyderabad, India, at Basavatharama Indo American Cancer Hospital on breast and colon rectal cancer.

In this study, *Pseudomonas aeruginosa* was isolated from the blood of Immuno compromised patients in Hyderabad, India, at Basavatharama Indo American Cancer Hospital and identified by 16S rRNA. Azurin gene was amplified using the suitable PCR programme and cloned in a suitable E.coli expression vector to study the apoptotic effect on mammalian cancer cells.

This study demonstrates customized methods to extract and purify azurin from different strains of *P. aeruginosa* with apparent homogeneity and their apoptotic effects on breast carcinoma cell lines and Human colon rectal cell lines. Biochemical analysis, and DGGE as well as DNA sequence analysis will enable to carry on future research.

The Objectives of the study are divided into three main categories.

1. **Isolation, Screening and Identification of *P.aeruginosa***
2. **Cloning, Expression and Purification of anticancer Azu protein of *P.aeruginosa***
3. **Functional characterization of azurin.**