

RESULTS AND DISCUSSION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Over 50% of all modern clinical drugs are of natural product origin and natural products play important roles in drug development in the pharmaceutical industry (Venkatachalam *et al.*, 2012).

The large majorities of the phytochemicals are redox active molecules and therefore defined as antioxidants. Antioxidants can eliminate free radicals and other reactive oxygen and nitrogen species and these reactive species contribute to most chronic diseases. It is hypothesized that antioxidants originating from foods may work as antioxidants in their own right *in vivo*, as well as bring about beneficial health effects through other mechanisms, related to antioxidant defense, longevity, cell maintenance and DNA repair (Carlsen *et al.*, 2010).

Cancer develops when normal cells in a particular part of the body begin to grow out of control. Cancer occurs in different cell types. Cancer kills or claims thousands of lives each day. Complex cell interactions in the tumor and its microenvironment play an important role in tumorigenesis and cancer progression (Soumya and Kumar, 2011).

Antimicrobial potential of medicinal plants are due to their active phytochemical constituents. The synthetic antibiotics are widely restricted because of their reported resistance to many pathogenic bacterial species. So, the researchers are turning their attention to natural products to develop new drugs for the microbial infections (Zain *et al.*, 2012). *In silico* studies will predict the antitumor and antimicrobial properties of the natural phytochemical constituents using molecular docking. So, knowledge of the orientation of molecule by molecular docking is preferred to follow the mechanism of action of compounds (Sanghani *et al.*, 2012).

Scientific investigation on antioxidant and antitumorigenic role of medicinal plants could lead to the development of potent, harmless and cost effective natural drugs for the treatment of cancer. Hence, the present study is 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 study was carried out in five phases,

The Phase I was designed to assess the *in vitro* antioxidative and antitumorigenic potential of methanolic extract of *Ficus racemosa* bark (MEFrB) fruits (MEFrF) and leaves (MEFrL). *In vitro* antioxidative role of different parts of *Ficus racemosa* was evaluated by their ability to scavenge the radicals such as ABTS, DPPH, Hydroxyl, Superoxide and non radicals such as Hydrogen peroxide and Nitric oxide. *In vitro* antitumorigenic potential was assessed by their cytotoxic effect to DLA tumor cells by MTT and Trypan blue exclusion assays.

In Phase II the assessment of the *in vivo* antioxidative and antitumorigenic efficacy of MEFrB, MEFrF and MEFrL in DLA transplanted Swiss albino mice were carried out. The antioxidative role was assessed against the standard antioxidant silymarin. The antioxidative and antitumorigenic effect was assessed in DLA tumor transplanted mice.

In Phase III evaluation of the antimicrobial activity of MEFrB, MEFrF and MEFrL was carried out using the selected bacterial species *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexneri* and *Staphylococcus aureus*.

The Phase IV involved the characterization of the phytochemical constituents of MEFrB by chromatographic and spectral studies.

In Phase V *in silico* Glide docking studies were carried out to characterize the antitumorigenic efficacy against the cancer target proteins Histone deacetylase (1T69) and Tubulin (1Z2B) and antimicrobial efficacy against microbial target proteins Deacetylase C synthase (1W2N) and Pantothenate kinase (ISQ5) of phytochemical constituents of MEFrB.

PHASE I

4.1. Effect of MEFrB, MEFrF and MEFrL on *in vitro* Antioxidative and Antitumorigenic Activity

4.1.1. Effect of MEFrB, MEFrF and MEFrL on *in vitro* antioxidative activity

- 4.1.1.1. Scavenging of ABTS radical
- 4.1.1.2. Scavenging of DPPH radical
- 4.1.1.3. Scavenging of Hydroxyl radical
- 4.1.1.4. Scavenging of Superoxide radical
- 4.1.1.5. Scavenging of Hydrogen peroxide
- 4.1.1.6. Scavenging of Nitric oxide

4.1.2. Effect of MEFrB, MEFrF and MEFrL on *in vitro* antitumorigenic activity to DLA tumor cells

- 4.1.2.1. Effect on the viability of DLA tumor cells in MTT assay
- 4.1.2.2. Effect on cytotoxicity of activity to DLA tumor cells in Trypan blue assay

PHASE II

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

4.2.1. Effect on the activities of the liver marker enzymes

- 4.2.1.1. Aspartate transaminase
- 4.2.1.2. Alanine transaminase
- 4.2.1.3. Alkaline phosphatase

4.2.2. Effect on the activities of enzymic antioxidants

- 4.2.2.1. Catalase
- 4.2.2.2. Glutathione peroxidase
- 4.2.2.3. Glutathione reductase
- 4.2.2.4. Superoxide dismutase

4.2.3. Effect on the levels of non enzymic antioxidants

- 4.2.3.1. Vitamin A
- 4.2.3.2. Vitamin E
- 4.2.3.3. Vitamin C
- 4.2.3.4. Reduced glutathione

4.2.4. Effect on the levels of TBARS

4.2.5. Effect on the life span of DLA tumor induced Swiss albino mice

4.2.6. Effect on the histological status of liver of DLA challenged Swiss albino mice

PHASE III

4.3. Effect of MEFrB, MEFrF and MEFrL on the Antibacterial Activity Against Different Microorganisms

PHASE IV

4.4. Phytochemical Constituents of MEFrB, MEFrF and MEFrL

4.4.1. Preliminary phytochemical analysis

4.4.2. Chromatographic and spectral analysis

4.4.2.1. Gas Chromatography - Mass Spectrometry

4.4.2.2. High Performance Thin Layer Chromatography

4.4.2.3. Fourier Transform - Infra Red Spectroscopy

PHASE V

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

4.5.1. Active site residues of tumorigenic and microbial target proteins

4.5.2. Glide docking

4.5.3. Prediction of ADME properties

PHASE I

4.1. Effect of MEFrB, MEFrF and MEFrL on *in vitro* Antioxidative and Antitumorigenic Activity

The Phase I was designed to assess the *in vitro* antioxidative and antitumorigenic potential of methanolic extract of *Ficus racemosa* bark (MEFrB), fruits (MEFrF) and leaves (MEFrL). *In vitro* antioxidative role of different parts of *Ficus racemosa* was evaluated by their ability to scavenge the radicals such as ABTS, DPPH, Hydroxyl, Superoxide and non radicals such as Hydrogen peroxide and Nitric oxide. *In vitro* antitumorigenic potential was assessed by their cytotoxic effect to DLA tumor cells by MTT and Trypan blue exclusion assays and also to assess the fifty percent effective dose (ED₅₀) at the minimum concentration.

4.1.1. Effect of MEFrB, MEFrF and MEFrL on *in vitro* antioxidative potential

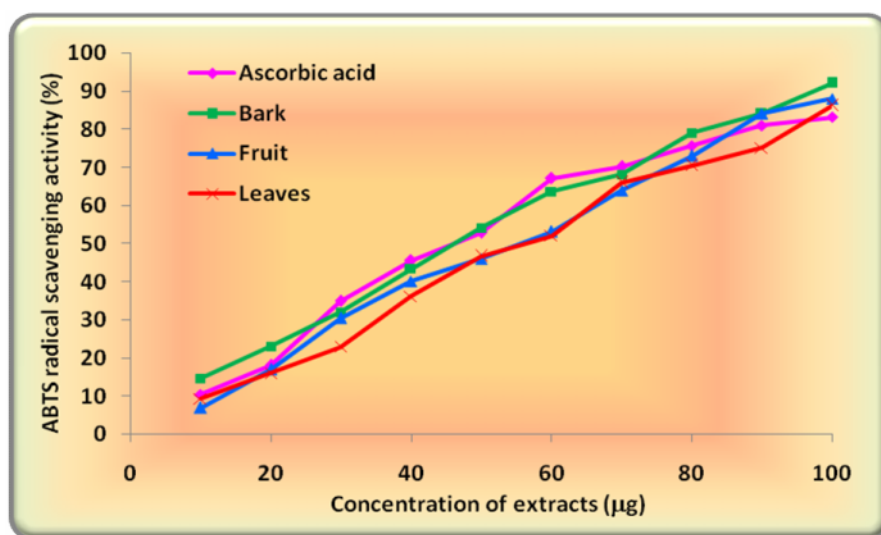
In vitro antioxidative role of MEFrB, MEFrF and MEFrL were evaluated by assessing their effect on the scavenging of ABTS, DPPH, hydroxyl, superoxide and non radicals such as hydrogen peroxide and nitric oxide against the standard antioxidant ascorbic acid. The results are shown in the Figures 26, 27,28,29,30 and 31.

4.1.1.1. Scavenging of ABTS radical

The ABTS radical scavenging activity of ascorbic acid the standard antioxidant, MEFrB, MEFrF and MEFrL was found to be dose dependent as shown in Figure 26. The ED₅₀ values were found to be 42µg (bark), 46µg (fruit), 43µg (leaves) and 49µg (ascorbic acid).

Figure 26

Effect of MEFrB, MEFrF and MEFrL on scavenging of ABTS radical



The ABTS radical is reactive towards most antioxidants including phenolics, thiols and Vitamin C. During this reaction the blue ABTS radical cation is converted back to its colorless neutral form. The scavenging activity of ABTS radical by the plant extract was found to be appreciable and this implies that the extract may be useful for treating radical related pathological damage especially at limited concentrations (Wang *et al.*, 1998). The ABTS model can be used to assess the scavenging activity of both the polar and non-polar samples (Re *et al.*, 1999). The

ABTS assay is based on the inhibition of the absorbance of the radical cation ABTS, which has a characteristic long, wave length absorption spectrum. The ABTS chemistry involves direct generation of ABTS radical mono cation with no involvement of any intermediary radical (Sasikumar *et al.*, 2010).

Naik *et al.* (2006a) also showed the scavenging ability of *Triphala*, an ayurvedic formulation towards different radicals such as hydroxyl radicals, superoxide radicals, DPPH and ABTS. The methanolic extract of the leaves and stem of *Calpurnia aurea* also showed effective scavenging of the ABTS radical (Pokorny, 2007). Khandhaker *et al.* (2008) reported that the methanolic extract of the leaves and stem of *Buddleja* were fast and effective scavengers of the ABTS radical. Gulcin *et al.* (2008) have shown that *Ligustroside* and *Oleuropein* isolated from the methanolic extracts of the root bark of *Chinonanthus virginicus* exhibited good ABTS scavenging activity.

The methanol extracts of *Celtis Africana* showed differential ABTS scavenging effect which was comparable to that of butylated hydroxytoluene, the standard antioxidant (Adedapo *et al.*, 2009). Jang *et al.* (2010) also reported the ABTS radical scavenging activity and antioxidant capacity of the acetone, ethanol, methanol and water extracts from fruit, seed and calyx parts of persimmon (*Diospyros kaki* cv. *Fuyu*).

Dharani *et al.* (2011) showed the ABTS inhibitory activity of the petroleum ether, benzene, chloroform, ethyl acetate, methanol and aqueous extracts of *Prosopis cineraria* leaves. Radha and Padma (2012) reported the dose dependent scavenging of ABTS radical by the aqueous, methanol and chloroform extracts of *Majorana hortonsis* leaves and methanol extract of *Aepinitos natensis* leaves.

4.1.1.2. Scavenging of DPPH radical

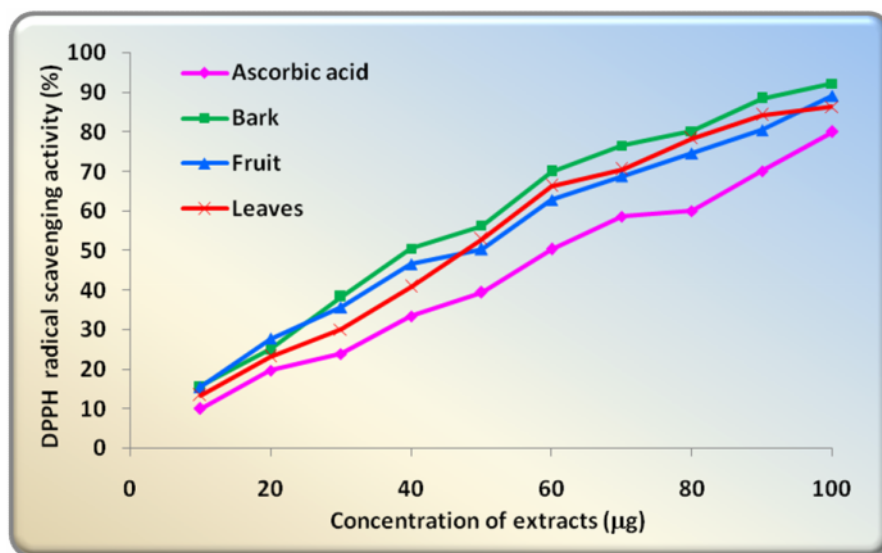
The MEFrB, MEFrF and MEFrL showed a dose dependent DPPH scavenging activity. Figure 27 shows the dose dependent DPPH radical scavenging activity of methanolic extract of various parts of *Ficus racemosa* and the ED₅₀ values of these extracts were found to be 40µg (bark), 49µg (fruit), 48µg (leaves) and 60µg (ascorbic acid).

Similarly Balasundram *et al.* (2005) also suggested that the ability of palm fruit methanolic extract of *Elaeis guineensis* to act as hydrogen donors as indicated by their ability to scavenge DPPH free radicals and in this respect the DPPH radical scavenging activity are comparable to that of ascorbic acid and gallic acid.

Our results are in tune with the investigations of Arokiaraj *et al.* (2008) and Makari *et al.* (2008) who reported that the methanol extracts of *Pterocarpus santalinns*, *Cordia wallichii* and *Celastrus paniculata* exhibited significant DPPH radical scavenging activity respectively. Adedapo *et al.* (2008) also have proved the antioxidant potential of the methanol extracts of the stems of *Acokanthera oppositifolia* and *Adenia gummifera* by their ability to scavenge DPPH radical.

Figure 27

Effect of MEFrB, MEFrF and MEFrL on scavenging of DPPH radical



The DPPH is stable nitrogen centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Dehpour *et al.*, 2009 and Prasad *et al.*, 2009). Sutharson *et al.* (2009) also reported the reduction of DPPH radical by the isolated compounds such as friedline 3 α - acetate and

β -amyrin from the methanolic extract of *Bischofia javanica* and *Fraxinus floribunda* due to their highest antioxidant activity.

The aqueous, methanolic and ethanolic extracts of *Melissa officinalis*, *Matricaria recutita* and *Cymbopogon citrates* were also found to possess DPPH scavenging activity (Pereira *et al.*, 2009). Hasan *et al.* (2009) by it's the DPPH radical scavenging activity of methanolic extract of seeds of *Syzygium cuminii* also showed stronger antioxidant role. Ebrahimzadeh *et al.* (2010b) also reported the pronounceable amount of DPPH radical scavenging activity of methanolic extracts of *Hyssopus officinalis*, *Viola odorata*, *Buxus hyrcana* and *Colchicum speciosum*. The Methanolic extract of *Njavara rice bran*, *Tephrosia purpurea* and *Lippia alba* also showed the DPPH radical scavenging activity (Saha *et al.*, 2010a). Siddique *et al.* (2010) also observed dose dependent DPPH radical scavenging effect of methanolic extracts of leaves, root and bark of *Aegle marmelos*.

The DPPH scavenging by the methanolic extract of fruits of *Ziziphus nummularia* Burm.F. was found to be more when compared to petroleum ether extract (Gupta *et al.*, 2011b). The methanolic extract of the *Hemidesmus indicus* showed potent radical scavenging of DPPH, superoxide radical and nitric oxide (Murali *et al.*, 2011a). Sreena *et al.* (2011) also reported the dose dependent scavenging of DPPH, NO, H₂O₂ and OH radicals by the methanolic extract of *Nerium indicum* leaves. Rajan *et al.* (2011) reported that the extracts of *Punica granatum* containing higher quantities of phenolic compounds responsible for the antioxidant and free radical scavenging activity and also chelates iron and possesses reducing power. Reddy *et al.* (2012) also reported the DPPH radical scavenging activity of methanol leaf extract of *Uvaria narum*.

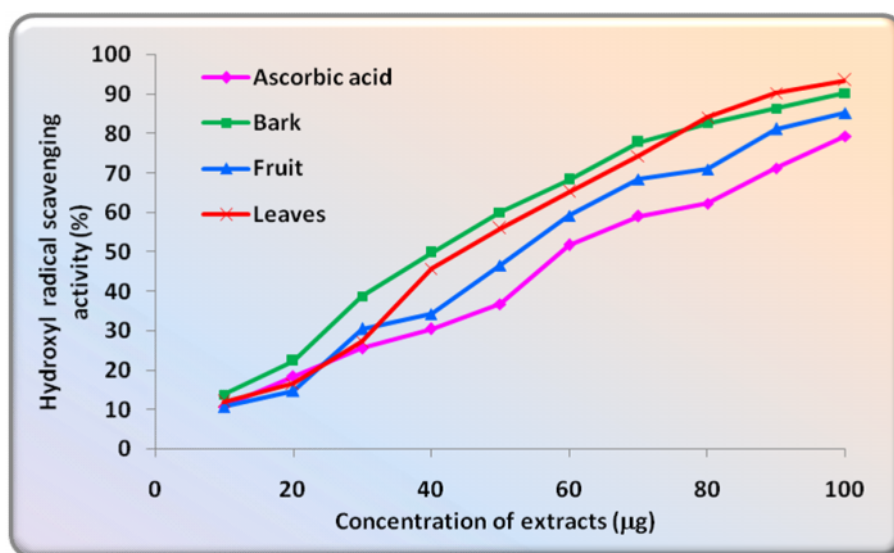
4.1.1.3. Scavenging of Hydroxyl radical

The hydroxyl radical scavenging activity of MEFrB, MEFrF and MEFrL was found to be dose dependent as shown in Figure 28. The ability of methanolic extract of various parts of *Ficus racemosa* to scavenge 50 percent of hydroxyl radical (ED₅₀) was found to be maximum with minimum concentration MEFrB (40 μ g) when compared to MEFrF (53 μ g), MEFrL (45 μ g).

Yin *et al.* (2008) showed increased radical scavenging activity with increasing concentration of roots of *Cirsium japonicum*. *Spondias pinnata* extract also showed the ability to inhibit hydroxyl radical mediated deoxy ribose degradation (Anour *et al.*, 2008). The methanol extract of *Lagerstroemia speciosa* L. showed higher hydroxyl radical scavenging activity when compared to the ethyl acetate, ethanol and water extracts (Priya *et al.*, 2008) and by the aqueous extract of *Wagatea spicata* flowers (Samak *et al.*, 2009). The methanolic extract of *Ficus mollis* leaves and stem bark showed significant inhibition of hydroxyl radical (Munna *et al.*, 2010).

Figure 28

Effect of MEFrB, MEFrF and MEFrL on scavenging of Hydroxyl radical



Hazra *et al.* (2010) also reported the radical scavenging activities of the methanolic extracts of fruits of *Terminalia chebula*, *Terminalia belerica* and *Embllica officinalis*. Balaji *et al.* (2011); Bala *et al.* (2011) and Savitha *et al.* (2011) also showed the scavenging of hydroxyl radical by methanol extract of *Ocimum tenuiflorum*, *Lxora coccinea*, *Manihot esculenta* and *Syzygium malaccinea* respectively.

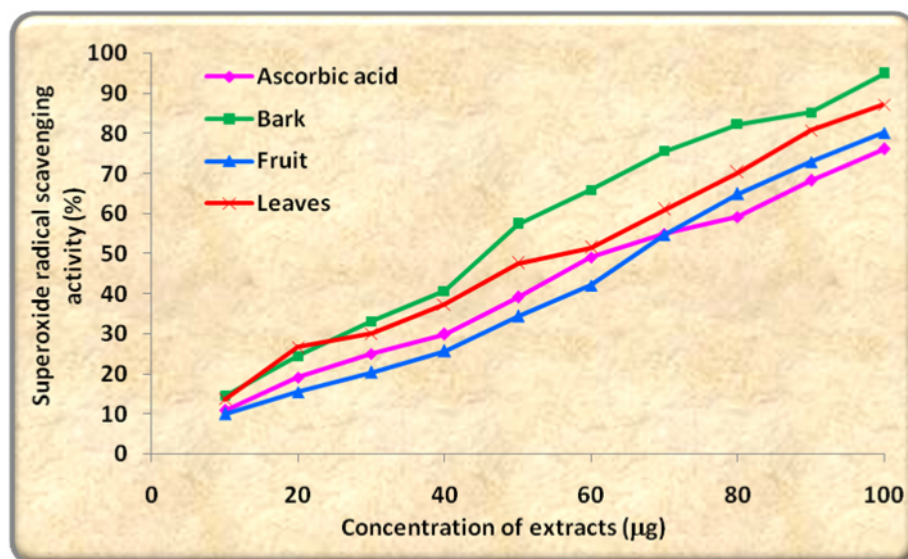
4.1.1.4. Scavenging of Superoxide radical

The effect of MEFrB, MEFrF and MEFrL on *in vitro* scavenging of superoxide radical is shown in Figure 29. The MEFrB, MEFrF and MEFrL inhibited

superoxide radical production *in vitro* in a dose dependent manner with the ED₅₀ of 47µg (bark), 55µg (fruit), 52µg (leaves) and 56µg (ascorbic acid).

Figure 29

Effect of MEFrB, MEFrF and MEFrL on scavenging of Superoxide radical



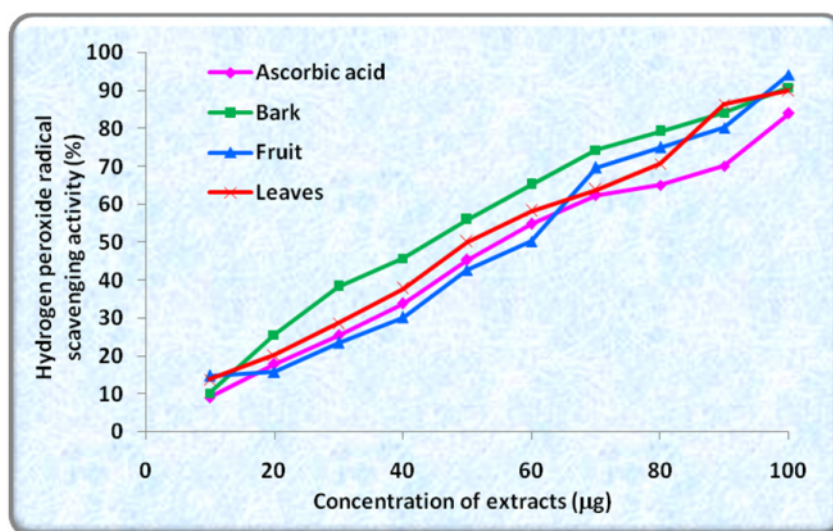
Superoxide anion is an oxygen-centered radical with selective reactivity. This species is produced by a number of enzyme systems in autooxidation reactions and by non enzymatic electron transfers that univalently reduce molecular oxygen (Gulcin *et al.*, 2005). Siddhuraju and Becker (2007) found the significant superoxide anion radical scavenging activity in the raw, dry heated seed extracts and the hydrothermally processed seed samples of cowpea (*Vigna unguiculata*). Mandal *et al.* (2011) reported the methanolic extract of the leaves and stem of *Drymaria diandra* efficiently inhibited superoxide anion.

4.1.1.5. Scavenging of Hydrogen peroxide

The methanolic extract of various parts of *Ficus racemosa* showed a dose dependent hydrogen peroxide scavenging activity. Figure 30 shows the dose dependent hydrogen peroxide scavenging activity of methanolic extract of various parts of *Ficus racemosa* and the ED₅₀ was found to be 43µg (bark), 53µg (fruit), 47µg (leaves) and 55µg (ascorbic acid).

Rai *et al.* (2007) showed that the plant extract *Psidium guajava* scavenged H_2O_2 more effectively than butylated hydroxyanisole. Shao *et al.* (2007) also showed the dose dependent hydrogen peroxide scavenging activity of all the organic fractions of *Garcinia kola*. The methanolic extracts of the plants *Cassia spectabilis* and *Cassia fistula* were identified as potential novel sources of hydrogen peroxide radical scavenging agents (Nehru *et al.*, 2008). Okoko (2009) also showed the dose dependent hydrogen peroxide scavenging activity of all the organic fractions of *Njavara* rice bran.

Figure 30
Effect of MEFrB, MEFrF and MEFrL on scavenging of Hydrogen peroxide



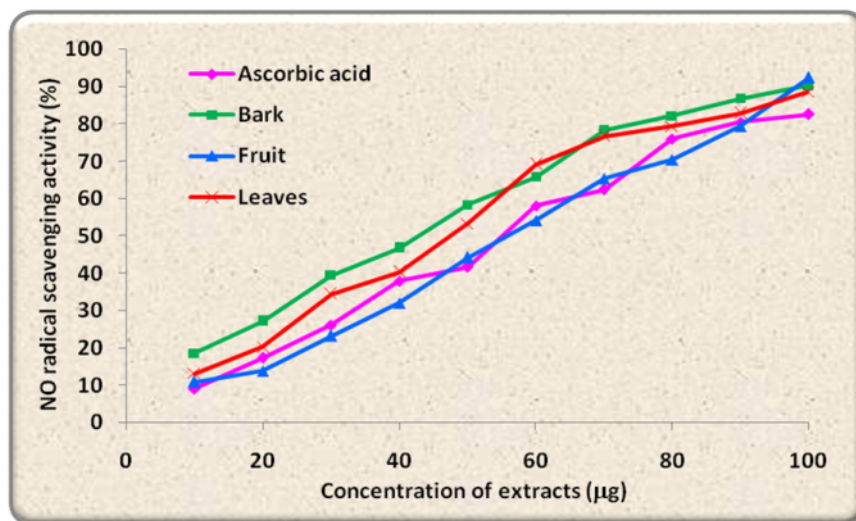
Yin *et al.* (2009) also showed the dose dependent scavenging of hydrogen peroxide by the ethanol extract of the leaves of *Stachytarpheta angustifolia* and the methanolic extract of *Picrasma quassiades* (Awah *et al.*, 2010). Ebrahimzadeh *et al.* (2010b) in *B. hyrcana* leaves crude extract and methanolic extract of *Garcinia kola* seeds and *H. officinalis* L. var. *angustifolius* aerial parts, *C. speciosum* flowers and *V. odorata*. Kwape and Chaturvedi (2012) have reported the hydrogen peroxide scavenging effects of methanol, chloroform and hexane extracts of *Zizius macronates*. Meena *et al.* (2012) also reported that the methanolic extract of leaves of *Baccopa monnieri* exhibited significant hydrogen peroxide radical inhibition.

4.1.1.6. Scavenging of Nitric oxide

The methanolic extract of MEFrB, MEFrF MEFrL and ascorbic acid showed dose dependent scavenging of nitric oxide (Figure 31). The ED₅₀ values were found to be 44µg (bark), 54µg (fruit), 48µg (leaves) and 55µg (ascorbic acid).

Figure 31

Effect of MEFrB, MEFrF and MEFrL on scavenging of Nitric oxide



Razali *et al.* (2008) reported the scavenging of superoxide anion and nitric oxide by the methanol, hexane and ethyl acetate extracts of shoots of *Anacardium occidentale*. Manikandan *et al.* (2009) also showed that the protective effect of ethylacetate, methanolic and ethanolic extracts of *Azadirachta indica* leaf fractions by scavenging superoxide, hydroxyl radicals and Nitric oxide non radicals.

Ebrahimzadeh *et al.* (2009) also showed the dose dependent DPPH, NO and H₂O₂ scavenging activities of methanolic extract of *Ornithogalum sinteisi*. Saha *et al.* (2010a) showed the highest nitric oxide scavenging activity of Njavara rice bran extracts when compared to other methanolic extract of rice bran such as Yamini, Jyothi and Vasumathi. Rao *et al.* (2010) have reported the antioxidant activity of the methanolic extract of Njavara rice bran by its scavenging effect on DPPH and nitric oxide radicals.

Arya and Yadav (2011) have reported that the nitric oxide radical scavenging activity of methanolic extract of the seeds of *Cassia occidentalis*.

Saradhadevi and Annapoorani (2011) reported that the methanolic extract of *Gloriosa superba* leaves showed increased scavenging activity of nitric oxide. The methanolic extract of the leaves of *Tabernaemontana divericata*, *Caesalpinia crista*, *Curcumin indica* and *Psidium guajava* showed promising scavenging of NO (Mandal *et al.*, 2011; Sivaprabha *et al.*, 2011; Rumzhum *et al.*, 2012 and Venkatachalam *et al.*, 2012).

From the above results, maximum free radical scavenging efficacy with minimum ED₅₀ of MEFrB was found to be in the order of OH (40µg) > ABTS (42µg) > H₂O₂(43µg) > NO (44µg) > O₂•⁻ (47µg) > DPPH (48µg); the ED₅₀of MEFrF was found to be in the order of ABTS (46µg) >DPPH (49µg) > H₂O₂ (53µg) > OH (53µg) > NO (54µg) > O₂•⁻(55µg) and the ED₅₀ of MEFrL was found to be in order of ABTS (43µg) > OH(45µg) > H₂O₂ (47µg) NO (48µg) > DPPH (48µg) > O₂•⁻ (52µg) and the ED₅₀ of standard ascorbic acid was found to be in the order of ABTS (49µg) > NO (55µg) > H₂O₂ (55µg) > O₂•⁻ (56µg) OH (59µg) > DPPH (60µg) respectively.

