3

EXPERIMENTAL PROCEDURE

Medicinal plants are valuable and used for the synthesis of numerous drugs. Indigenous plants are traditionally used against various ailments (Mahmood *et al.,* 2012). Indian medicinal plants are regularly used in various system of medicine because of minimal side effect and cost effectives. Medicinal plants containing active chemical constituent with high antioxidant property play an important role in the prevention of various degenerative diseases and have potential benefits to the society. Natural antioxidants from plant sources are potent and safe due to their harmless nature. Recently, interest has increased considerably in finding naturally occurring antioxidant for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity (Gupta *et al.,* 2011a).

Medicinal plants were found to contain various phytochemical compounds, which are used as natural medicines to treat common bacterial infections. The potential for developing antimicrobials from medicinal plants appears rewarding as it may lead to the development of phytomedicine against microbes (Poonam and Pratap, 2012).

This chapter presents in brief the experimental procedures for the evaluation of various parameters involved in the study. The antioxidant prevents the risk of several aging related diseases including cancer, cardiovascular disorder, diabetes, neurodegenerative disorders and others. In recent years, phytochemicals are increasingly purported to exert potent beneficial actions to support health and may play a role in reducing the side effects of the synthetic drugs used for the treatment of metabolic complications. To this effect, research has been focused on **"Antitumorigenic Effect in DLA Tumor Induced Mice and Antimicrobial Potential of *Ficus racemosa* and its Characterization by Spectral and *in silico* Studies".**

The present research was carried out in five phases.

PHASE I

3.1. Evaluation of MEFrB, MEFrF and MEFrL on *in vitro* Antioxidative and Antitumorigenic Activity

3.1.1 Assessment of MEFrB, MEFrF and MEFrL on *in vitro* antioxidative activity

3.1.1.1. ABTS radical scavenging assay

3.1.1.2. DPPH radical scavenging assay

3.1.1.3. Hydroxyl radical scavenging assay

3.1.1.4. Superoxide radical scavenging assay

3.1.1.5. Hydrogen peroxide scavenging assay

3.1.1.6. Nitric oxide scavenging assay

3.1.2 Assessment of MEFrB, MEFrF and MEFrL on *in vitro* antitumorigenic effect to DLA tumor cells

3.1.2.1. MTT assay

3.1.2.2. Trypan blue exclusion assay

PHASE II

3.2. Evaluation of MEFrB, MEFrF and MEFrL on *in vivo* Antioxidative and Antitumorigenic Potential in DLA Tumor Induced Swiss Albino Mice

3.2.1. Assessment of the activities of the liver marker enzymes in serum

3.2.1.1. Estimation of Aspartate transaminase

3.2.1.2. Estimation of Alanine transaminase

3.2.1.3. Estimation of Alkaline phosphatase

3.2.2. Assessment of activities of enzymic antioxidants

3.2.2.1. Estimation of Catalase

3.2.2.2. Estimation of Glutathione peroxidase

3.2.2.3. Estimation of Glutathione reductase

3.2.2.4. Estimation of Superoxide dismutase

3.2.3. Assessment of levels of non enzymic antioxidants

3.2.3.1. Estimation of Vitamin A

3.2.3.2. Estimation of Vitamin E

3.2.3.3. Estimation of Vitamin C

3.2.3.4. Estimation of Reduced glutathione

3.2.4. Evaluation of the levels of TBARS

3.2.5. Evaluation of the life span of DLA tumor induced Swiss albino mice

3.2.6. Evaluation of the histological status in the liver of DLA challenged Swiss albino mice

PHASE III

3.3. Assessment of MEFrB, MEFrF and MEFrL on the Antibacterial Activity against Different Microorganisms

PHASE IV

3.4. Phytochemical Constituents of MEFrB, MEFrF and MEFrL

3.4.1. Preliminary phytochemical analysis

3.4.2. Chromatographic and spectral analysis

3.4.2.1. Gas Chromatography- Mass Spectrometry

3.4.2.2. High Performance Thin Layer Chromatography

3.4.2.3. Fourier Transform-Infrared Spectroscopy

PHASE V

3.5. Antitumorigenic and Antimicrobial Efficacy of Phytochemical Constituents of MEFrB by *in silico* Studies

3.5.1. Active site residues of tumorigenic and microbial target protein

3.5.2. GLIDE docking

3.5.3. Assessment of ADME properties

PHASE I

Phase I involved the assessment of *in vitro* antioxidative and antitumorigenic potential of bark, fruits and leaves of *Ficus racemosa*.

Collection of plant material

Ficus racemosa bark, fruits and leaves were collected from Coimbatore district, Tamil Nadu. The collected plant parts were washed thoroughly in tap water, shade dried and finely powdered.

Preparation of organic extract of various parts of *Ficus racemosa*

Twenty gram of bark, fruits and leaf powder of *Ficus racemosa* was filled individually in the thimble and extracted with 200 ml of methanol using Soxhlet apparatus for 24 hours. The extract was then distilled and evaporated to dryness. The concentrated extracts were then accurately weighed and stored in small vials at -20^{0}C, for further studies.

3.1.1. Assessment of MEFrB, MEFrF and MEFrL on *in vitro* antioxidative and antitumorigenic activity

The *in vitro* antioxidative potential of MEFrB, MEFrF and MEFrL was evaluated using different concentrations by ABTS, DPPH, Hydroxyl, Superoxide radicals and non radicals such as Hydrogen peroxide and Nitric oxide scavenging assays.

3.1.1.1. ABTS radical scavenging assay

The ABTS radical scavenging activity was determined by the method described by Shiwaiker *et al.* (2006) as given in Appendix I.

3.1.1.2. DPPH radical scavenging assay

The DPPH radical scavenging activity was measured according to the method of Mensor *et al.* (2001) as given in Appendix II.

3.1.1.3. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity was analyzed according to the method of Elizabeth and Rao (1990) as given in Appendix III.

3.1.1.4. Superoxide radical scavenging assay

Superoxide radical scavenging activity was determined by the method of McCord and Fridovich (1968) as elaborated in Appendix IV.

3.1.1.5. Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging activity was determined according to the method described by Ruch *et al*. (1989) as given in Appendix V.

3.1.1.6. Nitric oxide scavenging assay

Nitric oxide radical scavenging activity was determined by the method described by Green *et al*. (1982) as given in Appendix VI.

3.1.2. Assessment of MEFrB, MEFrF and MEFrL on *in vitro* antitumorigenic effect to DLA tumor cells

The antitumorigenic effect was evaluated by the cytotoxic effect of MEFrB, MEFrF and MEFrL to DLA tumor cells. *In vitro* cytotoxic studies were carried out to find out the 50 percent effective dose (ED_{50}) of MEFrB, MEFrF and MEFrL by MTT and Trypan blue exclusion assays.

Maintenance of DLA tumor cells

Dalton's Lymphoma Asite (DLA) tumor cells were procured from Amala Cancer Research center, Thrissur, Kerala and were propagated in Swiss albino mice by intraperitoneal transplantation of $1x10^6$cells in 100µl of PBS. After 10 to 15 days, the cells were drawn from the intraperitoneal cavity and were used for the evaluation of *in vitro* antitumorigenic effect of MEFrB, MEFrF and MEFrL to DLA tumor cells.

3.1.2.1. MTT assay

The extent of viability of DLA cells with different concentration of MEFrB, MEFrF and MEFrL were analyzed by MTT assay by the method of Igarashi and Miyazawa (2001) as given in Appendix VII.

3.1.2.2. Trypan blue exclusion assay

The extent of DLA tumor dead cells with different concentration of MEFrB, MEFrF and MEFrL were analyzed by Trypan blue exclusion method of Salomi and Panikkar, 1989 (Appendix VIII).

PHASE II

3.2. Evaluation of MEFrB, MEFrF and MEFrL on *in vivo* Antioxidative and Antitumorigenic Potential in DLA Tumor Induced Swiss Albino Mice

In vivo studies were carried out by the intraperitoneal administration of 54 µg (ED_{50}) of bark, 60 µg (ED_{50}) of fruits and 58µg (ED_{50}) of leaves of *Ficus racemosa* to examine their antioxidative and antitumorigenic effect.

Maintenance of experimental animals

Swiss Albino Mice (20-25g) were procured from Small Animal Breeding Station, Kerala Agricultural University, Thrissur. Quarantined mice were housed in microloan boxes in a controlled hygienic environment at temperature 25 ± 2ºC and dark/light cycle (14/10h) and acclimatized to laboratory conditions for 15 days before the commencement of the experiment. The study was conducted as per the rules of institutional Animals Ethical Committee (Reg no: 623/02/b/CPCSEA). They were fed on standard pellet diet and water *ad libitium.*

Grouping of animals

The mice were divided into eleven groups with six mice in each group. In the present research intraperitoneal administration was carried out for a period of 20 days and 90 days.

Group 1 mice were administered with 100μl of PBS and are the vehicle control for DLA transplanted mice (Group 9).

Group 2 mice were administered with 100μl of DMSO and are the vehicle control for MEFrB, MEFrF and MEFrL (Groups 5, 6 and 7).

Group 3 mice were administered with 100μl of paraffin oil which constituted the vehicle control for the standard antioxidant silymarin (Group 5).

Group 4 mice were administered with 25mg/Kg body weight of standard antioxidant silymarin (Fraschini *et al.,* 2002) in 100μl of Paraffin oil.

Group 5 mice were administered with 54μg (ED_{50}) of MEFrB in 100μl of DMSO.