The dose dependent percentage scavenging of ABTS, DPPH, OH Superoxide radical and non radicals H₂O₂ and NO by MEFrB, MEFrF and MEFrL indicated their antioxidative potential. The hydroxyl radical is extremely reactive and can attack many cell constituents including lipids, nucleic acids and proteins. The dose dependent scavenging of the hydroxyl radical by all the methanolic extracts of *Ficus racemosa* may be due to their antioxidative role which may prevent the tumor formation and lipid peroxidation. MEFrB showed more efficient scavenging of free radicals and non radicals than that of MEFrF and MEFrL and the standard ascorbic acid.

The ED₅₀ of MEFrB was found to be more efficient with lower concentration (ED₅₀ 40 µg to 48 µg) than that of MEFrF (ED₅₀ 46 µg to 55 µg) and MEFrL (ED₅₀ 43 µg to 55 µg) and ascorbic acid (ED₅₀ 49 µg to 60 µg).This dose dependent scavenging of free radicals may be due to the antioxidative role of the secondary metabolites present in the methanolic extracts which may prevent the ill effects of excessive free radical generation. The antioxidative potential of MEFrB was found to be more significant than that of MEFrF and MEFrL. The antioxidative

efficacy may be due to the presence of highly active secondary metabolites of MEFrB.

Reactive oxygen species, which include free radicals such as superoxide anion radicals ($O_2^{\cdot-}$), hydroxyl radicals ($OH^{\cdot}$) and non free radical species like H_2O_2 and singlet oxygen ($^1O_2$), are various forms of activated oxygen. These molecules are exacerbating factors in cellular injury and aging process (Gulcin *et al.*, 2010). The results of the radical scavenging activity showed that all the extracts in this study were the effective radical scavengers. ABTS is based on hydrogen transfer reaction, while DPPH is based on electron transfer (Prior *et al.*, 2005). Nitric oxide is an essential molecule required for several physiological processes like neural signal transmission, vasodilation, immune response and blood pressure (Wink *et al.*, 1991). However, high concentration of NO may result in several pathological conditions including cancer and inflammation (Patil *et al.*, 2009). This may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric oxide there by inhibiting the generation of nitrite.

Superoxide can cause oxidation or reduction of solutes depending on their reduction potential. Both aerobic and anaerobic organisms possess superoxide dismutase enzymes, which catalyse the breakdown of superoxide radical (Shirwaiar *et al.*, 2007). Since, the conversion of superoxide and H_2O_2 into more reactive species, the hydroxyl radical, has been thought to be one of the unfavourable effects caused by superoxide radicals (Halliwell, 1991). The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cell (Singh *et al.*, 2002). Hydrogen peroxide is an important reactive oxygen species formed *in vivo* by many oxidizing enzymes such as superoxide dismutase. It has a strong oxidizing property with the ability to penetrate biological membranes. Hydrogen peroxide itself is not very reactive, but can sometimes be toxic to cells when it gives rise to hydroxyl radicals (Gulcin *et al.*, 2010). H_2O_2 scavenging property of these extracts may be attributed to their antioxidant contents that donate electrons to H_2O_2 and thus reducing it into water.

Present study and confirmed that all their antioxidant potential the extracts and free radical can be used for better inhibition of free radicals because scavenging activity of all the extracts were increased markedly with the increase of concentration in different antioxidant assays. But variation in the effectiveness among the three extracts may be depends upon the chemical composition of the extracts. Each extract contains generally different antioxidant compounds with different amount of antioxidant activity. The antioxidant activity has been attributed to various mechanisms, among which are the prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the prevention of continued hydrogen abstraction, the reductive capacity and radical scavenging (Zachariah *et al.*, 2012). Thus, the present results clearly indicated that the MEFrB, MEFrF and MEFrL possess antioxidant property and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. Among the three extracts of MEFrB exhibited the maximum protection against the selected radicals.

4.1.2. Effect of MEFrB, MEFrF and MEFrL on *in vitro* antitumorigenic activity to DLA tumor cells

In vitro antitumorigenic activity of MEFrB, MEFrF and MEFrL to DLA tumor cells was evaluated by assessing their *in vitro* cytotoxic effect in MTT assay and trypan blue exclusion assay.

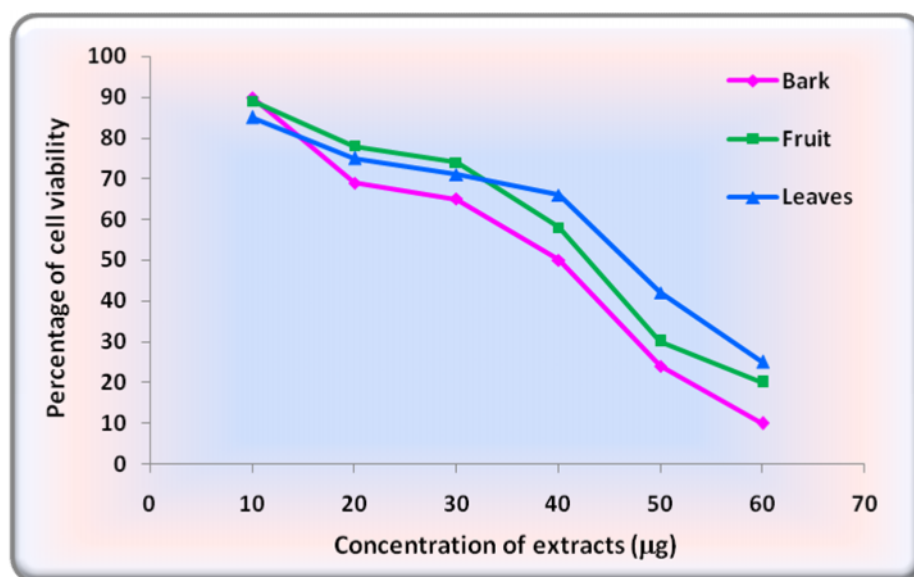
4.1.2.1. Effect on the viability of DLA tumor cells in MTT assay

The extent of cytotoxicity can be measured by MTT dye reduction assay which assess the maximum viability of cells. The percent viability was analysed by MTT assay after treatment with different concentration of MEFrB, MEFrF and MEFrL. MTT assay showed the decreased viability of DLA tumor cells with increased concentration of plant extracts and indicated the cytotoxic effect of MEFrB, MEFrF and MEFrL to DLA cells in concentration dependent manner (Figure 32). The extract killed 50 percent of DLA tumor cells at a concentration of 40µg (MEFrB), 42µg (MEFrF) and 46µg (MEFrL). This concentration was designated as fifty percent effective concentration (ED₅₀).

Our results are comparable to Naik *et al.* (2006a) who reported that 70% methanol extract of *Terminalia chebula* decreased the number of immortalized and cancer cell lines by inhibiting the rate of cell proliferation and induced cell death. Sivakumar *et al.* (2008) also reported that the *Abrus precatorius* seeds contain abrine, a poisonous principle similar to ricin from castor seeds, which suppresses *Ehrlich ascites* tumor growth in mice. Abrins are more toxic to tumor cells than normal cells.

Figure 32

Cytotoxic effect of MEFrB, MEFrF and MEFrL to DLA tumor cell in MTT assay



Thara and Zuhra (2012) showed an inhibition of the growth of DLA tumor cell in a dose dependent manner in MTT assay by the methanol fraction of *Saussurea lappa*. Motaal and Shaker (2011) showed a pronounced cytotoxic activity against MCF-7 cells by the pomegranate peel extract. The anticancer activity of the whole pomegranate fruit extract against HCT-116 colon cells was most prominent followed by the pulp extract. Sini *et al.* (2012) showed that the methanol extract from *Orthosiphon thymiflorus* is cytotoxic to DLA tumor cells. Senthilkumar *et al.* (2011) showed a significant antitumor effect of methanolic extract of *Indigofera linnaei* to EAC, DLA and human cancer cell lines. Subhadradevi *et al.* (2011) also reported the inhibition of the growth of DLA cells in

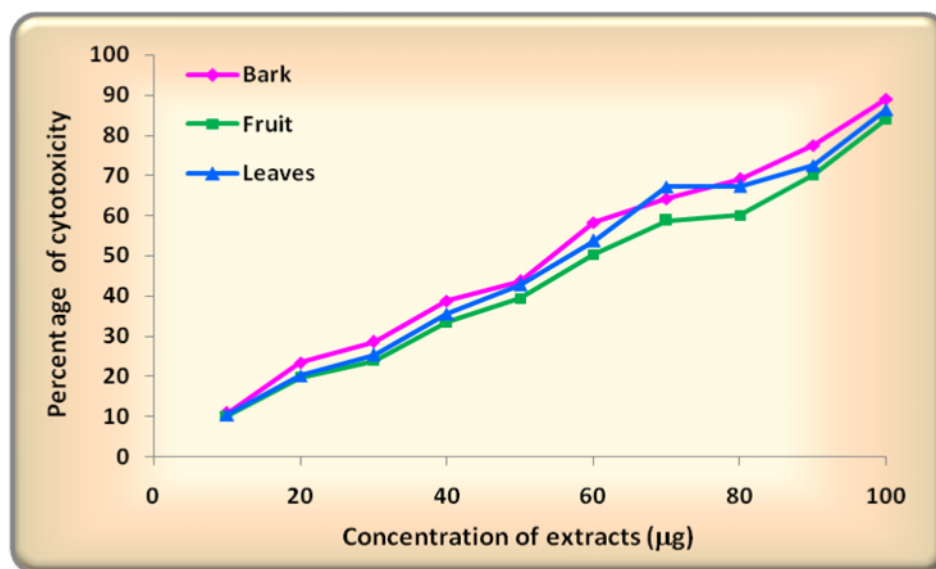
a dose dependent manner by the methanolic extract of *Apium graveolens* seed. Pradhan *et al.* (2012) reported the cytotoxic effect of *Limonia acidissima* Linn. against the SKBR3 and MDA-MB-435 breast cancer cell lines.

4.1.2.2. Effect on the cytotoxic activity to DLA tumor cells in Trypan blue assay

In vitro antitumorigenic effect of MEFrB, MEFrF and MEFrL was assessed by cytotoxic studies against DLA tumor cells using Trypan blue exclusion method. Plate III shows the antitumorigenic effect of MEFrB, MEFrF and MEFrL to DLA tumor cells. It is based on the principle that live cells possess intact cell membrane that exclude the dye while the dead cells do not and have blue colored cytoplasm under light microscope (Jayaseelan *et al.*, 2012). Figure 33 shows the dose dependent cytotoxic effect of MEFrB, MEFrF and MEFrL to DLA tumor cells.

Figure 33

Cytotoxic effect of MEFrB, MEFrF and MEFrL to DLA tumor cells in Trypan blue assay

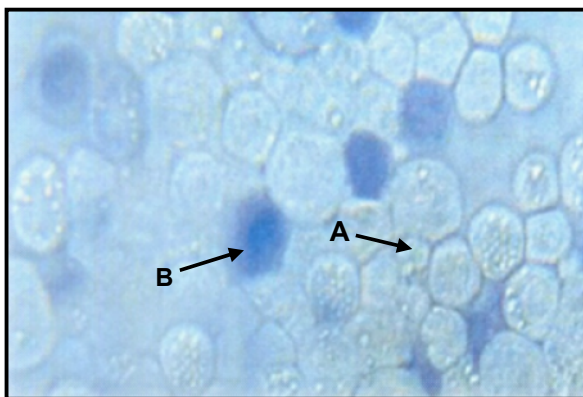


Incubation of DLA tumor cells with MEFrB, MEFrF and MEFrL showed a concentration dependent cytotoxic effect which was indicated by the increase in number of dead cells with increasing concentrations of MEFrB, MEFrF and MEFrL. The extract killed 50 percent of DLA tumor cells at a concentration of 54µg (MEFrB), 58µg (MEFrL) and 60µg (MEFrF). This concentration was designated as

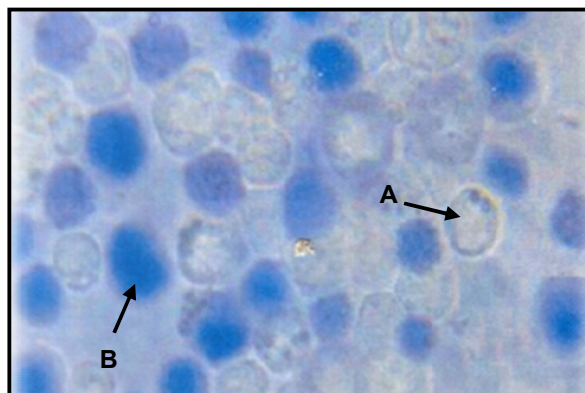
fifty percent effective concentration (ED₅₀) and was used in the following *in vivo* studies.

Plate III

Antitumorigenic effect of *Ficus racemosa* to DLA tumor cells



DLA tumor cells without plant extract



A - Live cells
B - Dead cells

DLA tumor cells with plant extract

Similar dose dependent cytotoxic activity of the fruit extract of *Piper logum* against DLA and ELA cells were reported by Sunila and Kuttan (2004). Similar to our results, the methanolic leaf extract of *Plumeria alba* also showed a concentration dependent cytotoxic activity to DLA and ELA cells (Radha *et al.*, 2008). Our observation was also supported by Adhvaryu *et al.* (2010) who showed maximum cytotoxicity to DLA tumor cells by the four ayurvedic herbs such as *Curcuma longa* L., *Ocimum sanctum* L., *Tinospora cordifolia* (Wild) and *Zizyphus mauritiana*. Chetan *et al.* (2010) revealed the potent antitumorigenic effect of

aqueous extract of *Areca catechu*. Mazumder *et al.* (2010) also reported the cytotoxic effect of methanolic extracts of stem and rhizomes of *Berberis aristata* and *Hemidesmus indicus*. in MCF7 breast cancer cell line.

Ravichandran *et al.* (2012) reported the cytotoxic potentials against EAC in higher concentration by the methanolic extract of *Phyllanthus maderaspatensis* and also reported that the activity might be due to the presence of secondary metabolites, particularly flavonoids which are known to implicate signal translation in cell proliferation and thereby prevent cancer.

The Trypan blue assay showed the dose dependent cytotoxic effect of the extracts MEFrB, MEFrF and MEFrL to DLA tumor cells with the fifty percent effective dose (ED₅₀) at 54µg, 58µg and 60µg respectively. The MTT assay also showed the dose dependent cytotoxic effect of the extract to DLA tumor cells with the ED₅₀ of 40µg (MEFrB), 42µg (MEFrF) and 46µg (MEFrL). Trypan blue and MTT assay thus showed their antitumorigenic effect.

PHASE II

A variety of plant secondary metabolites have been reported to act as antioxidants. Antioxidants are compounds that scavenge, dispose and suppress the formation of free radicals or oppose their actions to prevent the generation of free radicals and those that intercept any free radicals that are generated. They exist in both the aqueous and membrane compartment of cells and can be enzymes or non-enzymes to renew the process of life (Ebrahimzadeh *et al.*, 2010a; Jain *et al.*, 2011a; Maithreyi *et al.*, 2010 and Murali *et al.*, 2011b). Transplantable Dalton Lymphoma Ascites tumor cells into Swiss albino mice are an important experimental approach to study the antioxidative and antitumorigenic effect of medicinal plants (Segura *et al.*, 2000; Sundaresan and Subbiah, 2012 and Baskar *et al.*, 2012).

4.2. Effect of MEFrFB, MEFrF and MEFrL on *in vivo* Antioxidative and Antitumorigenic Potential in DLA Tumor Induced Swiss Albino Mice

Antioxidants play an important role in inhibiting and/or scavenging free radicals and thus provide protection against infections and degenerative diseases.

Antioxidative role of MEFrB, MEFrF and MEFrL was followed by assessing their effect on enzymic and non enzymic antioxidants and TBARS compared to silymarin the standard antioxidant. Antitumorigenic role of MEFrB, MEFrF and MEFrL was followed by assessing their effect on hepatic enzymic and non enzymic antioxidants in DLA tumor induced mice in the presence and absence of MEFrB, MEFrF and MEFrL. Before evaluating the antioxidative and antitumorigenic role, the normal functioning of the liver was assessed by evaluating the liver marker enzymes in the serum of controls and experimental groups.

4.2.1. Effect on the activities of the liver marker enzymes

To evaluate the normal functioning of the liver, the hepatocellular marker enzymes such as AST, ALT and ALP were assessed in the serum of all the vehicle controls, DLA tumor cells transplanted mice, silymarin, MEFrB, MEFrF and MEFrL treated and DLA+ MEFrB, DLA+ MEFrF and MEFrL treated Swiss albino mice.

4.2.1.1. Aspartate transaminase

The activity of AST was found to be significantly decreased in standard antioxidant silymarin administered mice when compared to paraffin oil control mice in all treatment periods. MEFrB, MEFrF and MEFrL mice showed significant decrease in AST activity when compared to DMSO control in all treatment periods. In MEFrB, MEFrF and MEFrL treated animals, the activity of AST was found to be significantly decreased when compared to silymarin in all the treatment periods. DLA tumor burden significantly enhanced the AST activity and indicated the membrane damage whereas the coadministration of MEFrB, MEFrF and MEFrL to DLA induced mice significantly decreased the AST activity and indicated the normal integrity of membrane of the liver.

The DLA tumor induced mice showed significant increase in AST activity when compared to all the controls and other experimental groups in 20 days treatment period. Administration of MEFrB, MEFrF and MEFrL to DLA tumor induced mice showed significant decrease in AST activity in 90 days when compared to 20 days treatment period as shown in Table 3 and Figure 34.

Table 3
Activity of AST in the serum of controls and experimental Swiss albino mice

Groups	Treatments	Activity of AST(U ^s / L) Treatment periods	
		20 days	90 days
1	PBS	49.11 ± 0.02	49.04 ± 0.02
2	DMSO	48.49 ± 0.08	48.34 ± 0.03
3	Paraffin oil	52.76 ± 0.03	52.56 ± 0.02
4	Silymarin	37.58 ± 0.35	34.62 ± 0.01
5	MEFrB	36.28 ± 0.18	31.30 ± 0.02
6	MEFrF	35.25 ± 0.11	32.36 ± 0.02
7	MEFrL	35.45 ± 0.36	32.52 ± 0.03
8	MEFrB+DLA	57.36 ± 0.03	50.46 ± 0.02
9	MEFrF+DLA	57.36 ± 0.24	55.86 ± 0.02
10	MEFrL+DLA	57.50 ± 0.04	53.57± 0.03
11	DLA	75.78 ± 0.04	-
One way ANOVA (with DLA) (P<0.05)		0.4452	-
One way without DLA (p<0.05)		0.4445	0.2249
Two way ANOVA Without DLA (p<0.05)		0.14876	

The values are mean ± SD of six animals

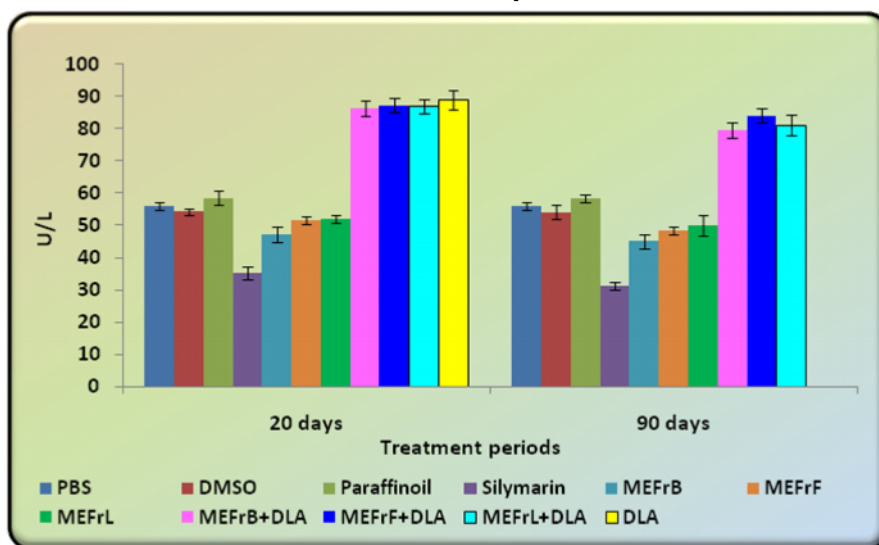
U^s - micromole of pyruvate formed /minute

These findings are in accordance with the findings of Dash *et al.* (2007) who also reported the significant decrease in the activity of biochemical markers such as AST and ALT in mice treated with chloroform and methanolic extracts of *Ichnocarpus frutescens*. Methanol extract of *Bixa orellana*, *Cajanus cajan*, *Glycosmis pentaphylla* and *Casuarina equisetifolia* exhibited the protective effect by lowering the activities of AST and ALT in the serum of CCl₄ induced rats (Ahsan *et al.*, 2009).

Tenpe *et al.* (2009) reported that administration of leaf organic extracts of *Oroxylum indicum* decreased the serum levels of AST, ALT and ALP. Which were found to be significantly increased in DLA tumor cells induced mice indicated the membrane damage. Thirupathi *et al.* (2009) and Radha (2010) also reported that the methanolic extract of leaves of *Balabites roxburghii* and *Bacopa monnieri* administration showed significant decrease in the level of biochemical markers such as AST, ALT and ALP against ethanol-CCl₄ induced toxicity and indicated their protective effect.

Figure 34

Effect of MEFrB, MEFrF and MEFrL on the activity of AST in the serum of controls and experimental mice



Sivaraj *et al.* (2011) reported a significant decrease in the levels of biochemical marker enzymes of liver like ALT, AST, ALP and bilirubin in plant extract administered animals. A similar significant decrease in AST, ALT and ALP was observed by Saroja and Annapoorani (2012a) by the administration of methanolic extract of *Cynodon dactylon* leaves against ELA induced Swiss albino mice.

4.2.1.2. Alanine transaminase

Methanolic extract of *Ficus racemosa* bark, fruit and leaves showed significant decrease in ALT activity when compared to DMSO in all the treatment

periods. Silymarin showed significant decrease in the activity of ALT when compared to paraffin oil control mice, MEFrB, MEFrF and MEFrL treated mice in 20 days and 90 days treatment periods.

Table 4
Activity of ALT in the serum of controls and experimental Swiss albino mice

Groups	Treatments	Activity of ALT (U [#] / L) Treatment periods	
		20 days	90 days
1	PBS	56.12 ± 0.05	56.03 ± 0.02
2	DMSO	54.22 ± 0.01	54.16 ± 0.02
3	Paraffin oil	58.63 ± 0.03	58.37 ± 0.02
4	Silymarin	35.19 ± 0.31	31.14 ± 0.02
5	MEFrB	47.22 ± 0.06	45.05 ± 0.02
6	MEFrF	51.61 ± 0.05	48.56 ± 0.01
7	MEFrL	52.15 ± 0.02	50.07 ± 0.06
8	MEFrB+DLA	86.40 ± 0.06	79.60 ± 0.01
9	MEFrF+DLA	87.34 ± 0.11	84.11 ± 0.04
10	MEFrL+DLA	87.04 ± 0.01	81.02 ± 0.01
11	DLA	89.04 ± 0.02	-
One way ANOVA (with DLA) (p<0.05)		0.3160	-
One way without DLA (p<0.05)		0.2967	0.3771
Two way ANOVA Without DLA (p<0.05)		0.22676	

The values are mean ± SD of six animals; U[#]- micromole of pyruvate formed /minute

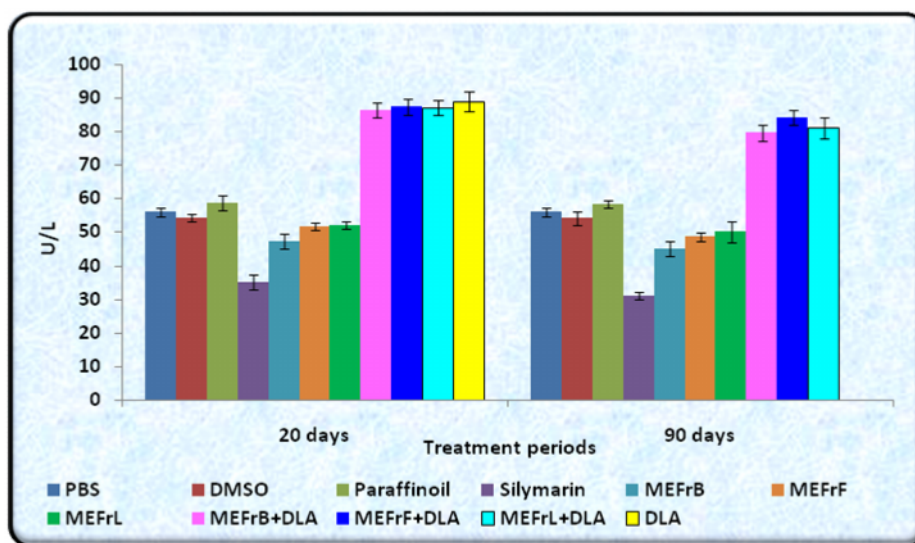
Significant decrease in the activity of ALT in 20 days and 90 days treatment periods in MEFrB treated mice was found to be more significant than that of MEFrF and MEFrL treated mice. DLA tumor induced mice showed significant increase in ALT activity when compared to all the controls and other experimental groups in 20 days treatment period. Coadministration of MEFrB, MEFrF and MEFrL to DLA tumor induced mice showed significant decrease in ALT activity in

90 days treatment periods when compared to 20 days treatment period as shown in Table 4 and Figure 35.

The above results are in accordance with the findings of Thamizh Selvam *et al.* (2010) who reported the abnormal high levels of liver function test parameters like SGOT, SGPT, ALP, bilirubin and protein levels in mice treated with the cellular damage of the hepatic cells. The CCl₄-induced hepatotoxicity in rats, as judged by the raised serum enzymes, was prevented by pretreatment with the aqueous and methanolic extracts of *Phyllanthus niruri* and demonstrated their hepatoprotective action (Harish and Shivanandappa, 2006). Opoku *et al.* (2007) showed that the administration of *Rhoicissus tridentate* extracts after CCl₄ intoxication in rats resulted in significantly reduced activities of ALT and ALP.

Figure 35

Effect of MEFrB, MEFrF and MEFrL on the activity of ALT in the serum of controls and experimental mice



Jothivel *et al.* (2007) also reported that the methanolic extract of *Costus pictus* showed significant decrease in the ALP, AST and ALT levels in the serum of rats. Qureshi *et al.* (2009) also investigated the similar significant decrease in the activity of AST, ALT and ALP by the leaf extract of *Cordia macleodii* in rat. Santhi and Annapoorani (2010) and Chandra Prabha and Annapoorani (2011) also reported that the coadministration of protein fraction of *Cynodon dactylon* and

Terminalia catappa to ELA tumor induced mice significantly decreased the activity of ALT in serum of mice.

4.2.1.3. Alkaline phosphatase

Methanolic extract of *Ficus racemosa* bark, fruit and leaves treated mice showed significant decrease in ALP activity when compared to DMSO in all treatment periods. Silymarin treated group showed significant decrease in serum ALP activity in when compared to paraffin oil administered mice in all treatment periods. ALP activity in MEFrB, MEFrF and MEFrL administered mice was found to be similar to that of the ALP activity found in silymarin in all the treatment periods. DLA tumor induced mice showed significant increase in ALP activity when compared to all the controls and other experimental groups in 20 days treatment period. Administration of MEFrB, MEFrF and MEFrL individually and coadministration to DLA tumor induced mice showed decrease in ALP activity in 90 days treatment period when compared to 20 days treatment period and the decrease was found to be significant only in MEFrF +DLA as shown in Table 5 and Figure 36.

Pradeep *et al.* (2007) also observed the significant increase in the levels of serum ALP, ACP and LDH in diethyl nitrosamine induced animals and significant decrease in *Cassia fistula* leaf extract administered animals. The decrease in ALT and AST activities by the methanolic fractions of *T. bangwensis* may be an indication of regeneration process and repair of hepatic tissue damage induced by CC1₄ (Suresh Kumar and Mishra, 2008 and Moselhy and Ali, 2009).

Santhi and Annapoorani (2010) also analysed the liver marker enzymes in serum namely GOT, GPT and ALP in all the experimental animals to assess the normal functioning of the liver. The activities of SGOT and SGPT were found to be significantly decreased by the administration of protein fraction of *Cynodon dactylon* and *Terminalia catappa*.

Chandra Prabha and Annapoorani (2011) also showed the increased activities of hepatospecific enzymes SGOT and SGPT in the liver of the mice challenged with the free radical enhancer CCl₄ and decreased activities by the

coadministration of protein fraction of *Cynodon dactylon* to CCl₄ induced mice and indicated the protective role of protein fraction of *Cynodon dactylon* against CCl₄.