Group 6 mice were administered with 60μg (ED_{50}) of MEFrF in 100μl of DMSO.

Group 7 mice were administered with 58μg (ED_{50}) of MEFrL in 100μl of DMSO.

Group 8 mice were transplanted with one acute dose of $1x10^6$ DLA tumor cells in 100μl of PBS and also administered with 54μg (ED_{50}) of MEFrB in 100μl of DMSO

Group 9 mice were transplanted with one acute dose of $1x10^6$ DLA tumor cells in 100μl of PBS and also administered with 60μg (ED_{50}) of MEFrF in 100μl of DMSO.

Group 10 mice were transplanted with one acute dose of $1x10^6$ DLA tumor cells in 100μl of PBS and also administered with 58μg (ED_{50}) of MEFrL in 100μl of DMSO.

Group 11 mice transplanted with one acute dose of $1x10^6$ DLA tumor cells in100μl of PBS.

Experimental groups 5 & 8, 6 & 9, 7 &10 were administered with MEFrB, MEFrF and MEFrL for the entire period of the study.

At the end of 20 days and 90 days of the study the mice were kept for overnight fasting and then sacrificed. The liver was quickly excised and plunged into sterile, ice cold saline for the removal of blood. The washed organ was blotted dry on sterile filter paper and immediately and stored in deep freezer at -20^0C. The blood was collected by heart puncture and the serum separated was used for the estimation of liver marker enzymes such as Aspartate transaminase, Alanine transaminase and Alkaline phosphatase. A part of the liver homogenate was prepared using PBS and used for the determination of enzymic antioxidants such as Catalase, Glutathione peroxidase, Glutathione reductase and Superoxide dismutase and non enzymic antioxidants such as, Vitamin A, Vitamin E, Vitamin C and Reduced glutathione. Another part of the liver homogenate was prepared using Tris HCl for the assessment of the level of TBARS. The above parameters were analyzed without elapse of time to avoid variation. The histological examinations of all the experimental mice liver were also carried out.

3.2.1. Assessment of the activities of the liver marker enzymes in serum

Liver plays a major role in detoxification and excretion of many exogenous and endogenous compounds. To evaluate the normal functioning of the liver treated with MEFrB, MEFrF and MEFrL in the presence and absence of DLA tumor cells, selected liver marker enzymes such as AST, ALT and ALP activities in the serum of mice were assessed.

3.2.1.1. Estimation of Aspartate transaminase

Aspartate aminotransaminase (AST) was assayed by the method of Reitman and Frankel (1957) as given in Appendix IX.

3.2.1.2. Estimation of Alanine transaminase

Alanine aminotransaminase (ALT) was assayed by the method of Reitman and Frankel (1957) as given in Appendix X.

3.2.1.3. Estimation of Alkaline phosphatase

Alkaline phosphatase (ALP) was assayed by the method of King (1965) as given in Appendix XI.

3.2.2. Assessment of activities of enzymic antioxidants

Enzymic antioxidants such as Catalase (CAT), Glutathione peroxidase (GPx) Glutathione reductase (GR) and Superoxide dismutase (SOD) were assessed in the liver homogenate of DLA challenged Swiss albino mice.

3.2.2.1. Estimation of Catalase activity

Catalase activity was assessed by the method of Luck (1974) as given in Appendix XII.

3.2.2.2. Estimation of Glutathione Peroxidase activity

The activity of GPx in the liver was assessed by the method of Rotruck *et al*. (1973) as expressed in Appendix XIII.

3.2.2.3. Estimation of Glutathione Reductase

The activity of Glutathione reductase in the liver was assessed by the method of David and Richard (1983) as expressed in Appendix XIV.

3.2.2.4. Estimation of Superoxide dismutase activity

The activity of SOD was estimated by the method of Misra and Fridovich (1972) as given in Appendix XV.

3.2.3. Assessment of levels of non enzymic antioxidants

The levels of the non enzymic antioxidants such as Vitamin A, Vitamin E, Vitamin C and Reduced glutathione were also assessed in the liver homogenate of DLA challenged Swiss albino mice.

3.2.3.1. Estimation of Vitamin A

Vitamin A was estimated by the method of Bayfield and Cole (1994) as given in Appendix XVI.

3.2.3.2. Estimation of Vitamin E

Vitamin E content was determined by the method of Rosenberg (1992) as expressed in Appendix XVII.

3.2.3.3. Estimation of Vitamin C

Vitamin C was estimated by the method of Roe and Keuther (1953) as given in Appendix XVIII.

3.2.3.4. Estimation of Reduced glutathione

The levels of reduced glutathione were determined by the method of Moron *et al.* (1979) as expressed in Appendix XIX.

3.2.4. Evaluation of the levels of TBARS

The TBARS play a major role in mediating oxidative damage in biological systems. Malondialdehyde, a major end product and index of TBARS, cross-links DNA and protein and nucleotides on the same and opposite strands thereby promoting carcinogenesis. Therefore, it is found to be increased in tumor conditions (Nichans and Samuelson, 1968). The rate of TBARS was assessed in the liver homogenate of different groups of mice using the method of Bishayee and Balasubramaniam (1971). The detailed procedure is given in Appendix XX.

3.2.5. Evaluation of the life span of DLA tumor induced Swiss albino mice

In vivo studies were carried out by the administration of MEFrB, MEFrF and MEFrL. To determine the antitumorigenic activity in terms of Increase in Life Span (ILS) of Swiss albino mice transplanted with DLA tumor cells. Four groups of (6 mice/group) Swiss albino mice were used for the *in vivo* studies. To the control group $1x10^6$ DLA tumor cells were administered intraperitoneally on the 1^{st} day of the experiment for the development of tumor. To the experimental groups one acute dose of $1x10^6$ DLA tumor cells and the MEFrB, MEFrF and MEFrL were administered intraperitoneally on the same day. Administration of the MEFrB,

MEFrF and MEFrL was continued for 90 days. The mortality of the animals dying off tumor was noted to find out the average life span (Geran *et al.,* 1972).

3.2.6. Evaluation of the histological status in the liver of DLA challenged Swiss albino mice

The liver samples of all the experimental mice and their controls were fixed in 10 per cent formalin and then embedded in paraffin. Microtome sections of 6µm thickness were prepared from each portion of liver and stained with haemotoxylin-eosin for histological observations using the method of Culling (1974) as shown in Appendix XXI.

PHASE III

3.3. Assessment of MEFrB, MEFrF and MEFrL on the Antibacterial Activity against Different Microorganisms

The antibacterial activity was evaluated by measuring the diameter of zone of inhibition around the well by agar well diffusion method (NCCLS, 1993) as given in Appendix XXII.

PHASE IV

3.4. Phytochemical Constituents of MEFrB, MEFrF and MEFrL

3.4.1. Preliminary phytochemical analysis

Phytochemical screening was performed using standard procedures. The procedurces for detection of alkaloids, flavonoids, saponins, phenols, glycosides (Raaman, 2006), tannins, carbohydrates (Iyengar, 1995), steroids and terpenoids (Siddiqui and Ali, 1997) are given in Appendix XXIII.

3.4.2. Chromatographic and spectral Analysis

3.4.2.1. Gas Chromatography and Mass Spectroscopy

Separation of the bioactive components of MEFrB by GC-MS was carried out by the method of Maciejewicz *et al.* (2007) as shown in Appendix XXIV.

3.4.2.2. High Performance Thin Layer Chromatography

Separation of flavonoids, phenolic and terpenoid constituents of MEFrB by HPTLC were carried out by the method of Wagner and Bladt (1996) as shown in Appendix XXV.

3.4.2.3. Fourier Transform-Infrared Spectroscopy

Determination of the functional groups of the bioactive components of MEFrB by FT-IR procedure was carried out by the method of Mohd Nasir *et al*. (2006) as shown in Appendix XXVI.

Plate I

***Ficus racemosa* and its parts**

Various parts of the tree

Bark

Leaves

Fruits

Plate II

Normal and tumor bearing Swiss albino mice

PHASE V

3.5. Antitumorigenic and Antimicrobial Efficacy of Phytochemical Constituents of MEFrB by *in silico* Studies

To predict the mechanism of action of the phytochemical constituents of MEFrB docking analysis was carried out using Glide. The Table 1 shows the tools and softwares used for *in silico* studies.

Table 1

Tools and softwares used for *in silico* studies

Tools and Softwares	Description
Pdb	Tumorigenic and microbial target proteins (1Z2B, 1T69, 1W2N, 1SQ5) downloaded with their corresponding co-crystal ligand.
Pdbsum	To identify the active site residues of target protein.
Ligplot	It automatically generates schematic diagrams of protein-ligand interactions for a given PDB file.
Glide	Software used for docking studies.
Ligprep	To prepare the ligands.
QikProp 3.2	Assessment of ADME properties.

3.5.1. Active site residues of tumorigenic and microbial target protein

Target-based drug discovery begins with the identification of a potential therapeutic drug target and understanding its role in the disease process. In the current study, Histone deacetylases (HDACs) and Tubulin (MTs) are the two tumorigenic target proteins and Deacetoxycephalosporin C Synthase (DAOCS) and Pantothenate kinase (CoA) are the two microbial target proteins chosen.

The drug targets for each tumorigenic and microbial activities were obtained from RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) with the following PDB ID Histone deacetylases (1T69), Tublin (1Z2B), Deacetoxycephalosporin C Synthase (1W2N) and Pantothenate kinase (1SQ5). The structural details of the target proteins are shown in the Table 2.