Table 5
Activity of ALP in the serum of controls and experimental Swiss albino mice

Groups	Treatments	Activity of ALP(U [@] / L) Treatment periods	
		20 days	90 days
1	PBS	115.70 ± 0.30	115.2 ± 0.03
2	DMSO	117.40 ± 0.37	117.2 ± 0.01
3	Paraffin oil	114.30 ± 0.37	114.3 ± 0.02
4	Silymarin	93.45 ± 0.35	93.24 ± 0.02
5	MEFrB	84.22 ± 0.06	80.07 ± 0.02
6	MEFrF	89.05 ± 0.03	85.05 ± 0.03
7	MEFrL	90.14 ± 0.02	93.34 ± 0.02
8	MEFrB+DLA	92.42 ± 0.12	89.24 ± 0.21
9	MEFrF+DLA	109.1 ± 0.01	91.29 ± 0.04
10	MEFrL+DLA	90.04 ± 0.01	86.13 ± 4.72
11	DLA	173.5 ± 0.38	-
One way ANOVA (with DLA) (P<0.05)		0.4444	-
One way without DLA (p<0.05)		0.4126	0.1346
Two way ANOVA Without DLA (p<0.05)		10.55414	

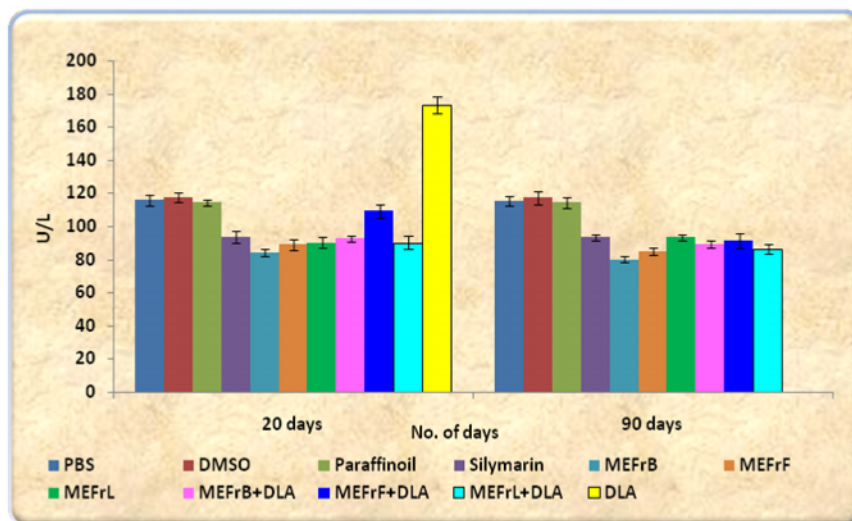
The values are mean ± SD of six animals; U[@] - micromole of phenol formed /minute

The AST, ALT and ALP activities were significantly increased in DLA tumor induced mice when compared to controls. The increase in the activities of these enzymes in serum may be due to the leakage of these cytosolic enzymes into the circulatory system due to hepatocellular damage in DLA tumor induced mice. DLA tumor burden might be responsible for the damage of liver cells and this damage was reverted by the administration of plant extracts. Treatment of plant extracts by themselves and to the DLA tumor induced mice caused a decrease in the AST, ALT and ALP activities to near normal level and showed their protective role and thereby maintained the normal functions of the liver. The study showed that

MEFrB, MEFrF and MEFrF have the potential to maintain the normal liver functions in mice.

Figure 36

Effect of MEFrB, MEFrF and MEFrL on the activity of ALP in the serum of controls and experimental mice



Transaminases are among a group of ubiquitous enzymes whose serum activity is elevated in many different diseased states, including myocardial infarction, viral and toxic hepatitis, muscular dystrophy and cancer (Nevin and Vijayammal, 2005). Free radicals and peroxidants have been implicated in the pathogenesis of toxic liver injury (Jalalpure *et al.*, 2003). AST, ALT and ALP are reliable determinants of liver parenchymal injury (Moss *et al.*, 1987). Increment in the activities of AST, ALT and ALP in serum may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro *et al.*, 1993). The elevation of these liver specific enzymes observed in tumor condition is a possible indicator of progression of tumor growth. The rise in the AST is usually accompanied by an elevation in the levels of ALT, which play a vital role in the conversion of amino acids to keto acids (Ibrahim and Nassar, 2008). The present study revealed the reversion of DLA tumor burden by the coadministration of MEFrB, MEFrF and MEFrF to DLA tumor induced mice and proved their antioxidative and anticarcinogenic role.

4.2.2. Effect on the activities of Enzymic Antioxidants

Oxygen free radicals are reactive species which are constantly generated in living cells as a part of normal metabolism. An excess production of ROS and RNS are harmful to cells, which is likely to exert toxic effects in the cells which in turn are involved in the pathogenesis of certain diseases and aging. To scavenge and neutralize these excess free radicals, the cells are endowed with the antioxidative defense enzymes such as catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD). Liver plays an important role in the metabolism of drug and nutrients. Because of its central role in drug metabolism, it is the most vulnerable tissue for drug toxicity. The enzymatic antioxidants such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and glutathione peroxidase (GPx) play a protective role against oxidative damage caused by free radicals through their free radical scavenging activity. They have been shown to inhibit the cancer causation in animals and in human trials. Hence, an attempt was made to study the effect of MEFrB, MEFrF and MEFrL on the activity of enzymic antioxidants in the liver of Swiss albino mice transplanted with and without DLA cells.

4.2.2.1. Catalase

Catalase activity was found to be significantly increased in mice treated with silymarin, MEFrB, MEFrF and MEFrL in all the treatment periods when compared to their respective controls. MEFrB, MEFrF and MEFrL administration showed more significant increase in catalase activity than that of silymarin administration in 90 days when compared to 20 days treatment period. DLA tumor induced mice showed a significant decrease in CAT activity in 20 days of treatment period when compared to all the controls and all the treatments. Catalase activity was found to be significantly increased in MEFrB, MEFrF and MEFrL administration individually and coadministration to DLA tumor induced mice in 90 days treatment periods when compared to 20 days treatment period as shown in Table 6 and Figure 37. MEFrB showed more significant increase in catalase activity than that of MEFrL and MEFrF.

The above observation was supported by Gupta *et al.* (2006) who showed a decrease in the activity of CAT in DLA tumor induced control group of mice liver.

Our finding was supported by Jain *et al.* (2008) who showed that CAT content have been significantly increased by the leaf extract of *Momordica dioica* in mice. Palani *et al.* (2009) also indicated the significant increased CAT activity in whole plant extract of *Pimpinella tirupatiensis* treated mice.

Table 6
Activity of CAT in the liver of controls and experimental Swiss albino mice

Groups	Treatments	Activity of Catalase (U ^{&} / mg protein) Treatment periods	
		20 days	90 days
1	PBS	21.65 ± 0.77	22.56 ± 0.42
2	DMSO	20.33 ± 0.30	21.38 ± 0.37
3	Paraffin oil	20.11 ± 0.76	21.18 ± 0.15
4	Silymarin	26.30 ± 1.16	29.36 ± 0.45
5	MEFrB	32.17 ± 3.18	38.24 ± 0.50
6	MEFrF	30.21 ± 2.74	36.45 ± 0.08
7	MEFrL	32.15 ± 1.85	36.21 ± 0.11
8	MEFrB+DLA	12.17 ± 1.30	19.62 ± 0.11
9	MEFrF+DLA	11.41 ± 1.09	17.66 ± 0.10
10	MEFrL+DLA	11.36 ± 1.18	18.23 ± 0.13
11	DLA	9.80 ± 1.11	-
One way ANOVA (with DLA) (P<0.05)		0.0710	-
One way without DLA (p<0.05)		0.0668	0.0549
Two way ANOVA Without DLA (p<0.05)		0.06261	

The values are mean ± SD of six animals

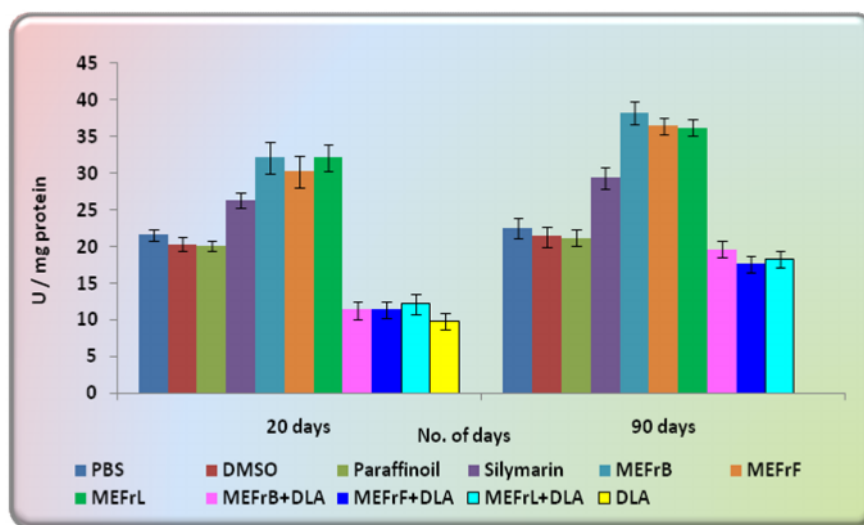
U[&] - The amount of enzyme required to decrease the absorbance by 0.05 units at 240nm

Rao *et al.* (2010) also stated that higher superoxide dismutase and catalase activities were detected in *Clitoria ternate* and *Eclipta prostrata* treated mice. Adhvaryu *et al.* (2010) also showed increased level of enzymic antioxidants (CAT,

SOD and GPx) in the liver of DLA mice treated with four ayurvedic herbs such as *Curcuma longa L.*, *Ocimum sanctum L.*, *Tinospora cordifolia (wild)* and *Zizyphus mauritiana Lam.* Gupta *et al.* (2010) also showed significant increase in CAT activity by the methanol extract of *Caesalpinia bonducella* leaves in Ehrlich Ascites Carcinoma bearing Swiss albino mice.

Figure 37

Effect of MEFrB, MEFrF and MEFrL on the activity of CAT in the liver of controls and experimental mice



4.2.2.2. Glutathione peroxidase

The activity of GPx was found to be increased significantly by the administration of MEFrB, MEFrF, MEFrL and silymarin in all the treatment periods when compared to the controls. GPx activity was found to be increased in MEFrB, MEFrF and MEFrL treated mice in all treatment periods when compared to silymarin.

The activity of GPx in DLA tumor induced mice was found to be significantly decreased in 20 days treatment period when compared to all the controls and all the treatments. The activity of GPx in DLA tumor induced mice administered with MEFrB, MEFrF and MEFrL was found to be significantly increased in the 90 days treatment periods when compared to 20 days treatment period (Table 7 and Figure 38).

Table 7

Activity of GPx in the liver of controls and experimental Swiss albino mice

Groups	Treatments	Activity of Glutathione peroxidase (U [*] /nmoles) Treatment periods	
		20 days	90 days
1	PBS	46.97 ± 0.53	47.17 ± 0.04
2	DMSO	46.70 ± 0.43	46.95 ± 0.02
3	Paraffin oil	46.83 ± 0.62	47.13 ± 0.01
4	Silymarin	57.93 ± 0.27	58.83 ± 0.01
5	MEFrB	47.56 ± 0.06	49.36 ± 0.02
6	MEFrF	67.32 ± 0.04	71.13 ± 0.02
7	MEFrL	77.44 ± 0.39	78.64 ± 0.02
8	MEFrB+DLA	44.56 ± 0.28	50.50 ± 0.00
9	MEFrF+DLA	44.45 ± 0.09	49.25 ± 0.02
10	MEFrL+DLA	74.71 ± 0.17	76.51 ± 0.01
11	DLA	23.03 ± 0.51	-
One way ANOVA (with DLA) (P<0.05)		4.8459	-
One way without DLA (p<0.05)		4.8456	0.1772
Two way ANOVA Without DLA (p<0.05)		0.353345	

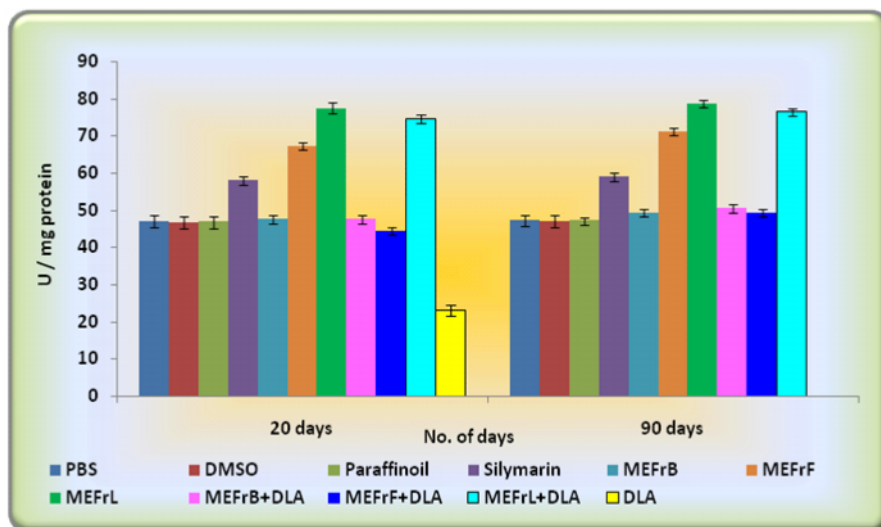
The values are mean ± SD of six animals

U^{*} - nmoles of GSH utilized /minute

Gupta *et al.* (2006) showed increased levels of GPx, GR, SOD, CAT activity and GSH level in DLA induced mice liver injury by the methanolic extract of capitula of *Chamomile recutita*. Gupta and Singh (2007) observed that the administration of *Enicostemma littorale* and silymarin restored the decreased level of antioxidant profile in CCl₄ induced oxidative stress in rats.

Figure 38

Effect of MEFrB, MEFrF and MEFrL on the activity of GPx in the liver of controls and experimental mice



The results are in tune with the report of Santhi *et al.* (2010) who reported that the activities of hepatic GPx, SOD and CAT increased by the administration of *Cynodon dactylon* protein fraction on ELA induced toxicity in mice model. Ethanolic extract of *Tinospora cordifolia* increased the activities of GPx, GR, SOD and GST in the liver and kidney of mice treated with aflatoxin B1 and proved its antioxidative potential (Gupta *et al.*, 2011c). In another study by Sharma and Gupta (2011) also reported that the activities of GPx, SOD, GR, GST and CAT were significantly increased in the liver of aflatoxin B1 induced mice by the administration of ethanolic extract of *Tinospora cordifolia* root. In another study postulated by Saroja *et al.* (2012) reported that the activities of GPx, SOD and CAT were significantly increased in the liver of EAC induced mice by the administration of flavonoid fraction of *Terminalia catappa*.

4.2.2.3. Glutathione reductase

The GR activity was found to be significantly increased in mice administered with silymarin, MEFrB, MEFrF and MEFrL when compared to their controls in all treatment periods.

Table 8

Activity of GR in the liver of controls and experimental Swiss albino mice

Groups	Treatments	Activity of Glutathione reductase (U [#] / microgram) Treatment periods	
		20 days	90 days
1	PBS	5.58 ± 0.38	5.68 ± 0.05
2	DMSO	6.77 ± 0.20	6.29 ± 0.02
3	Paraffin oil	5.83 ± 0.08	6.37 ± 0.01
4	Silymarin	10.66 ± 0.24	18.55 ± 0.03
5	MEFrB	33.15 ± 1.40	36.05 ± 0.05
6	MEFrF	31.52 ± 0.41	33.38 ± 0.01
7	MEFrL	31.99 ± 0.94	34.19 ± 0.03
8	MEFrB+DLA	10.88 ± 0.42	14.78 ± 0.08
9	MEFrF+DLA	10.47 ± 0.38	13.94 ± 0.01
10	MEFrL+DLA	12.15 ± 0.61	16.13 ± 0.01
11	DLA	13.60 ± 0.36	-
One way ANOVA (with DLA) (P<0.05)		0.1803	-
One way without DLA (p<0.05)		0.1182	0.2220
Two way ANOVA Without DLA (p<0.05)		0.17493	

The values are mean ± SD of six animals

U[#] - microgram of NADPH oxidized per minute

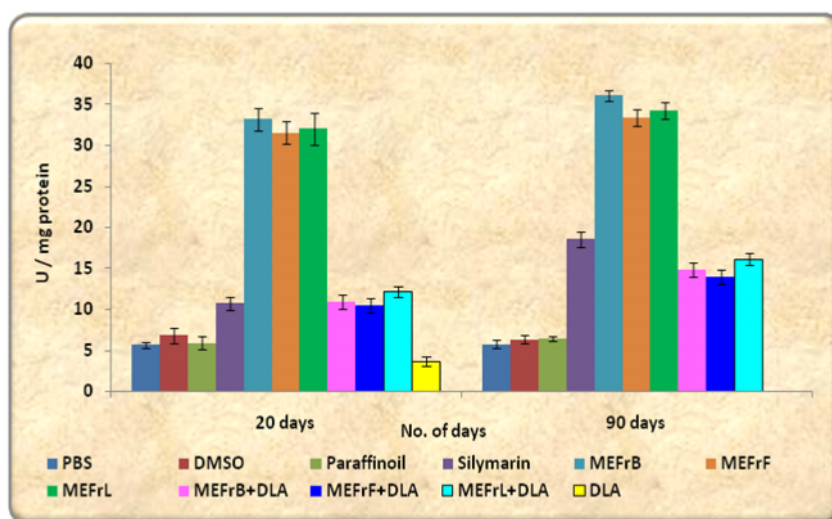
MEFrB, MEFrF and MEFrL administered mice showed more significant increase in GR activity than that found in silymarin treatment. DLA tumor induced mice showed a significant decrease in GR activity in 20 days treatment period when compared to all the controls and all the treatment groups. Administration of MEFrB, MEFrF and MEFrL individually and to DLA tumor induced mice showed significant increase in the activity of GR in 90 days when compared to 20 days treatment period as shown in Table 8 and Figure 39.

The above observations were agreed with those of Venukumar and Latha (2002) who investigated the antioxidant activity of *Curculigo orchiodes* in CCl₄

induced hepatopathy in rats. Gupta *et al.* (2004) also showed the enhancement of the activities of CAT and SOD in Ehrlich Ascitic Carcinoma bearing mice upon administration of the methanol extract of *Caesalpinia bonducella* leaves. Similarly, the methanol extract of *Solanum trilobatum* significantly increased the activities of enzymic antioxidants (GPx, CAT and SOD) in the liver of CCl₄ intoxicated rats (Shahjahan *et al.*, 2004). Mahmoud *et al.* (2006) also showed that the methanolic extract of *Urtica pilulifera* increased the activity of enzymic antioxidants (GR, GST, GPx and SOD) in mice liver.

Figure 39

Effect of MEFrB, MEFrF and MEFrL on the activity of GR in the liver of controls and experimental mice



Natansen *et al.* (2007) also reported the restoration of hepatic activity towards the normal level in DLA tumor bearing mice by the administration of methanol extract of *Careya arborea* bark. The methanolic fraction of *Jatropha curcas* was found to normalize the decreased activities of SOD, CAT, GPx, GST and GR in hepatic carcinoma induced rats (Balaji *et al.*, 2009).

Gutathione reductase is one of the GSH related enzymes which play detoxifying and antioxidant roles by metabolizing xenobiotics through the conjugation with glutathione or reduction of free radicals (Reddy *et al.*, 2010). Decrease in the activity of GR observed in DLA tumor mice may be due to its decreased expression during hepatocellular damage by ROS. It is likely that

methanolic extract of various parts of *Ficus racemosa* maintained GR activity in the liver by inhibiting lipid peroxidation and by maintaining GSH levels.

4.2.2.4. Superoxide dismutase

The SOD activity was found to be significantly increased in mice administered with silymarin, MEFrB, MEFrF and MEFrL when compared to their controls in all treatment periods. MEFrB, MEFrF and MEFrL administered mice showed more significant increase in SOD activity than that found in silymarin treatment. DLA tumor induced mice showed a significant decrease in SOD activity in 20 days treatment period when compared to all the controls and all the treatments. Administration of MEFrB, MEFrF and MEFrL individually and to DLA tumor induced mice showed significant increase in the activity of SOD activity in 90 days treatment period when compared to 20 days treatment period as shown in Table 9 and Figure 40.

Similar significant increase in the activity of SOD was observed in the mice liver by the administration of aqueous extract of *Ocimum sanctum* (Panda and Kar, 1998) Administration of *Withania somnifera* extract also showed significant enhancement in enzymic antioxidants when compared with the control (Davis and Kuttan, 2001). Increased activities of CAT and SOD were also reported by Natasen *et al.* (2007) in DLA induced animal administration by the methanolic extract of *Careya arborea* bark. Turk *et al.* (2008) also revealed the significant decrease in malondialdehyde level and marked increase in glutathione, glutathione peroxidase, catalase activities and Vitamin C levels in rats treated with different doses of pomegranate juice.

The SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical (Palanivel *et al.*, 2008).

Table 9

Activity of SOD in the liver of controls and experimental Swiss albino mice

Groups	Treatments	Activity of Superoxide dismutase (U ^s / mg protein) Treatment periods	
		20 days	90 days
1	PBS	4.16 ± 0.07	4.02 ± 0.07
2	DMSO	4.20 ± 0.04	4.26. ± 0.03
3	Paraffin oil	4.18 ± 0.02	4.24 ± 0.03
4	Silymarin	9.23 ± 0.11	12.29 ± 0.04
5	MEFrB	12.84 ± 0.03	15.21 ± 0.02
6	MEFrF	12.30 ± 0.02	15.87 ± 0.04
7	MEFrL	12.41 ± 0.07	13.02 ± 0.07
8	MEFrB+DLA	11.68 ± 0.07	14.08 ± 0.03
9	MEFrF+DLA	11.54 ± 0.10	13.95 ± 0.05
10	MEFrL+DLA	11.67 ± 0.10	14.07 ± 0.02
11	DLA	1.22 ± 0.07	-
One way ANOVA (with DLA) (P<0.05)		0.2775	-
One way without DLA (p<0.05)		0.2645	0.1266
Two way ANOVA Without DLA (p<0.05)		0.21401	

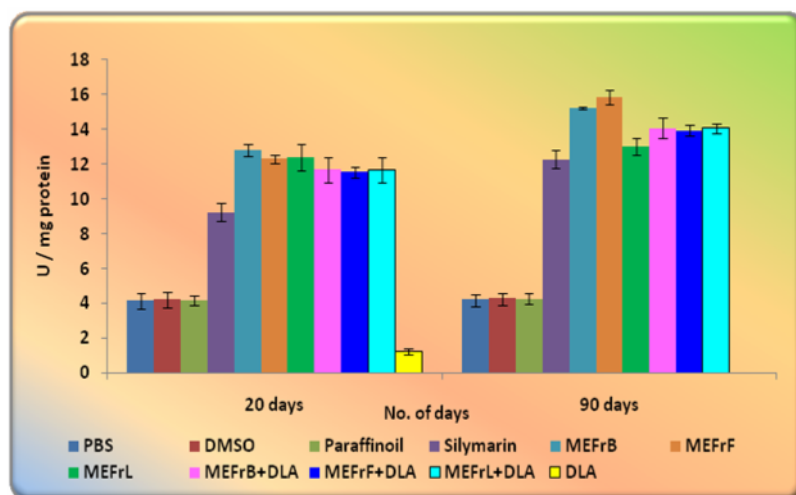
The values are mean ± SD of six animals

U^s - The amount of enzyme that gives 50 percent inhibition of the extent of NBT reduction

Ehrenbergerova *et al.* (2009) also reported that the green biomass of young barely plants in mice exhibited statistically higher activity of superoxide dismutase and catalase. Ilango and Chitra, (2010) also observed an increase in the activities of the antioxidant enzymes, namely superoxide dismutase and catalase in the *Limonia acidissima* Linn. extract treated rats.

Figure 40

Effect of MEFrB, MEFrF and MEFrL on the activity of SOD in the liver of controls and experimental mice



Karuppusamy *et al.* (2011) also suggested that wild fruits are promising sources of potential antioxidants and may be efficient as preventive agents in some diseases. The activity of CAT, SOD and GR were found to be significantly increased in the liver of mice treated with methanolic extract of *Gloriosa superba* (Saradhadevi and Annapoorani, 2011).

The body has an effective mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes, CAT, GPx and SOD which constitute a mutually supportive team of defense against ROS. Our results revealed the significant increase in the antioxidant enzymes CAT, GPx, GR and SOD in the liver of mice treated with DLA along with plant extracts. CAT, GPx and SOD are involved in the clearance of superoxide and hydrogen peroxide (Rajeshwar *et al.*, 2005). CAT and SOD can counteract the deleterious action of reactive oxygen metabolites and protect from cellular and molecular damage (Abei, 1984). They also can act as anticarcinogens and inhibitors at initiation and promotion/transformation stage in carcinogenesis (Chen *et al.*, 2012). SOD converted O_2 into H_2O_2 and GPx and catalase metabolize H_2O_2 to non-toxic products (Yan *et al.*, 2011). Further, it has been reported that a decrease in SOD activity in DLA bearing mice might be due to the loss of Mn^{2+} containing SOD activity in DLA cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver (Sun *et al.*, 1989). A decrease in SOD and CAT activities described in tumors is regarded as a marker

of malignant transformation. Similar significant decrease in SOD and CAT activities were observed in our present study in DLA bearing mice.

It was reported that plant derived extracts containing antioxidant principles reduced the tumor volume and tumor cell count and showed its antitumor activity in experimental animals (Gupta *et al.*, 2004). Antitumor activity of these antioxidants is either through induction of apoptosis (Liu *et al.*, 1998) or by inhibition of neovascularisation (Maity *et al.*, 2000). The observed properties in the present study may be attributed to the antioxidant and antitumor principles present in the MEFrB, MEFrF and MEFrL.

The GPx is an equally important antioxidant, which reacts with hydrogen peroxide thus preventing intracellular damage caused by the same. Several studies have reported the decreased activities of GPx in various cancerous conditions. There was a decline in the activities of GPx in the DLA treated groups in the present study, which may be due the altered antioxidant defense system caused by enormous production of free radicals in DLA induced carcinogenesis (Padmavathi *et al.*, 2006). The decrease in GPx levels in DLA induced mice could also be due to insufficient disposal of peroxides and results in elevated lipid peroxidation (Ramesh and Satakopan, 2010). GR, the most important biomolecule protecting against chemical induced toxicity, participates in the elimination of reactive intermediates by reduction of hydroperoxides in the presence of GSH (Garg *et al.*, 2009). Activities of GPx and GR also found to be elevated in the plant extract treated groups.

The MEFrB, MEFrF and MEFrL were found to significantly enhance the activities of all the enzymes analyzed. Significant increase in the activity of SOD was found to be more significant in DLA + MEFrB than that was found in MEFrL and MEFrF and DLA + MEFrL and DLA + MEFrF in 20 days treatment period where as in 90 days period the order was found to be MEFrF > MEFrB > MEFrL individually and in DLA induced mice it was MEFrB > MEFrF > MEFrL. The MEFrB showed maximum significant activities of all the enzymic antioxidants followed by the MEFrL and MEFrF.

4.2.3. Effect on the levels of non enzymic antioxidants

Antioxidants are important substances with the ability to protect the body from damage caused by free radical induced oxidative stress. A variety of free radicals scavenging antioxidants exist within the body. The non enzymic antioxidants such as Vitamin A, E, C and reduced glutathione act as the secondary line of defense to cope up with the deleterious effects of free radicals. So, the levels of non enzymic antioxidants such as Vitamin A, C, E and Reduced glutathione in the liver of Swiss albino mice in different treatment periods in all the treatment groups were assessed.

4.2.3.1. Vitamin A

Table 10 and Figure 41 show the effect of MEFrB, MEFrF and MEFrL on the levels of Vitamin A in the liver of controls and experimental mice. Silymarin treated group showed significantly increased level of Vitamin A in 20 days and 90 days of treatment periods when compared to paraffin oil treated mice. A significant increase in the level of Vitamin A was noticed in MEFrB, MEFrF and MEFrL administered mice when compared to DMSO and silymarin treated mice in all the treatment periods.

The DLA tumor induced mice showed significantly decrease in the level of Vitamin A in 20 days of treatment period when compared with all the controls. The level of Vitamin A was found to be increased in DLA tumor induced mice administered with MEFrB, MEFrF and MEFrL in 90 days treatment period when compared to 20 days treatment period. The significant effect of extract on Vitamin A levels were found to be MEFrB and DLA +MEFrB which was followed by MEFrL and DLA+MEFrL and MEFrF and DLA+ MEFrF in 20 days treatment periods. In 90 days treatment peroid the order was found to be MEFrB> MEFrF and MEFrL and DLA + MEFrF> DLA + MEFrB and DLA + MEFrL.