Table 2

Tumorigenic and microbial target proteins

Structural Details	Tumorigenic targets		Microbial targets	
	1T69	1Z2B	1W2N	1SQ5
Resolution [Å]	2.91	4.10	2.70	2.20
R-Value	0.249	0.212	0.249	0.187
R-Free	0.310	0.269	0.287	0.225
Chains	A	A,B,C,D,E	A	A,B,C,D

Construction of ligand library

The drug is most commonly an organic small molecule which activates or inhibits the function of a biomolecule such as a protein which in turn results in a therapeutic benefit to the patient. In the most basic sense, drug design involves design of small molecules that are complementary in shape and charge to the bimolecular target to which they interact and therefore will bind to it (Cohen and Claude, 1996).

About 24 phytochemical constituents identified in GC-MS from MEFrB were docked against the tumorigenic and microbial targets. The canonical structure or PDB files of the compounds were used for docking.

Prediction of active site residues of target protein

The LIGPLOT program automatically generates schematic 2-D representations of protein-ligand complexes from standard Protein Data Bank file input. The output is a color, or black-and-white, PostScript file giving a simple and informative representation of the intermolecular interactions and their strengths, including hydrogen bonds, hydrophobic interactions and atom accessibilities. The program is completely general for any ligand and can also be used to show other types of interaction in proteins and nucleic acids. It was designed to facilitate the rapid inspection of many enzyme complexes, but has found many other applications.

The program was carried out by

a) Opening the PDB homepage (http://www.ebi.ac.uk/pdbsum/) and entering the PDB ID (1Z2B, 1T69, 1W2N, 1SQ5).

b) Selecting the ligand it automatically generates schematic diagrams of protein-ligand interactions.

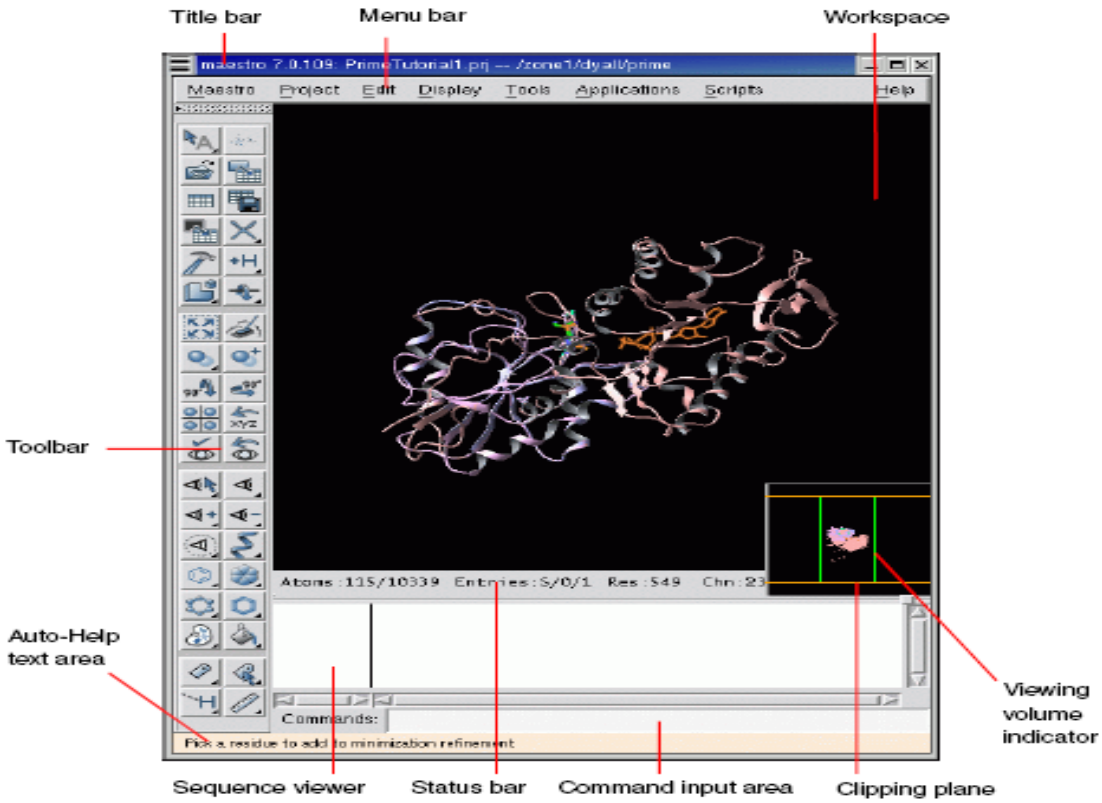
3.5.2. Glide docking

Maestro is the graphical user interface for all of Schrödinger's products: CombiGlide™, Epik™, Glide™, Impact™, Liaison™, Ligprep™ MacroModel™, Phase™, Prime™, QikProp™, Qsite™, and Strike™. Schrödinger developed state-of-the-art chemical simulation software for use in pharmaceutical, biotechnology and materials science research.

Grid based Ligand Docking with Energetics (GLIDE) searches for favorable interactions between one or more typically small ligand molecules and a larger receptor molecule usually a protein. Each ligand must be a single molecule, while the receptor may include more than one molecule. GLIDE can be run in rigid or flexible docking modes. The later automatically generates conformation for each input ligand. The combination of positions and orientation of the ligand relative to the receptor, along with its conformation in flexible docking, is referred to as a ligand pose. The Figure 17 shows the Maestro workspace.

Figure 17

Maestro window

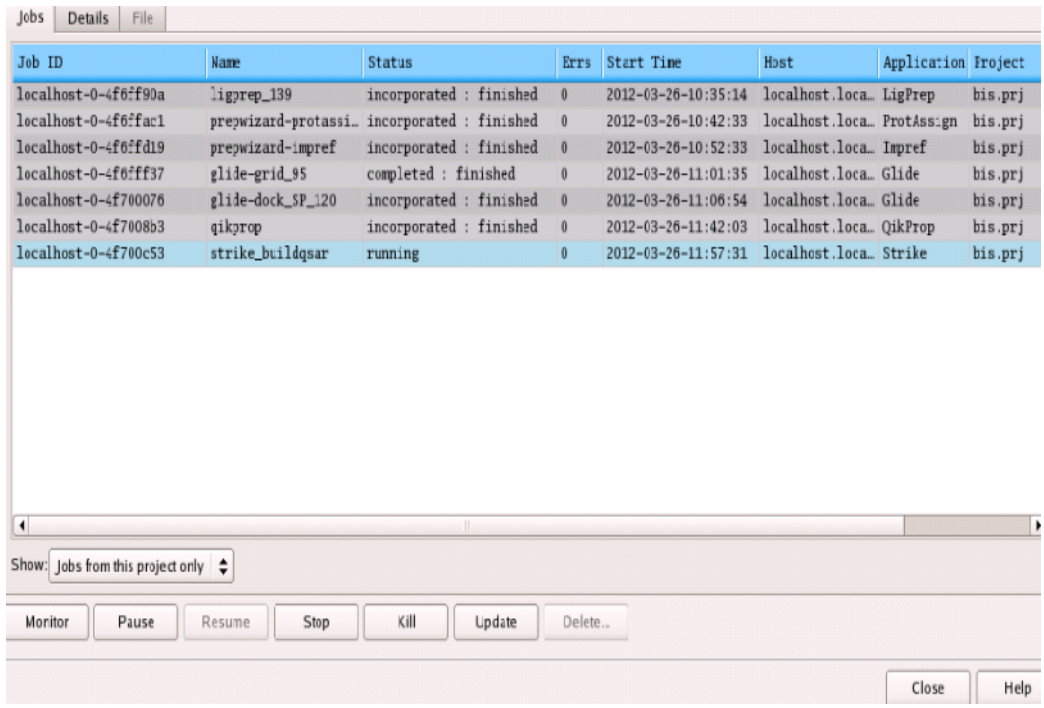


Running and monitoring jobs

Maestro has panels for each product for preparing and submitting jobs. To use these panels, appropriate product and task from the Applications menu and its submenus should be chosen. Set the appropriate options in the panel and then click Start to set options for running the job. The Monitor panel is the control panel for monitoring the progress of jobs and for pausing, resuming or ending jobs. The text pane shows various output information from the monitored job, such as the contents of the log file. While jobs are running, the Detach, Pause, Resume, Stop, Kill and Update buttons are active. When no jobs currently running, only the Monitor and Delete buttons are active, when a monitored job ends, the results are incorporated into the project according to the settings used to launch the job. The Figure 18 shows the panel for running and monitoring jobs.

Figure 18

Panel for running and monitoring jobs



Preparation of protein target structures

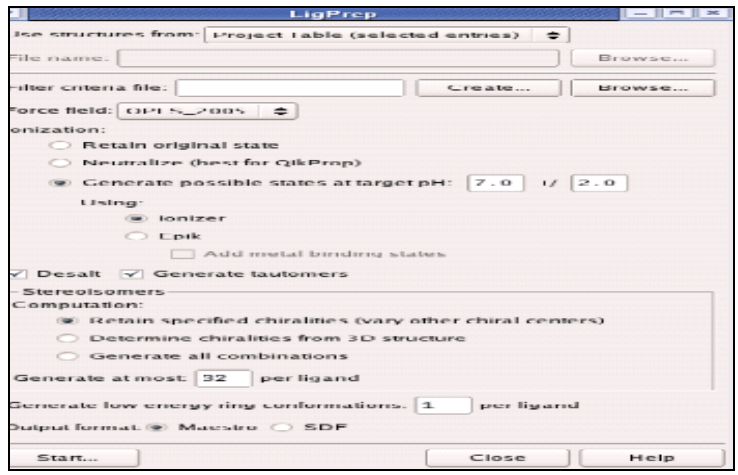
The three-dimensional structures of protein for the selected tumorigenic and microbial targets were taken from the PDB (1Z2B, 1T69, 1W2N and 1SQ5) and modified for Glide Docking calculations. For Glide calculations, corresponding proteins complex with ligand was imported to Maestro (Schrödinger) and was refined using the Amber force field. The protein contains many chains. Only the chain that is in complex with the ligand will be prepared for further process and the other chains were deleted.