Table 10
Levels of Vitamin A in the liver of controls and experimental
Swiss albino mice

Groups	Treatments	Level of Vitamin A ($\mu\text{g/g}$ tissue) Treatment periods	
		20 days	90 days
1	PBS	3.93 ± 0.46	4.05 ± 0.09
2	DMSO	3.35 ± 0.33	3.46 ± 0.28
3	Paraffin oil	3.22 ± 0.22	3.32 ± 0.10
4	Silymarin	4.46 ± 0.02	4.61 ± 0.07
5	MEFrB	5.79 ± 0.08	8.69 ± 0.34
6	MEFrF	5.22 ± 0.06	7.79 ± 0.14
7	MEFrL	5.60 ± 0.17	6.83 ± 0.11
8	MEFrB+DLA	3.61 ± 0.10	5.89 ± 0.07
9	MEFrF+DLA	3.26 ± 0.23	6.08 ± 0.21
10	MEFrL+DLA	3.47 ± 0.05	4.77 ± 0.06
11	DLA	1.50 ± 0.17	-
One way ANOVA (with DLA) ($P < 0.05$)		0.2847	-
One way without DLA ($p < 0.05$)		0.2682	0.2260
Two way ANOVA Without DLA ($p < 0.05$)		0.22867	

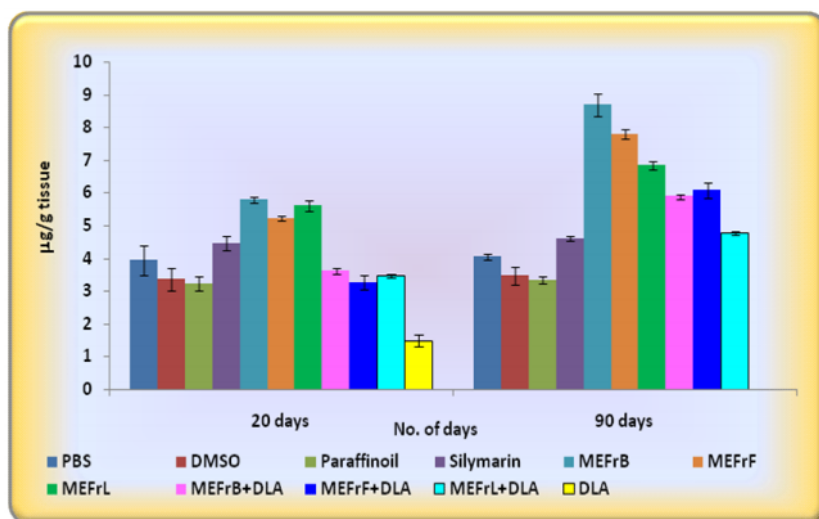
The values are mean $\pm$ SD of six animals

The decreased levels of Vitamin A in the liver of tumor induced mice may be due to the increased levels of ROS and RNS in tissues. The significant increase in Vitamin A levels in the tumor bearing mice by MEFrB, MEFrF and MEFrL may be due to the regeneration of Vitamin A by their antioxidative role.

Administration of *Cynodon dactylon* leaf protein also enhanced the levels of non enzymic antioxidants Vitamin A, E and reduced glutathione in ELA induced mice (Santhi *et al.*, 2010). The decreased level of GSH in ELA induced mice may be due to the over utilization of this antioxidant to combat the free radicals. The protective action was exerted by the coadministration of flavonoid fraction of *Cynodon dactylon* and *Terminalia catappa* to ELA induced mice by significantly increasing GSH levels (Saroja and Annapoornai, 2012b).

Figure 41

Effect of MEFrB, MEFrF and MEFrL on the levels of Vitamin A in the liver of controls and experimental mice



4.2.3.2. Vitamin E

Table 11 and Figure 42 show the significant increase in the level of Vitamin E in MEFrB, MEFrF and MEFrL administered mice when compared to DMSO control mice in 20 days and 90 days of treatment periods. When compared to paraffin oil control the silymarin treated mice showed significant increase in the level of Vitamin E in all the treatment periods. Silymarin treated groups showed a significant increase in the Vitamin E levels on 20 days and 90 days treatment periods when compared to MEFrB, MEFrF and MEFrL treated groups.

The level of Vitamin E was found to be significantly decreased in DLA tumor induced mice in 20 days of treatment period. Level of Vitamin E in 90 days treatment periods was found to be increased significantly in DLA induced mice coadministered with MEFrB, MEFrF and MEFrL when compared to the 20 days treatment period. More significant increase in the level of Vitamin A was noticed in MEFrB than that found in MEFrF and MEFrL. Significant increase in Vitamin E level in DLA induced mice coadministered with MEFrL was found to be more than that found in DLA+MEFrF followed by DLA+MEFrL and DLA+MEFrB.

Table 11

Levels of Vitamin E in the liver of controls and experimental Swiss albino mice

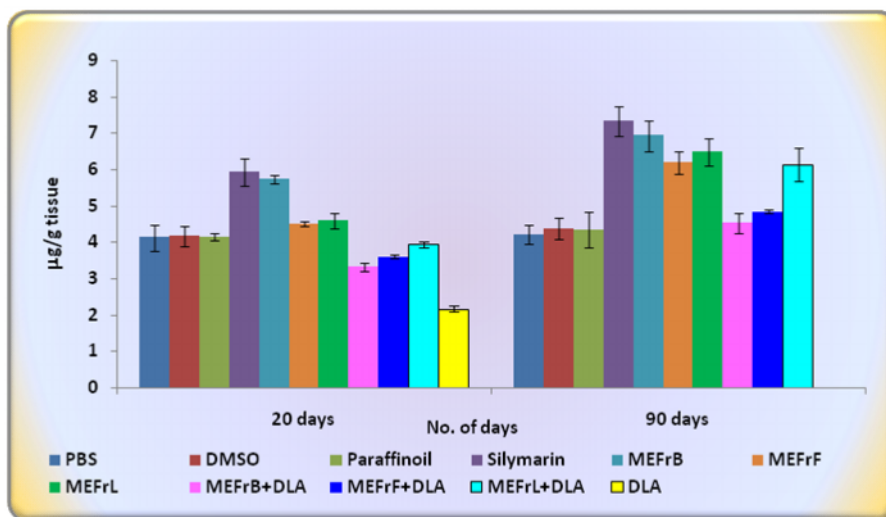
Groups	Treatments	Level of Vitamin E ($\mu\text{g/g}$ tissue) Treatment periods	
		20 days	90 days
1	PBS	4.12 ± 0.51	4.20 ± 0.11
2	DMSO	4.16 ± 0.09	4.38 ± 0.10
3	Paraffin oil	4.13 ± 0.51	4.35 ± 0.07
4	Silymarin	5.92 ± 0.04	7.32 ± 0.15
5	MEFrB	5.72 ± 0.03	6.93 ± 0.04
6	MEFrF	4.51 ± 0.02	6.19 ± 0.14
7	MEFrL	4.58 ± 0.18	6.47 ± 0.46
8	MEFrB+DLA	3.31 ± 0.44	4.52 ± 0.49
9	MEFrF+DLA	3.60 ± 0.04	4.83 ± 0.07
10	MEFrL+DLA	3.93 ± 0.14	6.14 ± 0.07
11	DLA	2.16 ± 0.57	-
One way ANOVA (with DLA) ($P < 0.05$)		0.3643	-
One way without DLA ($p < 0.05$)		0.3288	0.8902
Two way ANOVA Without DLA ($p < 0.05$)		0.28625	

The values are mean $\pm$ SD of six animals

Manju and Nalini (2005) have reported a significant increase in the levels of non enzymic antioxidants when compared to the control group in male Wister rats treated with methanolic extract of *Zingiber officinalis*. Sambathkumar *et al.* (2005) also have reported the significant increase in hepatic antioxidative Vitamins E and C by the administration of methanolic extract of *Careya arborea* Roxb and silymarin in rats with CCl_4 induced liver damage. Santhi and Annapoorani (2009) who showed a similar kind of increase in the levels of Vitamin E in the liver of ELA tumor induced mice due to the administration of *Terminalia catappa* protein fraction.

Figure 42

Effect of MEFrB, MEFrF and MEFrL on the levels of Vitamin E in the liver of controls and experimental mice



4.2.3.3. Vitamin C

Silymarin treated group showed significant increased level of Vitamin C in 20 days and 90 days treatment periods when compared to paraffin oil treated mice. A significant increase in the level of Vitamin C was noticed in MEFrB, MEFrF and MEFrL administered mice when compared to DMSO and silymarin treated mice in all the treatment periods. The increase in Vitamin A was found to be more significant in MEFrB than that of MEFrF and MEFrL in 20 days treated mice. In 90 days treatment period the order was found to be MEFrB > MEFrF and MEFrL and DLA + MEFrB > DLA + MEFrF and DLA + MEFrL.

The DLA tumor induced mice showed a decrease in the level of Vitamin C in 20 days of treatment period when compared to all the controls. The level of Vitamin C was found to be increased in DLA tumor induced mice when administered with the MEFrB, MEFrF and MEFrL in 90 days treatment periods when compared to 20 days treatment period as shown in Table 12 and Figure 43. The significant increase in Vitamin A levels was found to be in the order of MEFrB, MEFrL and MEFrF in DLA induced mice in both the treatment periods.

Table 12

Levels of Vitamin C in the liver of controls and experimental Swiss albino mice

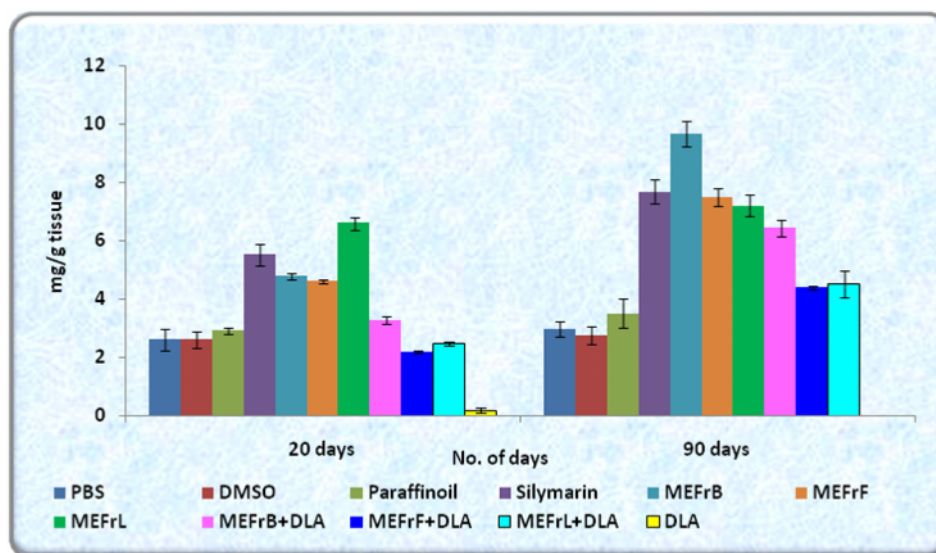
Groups	Treatments	Level of Vitamin C (mg/g tissue) Treatment periods	
		20 days	90 days
1	PBS	2.58 ± 0.35	2.95 ± 0.02
2	DMSO	2.59 ± 0.30	2.75 ± 0.03
3	Paraffin oil	2.90 ± 0.09	3.49 ± 0.48
4	Silymarin	5.51 ± 0.36	7.77 ± 0.40
5	MEFrB	4.77 ± 0.11	9.67 ± 0.04
6	MEFrF	4.59 ± 0.06	7.49 ± 0.03
7	MEFrL	6.58 ± 0.21	7.19 ± 0.01
8	MEFrB+DLA	3.27 ± 0.11	6.41 ± 0.02
9	MEFrF+DLA	2.16 ± 0.05	4.38 ± 0.03
10	MEFrL+DLA	2.46 ± 0.07	4.50 ± 0.04
11	DLA	0.18 ± 0.07	-
One way ANOVA (with DLA) (P<0.05)		0.1777	-
One way without DLA (p<0.05)		0.0778	0.0392
Two way ANOVA Without DLA (p<0.05)		0.13766	

The values are mean ± SD of six animals

Chakraborty and Verma (2010) showed that the administration of aqueous extract of *Embllica officinalis* caused a significant amelioration in the ochratoxin induced lipid peroxidation in mouse liver by enhancing the concentration of non enzymic antioxidants Vitamin C and GSH. Vitamin C is a potent scavenger of singlet oxygen.

Figure 43

Effect of MEFrB, MEFrF and MEFrL on the levels of Vitamin C in the liver of controls and experimental mice



4.2.3.4. Reduced glutathione

Administration of Silymarin, MEFrB, MEFrF and MEFrL significantly increased the level of GSH in 20 days and 90 days treatment periods when compared to their controls. The level of GSH in MEFrB, MEFrF and MEFrL treated mice was found to be increased significantly when compared to silymarin treated group in 20 days and 90 days treatment periods as shown in Table 13 and Figure 44.

The body has an effective defense mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as SOD, CAT, GRD and GPx. These enzymes constitute a mutually supportive team of defense against ROS and RNS (Venukumar and Latha, 2002). Gupta *et al.* (2004) also reported that the administration of methanolic extract of *Bhuhina racemosa* elevated the levels of reduced glutathione in DLA and ELA tumor bearing Swiss albino mice.

The level of reduced glutathione in the DLA tumor induced mice was found to be significantly decreased in 20 days treatment period when compared to all controls and all the treatments. DLA tumor induced mice treated with MEFrB, MEFrF and MEFrL showed a significant increase in GSH levels in 90 days when compared to the 20 days treatment periods.

Table 13

Levels of GSH in the liver of controls and experimental Swiss albino mice

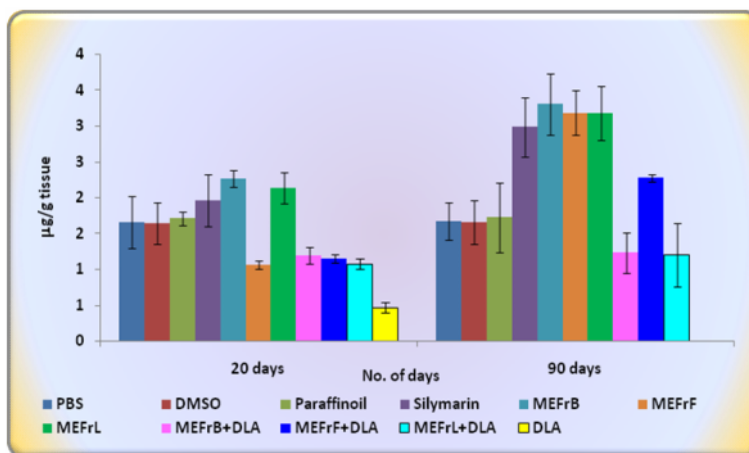
Groups	Treatments	Level of Reduced glutathione (mg/g tissue) Treatment periods	
		20 days	90 days
1	PBS	1.65 ± 0.03	1.77 ± 0.02
2	DMSO	1.64 ± 0.02	1.65 ± 0.01
3	Paraffin oil	1.70 ± 0.01	1.71 ± 0.01
4	Silymarin	1.96 ± 0.02	2.99 ± 0.00
5	MEFrB	2.26 ± 0.02	3.29 ± 0.01
6	MEFrF	1.05 ± 0.03	3.18 ± 0.00
7	MEFrL	2.13 ± 0.02	3.17 ± 0.03
8	MEFrB+DLA	1.19 ± 0.05	1.22 ± 0.02
9	MEFrF+DLA	1.14 ± 0.02	2.28 ± 0.01
10	MEFrL+DLA	1.07 ± 0.01	1.19 ± 0.07
11	DLA	0.46 ± 0.02	-
One way ANOVA (with DLA) (P<0.05)		0.0327	-
One way without DLA (p<0.05)		0.0313	0.0409
Two way ANOVA Without DLA (p<0.05)		0.03690	

The values are mean ± SD of six animals

Neoplastic cells may sequester essential antioxidants from circulation to supply the demands of growing tumor. Antioxidant vitamins have a number of biological activities such as immune stimulation, scavenging the free radicals and alteration in metabolic activation of carcinogens (Poppel and Berg, 1997). They can utilize reactive oxygen metabolites, protecting biopolymers and reduce oxidative DNA damage (Halliwell, 1996). The vitamins A, E and C also exist in inter convertible forms and participate in neutralizing free radicals (Lastra *et al.*, 2000).

Figure 44

Effect of MEFrB, MEFrF and MEFrL on the levels of GSH in the liver of controls and experimental mice



Vitamin A and its derivatives are lipophilic compounds that can inhibit free radical reactions by reducing $\text{ROO}^\cdot$ thereby limiting oxidative membrane damage and alteration of membrane structure integrity (Stahl and Sies, 2007). Vitamin C is the major water-soluble antioxidant and acts as first defense against free radicals in whole blood and plasma. It is a powerful inhibitor of lipid peroxidation and regenerates Vitamin E in lipoproteins and membranes (Fusco *et al.*, 2007). Vitamin C is an electron donor and therefore a reducing agent. It is called an antioxidant because, by donating its electrons, it prevents other compounds from being oxidized (Padayatty *et al.*, 2003). The major antioxidant that protects the cell is Vitamin E. It is a potent oxygen radical scavenger that protects cells from carcinogenic chemicals by inhibiting LPO and free radical-mediated consequences (Padmavathi *et al.*, 2006). Glutathione, a potent inhibitor of neoplastic process plays an important role as an endogenous antioxidant system that is found particularly in high concentration in liver and is known to have key function in the protective process (Sinclair *et al.*, 1990). GSH serves as an electron donor for glutathione peroxidase and as a substrate for glutathione -S- transferases. It also contributes to the detoxification and elimination of toxic electrophilic metabolites and xenobiotics (Qiao *et al.*, 2010).

The significant depressed levels of the non enzymic antioxidants could be due to the increased utilization of these antioxidants in combating the oxidative stress due to tumor burden. Significant improvement in vitamins A, E, C and GSH levels in mice supplemented with silymarin, MEFrB, MEFrF and MEFrL individually and with DLA tumor induced mice could be due to their antioxidative role. The decreased non enzymic antioxidants in DLA tumor treated mice in our study might due to excess utilization of these antioxidants by the tumor cells.

The present findings very well indicated that MEFrB, MEFrF and MEFrL had very good potential to improve human antioxidant status and could prevent normal oxidative stress that happens daily due to normal exposure to many causal chemicals and conditions. In recent years the importance of oxidative stress in the pathophysiology of many human disorders has been highlighted. So, use of these plant extracts, as herbal medicine, is highly recommended. The above results of the activities of enzymic antioxidants (CAT, GPx, GR, and SOD) and the levels of non enzymic antioxidants (Vitamins A, E, C and GSH) revealed that MEFrB is most effective followed by MEFrF and MEFrL and silymarin, the standard antioxidant. These results clearly indicated that *Ficus racemosa* is a very good inducer of enzymic and non enzymic antioxidants.

4.2.4. Effect on the Levels of TBARS

The measurement of lipid peroxidation as Thio Barbituric acid Reacting Substances (TBARS) is a convenient method to monitor oxidative damage in tissues. ROS cause peroxidation of membrane lipids with devastating effect on functional status. The preservation of cellular membrane integrity depends on protection or repair mechanisms capable of neutralizing oxidative reactions. Free radicals, mostly ROS, cause cellular injury, the consequences of which are often exhibited and measured as TBARS.

Table 14
Levels of TBARS in the liver of controls and experimental Swiss albino mice

Groups	Treatments	Level of TBARS (nmoles of MDA/mg protein)
--------	------------	---

		Treatment periods	
		20 days	90 days
1	PBS	1.89 ± 0.19	1.77 ± 0.05
2	DMSO	1.46 ± 0.24	1.35 ± 0.02
3	Paraffin oil	1.72 ± 0.02	1.03 ± 0.01
4	Silymarin	1.27 ± 0.23	0.91 ± 0.01
5	MEFrB	1.61 ± 0.10	1.11 ± 0.04
6	MEFrF	1.50 ± 0.17	1.29 ± 0.00
7	MEFrL	1.37 ± 0.15	1.01 ± 0.01
8	MEFrB+DLA	4.22 ± 0.06	3.62 ± 0.01
9	MEFrF+DLA	4.36 ± 0.20	3.76 ± 0.04
10	MEFrL+DLA	4.47 ± 0.20	3.66 ± 0.03
11	DLA	5.53 ± 0.14	-
One way ANOVA (with DLA) (P<0.05)		0.0587	-
One way without DLA (p<0.05)		0.0514	0.4920
Two way ANOVA Without DLA (p<0.05)		0.04866	

The values are mean ± SD of six animals

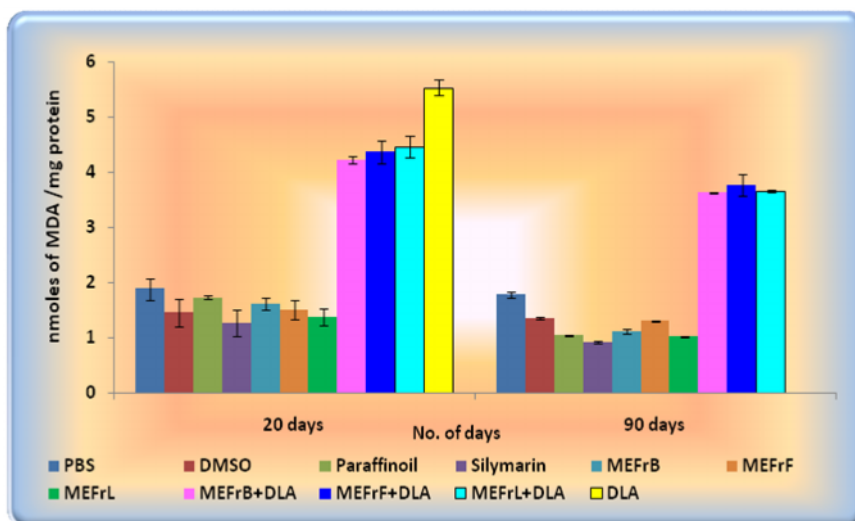
TBARS is the oxidative deterioration of polyunsaturated lipids to form radical intermediates that cause oxidative damage. TBARS, which involves a series of free radical mediated chain reactions and also associated with several types of biological damages. TBARS in turn gives product like malondialdehyde (MDA) that cause damage to the membrane (Edwards and Fuller, 1996). The effect of MEFrB, MEFrF and MEFrL against vehicle controls, silymarin and DLA tumor induce mice on the levels of TBARS in the liver of Swiss albino mice are given in Table 14 and Figure 45.

The level of TBARS in the liver was found to be decreased in all the treatment periods by the administration of MEFrB, MEFrF and MEFrL when compared to DMSO the vehicle control group. The level of TBARS in the standard antioxidant silymarin treated mice was found to be significantly decreased when

compared to paraffin oil treated mice in all treatment periods. MEFrB administration showed more significantly decreased levels of TBARS in 20 days and 90 days treatment periods than that of the TBARS level in MEFrF, MEFrL and silymarin administered mice. The mice transplanted with DLA tumor cells showed a significant increase in the level of TBARS when compared to all the controls and experimental groups in 20 days treatment period. Coadministration of MEFrB, MEFrF and MEFrL to DLA tumor induced mice showed significant decrease in the levels of TBARS in 90 days treatment period when compared to 20 days treatment period.

Figure 45

Effect of MEFrB, MEFrF and MEFrL on the levels of TBARS in the liver of controls and experimental mice



The TBARS, which involves a series of free radical mediated chain reaction processes, is also associated with several types of biological damage. TBARS has been postulated to the destructive process of liver injury. Our data showed the significant reduced levels of TBARS in mice supplemented with the MEFrB, MEFrF and MEFrL individually and in DLA tumor induced mice which indicated the inhibition of lipid peroxidation and enhancement of antioxidative defense mechanisms to prevent the formation of excessive free radicals and also confirmed their antilipidperoxidative role.

The methanolic extract of the plant *Hedyotis corymbosa* significantly reduced the accumulation of lipid peroxides in rat liver homogenate (Sadasivan *et al.*, 2006). Natsen *et al.* (2007) found decreased levels of TBARS in serum, liver and in Kidney in DLA tumor induced mice treated with methanolic extract of bark of *Careya arborea* Roxb. The methanolic extract of whole plant of *Amaranthus spinosus* showed potent hepatoprotective activity against carbon tetrachloride induced hepatic damage in rats and significantly altered malondialdehyde levels (Zeashan *et al.*, 2008).

Similar was reported by Ahamed (2008) in methanolic extract of *Feronia limonia* and Chandrashekhar *et al.* (2009) in the methanolic extract of *Stereospermum suaveolens* showed dose dependent reduction in the *in vivo* lipid peroxidation in CCl₄ induced mice. The flowers of the plant *Dendrobium nobile* inhibited the TBARS level significantly in both liver homogenate and RBC ghosts (Devi *et al.*, 2009). A high degree of inhibition of TBARS was shown by the polar and non-polar extracts of *Cyanthillium cinereum* (Less.) H. Rob (Guha *et al.*, 2009). *Moringa oleifera* leaf extract inhibited the amount of MDA generated in liver homogenate (Sreelatha and Padma, 2009).

Lou *et al.* (2010) reported that the fractions of burdock leaves in combination with tertiary butylhydroquinone exhibited high TBARS inhibitory activity. The methanolic extract of *Aphanes arvensis* extract significantly reduced TBARS formation, indicating significant anti-lipid peroxidation in a concentration dependent manner and their inhibitory effects were comparable to the reference compound, Trolox (Hamad *et al.*, 2010).

Lung-Yuan *et al.* (2011) also reported that treatment with methanolic leaf extract of *Dendrobium tosaense* and *Ephemerantha fimbriata* significantly decreased on the level of TBARS in carbon tetrachloride induced acute liver injury in rats. Sivaraj *et al.* (2011) reported that the aqueous leaf extract of *Andrographis paniculata* significantly decreased the levels of TBARS in the liver tissue of ethanol induced liver toxicity in male albino rats. Saroja and Annapoorani (2012b) also reported the significant reduced levels of TBARS in mice supplemented with the

flavonoid fractions of *Cynodon dactylon* individually and in ELA tumor induced mice which indicated the inhibition of lipid peroxidation and enhancement of antioxidative defense mechanisms. The DLA administered mice enhanced the levels of TBARS which reflected to enhance the lipid peroxidation which in turn leads to the membrane damage. This membrane damage was repaired by the administration of MEFrB, MEFrF and MEFrL individually and to the DLA tumor induced mice.

4.2.5. Effect on the Life Span of DLA Tumor Induced Swiss Albino Mice

The *in vivo* study was carried out to follow the antitumor activity in terms of average life span of DLA tumor bearing mice treated with MEFrB, MEFrF and MEFrL and the results are shown in Table 15.

Table 15
Average life span of DLA tumor induced mice treated with
MEFrB, MEFrF and MEFrL

Treatment	EC ₅₀ in µg / 100 µl / g.b.wt / 1 x 10 ⁶ DLA tumor cells	Average number of mice that survived after transplantation of DLA tumor cells (days)									Average life span (days)
		10	20	30	40	50	60	70	80	90	
DLA	-	6/6	3/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	19.5
MEFrB+DLA	54	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	90
MEFrF+DLA	60	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	5/6	81.5
MEFrL+DLA	58	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	5/6	81

The life span of DLA tumor bearing mice was found to be ranged from 18-21 days with the average life span of 19.5 days. Coadministration of MEFrB, MEFrF and MEFrL to DLA induced mice inhibited the growth of DLA tumor cells and increased the average life span to 90 days for MEFrB, 81.5 days for MEFrF and 81 days for MEFrL and indicated their antitumorigenic effect.

Radha and Sivakumar (2009) also showed increased the lifespan of DLA and ELA tumor induced Swiss albino mice by the administration of the methanolic extract of *Plumeria alba* leaves. Similar antitumor properties of *Jasminum sambac* (Linn) was investigated *in vitro* and *in vivo* using Dalton's ascites lymphoma model (Kalaiselvi *et al.*, 2012) and methanolic extract of *Argemone mexicana* Linn against the HeLa and MCF-7 cells. *In vivo* antitumor activity of crude methanolic extract of the leaves of *Leea indica* (*L. indica*) was reported against Ehrlich ascites carcinoma cells in Swiss albino mice (Raihan *et al.*, 2012).

4.2.6. Effect of the histological status of liver of DLA challenged Swiss albino mice

Plate IV illustrates the histological status of the liver of all the controls and experimental groups. The hepatic cells of PBS group showed, normal liver parenchyma with central vein and blood vessels. Histological appearance of hepatic cells of DMSO treated mice showed normal architecture of portal triads, sinusoids, kupffa cells and central vein. The hepatic cell nuclei of paraffin oil group were found to be normal. Histological sections of liver of silymarin treated group exhibited normal architecture. The hepatic cells of MEFrB, MEFrF and MEFrL treated mice were found to be normal with congestion of blood vessel. The portal triads, parenchyma with nuclei of hepatic cells were found to be normal with marked sinusoidal and central vein congestion in DLA+MEFrB, DLA+MEFrF and DLA+MEFrL. The hepatic cells of DLA treated mice showed increased size of hepatocytes, macrovesicular fatty changes, enlarged hyper chromatic nuclei, dilated sinusoidal and central vein congestion including severe necrosis.