Preparation of ligand compounds

All the 24 phytochemical constituents of MEFrB were the ligand molecules to be docked into the active site of target proteins. The structures of all the small ligand molecules were drawn using the builder panel available in the Schrödinger software. The structures were then energy minimized using the OPLS-2005 force field until it reaches the RMSD 0.0018 Kcal/mol. The Figure 19 shows the LigPrep panel.

Figure 19

LigPrep panel

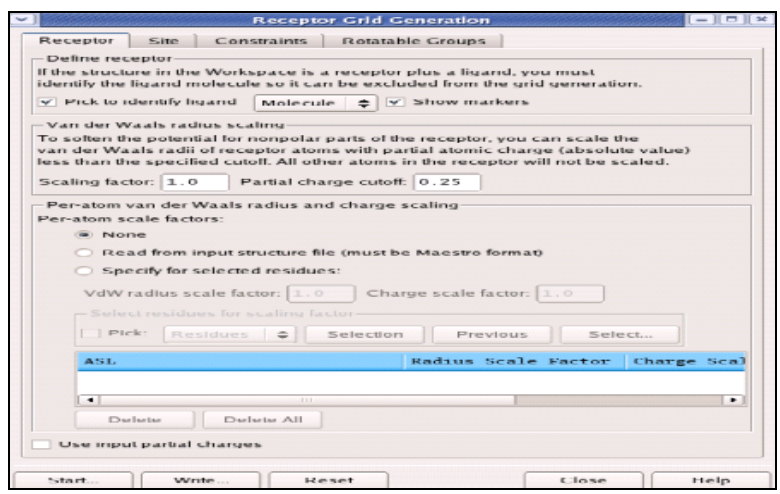


Grid generation

Glide searches for favorable interactions between one or more typically small ligand molecules and a typically larger receptor molecule usually a protein. Each ligand must be a single molecule, while the receptor may include more than one molecule that is a protein and a cofactor. Choose Receptor Grid Generation from the Glide submenu of the Applications menu. The Receptor Grid Generation panel has three tabbed folders, to specify settings for the receptor grid generation job such as **a) receptor b) site and c) constraints** as shown in Figure 20.

Figure 20

Receptor grid generation panel



To specify the receptor grid for the docking job, click Browse in the Receptor grid section of the Settings folder to open a file selector and choose a grid file (.grd). The file name, without the .grd extension, is displayed in the Receptor grid base name text box.

Protein-Ligand docking

The Three types of Docking Algorithms used for the current studies are (i) **Standard Precision (SP) docking**, (ii) **Extra Precision (XP) docking and** (iii) **Induced Fit (IF) docking.** In the current study, ligands were docked using all the three types of docking. The SP docking is appropriate for screening ligands of unknown quality in large numbers. The XP docking and scoring is a more powerful and discriminating procedure, which takes longer to run than SP. XP docking is designed to be used on ligand poses that have a high score using SP docking. It used to perform the more expensive docking simulation on worthwhile poses. The induced fit docking allows the receptor to alter its binding sites so that it more closely conforms to the shape and binding mode of the ligand. The Figure 21 and 22 shows the Ligand docking panel and window for induced fit docking.