The pathological cellular architecture of hepatic cells observed in DLA tumor induced mice could be due to the formation of highly reactive radicals because of oxidative threat caused by DLA tumor burden. DLA tumor induced liver

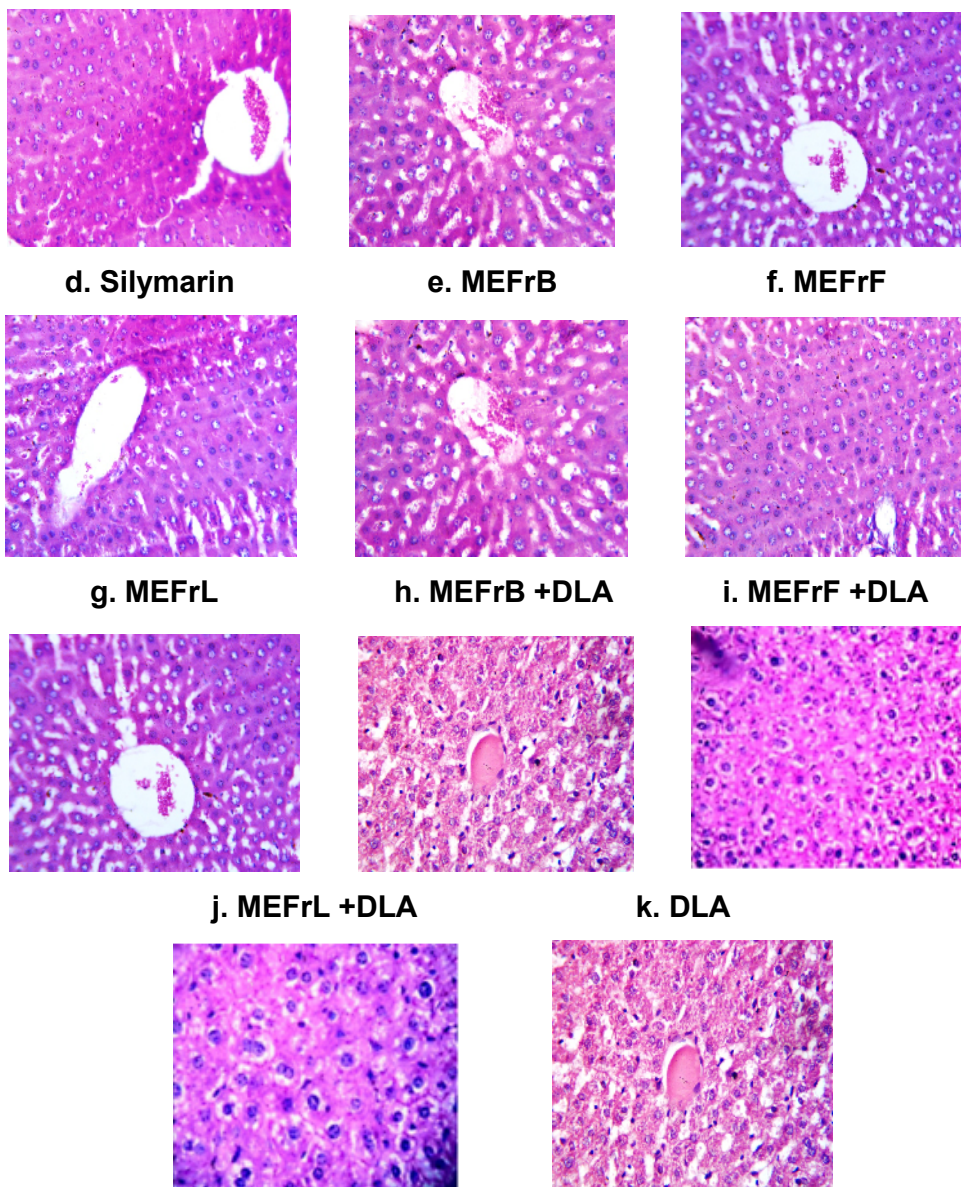
Plate IV

Histological appearance of Hepatic cells of Swiss albino mice (H and E 100X)

a. PBS

b. DMSO

c. Paraffin oil



sections indicated the malignant changes. All these changes were very much reduced in mice treated with MEFrB, MEFrF and MEFrL individually and to DLA tumor induced mice. These observations showed the protective role of MEFrB, MEFrF and MEFrL against DLA tumor induced hepatic damage.

Chaves *et al.* (2004) reported similar findings in the hepatic cells of Swiss albino mice treated with the two strains of *Candida albicans*. Chaudhari *et al.* (2009) with hydroalcoholic extract of leaves of *Momordica charantia* showed

significant recovery with disappearance of fatty deposition and necrosis in rats induced with CCl₄. Hassan and Abdel-Gawad (2010) also reported that the *Zizypus* leaves extract diminished pathological alterations induced by EAC cells.

In Phase II, the antitumorigenic effect of MEFrB, MEFrF and MEFrL was evaluated by the assessment of liver marker enzymes, enzymic and non enzymic antioxidants and TBARS along with histological analysis of the liver compared to the standard antioxidant silymarin. The activities of all the three liver marker enzymes were found to be significantly decreased by the administration of MEFrB, MEFrF and MEFrL in the presence and absence of DLA tumor cells and indicated their role in maining the normal functions of the liver. In DLA tumor cells induced mice liver significant decrease in the enzymic and non enzymic antioxidants and increase in the level of TBARS were noticed. Administration of MEFrB, MEFrF and MEFrL individually and to DLA tumor induced mice significantly enhanced the enzymic and non enzymic antioxidant status and decreased the levels of TBARS in all the treatment periods. DLA tumor bearing mice showed an average life span of 20 days whereas the administration of MEFrB, MEFrF and MEFrL to DLA transplanted mice increased the average life span to 90 days for MEFrB, 82 days for MEFrF and 81 days for MEFrL and indicated their antitumorigenic effect.

Histological examination of the liver of control groups, silymarin, MEFrB, MEFrF and MEFrL treated groups exhibited normal architecture. Histological observation of the liver of DLA tumor induced mice showed necrosis and a marked microvesicular fatty changes which were reverted by the coadministration of MEFrB, MEFrF and MEFrL. These results clearly revealed the antioxidative and antitumorigenic role of MEFrB, MEFrF and MEFrL. The antioxidative potential of all the three extracts were found to be more significant than that of the standard antioxidant silymarin. The effects may be due to their phytochemical constituents. So, the characterization of the phytochemical constituents of MEFrB, MEFrF and MEFrL was carried out in Phase III.

PHASE III

4.3. Effect of MEFrB, MEFrF and MEFrL on the Antibacterial Activity against Different Microorganisms

The plant extracts were evaluated for their antibacterial activity by assessing zone of inhibition against the standard streptomycin for both Gram positive and Gram negative bacterial isolates namely *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexneri*, and *Staphylococcus aureus*.

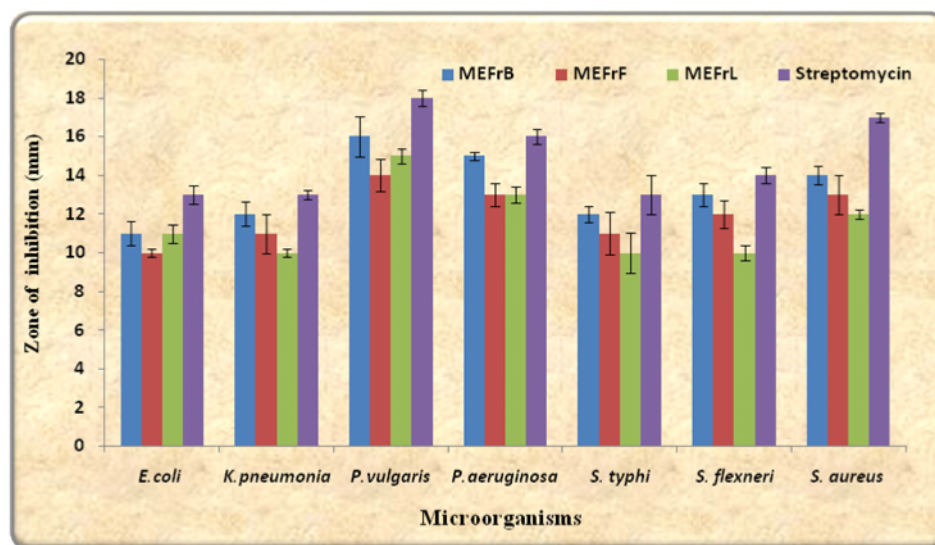
The antibacterial activity of MEFrB, MEFrF and MEFrL against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexneri* and *Staphylococcus aureus* are represented in Table 16 and Figure 46.

Table 16
Antibacterial activity of MEFrB, MEFrF and MEFrL

Microorganisms	Zone of Inhibition (mm)			
	MEFrB	MEFrF	MEFrL	Streptomycin
<i>Escherichia coli</i>	11 ± 0.62	10 ± 0.22	11 ± 0.47	13 ± 0.70
<i>Klebsiella pneumonia</i>	12 ± 0.62	11 ± 1.02	10 ± 0.23	13 ± 0.40
<i>Proteus vulgaris</i>	16 ± 1.02	14 ± 0.84	15 ± 0.40	18 ± 0.47
<i>Pseudomonas aeruginosa</i>	15 ± 0.23	13 ± 0.62	13 ± 0.40	16 ± 0.40
<i>Salmonella typhi</i>	12 ± 0.40	11 ± 1.02	10 ± 1.02	13 ± 0.23
<i>Shigella flexneri</i>	13 ± 0.62	12 ± 0.70	10 ± 0.40	14 ± 0.62
<i>Staphylococcus aureus</i>	14 ± 0.47	13 ± 1.02	12 ± 0.23	17 ± 1.02

Values are means of ± SD triplicates

Figure 46
Effect of MEFrB, MEFrF and MEFrL on antibacterial activity



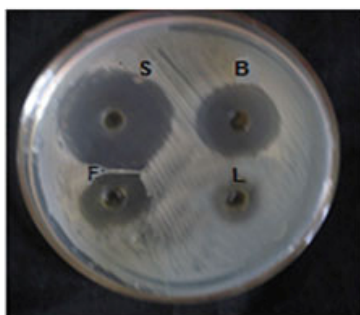
The MEFrB and MEFrF showed the zone of inhibition against the Gram negative bacteria *Proteus vulgaris* (16mm and 14mm) which was followed by *Pseudomonas aeruginosa* (15mm and 13mm), *Shigella flexneri* (13mm and 12mm), *Klebsiella pneumonia* (12mm and 11mm), *Salmonella typhi* (12mm and 11mm) and *Escherichia coli* (11mm and 10 mm) respectively.

The MEFrL showed maximum zone of inhibition against the Gram negative bacteria *Proteus vulgaris* (15mm) which was followed by *Pseudomonas aeruginosa* (13mm), *Staphylococcus aureus* (12mm), *Escherichia coli* (11mm) and all the three bacteria as *Klebsiella pneumonia*, *Salmonella typhi* and *Shigella flexneri* showed (10mm).

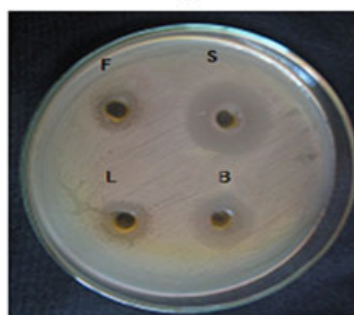
The MEFrB was found to exhibit the maximum zone of inhibition against all the selected Gram negative bacterial pathogens namely *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella flexneri* and Gram positive pathogen *Staphylococcus aureus* which was followed by MEFrF and MEFrL. The MEFrB showed maximum zone of inhibition against the Gram positive bacteria *Staphylococcus aureus* (14mm) which was followed by MEFrF and MEFrL.

Plate V
Antibacterial activity of MEFrB, MEFrF and MEFrL

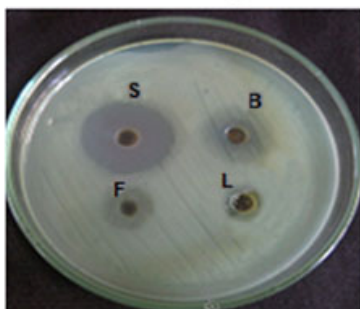
Escherichia coli



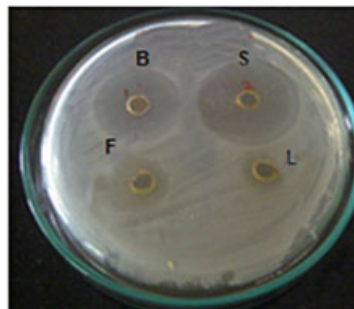
Klebsiella pneumonia



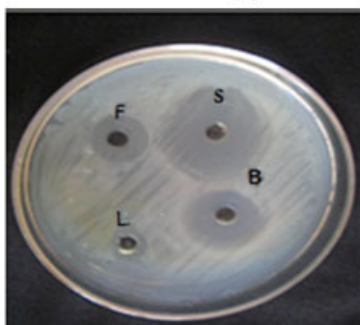
Proteus vulgaris



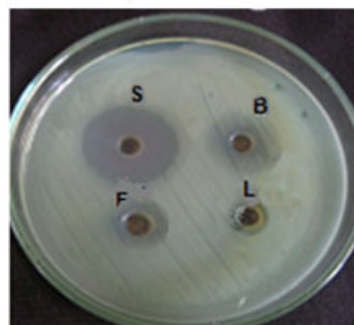
Pseudomonas aeruginosa



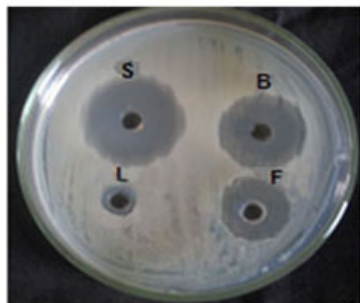
Salmonella typhi



Shigella flexneri



Staphylococcus aureus



S – Streptomycin

B – Bark

F – Fruit

L – Leaves

The aminoglycosides are products of *Streptomyces* species and are represented by streptomycin, kanamycin, tobramycin and gentamicin. These

antibiotics exert their activity by binding to bacterial ribosomes and preventing the initiation of protein synthesis. Streptomycin is the antibiotic synthesized by *Streptomyces griseus*. The drug works by interfering with a microorganism's ability to synthesize certain vital proteins. It inhibits growth, rather than killing the infectious agent. Because it was effective against so many diseases, it was used frequently and resulted in the resistance of many of the bacteria, including the one that causes tuberculosis. Streptomycin can be used with penicillin to treat different heart valve infections and with tetracyclines to treat the plague, tularemia, and brucellosis (<http://cbassandchuck.pbworks.com/w/page/6344093/Medicinal%20Uses>).

Proteus vulgaris is a rod-shaped Gram negative chemoheterotroph bacterium. *P. vulgaris* possesses peritrichous flagella, making it actively motile. It inhabits the soil, polluted water, raw meat, gastrointestinal tracts of animals and dust. In humans, *Proteus* species most frequently cause urinary tract infections, but can also produce severe abscesses (http://en.citizendium.org/wiki/proteus_vulgaris).

Pseudomonas aeruginosa is a Gram negative, motile, rod shaped bacterium. The bacterium is capable of both aerobic and anaerobic growth. It is abundant in various types of moist environments and can adapt to numerous others. This opportunistic pathogen is commonly associated with hospital-acquired infection, most notably in immunocompromised individuals. *Pseudomonas aeruginosa* accounts for 10% of all hospital-acquired infections. Specifically, this bacterium is the second most frequently recovered pathogen from intensive care unit patients (<http://www.specialpathogenslab.com/SPL-Advantage/FactSheetPseudomonas.pdf>).

Shigella flexneri is a Gram negative bacterium which causes the most communicable of bacterial dysenteries and shigellosis. Shigellosis causes 1.1 million deaths and over 164 million cases each year, with the majority of cases occurring in the children of developing nations. The pathogenesis of *S. flexneri* is based on the bacterium's ability to invade and replicate within the colonic epithelium,

which results in severe inflammation and epithelial destruction (Jennison and Verma, 2004).

Salmonella typhi is an important intracellular pathogen. Among the more than 2,300 closely-related *Salmonella* serovars bacteria recognized, *S. typhi* is the only one that is pathogenic exclusively for humans, in whom it causes typhoid or enteric fever (Zhang *et al.*, 2008). *Escherichia coli* are a common, rod-shaped bacterium that lives in human and animal intestines, which virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis. *Klebsiella pneumoniae*, which is the causative organism of *pneumoniae* (Shihabudeen *et al.*, 2010).

Prashanth *et al.* (2001) also reported that the methanolic extracts exhibited a higher degree of antimicrobial activity than that of hexane extracts. Rajakaruna *et al.* (2002) reported that *Syzygium cuminii* showed good activity against *Staphylococcus aureus* and *Bacillus subtilis*. Parekh *et al.* (2005) reported that different extracts of *Punica granatum* fruit showed antibacterial activity against *P.vulgaris* and *B.subtilis*.

Parekh and Chanda (2007) investigated thirty four medicinal plants for potential antibacterial activity and found that the ethanol and methanol were more effective than aqueous extracts against *C. fruendii*, *E. aerogenes*, *E. coli*, *P. vulgaris*, *P. aeruginosa* and *S. typhimurium* bacterial strains. Anthony and Bukola (2009) have indicated that methanolic leaf extracts of *Lasitheria africanum* exhibited higher degree of antibacterial activities against *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Staphylococcus aureus* than aqueous extract.

The methanolic extract of shoots and leaves from explants of *Chelidonium majus* L. grown under *in vitro* conditions showed higher antibacterial activity against *B.subtilis*, *S.enteritidis* and *S.aureus* than the plant grown under *in vivo* condition (Citric *et al.*, 2009). Patil *et al.* (2009) reported that the methanolic

extract of *Solanum surattense* whole plant showed antibacterial activity against the Gram positive bacteria *B. subtilis* and *S. aureus*.

Denolix elata showed strong inhibition to *B.subtilis* and *E.coli*, while *Mollugo cerviana* and *Solanum incanum* showed inhibition to same organism in addition, it also inhibited *K.pneumonia*. The *Merrenia tridentate* extract showed high inhibitory activity against *S.aureus* (Pavithra *et al.*, 2009). In developing countries about three quarters of the population relies on plant-based preparations used in their traditional medicinal system and as the basic needs for human primary health care. Therefore, several medicinal plants have been evaluated for possible antimicrobial activity and to get remedy for a variety of microbial origin (Kanerla *et al.*, 2009). Antimicrobial susceptibility of *Gmelina arborea* Roxb revealed that the methanol and chloroform bark extracts have effective antimicrobial activity against *S.typi*, *S.aureus*, *Mycobacterium Sp* (Audipudi and Bhaskar, 2010).

Methanol and hexane leaf extracts of *Artemisia nilaggirica* were screened by Ahameethunisa and Hopper (2010) for the potential antimicrobial activity against phytopathogens and clinically important standard reference bacterial strains such as *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhi*, *Proteus vulgari*, *Pseudomonas aeruginosa* and *Yersinia enterocolitica* and revealed the greater antimicrobial activity.

Klebsiella pneumoniae is a Gram negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin and intestines (http://en.wikipedia.org/wiki/Klebsiella_pneumoniae). *Klebsiella pneumoniae* is the major cause of liver abscess, Gram negative bacillary meningitis, brain abscess, lung abscess, thoracic emphysema, prostatic abscess, deep neck infection and complicated skin and soft tissue infections (Lin *et al.*, 2010).

Pavithra *et al.* (2010) have shown that chloroform and methanol extracts of *Denolix elata* exhibited significant antibacterial activity against Gram positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* and Gram negative bacteria such as *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas*

aeruginosa. The antibacterial activity of water and methanol extract of *Annona muricata* showed the maximum antibacterial activity than aqueous extract against *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella pneumonia* and *Bacillus subtilis* (Pathak and Saraswathy, 2010). Alavijeh *et al.* (2012) showed the antibacterial activity the methanolic extracts of *Curcuma longa* against *Bacillus subtilis*, *Escherchia coli*, *Stphaylocuccus aureus* and *Pseudomonas fluorescence*

Joglekar *et al.* (2012) also observed of antibacterial activity of the methanolic extract of *Aegle marmelos* against *Klebsiella pneumoniae* which showed maximum zone of inhibition followed by *C.sativum* and *T. foenum greacum*. Lagnika *et al.* (2012) compared the antibacterial activity of *Pteleopsis suberosa* and *Bridellia ferrugenea*. Of these *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were found to have maximum sensitivity to *Bridellia ferrugenea* than *Pteleopsis suberosa*. Oshomoh and Idu *et al.* (2012) reported the antibacterial activity of the aqueous extract of *P.curatilloila* which was found to be more sensitive than that of the ethanolic extract of *Microsporum gypseum*.

The methanolic extract of *Andrographis paniculata* (leaves), *Eugenia jambolana* (kernel), *Cassia auriculata* (flowers), *Murraya koenigii* (leaves), *Salvadora persica* (stem) and *Ipomoea batatas* (leaves) showed antimicrobial activity against two Gram positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermitis*) and three Gram negative *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Mohamed *et al.*, 2010). Antimicrobial activities have been reported with the methanolic extracts of *Abutilon indicum*, *Adenocalymma alliaceum*, *Carica papaya*, *Crotolaria laburnifolia*, *Croton bonplandianum*, *Derris scandens*, *Eichornia crassipes*, *Iopomea hispida*, *Moringa heterohylla* and *Peltophorum pterocarpum* against Gram positive and Gram negative bacteria (Vadlapudi and Chandrashekar Naidu, 2010).

The Methanolic extract of *Gymnostachyum febrifugum* and their antibacterial activity against *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *E. coli* and *P. mirabilis* were investigated by Arunachalam and Parimelazhagan (2011). The

methanolic and ethyl acetate extracts of *Tridax procumbens* showed significant antibacterial activity against *E. coli*, *K. pneumoniae*, *S. typhi*, *B. cereus* and *S. aureus* (Bharathi *et al.*, 2012). Antibacterial activity of methanolic and ethanolic extract of *Solanum nigrum* against *B. subtilis*, *E.coli*, *K. pneumoniae* and *P. aeruginosa* were reported by Parameswari *et al.* (2012). Methanolic and chloroform extracts of *Saussurea lappa* also showed antibacterial properties against *S. aureus*, *E. coli*, *P. aureginosa*, *P. vulgaris* and *K. pneumoniae* (Thara and Zuhra, 2012). The results of phase I, phase II and phase III indicated the antioxidative, antitumorigenic and antimicrobial activity of MEFrB, MEFrF and MEFrL. These activities may be due to their phytochemical constituents the activity of MEFrB on the antioxidative, antitumorigenic and antimicrobial activity were found to be more pronounced in MEFrB than that of MEFrF and MEFrL.

PHASE IV

4.4. Phytochemical Constituents of MEFrB, MEFrF and MEFrL

Phytochemistry is the branch of chemistry which deals with the isolation and characterization of the available primary and secondary metabolites in plants using the modern techniques like GC-MS, HPTLC and FT-IR. Secondary metabolites play an important role as antioxidants and antitumorigenic agents.

4.4.1. Preliminary phytochemical analysis

The phytochemical constituents are the potential source of preursors in the development of synthetic drugs. So, in the present investigation, preliminary phytochemical screening has been done in the MEFrB, MEFrF and MEFrL. All the three extracts of MEFrB, MEFrF and MEFrL showed the presence of phytochemical constituents namely, fats, fixed oils, flavonoids, glycosides, phenolics, saponins, steroids, tannins and terpenoids (Table 17).

Edeoga *et al.* (2005) also showed the presence of different types of constituents like flavonoids, steroids, terpenoids, carbohydrates and alkaloids in Nigerian plants such as *Cleome nutidosperma* and *Emilia coccinea*. Phytochemical screening of the different extracts of fruit of *Hugonia mystax* L. showed the presence of various classes of secondary metabolites such as flavonoids, phenols, saponins, steroids, tannins and terpenoids. The methanolic extract of *Evolvus nummularis* showed the presence of alkaloid, triterpenoids, tannins, cardiac

glycosides and flavonoids. The stem and bark of *Thespesia populnea*, commonly known as the “Tulip tree or portia tree” showed the presence of alkaloids, terpenoids, phenols, tannins and flavonoids (Ananjiwala *et al.*, 2007).

Table 17
Phytochemical constituents of MEFrB, MEFrF and MEFrL

Phytochemical constituents	<i>Ficus racemosa</i>		
	Bark	Fruit	Leaves
Fats	+	++	+
Fixed oils	+++	++	+
Flavonoids	++++	++++	+++
Glycosides	+	+	+
Phenolics	+++	+	++
Saponins	+	+	+
Steroids	+	+	+
Tannins	+	+	+
Terpenoids	+++	+	+

+ - Trace, ++ - Minimum, +++ - Moderate, ++++ - High

Mahour *et al.* (2008) also carried out the preliminary qualitative chemical test of fruit pulp of *Feronia elephantum* Corr. and revealed the presence of flavonoids, tannin, polysterol, aminoacids, carbohydrates, flavonoids and triterpenoids which are responsible for the various biological activities like immunomodulation, antiulcer and antioxidant. The root and leaf sample of *Phyllanthus niruri* showed the presence of phenolic compounds, anthroquinone, flavonoids, saponins, steroids, tannins and triterpenoids (Chitravadivu *et al.*, 2009b). Igbinosa *et al.* (2009) revealed the presence of saponins, steroids, tannins, glycosides, alkaloids and flavonoids of *J.curcas* stem bark. Mothana *et al.* (2009) showed the presence of different types of active constituents like flavonoids, terpenoids and tannins in methanolic extract of *Rhus thyrsiflora*. Tiwari *et al.* (2009) reported the presence of alkaloids, saponin, steroids, tannins, flavonoids, protein and carbohydrate in both crown as well as in fruit of *Solanum melongina*.

Phytochemical screening of methanol extracts of *Pergularia daemia* leaves revealed the presence of alkaloids, carbohydrates, flavonoids, tannins and

terpenoids (Karthishwaran *et al.*, 2010). The Preliminary phytochemical screening of the root and leaf of *F. strobilifera* showed the presence of phytosterols, lipids, phenolic compounds, carbohydrates, flavonoids and tannins (Madan *et al.*, 2010). Herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Much of the protective effect of fruits and vegetables has been attributed by their phytochemicals, which are the non-nutrient plant compounds (Gunasekaran and Anitha, 2011).

Jain *et al.* (2011a) determined the phytochemicals in the methanolic extract of *Feronia limonia*. L and showed the presence of phenols, saponin and tannin. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, glycosides, saponins, flavonoids, tannins and alkaloids have hypoglycemic activities and anti-inflammatory activities. Saponins possess hypocholesterolemic and antidiabetic properties. The terpenoids have also been shown to decrease blood sugar level in animal studies. Steroids, triterpenoids and saponins showed the analgesic properties and control the central nervous system activities (Khan *et al.*, 2011). Mann *et al.* (2012) reported that *Trapa bispinosa* was found to be rich in proteins, carbohydrate and minerals. The presence of secondary metabolites in plants produces some biological activities and are the major source of pharmaceuticals, food additives, fragrance and pesticides (Vimalvady *et al.*, 2012). The above results indicated that the total content of phytochemical constituents was found to be more in MEFrB than that of MEFrF and MEFrL for the identification of the bioactive compounds and *in silico* studies were carried out using MEFrB.

4.4.2. Chromatographic and spectral analysis

The GC-MS and HPTLC procedure could be useful for the qualitative and quantitative analysis. Spectral analysis may provide molecular level information of functional groups, bonding types and molecular conformations. Spectral bands are molecules specific and provide direct information about the biochemical composition. These bands are relatively narrow, easy to resolve and sensitive to molecular structure, conformation and environment (Prasad *et al.*, 2011).

4.4.2.1. Gas Chromatography - Mass Spectroscopy (GC-MS)

The major constituents of MEFrB were identified using GC-MS analysis. The GC-MS analysis revealed several peaks for MEFrB. The constituents were identified by matching the peaks with National Institute of Standards and Technology spectral library (NIST). The 24 phytochemical constituents present in MEFrB as identified by GC-MS are listed in Table 18 and Figure 47.

Table 18
Phytochemical constituents of MEFrB identified in GC-MS analysis

S.No	Retention time	Compound name	Molecular formula	Molecular weight	Area %
1	5.43	(S)-2-Benzylloxycarbonylamino-4-oxo-4-phenylbutanoyl azetidine	C ₂₁ H ₂₂ N ₂ O ₄	366	0.76
2	5.43	1-[N (Benzylloxycarbonyl)aminomethyl] benzotriazole	C ₁₅ H ₁₄ N ₄ O ₂	282	0.76
3	5.43	Benzyl propenoate	C ₁₀ H ₁₀ O ₂	162	0.76
4	33.71	(Z)-(2S,3S)-2,3-bis[(methoxymethyl)oxy]-5-(4-methoxyphenyl) pent-4-enol	C ₁₆ H ₂₄ O ₆	312	0.80
5	33.71	(E)-(2S,3S)-2,3-bis[(methoxymethyl)oxy]-5-(4-methoxyphenyl) pent-4-enal	C ₁₆ H ₂₂ O ₆	310	0.80
6	32.70	bis(2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	390	0.77
7	32.70	i-Propyl 3-(phenylamino)-2-(phenylseleno)-3-(phenyl)propanoate	C ₂₄ H ₂₅ NO ₂ Se	439	0.77
8	32.70	1,2-Benzenedicarboxylic acid, dicyclohexyl ester	C ₂₀ H ₂₆ O ₄	330	0.88
9	23.52	1-methoxy-5-methyl-1-phenylhex-4-ene	C ₁₄ H ₂₀ O	204	1.20
10	24.03	1-Hexyl-2-nitrocyclohexane	C ₁₂ H ₂₃ NO ₂	213	0.95
11	30.73	2,2-Diphenyl-1(4-methoxyphenyl)-1,2-dihydroazeto[2,1-b] quinazolin-8-one	C ₂₉ H ₂₂ N ₂ O ₂	430	4.20
12	30.73	(2S)-2-(2,3-Dimethoxyphenyl)carbonylamino-N-(2,3-dimethoxy benzyl)-3 methylbutylamide	C ₂₃ H ₃₀ N ₂ O ₆	430	4.20
13	28.72	13-Carbethoxy-12-carbethoxymethyl-pyrrolo[1,2-a:4,3-b']diquinoline-14-ium-chloride	C ₂₆ H ₂₃ ClN ₂ O ₄	462	0.84
14	28.72	2-(1-ethyl-1-butenyl)-1-nitrocyclohexane	C ₁₂ H ₂₁ NO ₂	211	0.84

S.No	Retention time	Compound name	Molecular formula	Molecular weight	Area %
15	32.70	1,2-Benzenedicarboxylic acid, dioctyl ester	C ₂₄ H ₃₈ O ₄	390	0.88
16	32.70	1,2-Benzenedicarboxylic acid, disooctyl ester	C ₂₄ H ₃₈ O ₄	390	0.88
17	32.70	i-Propyl 3-(phenylamino)-2-(phenylseleno)-3-(phenyl)propanoate	C ₂₄ H ₂₅ NO ₂ Se	439	0.77
18	23.52	Di-isodecyl phthalate	C ₂₈ H ₄₆ O ₄	446	1.05
19	20.86	Methyl 4-Phenyl-3-oxahept-6-enoate	C ₁₃ H ₁₆ O ₃	220	4.46
20	20.86	1-Methyl-2-methyleneacenaphthene-1-carboxaldehyde	C ₁₅ H ₁₂ O	208	4.46
21	26.21	Pentadecanoic acid, methyl ester	C ₁₆ H ₃₂ O ₂	256	3.73
22	7.77	Undecane, 2-methyl	C ₁₂ H ₂₆	170	1.57
23	26.95	11,14, 17- Eicosatrienoic acid, methyl ester	C ₂₁ H ₃₆ O ₂	320	6.85
24	32.71	(+) – Cis – (1S, 4R)-4-(Tetrahydropyran – 2-yloxy)cyclohex – 2- enol	C ₁₁ H ₁₈ O ₃	198	1.27

Figure 47
Gas chromatogram of MEFrB

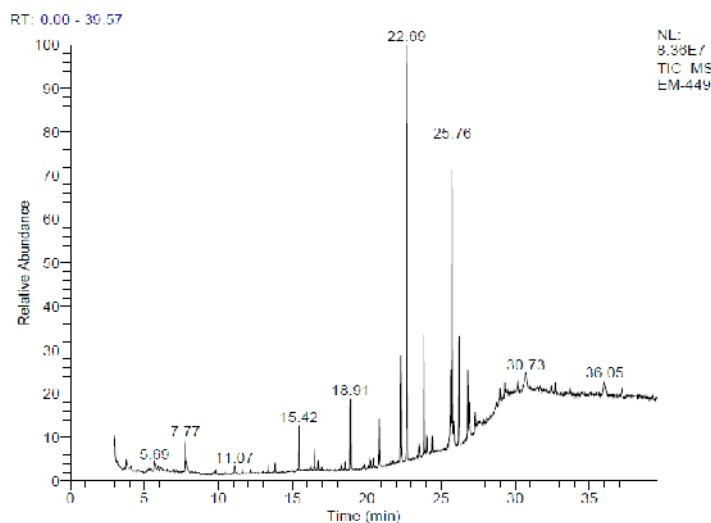
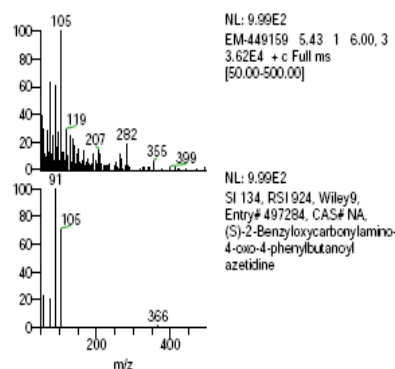
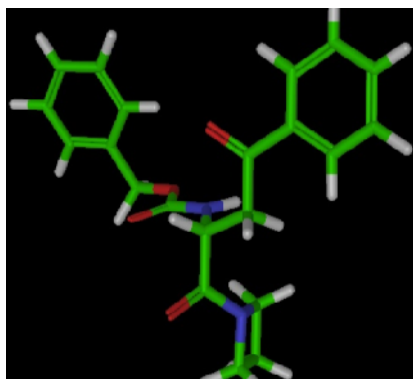


Figure 48

Structure and mass spectra of major and minor constituents of MEFrB

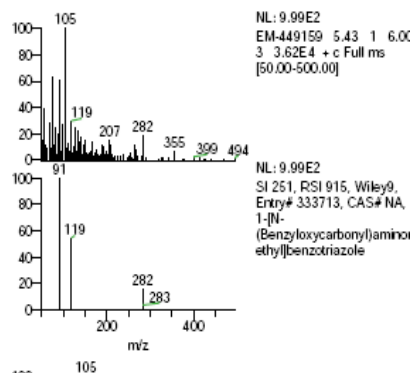
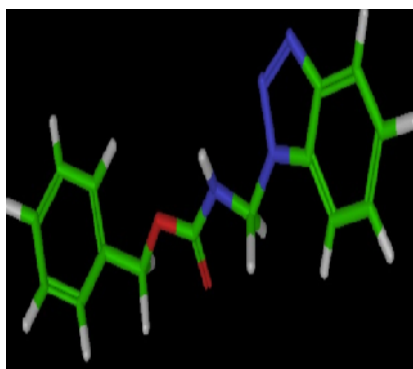
Compound 1

(S)-2-Benzyloxycarbonylamino-4-oxo- 4-phenylbutanoyl azetidine



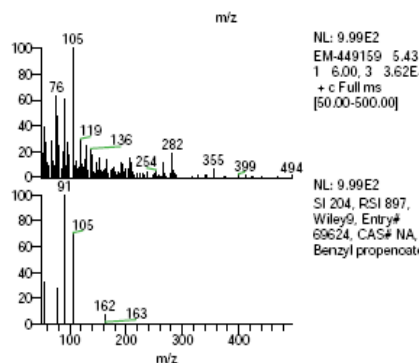
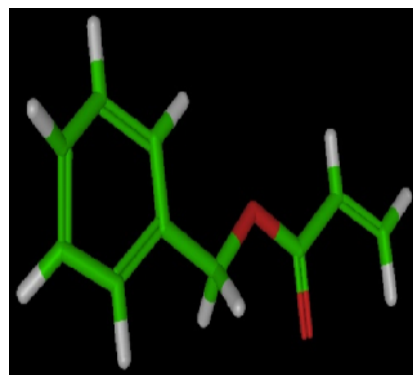
Compound 2

1-[N-(Benzyloxycarbonyl) aminomethyl] benzotriazole



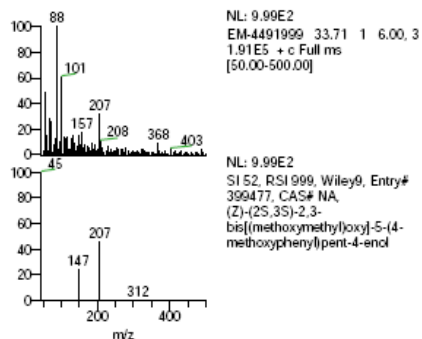
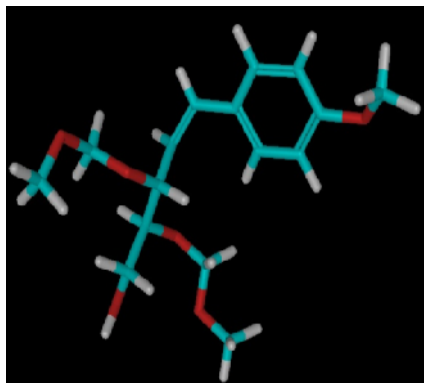
Compound 3

Benzyl propenoate



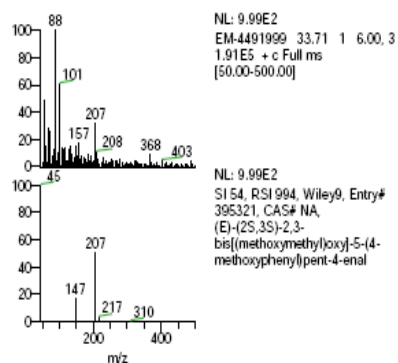
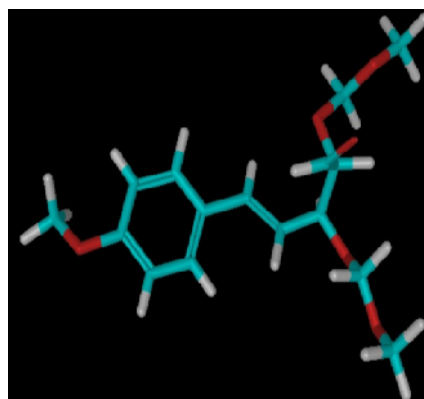
Compound 4

(Z)-(2S,3S)-2,3-bis[(methoxymethyl)oxy]-5-(4-methoxyphenyl) pent-4-enol



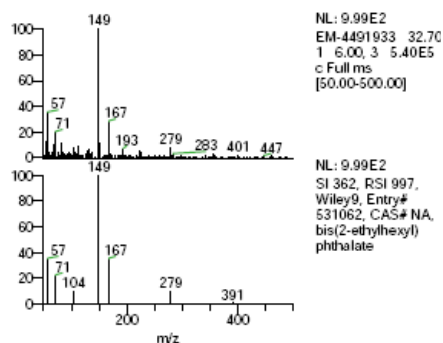
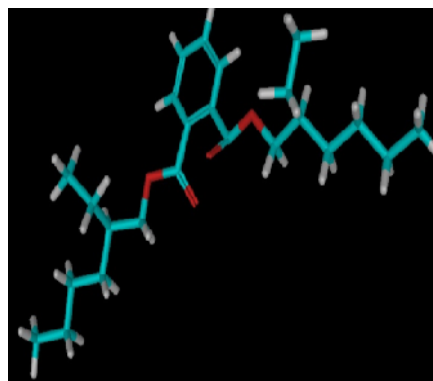
Compound 5

(E)-(2S,3S)-2,3-bis[(methoxymethyl)oxy]-5-(4-methoxyphenyl) pent-4-enal



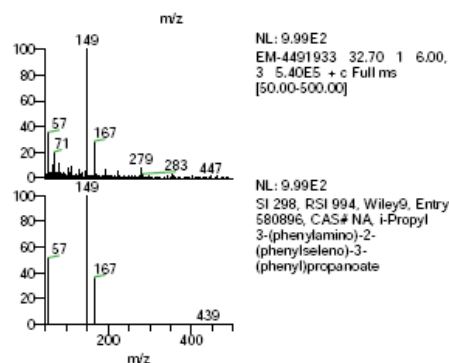
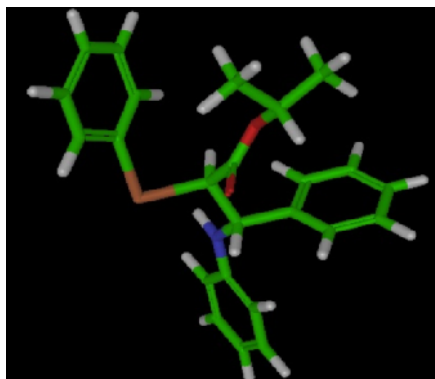
Compound 6

Bis (2-ethylhexyl) phthalate



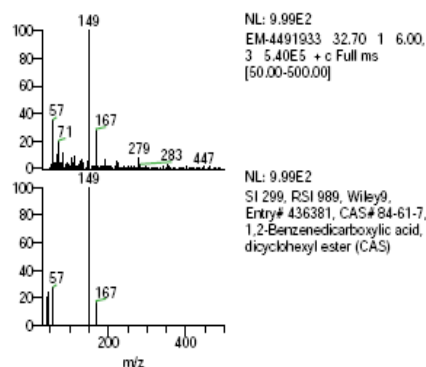
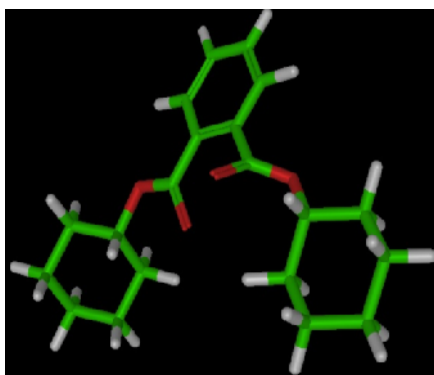
Compound 7

1- Propyl 3-(phenylamino)-2-(phenylseleno)-3-(phenyl)propanoate



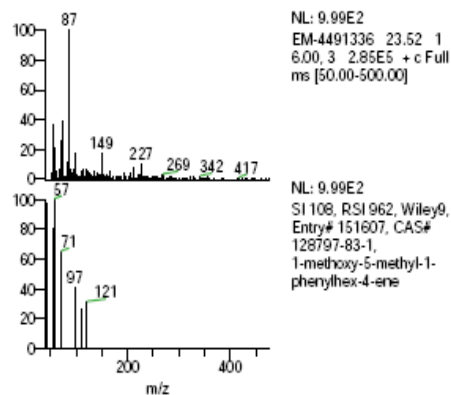
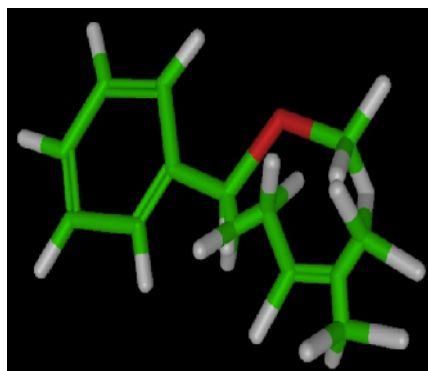
Compound 8

1,2-Benzenedicarboxylic acid, dicyclohexyl ester



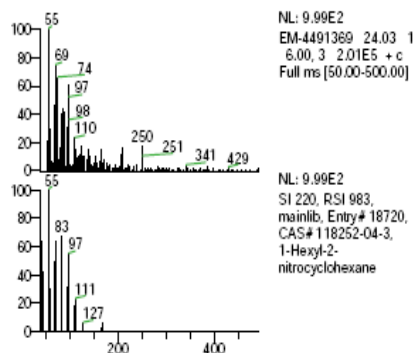
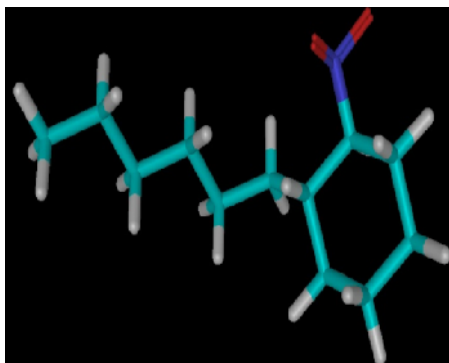
Compound 9

1-methoxy-5-methyl-1-phenylhex-4-ene



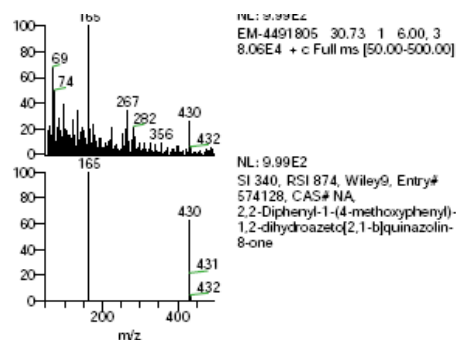
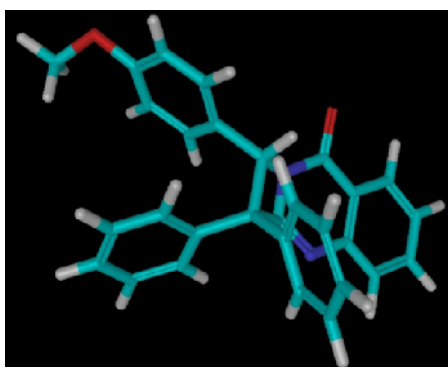
Compound 10

1-Hexyl-2-nitrocyclohexane



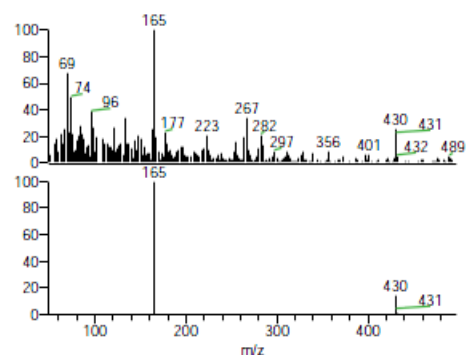
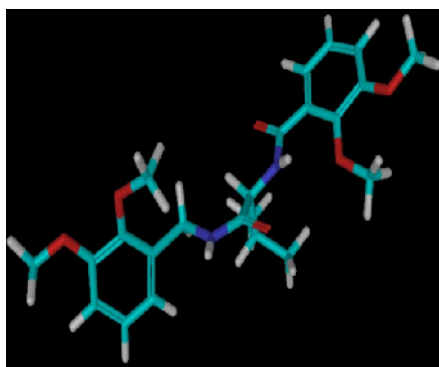
Compound 11

2,2-Diphenyl-1(4-methoxyphenyl)1,2-dihydroazeto[2,1-b]quinazolin-8-one



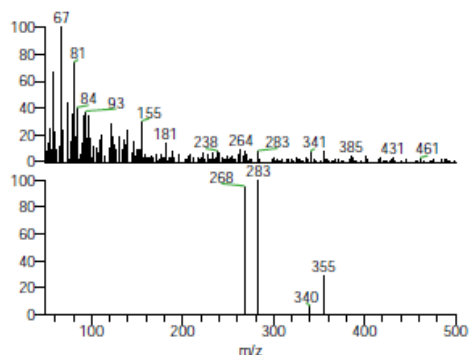
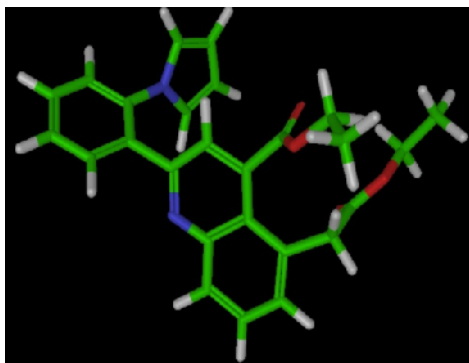
Compound 12

(2S)-2-(-2,3-Dimethoxyphenyl)carbonylamino-N-(2,3-dimethoxy benzyl)-3 methylbutylamide



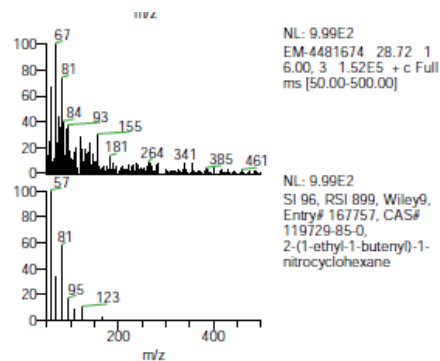
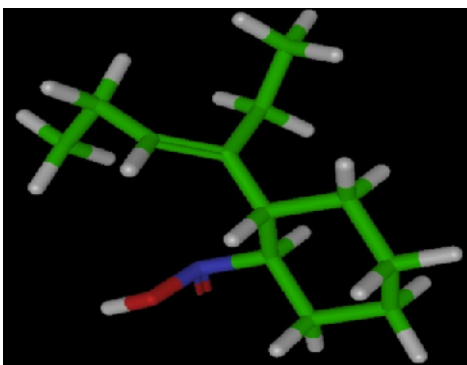
Compound 13

13-Carbethoxy-12-carbethoxymethyl-pyrrolo [1,2-a:4,3-b']diquinoline-14-ium chloride



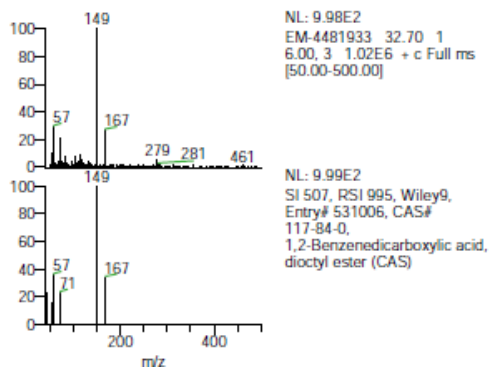
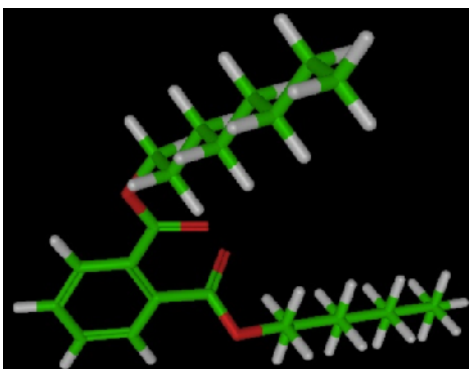
Compound 14

2-(1-ethyl-1-butenyl)-1-nitrocyclohexane



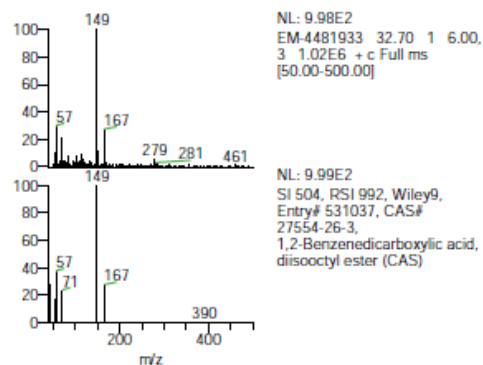
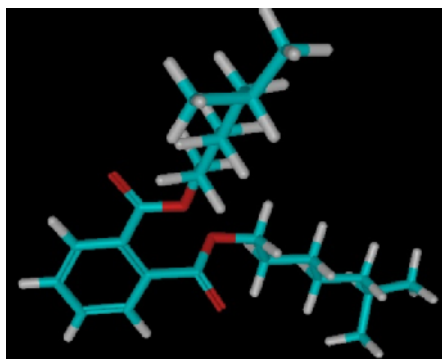
Compound 15

1,2-Benzenedicarboxylic acid, dioctyl ester



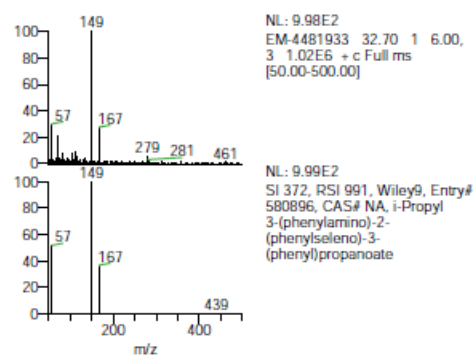
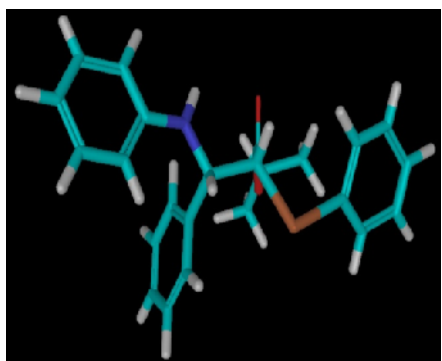
Compound 16

1, 2-Benzenedicarboxylic acid, diisooctyl ester



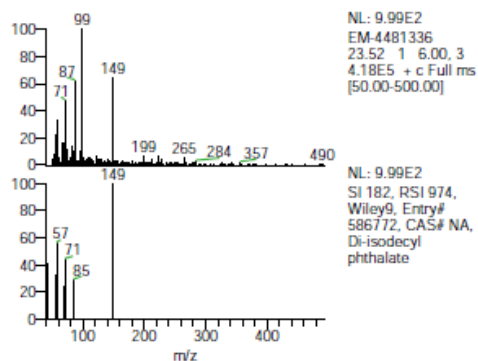
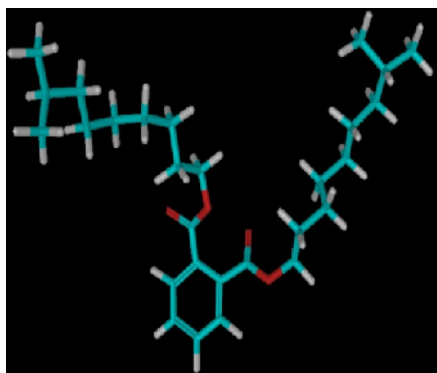
Compound 17

i- Propyl 3-(phenylamino)-2-(phenylseleno)-3-(phenyl)propanoate



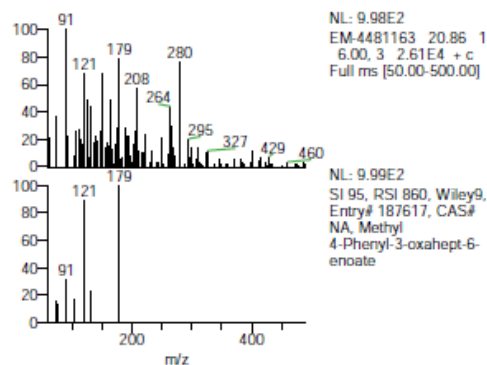
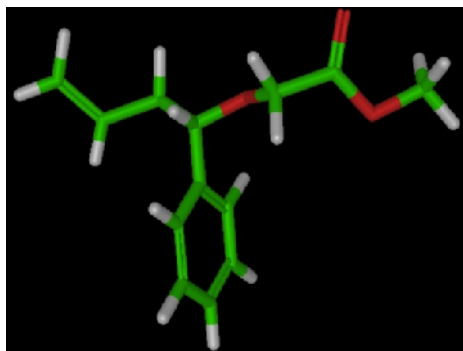
Compound 18

Di-isodecyl phthalate



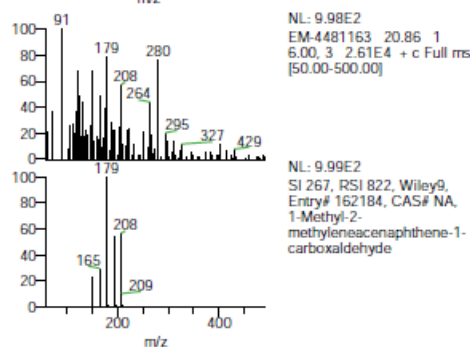
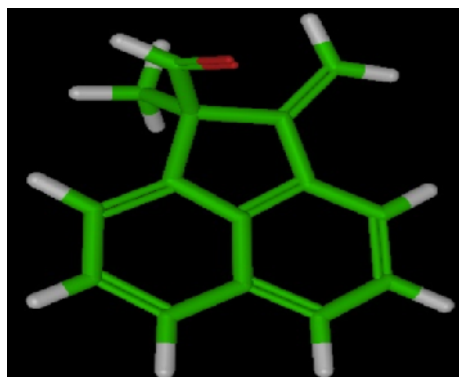
Compound 19

Methyl 4-Phenyl -3-oxahept-6-enoate



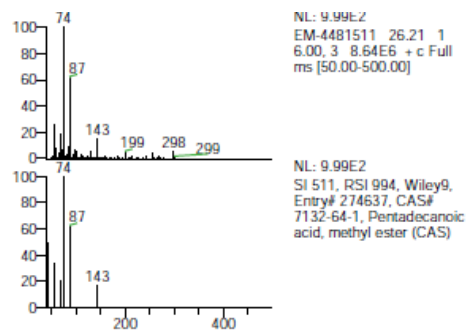
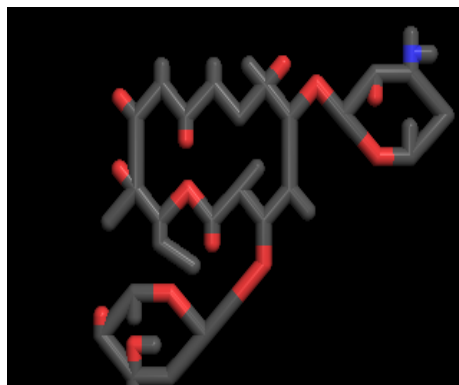
Compound 20

1-Methyl-2-methyleneacenaphthene-1-carboxaldehyde



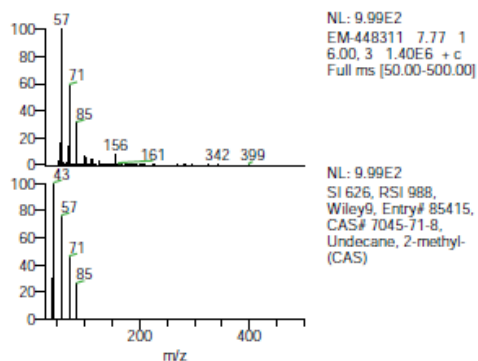
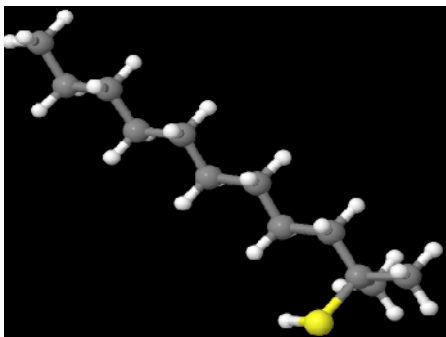
Compound 21

Pentadecanoic acid,methyl ester



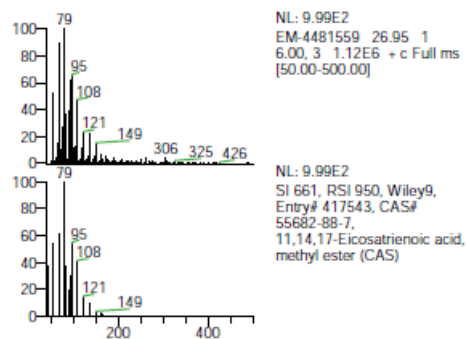
Compound 22

Undecane, 2-methyl



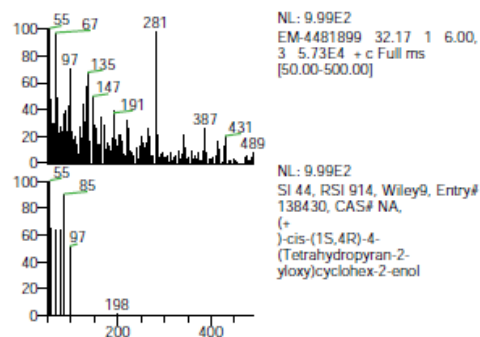
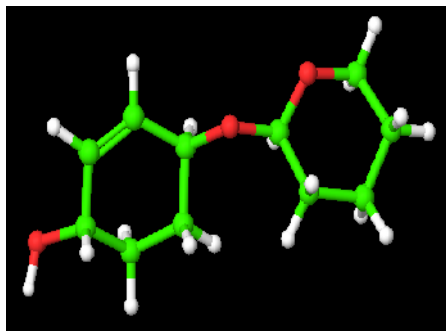
Compound 23

11,14, 17- Eicosatrienoic acid, methyl ester



Compound 24

(+) – Cis – (1S, 4R)-4- (Tetrahydropyran – 2- yloxy)cyclohex – 2- enol



Runa and Li (2007) reported the presence of Bis (2-ethyl hexyl) phthalate in the roots of *Euphorbia hylonoma* Mazz and Ramalakshmi and Muthuchelian (2011) showed the antimicrobial and pharmacological activities of phthalates. The GC-MS analysis of the aqueous extracts of the fresh and the dry *Cleistanthus collinus* leaves indicated the presence of several bioactive compounds (Parasuraman *et al.*, 2009). The medicinal properties of MEFrB such as anticancer and antimicrobial role may be due to the synergistic activity of these constituents. Bayoub *et al.* (2010) and Abhishek *et al.* (2011) showed identified the major components responsible for the antibacterial effect of methanol extracts of thirteen plants and *Murraya koenigii* leaves by gas chromatography coupled with mass spectrometry (GC/MS) analysis respectively. Ganesh and Jannet (2011) showed the presence of several bioactive constituents in the methanolic extracts of leaves of *Acanthus ilicifolius* and *Avicennia officinalis*.