Figure 21

Ligand docking panel

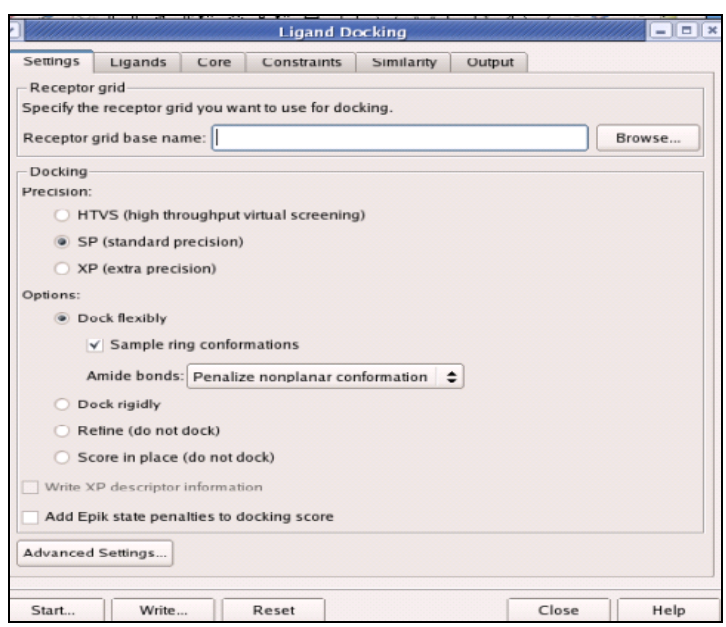
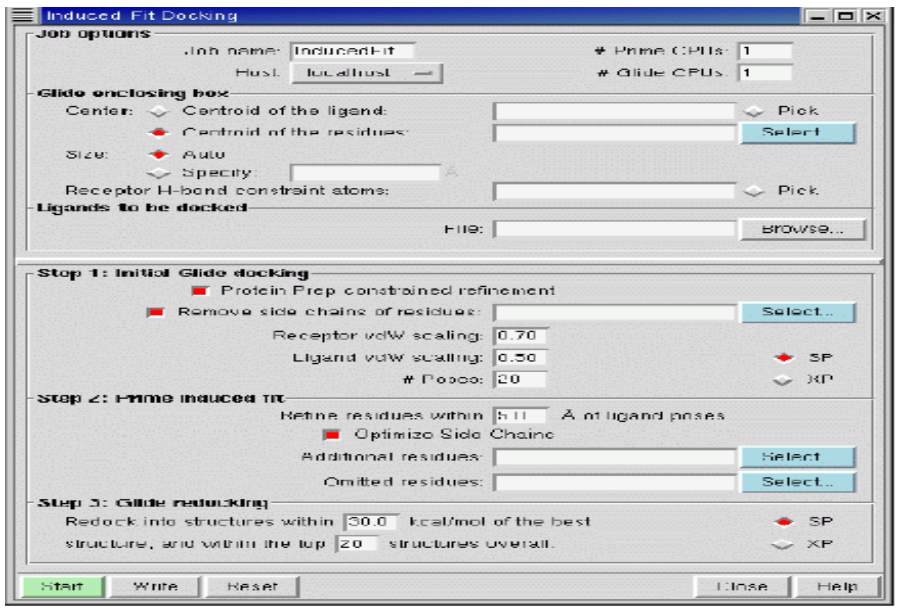


Figure 22

Window for induced fit docking



Docking output job files

Jobname_lig.mae : The input ligand structure file

Jobname_lig_prep.mae : The post preparation ligand structure file

Jobname_lig_ref.mae : The post refinement ligand structure file if present, The Receptor structure file contains only the receptor file.

Jobname_prot.mae : The input receptor structure files.

Jobname_prot_prep.mae : The post preparation receptor structure file

Jobname_prot_ref.mae : The post refinement receptor structure file. Contains the Receptor and ligand structure unless there is a separate Ligand structure file

Jobname.log : The log files for the complete preparation and Refinement job.

The Figures 23 and 24 shows the Schematic representation of ligand construction and receptor ligand docking.

Figure 23

Ligand construction

Preliminary phytochemical screening of MEFrB and identification of plant constituents by GC-MS analysis.

↓

In Glide, the structure of phytochemical compounds were drawn and saved in Maestro format

↓

The saved structures were minimized using LigPrep

Figure 24

Receptor ligand docking

Target structures (1T69, 1Z2B, 1W2N, 1SQ5) was loaded into Glide software

↓

The minimized inhibitor compounds (ligand) was loaded

Binding site specified

↓

Ligands docked with receptor

↓

Results and docking score were saved

3.5.3. Assessment of ADME properties

The increase in the number of new structures generated each year has not resulted in the expected increase in the number of marketed new drugs. Nearly 40% of drug candidates fail in clinical trials due to poor ADME (Absorption, Distribution, Metabolism and Excretion) properties (Clark and Grootenhuis, 2002).The ability to detect problematic candidates early can dramatically reduce the amount of wasted time and resources.

Qikprop 3.2 analysis

The QikProp 3.2 efficiently evaluates pharmaceutically relevant ADME properties for over half a million compounds per hour, making it an indispensable lead generation and lead optimization tool (Waterbeemd and Gifford, 2003). QikProp 3.2 panel is shown in Figure 25.

Figure 25

QikProp 3.2 panel

Statistical analysis

The data presented in this research are mean ± SD of 6 mice in each group. The results of Phase II *in vivo* studies for 20 days treatment period are subjected to one-way ANOVA including DLA groups and the results of 20 days and 90 days excluding DLA groups treatment periods are subjected to two way ANOVA using Sigma Stat Statistical Package to test the level of statistical significance at $p<0.05$.

The results obtained from the five phases of the study are presented in the next chapter.