4.4.2.2. High Performance Thin Layer Chromatography (HPTLC)

The HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. The MEFrB were subjected to HPTLC analysis to confirm the presence of flavonoids, phenolics and terpenoids. First, the flavonoid profile of MEFrB was analysed using catechin as reference standard and 1% ethanolic aluminium chloride reagent as spray reagent. The plate was photo-documented at UV 366 nm using Photo-documentation (CAMAG REPROSTAR 3) chamber. The MEFrB showed the presence of 12 flavonoids against the standards catechin. The Table 19 and Figure 49 show the flavonoids of MEFrB.

Yellow and yellow green fluorescence zone at UV366 nm was observed from the chromatogram, which confirmed the presence of flavonoids in the bark of *Ficus racemosa* as given in Plate VI a.

Table 19
Peaks of flavonoids of MEFrB in HPTLC

Track	Peak	Rf	Height	Area	Flavonoids
STD	1	0.45	470.9	19455.1	Catechin
MEFrB	1	0.01	419.3	8038.6	Unknown
	2	0.05	171.2	4198.1	Unknown
	3	0.24	11.2	271.4	Unknown
	4	0.32	18.4	230.1	Unknown
	5	0.52	27.2	697.4	Flavonoid 1
	6	0.55	38.2	1718.1	Unknown
	7	0.62	36.0	525.8	Unknown
	8	0.66	34.1	723.8	Unknown
	9	0.72	24.1	598.6	Unknown
	10	0.83	25.3	843.1	Unknown
	11	0.92	320.6	8446.7	Unknown
	12	0.95	273.0	7127.5	Unknown

Figure 49
Peak densitogram of flavonoids of MEFrB in HPTLC

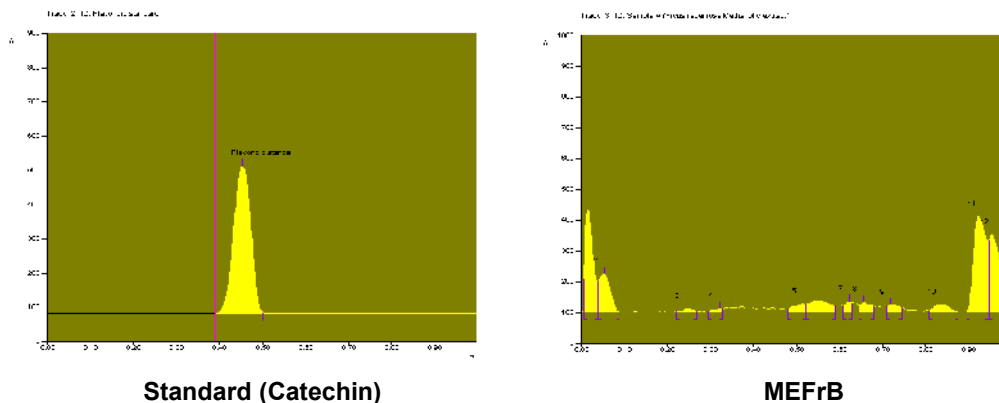
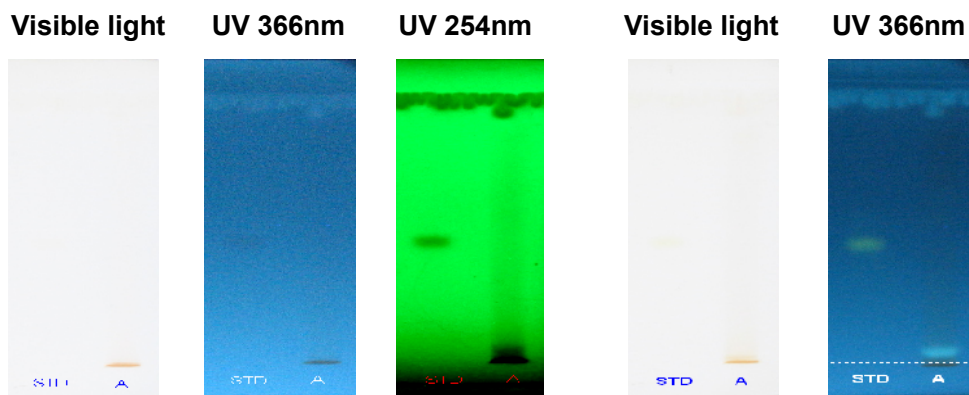


Plate VI
HPTLC chromatograms of MEFrB

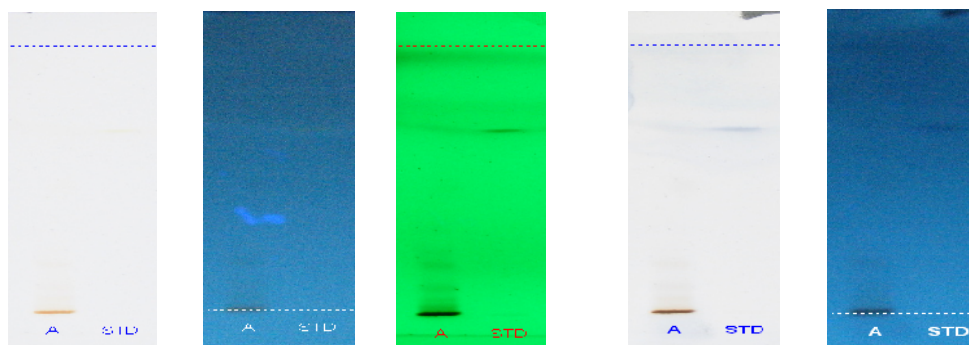
Before derivatization

After derivatization

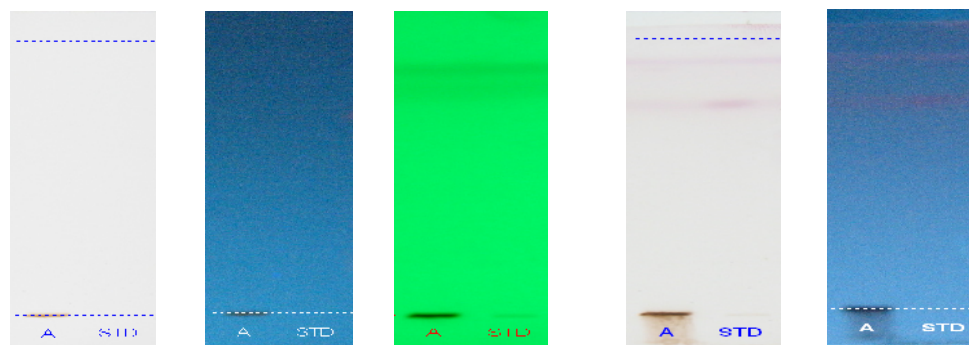
a. Flavonoid profile and Standard - Catechin



b. Phenolic profile and Standard - Rutin



c. Terpenoid profile and Standard - Solanesol



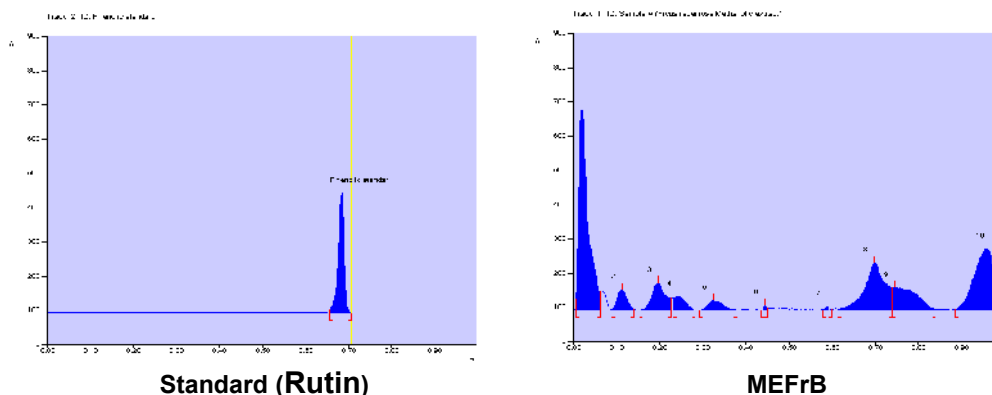
The Phenolic profile of MEFrB was analysed using rutin as reference standard and 20% Sodium carbonate solution followed by Folin Cio-calteu spray reagent. The plate was photo-documented at UV366 nm using Photo-documentation (CAMAGREPROSTAR 3) chamber. Blue, Brown coloured zone at visible light was observed from the chromatogram, which confirmed the presence of phenolic compounds in the bark of *Ficus racemosa* as given in Plate VI b Table 20 and Figure 50. The MEFrB showed the presence of 10 phenolic compounds against the standard rutin.

Table 20
Peaks of phenolic compounds of MEFrB in HPTLC

Track	Peak	Rf	Height	Area	Phenolic compounds
STD	1	0.69	414.0	9581.0	Rutin
MEFrB	1	0.02	654.2	14980.2	Unknown
	2	0.11	79.7	2025.2	Unknown
	3	0.20	80.3	2378.3	Unknown
	4	0.24	41.4	1030.8	Unknown
	5	0.33	30.7	911.2	Unknown
	6	0.44	16.3	105.4	Unknown
	7	0.59	12.6	72.3	Unknown
	8	0.70	140.5	6046.7	Phenolic 1
	9	0.75	67.0	3476.2	Unknown
	10	0.96	151.7	5845.3	Unknown

Figure 50

Peak densitogram of phenolic compounds of MEFrB in HPTLC

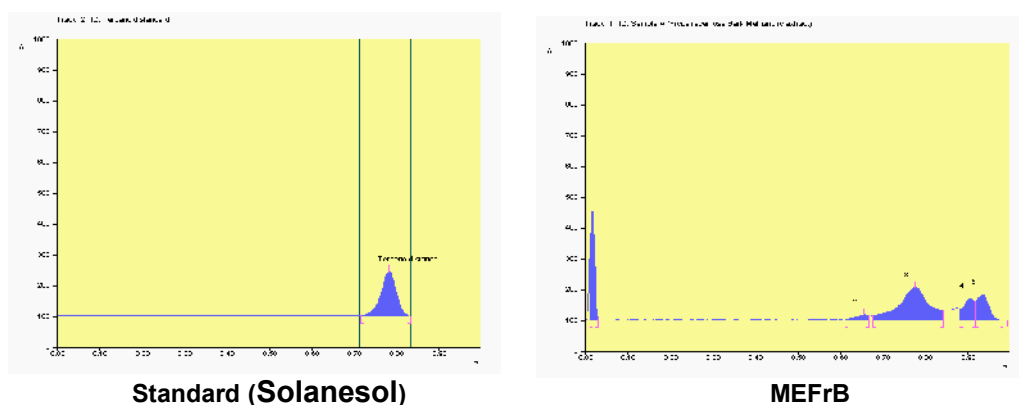


The terpenoid profile of MEFrB was analysed using solanesol as reference standard and anisaldehyde sulphuric acid spray reagent. The plate was photo-documented at UV 366nm using Photo- documentation (CAMAG REPROSTAR 3) chamber. Blue, Violet coloured zone at Visible light mode was present in the track. The chromatogram after derivatization confirmed the presence of terpenoids in the bark of *Ficus racemosa* as given in Plate VI c and Table 21 and Figure 51 was observed. The MEFrB showed the presence of 5 terpenoids against the standard solanesol.

Table 21
Peaks of terpenoids of MEFrB in HPTLC

Track	Peak	Rf	Height	Area	Terpenoids
STD	1	0.78	170.4	8193.9	Solanesol
MEFrB	1	0.02	356.3	3593.0	Unknown
	2	0.66	17.3	523.2	Unknown
	3	0.79	105.1	6983.1	Terpenoid
	4	0.91	70.3	1710.6	Unknown
	5	0.94	83.5	2124.8	Unknown

Figure 51
Peak densitogram of terpenoids of MEFrB in HPTLC

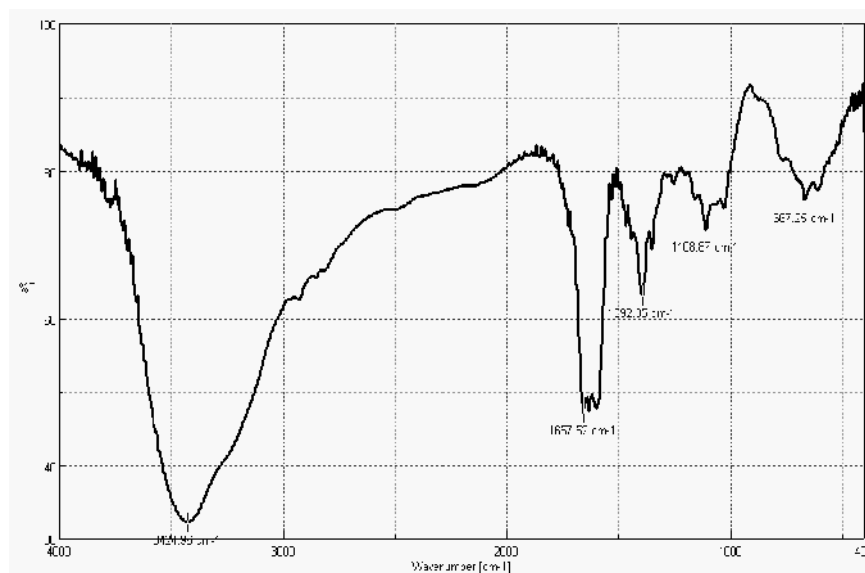


Sasikumar *et al.* (2009) showed the presence of phenolics and flavonoids using HPTLC in the methanolic and aqueous extracts of *Pandanus odoratissimus* Linn. Sophia *et al.* (2011) reported the presence of terpenoids in *Emilia sonchifolia* by HPTLC analysis. The HPTLC analysis of MEFrB showed the presence of 12 flavonoids, 10 phenolic compounds and 5 terpenoids against the standards catechin, rutin and solanesol respectively. Mishra *et al.* (2012) also reported quercetin in the methanolic extract of *Phaseolus vulgaris* (common bean). Further phytochemical studies by FT-IR could reveal the presence of functional side chains of its secondary metabolites responsible for the observed bioactivities of MEFrB.

4.4.2.3. Fourier Transform Infra Red Spectroscopy (FT- IR)

The absorption bands, the wave number (cm^{-1}) of dominant peak obtained from absorption spectra and Figure 52 shows the FT-IR spectra of MEFrB.

Figure 52
FT-IR spectra of MEFrB



The FT-IR analysis of MEFrB showed the peak at 3424.96 cm^{-1} revealed C-H, O-H and N-H stretching variations of alcohols and phenols. The peak at 1657.52 cm^{-1} revealed C=O stretching variations of aldehyde / ketones / carboxylic acids / esters and NH primary amines. The peak at 1392.35 cm^{-1} revealed C-H

stretching variations of aromatic bonding and 667.25 cm^{-1} revealed C-H stretching variations of alkanes. The peak at 1108.87 cm^{-1} revealed C-O stretching variations of alcohols / ethers / carboxylic acids and esters, the absorbance bands known to be associated with the stretching vibrations for -C, C-CO, -C-C (aromatic ring), C-O (esters and ethers) and C-O (polyols). The presence of characteristic functional groups such as hydroxyl, aldehyde and halogen compounds are responsible for various medicinal properties of MEFrB. At present, particularly in phytochemistry, FT-IR has been exercised to identify the functional groups such as C-O, C-H, COO^- , ester, amide, carbonyl groups of certain plant secondary metabolites (Yang and Yen, 2002; Ivanova and Singh, 2003 and Stehfest *et al.*, 2005). Similar results for the presence of functional groups such as -OH, C-O, C-H and carbonyl groups in FT-IR spectra were reported by Nirmala Devi *et al.* (2010) in the methanolic extract of the leaves of *Rhinacanthus nasutus* and Karthishwaran *et al.* (2010) in the methanolic extract of *Pergularia daemia*.

Ragavendran *et al.* (2011) also showed the presence of characteristic functional groups such as carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons, halogens are responsible for various medicinal properties of aqueous, ethanol and aqueous ethanol extracts of *Aerva lanata*. Thenmozhi *et al.* (2011) showed that the methanolic extract of *Eclipta alba* and *Emilia sonchifolia* identified the compounds like mono fluorinated compounds, poly fluorinated compounds, amide II band, ketones- dialkylketone groups, amine salts, polymeric hydroxyl compounds and primary alcohols. The FT-IR analysis of the methanolic extract of *Annona squamosa*, *Catharanthus roseus*, *Sapindus emarginatus* and *Wrightia tinctoria* were reported by Shalini and Sampathkumar (2012) and Pramila *et al.*, (2012) indicated the methanolic extract of *Mentha piperita* by molecular configuration of different functional groups.

In Phase IV the preliminary phytochemical analysis of the MEFrB, MEFrF and MEFrL revealed the presence of flavonoids, steroids, phenolics, terpenoids, fixed oils, fats, saponins and tannins. GC-MS analysis identified 24 compounds in MEFrB. HPTLC analysis of MEFrB showed the presence of 12 flavonoids, 10 phenolic compounds and 5 terpenoids against the standards catechin, rutin and

solanesol respectively. The FT-IR spectra revealed the major functional groups of the phytochemical constituents of MEFrB. However, further research on the phytochemical constituents and their functional groups has to be carried out.

PHASE V

4.5. Antitumorigenic and Antimicrobial efficacy of Phytochemical Constituents of MEFrB by *in silico* Studies

4.5.1. Active site residues of tumorigenic and microbial target proteins

4.5.2. Glide docking

4.5.3. Prediction of ADME properties

4.5.1. Active site residues of tumorigenic and microbial target proteins

The ligplot automatically generates schematic diagrams of protein-ligand interactions for a given PDB file. The Figure 53 and 54 depicts the active site residues and interactions of co-crystal ligands SHH and VLB with tumorigenic target proteins (1T69 and 1Z2B) and PN1 and PAU with microbial (1W2N and 1SQ5) target proteins.

4.5.2. Glide docking

In GLIDE the crystal structure of various target protein complexes with ligand is downloaded from Protein Data Bank (PDB ID -1T69, 1Z2B, 1W2N and 1SQ5) and saved. The downloaded protein is imported into the Maestro work space and water molecules are deleted and energy minimization procedures were performed for all the target proteins and the four ligand compounds. All the 24 bioactive compounds of MEFrB identified in GC- MS analysis were chosen for the docking studies.

SP docking

All the prepared ligands were subjected to SP docking with all the four target proteins. Based on glide score, glide energy and number of hydrogen bonds, the best ligands can be identified in comparison with corresponding co-crystal ligands (1T69-SHH, 1Z2B-VLB, 1W2N-PN1 and 1SQ5-PAU). All the 24 compounds identified in GC-MS were selected for SP and XP docking. SP docking results are shown in Table 22.

Ligplot interactions of tumor target proteins and their active sites



Ligplot interactions of microbial target proteins and their active sites

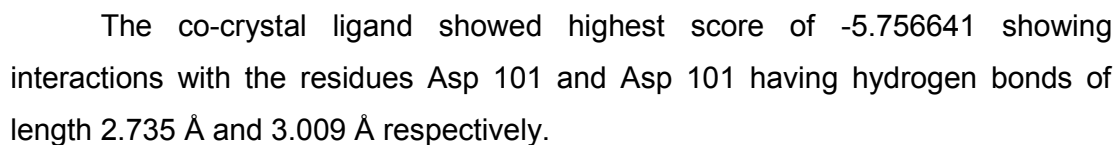


Table 22

SP docking of tumor target protein Histone deacetylase (1T69) with bioactive compounds of MEFrB

Docking of Tumor target (1T69)	Glide score	Glide energy (Kcal / Mol)	Hydrogen bond interactions (DH...A)	Bond distance (Å)
Co-crystal (SHH)	-5.756641	-35.742488	Asp101 (OH...O) Asp 101 (NH...O)	2.735 3.009
Compound 2	-7.035352	-41.400277	Tyr 306 (OH...O) Gly 151 (NH...O)	2.917 3.074
Compound 4	-5.320918	-37.999418	Tyr 306 (OH...O) Phe 208 (NH...O)	2.662 2.812
Compound 5	-5.133904	-33.495655	Tyr 306 (OH...O) Phe 208 (NH...O)	2.732 2.983
Compound 10	-5.330918	-31.641964	Tyr 302 (OH...O) Phe 199 (NH...O)	2.701 2.886
Compound 18	-5.226839	-45.906771	Tyr 298 (OH...O) Phe 192 (NH...O)	2.644 2.902
Compound 24	-6.727805	-30.855328	Tyr207 (OH....O)	2.345

The compounds 2 and 24 showed highest score of -7.035352 and -6.727805 which was found to be greater than co-crystal. The compound 2 showed strong interaction with the residues Tyr 306 and Gly 151 having hydrogen bonds of length 2.917 Å and 3.074Å respectively.

The compound 24 showed strong interaction with the residue Tyr 207 having hydrogen bond of length 2.345 Å. SP docking of the tumor target protein histone deacetylase (1T69) with the 24 compounds identified in GC-MS in comparison with the co-crystal ligand SHH showed highest score for compound 2 and 24. The scores were found to be high than that of the co-crystal SHH.

The SP docking of tumor target protein tubulin (1Z2B) with the 24 compounds in comparison with co-crystal ligand (VLB) is shown in Table 23.

Table 23

SP docking of tumor target protein Tubulin (1Z2B) with bioactive compounds of MEFrB

Docking of Tumor target (1Z2B)	Glide score	Glide energy (Kcal / Mol)	Hydrogen bond interactions (DH...A)	Bond distance (Å)
Co-crystal (VLB)	-3.506094	-29.395245	Thr 349 (OH...O) Asn 329 (OH...O)	2.818 2.853
Compound 2	-6.727805	-30.855328	Asn 199 (OH...O) Val 151 (NH...O)	2.001 1.682
Compound 4	-5.927351	-26.041248	Phe 321 (OH...O) Asn 208 (NH...O)	2.132 2.812
Compound 5	-5.690095	-38.596353	Asp 102 (OH...O) Asn 99 (NH...O)	2.204 1.440
Compound 10	-5.568694	-34.622173	Asn 302 (OH...O) Asp199 (NH...O)	2.402 2.188
Compound 18	-5.330918	-31.641964	Asn 174 (OH...O) Asn 177 (NH...O)	2.102 2.504
Compound 24	-6.432191	-31.061938	Ala 204 (OH....O)	2.198

The co-crystal ligand showed highest score of -3.506094 showing two strong interactions with the residues Thr 349 and Asn 329 having hydrogen bonds of length 2.818 Å and 3.853 Å respectively.

The compound 2 showed strong interaction with the residues Asn 199 and Val151 having hydrogen bonds of length 2.001 Å and 1.682Å respectively. The compound 4 showed interactions with the residues Phe 321 and Asn 208 having hydrogen bonds of length 2.132 Å and 2.812 Å.

The compound 5 showed two interactions with the residues Asn102 and Asn 99 having hydrogen bonds of length 2.204Å and 1.440Å respectively.

The compound 10 showed two interactions with Asn 302 and Asp 119 having hydrogen bonds of length 2.402 Å and 2.188 Å respectively.

The compound 18 showed two interactions with Asn 174 and Asn177 having hydrogen bonds of length 2.102 Å and 2.504 Å respectively. The compound 24 showed interaction with the residue Ala 204 having hydrogen bond of length 2.198 Å.

The six compounds (2, 4, 5, 10, 18 and 24) showed higher glide score than that of the co-crystal (SHH).

The SP docking of microbial target protein Deacetoxy C synthase (1W2N) with 24 compounds showed six best compounds (1, 4, 5, 10, 18 and 24) in comparison with co-crystal ligand (PN1). The six compounds docked with the microbial target protein Deacetoxy C synthase (1W2N) are shown in Table 24.

Table 24
SP docking of microbial target protein Deacetoxy C synthase (1W2N)
with bioactive compounds of MEFrB

Docking of Microbial target (1W2N)	Glide score	Glide energy (Kcal / Mol)	Hydrogen bond interactions (DH...A)	Bond distance (Å)
Co-crystal (PN1)	-3.531908	-25.767654	Arg162 (NH...O) His 183 (NH...O)	3.093 3.033
Compound 1	-7.616970	-35.875466	Leu 152 (OH...O) Asp 162 (NH...O)	3.001 3.692
Compound 4	-5.849041	-33.052920	Asp 185 (OH...O)	2.132
Compound 5	-6.602465	-35.914890	Asp 102 (OH...O) Arg 99 (NH...O)	2.320 2.120
Compound 10	-5.103817	-32.529910	Ser 282 (OH...O) His199 (NH...O)	2.402 2.015
Compound 18	-5.031325	-40.474897	Arg 182 (OH...O)	2.812
Compound 24	-6.411624	-30.128754	Arg185 (OH....O)	2.552

The co-crystal ligand showed highest score of -3.531908 showing two strong interactions with the residues Arg 162 and His 183 having hydrogen bonds of length 3.093Å and 3.033 Å.

The compound 1 showed highest score -7.616970 and strong interaction with the residues Leu 152 and Asp 162 having hydrogen bonds of length 3.001 Å and 3.692Å.

Other compound 4 showed highest score -5.849041 and strong interaction with the residues Asp 185 having hydrogen bond of length 2.132 Å.

The compound 5 showed highest score -6.602465 and interactions with the residues Asp 102 and one interaction with Arg 99 having hydrogen bonds of length 2.320 Å and 2.120 Å.

The compound 10 showed highest glide score, glide energy and interaction with Ser 282 and His 199 residues having hydrogen bonds of length 2.402 Å and 2.015 Å respectively.

The compound 18 showed high glide score, glide energy and interaction with Arg 182 residue having hydrogen bond of length 2.812 Å.

The compound 24 showed highest score -6.411624 and interaction with Arg 185 having hydrogen bond of length 2.552 Å respectively.

The SP docking of microbial target protein Pantothenate kinase (1SQ5) with 24 compounds showed one best compound 1 in comparison with co-crystal ligand (PAU). The six compounds docked with the microbial target protein Pantothenate kinase (1SQ5) are shown in Table 25.

Table 25
SP docking of microbial target protein Pantothenate kinase (1SQ5) with bioactive compounds of MEFRB

Docking of Microbial target (1SQ5)	Glide score	Glide energy (Kcal / Mol)	Hydrogen bond interactions (DH...A)	Bond distance (Å)
Co-crystal (PAU)	-6.072888	-46.493287	Lys1101 (NH...O) Lys1101 (NH...O) Ser1102 (NH...O) His1177 (OH...N) His1177 (OH...N)	3.321 2.900 3.006 2.778 3.028
Compound 1	-6.211437	-25.854368	Ala 213 (OH...O)	1.667
Compound 4	-5.624310	-24.116293	Lys 68 (OH...O)	2.097
Compound 5	-4.836510	-43.624932	Asp 183 (OH...O) Lys 145 (NH...O)	1.846 1.961
Compound 10	-4.709001	-47.063398	Lys 43 (NH...O)	1.817
Compound 18	-4.082662	-39.014602	Try 176 (NH...O) Gly 81 (OH...O)	2.101 2.217
Compound 24	-4.445406	-24.794020	Ala 151 (OH...O)	1.838

The co-crystal ligand showed highest score of -6.072888 showing two strong interactions with the residues Lys 1101, Lys 1101, Ser 1102, His 1177 and His 1177 having hydrogen bonds of length 3.321 Å, 2.900 Å, 3.006 Å, 2.778 Å and 3.028 Å.

The compound 1 showed highest score of -6.211437 and strong interaction with the residue Ala 213 having hydrogen bond of length 1.677 Å.

The compound 4 showed highest score of -5.624310 and strong interaction with the residue Lys 68 having hydrogen bond of length 2.097 Å.

XP docking

The 24 bioactive compounds were screened by XP docking for the entire four target proteins in comparison with corresponding co-crystal ligands (1T69-SHH, 1Z2B-VLB, 1W2N-PN1, 1SQ5-PAU). The four compounds docked with Histone deacetylase (1T69) are shown in Table 26.

Table 26
XP docking of tumor target protein Histone deacetylase (1T69)
with bioactive compounds of MEFrB

Docking of Tumor target (1T69)	Glide score	Glide energy (Kcal / Mol)	Hydrogen bond interactions (DH...A)	Bond distance (Å)
Co-crystal (SHH)	-5.756641	-35.742488	Asp101 (OH...O) Asp 101 (NH...O)	2.735 3.009
Compound 2	-4.616970	-35.875466	Tyr 306 (OH...O) Gly 151 (NH...O)	2.917 3.074
Compound 4	-6.602465	-35.914890	Tyr 306 (OH...O) Phe 208 (NH...O)	2.732 2.983
Compound 5	-6.210359	-30.8575466	Tyr306 (OH....O)	2.450
Compound 24	-5.849041	-33.052920	Tyr 306 (OH...O) Phe 208 (NH...O)	2.662 2.812

Only three compounds showed high score in XP docking for histone deacetylase (1T69) with co-crystal ligand (SHH). The co-crystal ligand showed

highest score of -5.756641 showing two strong interactions with Asp 101 having hydrogen bonds of length 2.735 Å and 3.009 Å.

The compound 4 showed highest score of -6.602465 greater than co-crystal showing strong interaction with the residues Tyr 306 and Phe 208 having hydrogen bonds of length 2.732 Å and 2.983 Å.

The compound 5 showed the highest score of -6.210359 greater than co-crystal showing strong interaction with the residues Tyr 306 having hydrogen bonds of length 2.450 Å respectively.

The compound 24 showed highest score of -5.849041 greater than co-crystal showing strong interaction with the residues Tyr 306 and Phe 208 having hydrogen bonds of length 2.662 Å and 2.812 Å.

The XP docking for Tubulin (1Z2B) showed five best compounds with high score and energy in comparison with their co-crystal ligand (VLB). The five compounds docked with Tubulin (1Z2B) are shown in Table 27.

The co-crystal ligand showed highest score of -3.506094 and two strong interactions with the residues Thr 349 and Asn 329 having hydrogen bonds of length 2.818 Å and 2.853 Å respectively.

The compound 4 showed highest score of -6.860716 greater than co-crystal showing strong interaction with the residues Asn 206, Ala174 and Val17 having hydrogen bonds of length 2.021 Å, 2.135 Å and 1.766 Å respectively.

The compound 5 showed highest score of -4.423304 greater than co-crystal showing strong interaction with the residues Phe 351, Asn 329 and Asn 249 having hydrogen bonds of length 3.050 Å, 2.916 Å and 2.997 Å respectively.

The compound 10 showed highest score of -3.930545 greater than co-crystal showing two interactions with the residues Asn 329 and one interaction with Asn 249 having hydrogen bonds of length 3.125 Å, 2.891 and 3.456 Å respectively.

The compound 18 showed highest score of -3.76100 greater than co-crystal showing one interaction with the residue Asn 101 having hydrogen bond of length 2.265 Å. The Figures 55 and 56 depict the interaction of highly scored compounds selected from XP docking.

The compound 24 showed highest score of -4.36700 than co-crystal showing two interactions with the residues Asp 98 and one interaction with Asn 101 having hydrogen bonds of length 1.540Å and 2.408 Å respectively.

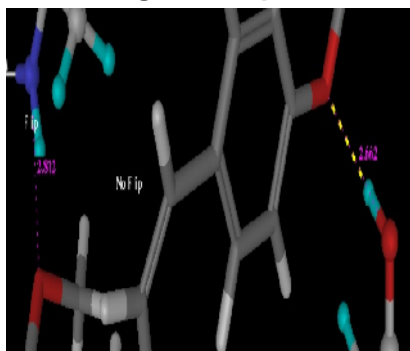
Table 27

XP docking of tumor target protein Tubulin (1Z2B) with bioactive compounds of MEFrB

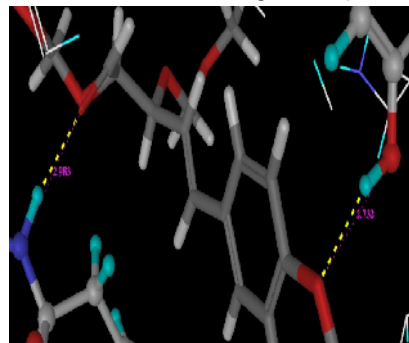
Docking of Tumor target (1Z2B)	Glide score	Glide energy (Kcal / Mol)	Hydrogen bond interactions (DH...A)	Bond distance (Å)
Co-crystal (VLB)	-3.506094	-29.395245	Thr 349 (OH...O) Asn 329 (OH...O)	2.818 2.853
Compound 4	-6.860716	-23.287357	Asn 206 (NH...O) Ala 174 (NH...O) Val 177 (NH...O)	2.021 2.135 1.766
Compound 5	-4.423304	-30.851479	Phe 351 (OH...O) Asn 329 (NH...O) Asn 249 (NH...O)	3.050 2.916 2.997
Compound 10	-3.930545	-30.183197	Asn 329 (NH...O) Asn 329 (NH...O) Asn 249 (NH...O)	3.125 2.891 3.456
Compound 18	-3.76100	-49.76400	Asn 101 (NH...O)	2.265
Compound 24	-4.36700	28.045210	Asp 98 (OH...O) Asn 101 (NH...O)	1.540 2.408

Figure 55

XP docking of compounds 4 and 5 with Histone deacetylase (1T69)



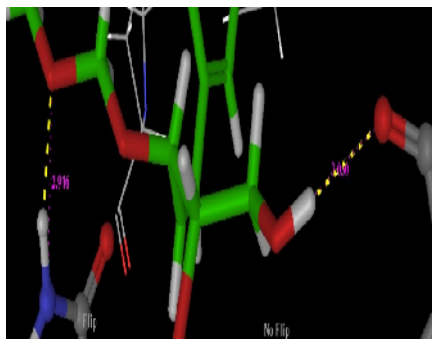
Compound 4



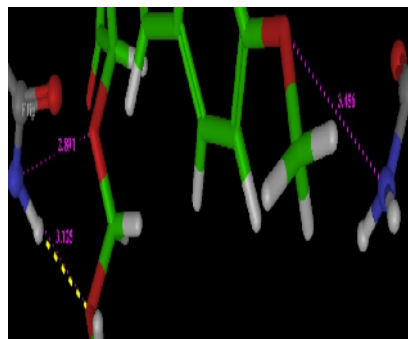
Compound 5

Figure 56

XP docking of compounds 4 and 5 with Tubulin (1Z2B)



Compound 4



Compound 5

Four compounds showed high score and energy in XP docking for Deacetoxy C synthase (1W2N) in comparison with co-crystal ligand (PN1). The four compounds docked with Deacetoxy C synthase (1W2N) are shown in Table 28.

Table 28

XP docking of microbial target protein Deacetoxy C synthase (1W2N) with bioactive compounds of MEFrB

Docking of Microbial target (1W2N)	Glide score	Glide energy (Kcal / Mol)	Hydrogen bond interactions (DH...A)	Bond distance (Å)
Co-crystal (PN1)	-3.531908	-25.767654	Arg162 (NH...O) His 183 (NH...O)	3.093 3.033
Compound 1	-4.975305	-27.913172	Asp 185 (OH...O) Arg 162 (NH...O)	3.027 2.860
Compound 4	-6.210359	-30.875466	Asp 185 (OH...O)	2.450
Compound 5	-6.080349	-45.155257	Leu 186 (NH...O) Leu 186 (NH...O) His 183 (NH...O) Asp 185 (NH...O)	3.309 3.037 3.026 3.018
Compound 24	-4.374576	-31.680903	Arg 162 (NH...O) His 183 (NH...O) His 243 (NH...O) Ser 203 (OH...O)	2.983 3.230 2.744 2.676

The co-crystal ligand showed the highest score of -3.531908 and strong interactions with the residues Arg 162 and His 183 having hydrogen bonds of length 3.093 Å and 3.033 Å respectively.

The compound 1 showed highest score of -4.975305 greater than co-crystal and strong interaction with the residues Asp185 and Arg 162 having hydrogen bonds of length 3.027 Å 2.860 Å respectively.

The compound 4 showed highest score of -6.210359 greater than co-crystal showing strong interaction with Asp185 having hydrogen bond of length 2.450 Å.

The compound 5 showed high score of -6.080349 which was found to be greater than co-crystal score and strong interaction with the residues Leu 186 , Leu 186, His 183 and Asp 185 having hydrogen bonds of length 3.309 Å 3.037 Å, 3.026 Å and 3.018 Å respectively.

The compound 24 showed highest score of -4.374576 greater than co-crystal and strong interaction with the residues Arg 162, His 183, His 243 and Ser 203 having hydrogen bonds of length 2.983 Å, 3.230 Å, 2.744 Å and 2.676 Å respectively.

The XP docking showed three best compounds with high score and energy for Pantothenate Kinase (1SQ5) in comparison with co-crystal ligand (PAU). The three compounds docked with Deacetoxy C synthase (1W2N) are shown in Table 29.

The co-crystal ligand (PAU) showed the highest score of -6.072888 showing five strong interactions, two interactions with the same residue Lys 1101, one interaction with the residue Ser 1102 and two interactions with the same residue His 1177 having hydrogen bonds of length 3.321 Å , 2.900 Å ,3.006 Å, 2.778 Å and 3.028 Å.

The compound 1 showed the highest score of -6.777798 greater than co-crystal and one interaction with the residue Arg 1243, two interactions with the same residue Tyr 1240 and one interaction with the residue Asn 1282 having hydrogen bonds of length 3.080 Å 3.039 Å, 3.395 Å and 2.839 Å respectively.

The compound 4 showed the highest score of -8.744492 greater than co-crystal and strong interaction with the residues Arg 1243, Asn 1282, Tyr 1240 and Tyr 1240 having hydrogen bonds of length 2.927 Å 2.899 Å, 2.743 Å and 2.334 Å.

The compound 5 showed the highest score of -7.159384 greater than co crystal and strong interactions with the residues Asp 1127, Arg 1243 and Tyr 1240 having hydrogen bonds of length 2.710 Å, 2.591 Å and 2.930 Å respectively. The Figures 57 and 58 depict the interaction of highly scored compounds selected from XP docking.

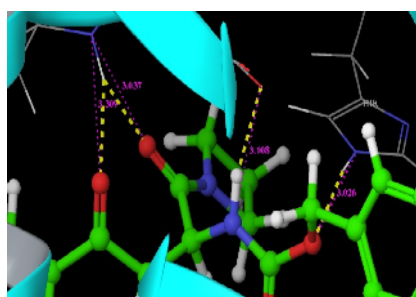
Table 29

**XP docking of microbial target protein Pantothenate Kinase (1SQ5)
with the bioactive compounds of MEFrB**

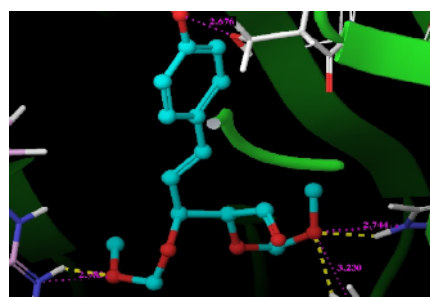
Docking of Microbial target (1SQ5)	Glide score	Glide energy (Kcal / Mol)	Hydrogen bond interactions (DH...A)	Bond distance (Å)
Co-crystal (PAU)	-6.072888	-46.493287	Lys1101 (NH...O)	3.321
			Lys1101 (NH...O)	2.900
			Ser1102 (NH...O)	3.006
			His1177 (OH...N)	2.778
			His1177 (OH...N)	3.028
Compound 1	-6.777798	-30.159645	Arg1243 (NH...O)	3.080
			Tyr1240 (OH...O)	3.039
			Tyr1240 (OH...O)	3.395
			Asn1282 (NH...O)	2.839
Compound 4	-8.744492	-42.031084	Arg1243 (NH...O)	2.927
			Asn1282 (NH...O)	2.899
			Tyr1240 (OH...O)	2.743
			Tyr1240 (OH...O)	2.834
Compound 5	-7.159384	-41.891399	Asp1127 (OH...O)	2.710
			Arg1243 (NH...O)	2.591
			Tyr1240 (OH...O)	2.930

Figure 57

XP docking of compounds 4 and 5 with Deacetoxy C synthase (1W2N)



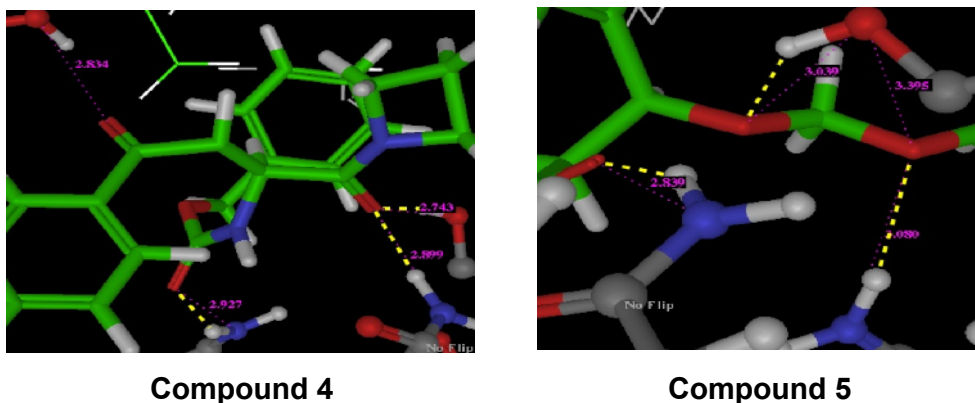
Compound 4



Compound 5

Figure 58

XP docking of compounds 4 and 5 with Pantothenate Kinase (1SQ5)



Induced fit docking

Induced fit docking were used to perform an interactive study, where more number of poses can be generated for a single ligand that can be docked with the target receptor molecule. From the results of XP docking, highly scored compounds from tumorigenic and microbial targets were subjected to induced fit docking. The present study on SP and XP docking for bioactive compounds on tumorigenic targets (1T69 and 1Z2B) and microbial targets (1W2N and 1SQ5) showed high score and interactions for the seven compounds 1, 2, 4, 5, 10, 18 and 24. So, these highly scored compounds selected from both the targets were used to perform induced fit docking with their corresponding targets.

In induced fit docking, each compound was obtained with 3 poses and the best pose was pictorially represented. The Table 30 shows the induced fit docking results for tumor target protein Histone deacetylase (1T69) with highly scored compounds (4 and 5) of three poses each.

The co-crystal (SHH) showed the score of -4.547854 and strong interactions with the residues Asp178, Asp 267 and Tyr 306 having hydrogen bonds of length 2.660 Å, 2.769 and 2.810 Å respectively (Figure 59).

The compound 4 was obtained with 3 poses and all the 3 poses showed highest score compared to the co-crystal ligand. The best pose showed the highest score of -6.094091 and strong interactions with the residues Tyr 306, Lys

33 and His 180 having hydrogen bonds of length 2.783 Å, 3.198 Å and 2.942 Å respectively.

Table 30

Induced fit docking of tumor target protein Histone deacetylase (1T69) with the bioactive compounds of MEFrB

Docking of Tumor target (1T69)	Pose Number	Glide score	Glide energy (Kcal / Mol)	Hydrogen bond interactions (DH...A)	Bond distance (Å)
Co-crystal (SHH)	Pose 1	-4.547854	-42.202789	Asp178 (OH...O)	2.660
				Asp 267 (NH...O)	2.769
				Tyr 306 (OH...O)	2.810
Compound 4	Pose 1	-6.094091	-45.473108	Tyr 306 (OH...O)	2.783
				Lys 33 (NH...O)	3.198
				His 180 (NH...O)	2.942
	Pose 2	-5.845370	-45.878405	Gly 305 (NH...O)	3.003
	Pose 3	-5.798657	-45.301232	Tyr 306 (OH...O)	3.137
Tyr 306 (OH...O)				2.790	
Asp 267 (OH...O)				3.084	
Compound 5	Pose 1	-6.762940	-42.202323	Gly 305 (NH...O)	3.218
				Trp 141 (NH...O)	2.721
	Pose 2	-6.523717	-43.057300	His 180 (NH...O)	2.977
	Pose 3	-5.451383	-34.974048	His 143 (NH...O)	2.938

The compound 5 was obtained with 3 poses and all the 3 poses showed highest score compared to the co-crystal ligand. The best pose showed the highest score of -6.762940 and strong interactions with the residues Gly 305 and Trp 141 having hydrogen bonds of length 3.218 Å and 2.721 Å respectively (Figure 60).

Figure 59

IF docking of co-crystal ligand (SHH) with Histone deacetylase (1T69)

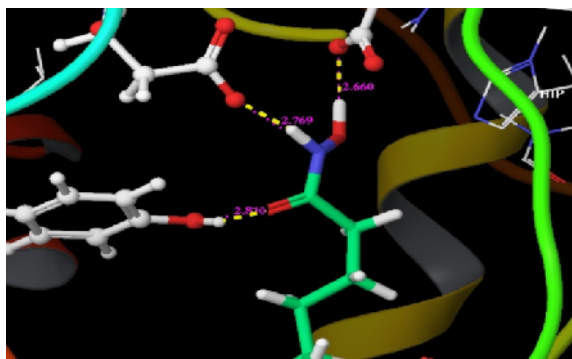
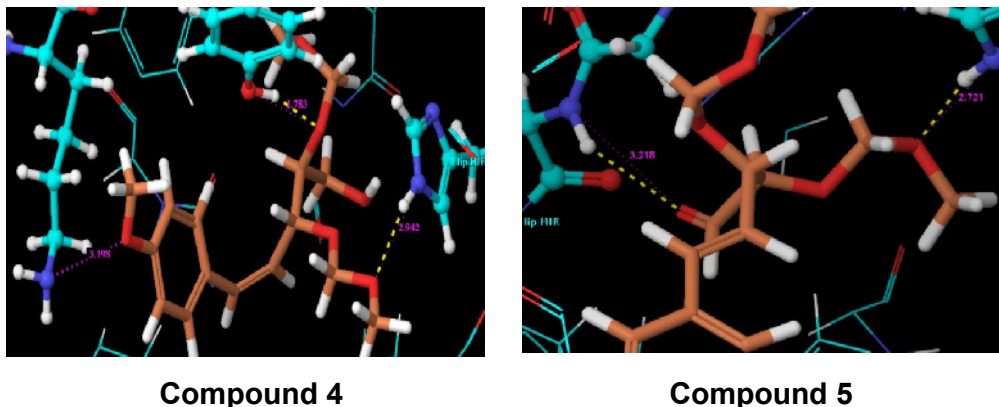


Figure 60

IF docking of compounds 4 and 5 with Histone deacetylase (1T69)



The Table 31 shows the induced fit docking results of the tumor target protein Tubulin (1Z2B) with highly scored compounds (4 and 5) of three poses each.

Table 31

Induced fit docking of tumor target protein Tubulin (1Z2B) with the bioactive compounds of MEFrB

Docking of Tumor target (1Z2B)	Pose number	Glide score	Glide energy (Kcal/Mol)	Hydrogen bond interactions (DH...A)	Bond distance (Å)
Co-crystal (VLB)	Pose 1	-3.686134	-32.534403	Lys 336(NH...O)	2.758
Compound 4	Pose 1	-5.245243	-34.762822	Asn 329 (NH...O)	2.935
				Lys 336 (NH...O)	2.771
				Lys 336 (NH...O)	2.983
				Lys 336 (NH...O)	2.982
				Gly 350 (OH...O)	2.814
	Pose 2	-4.770638	-35.405276	Asn 329 (NH...O)	3.404
Compound 5	Pose 1	-4.614675	-35.631538	Lys 336 (NH...O)	2.703
				Lys 336 (NH...O)	2.880
				Lys 336 (NH...O)	2.844
				Lys 336 (NH...O)	2.955
				Lys 336 (NH...O)	3.079
	Pose 3	-4.226431	-33.514821	Gly 350 (OH...O)	2.859
Compound 5	Pose 1	-4.567160	-32.091455	Lys 336 (NH...O)	2.885
				Lys 336 (NH...O)	2.780
				Asn 329 (NH...O)	3.226
				Lys 336 (NH...O)	3.064
				Lys 336 (NH...O)	2.848
	Pose 3	-4.386168	-34.784741	Thr 349 (OH...O)	2.922
				Lys 336 (NH...O)	3.034

The co-crystal (VLB) showed the score of -3.686134 and strong interactions with the residue Lys 336 having hydrogen bonds of length 2.758 Å. The compound 4 was obtained with 3 poses and all the 3 poses showed highest score compared to the co-crystal ligand. The best pose showed the highest score of -5.245243 and 1 interaction with the residue Asn 329, 3 interactions with same residue Lys 336 and 1 interaction with residue Gly 350 having hydrogen bonds of length 2.935 Å, 2.771 Å, 2.983 Å, 2.982 Å and 2.814 Å respectively (Figure 61).

The compound 5 was obtained with 3 poses and all the 3 poses showed highest score compared to the co-crystal ligand. The best pose showed the highest score of -4.614675 and 2 strong interactions with same residue Lys 336 and 1 interaction with the residue Asn 329 having hydrogen bonds of length 2.885 Å, 2.780 Å and 3.226 Å respectively (Figure 62).

Figure 61

IF docking of co-crystal ligand (VLB) with Tubulin (1Z2B)

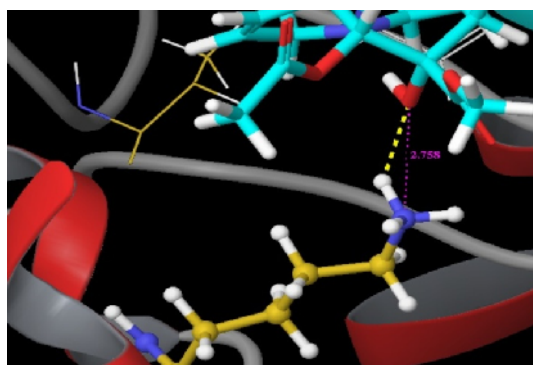
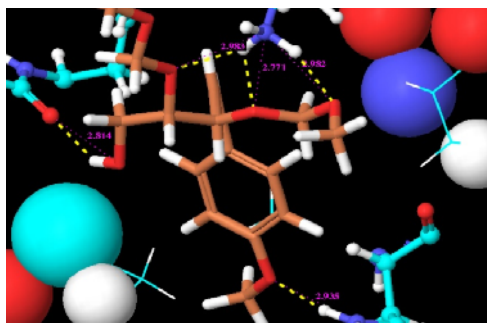
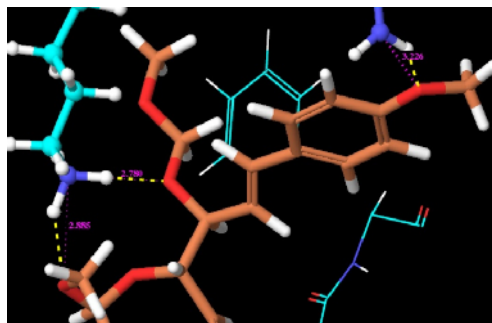


Figure 62

IF docking of compounds 4 and 5 with Tubulin (1Z2B)



Compound 4



Compound 5

The Table 32 shows the induced fit docking results for the microbial target - Deacetoxy C synthase (1W2N) with highly scored compounds (5) of three poses each. The co-crystal (PN1) showed the score of -4.196098 and 2 strong interactions with same residue Arg 74, 1 interaction with the residue Arg 266 and 1 interaction with the residue Glu 156 having hydrogen bonds of length 3.027 Å, 2.545 Å, 2.735 Å and 3.059 Å respectively.

Table 32

Induced fit docking of microbial target protein Deacetoxy C synthase (1W2N) with the bioactive compound of MEFrB

Docking of Microbial target (1W2N)	Pose Number	Glide score	Glide energy (Kcal/ Mol)	Hydrogen bond interactions (DH...A)	Bond distance (Å)
Co-crystal (PN1)	Pose 1	-4.196098	-30.839591	Arg 74 (NH...O)	3.027
				Arg 74 (NH...O)	2.545
				Arg 266 (NH...O)	2.735
				Glu156 (NH...O)	3.059
Compound 5	Pose 1	-4.715181	-31.482914	Arg 74 (NH...O)	3.005
				Arg 74 (NH...O)	3.186
				Arg 74 (NH...O)	3.229
				Arg 160(NH...O)	2.932
				Leu 186(NH...O)	2.980
	Pose 2	-4.489158	-38.163138	Arg 266(NH...O)	3.209
				Arg 266(NH...O)	3.034
				Arg 74(NH...O)	2.778
	Pose 3	-4.435128	-33.019959	Arg 74 (NH...O)	2.877
				Leu 186 (NH...O)	2.818

The compound 5 was obtained with 3 poses and all the 3 poses showed highest score compared to the co-crystal ligand. The best pose 1 showed the highest score of -4.715181 and 3 interactions with same residue Arg 74, 1 interaction with the residue Arg 160 and 1 interaction with the residue Leu 186 having hydrogen bonds of length 3.005 Å, 3.186 Å, 3.229 Å, 2.932 Å and 2.980 Å respectively (Figures 63 and 64).

Figure 63

**IF docking of co-crystal ligand (PN1) with
Deacetoxy C synthase (1W2N)**

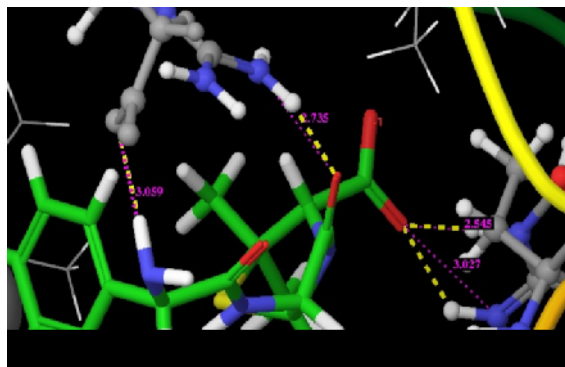
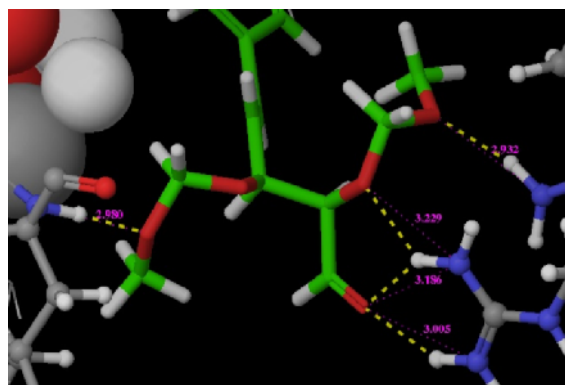


Figure 64

IF docking of compound 5 with Deacetoxy C synthase (1W2N)



The table 33 shows the induced fit docking results of microbial target - Pantothenate Kinase (1SQ5) with highly scored compound 5 in three poses. The co-crystal (PAU) showed the score of -5.387694 and 4 interactions with the residues Lys 1101, Lys 1101, Arg 1243 and Asp 1127 having hydrogen bonds of length 2.759 Å , 3.051 Å , 3.065 Å and 2.615 Å respectively.

The compound 5 was obtained with 3 poses and all the 3 poses showed highest score compared to the co-crystal ligand. The best pose 1 showed the highest score of -6.501589 and 4 interactions with residues Arg 1243, Arg 1243, Tyr 1240 and Tyr 1175 having hydrogen bonds of length 2.828 Å , 2.740 Å , 2.810 Å and 2.790 Å respectively (Figures 65 and 66).

Table 33

Induced fit docking of microbial target protein Pantothenate Kinase (1SQ5) with the bioactive compound of MEFrB

Docking of Microbial target (1SQ5)	Pose Number	Glide score	Glide energy (Kcal/ Mol)	Hydrogen bond interactions (DH...A)	Bond distance (Å)
Co-crystal (PAU)	Pose 1	-5.387694	-43.643297	Lys1101 (NH...O)	2.759
				Lys1101 (NH...O)	3.051
				Arg1243 (NH...O)	3.065
				Asp1127 (OH...O)	2.615
Compound 5	Pose 1	-6.501589	-47.253442	Arg1243 (NH...O)	2.828
				Arg1243 (NH...O)	2.740
				Tyr 1240 (OH...O)	2.810
				Tyr 1175 (OH...O)	2.790
	Pose 2	-5.901405	-45.539488	Tyr 1180 (OH...O)	2.846
				Arg1243 (NH...O)	3.124
				Arg1243 (NH...O)	2.922
				Tyr 1258 (OH...O)	3.186
	Pose 3	-5.394163	-44.709873	Arg 1243 (NH...O)	3.210
				Lys 1101 (NH...O)	2.988

Figure 65

IF docking of co-crystal ligand (PAU) with Pantothenate Kinase (1SQ5)

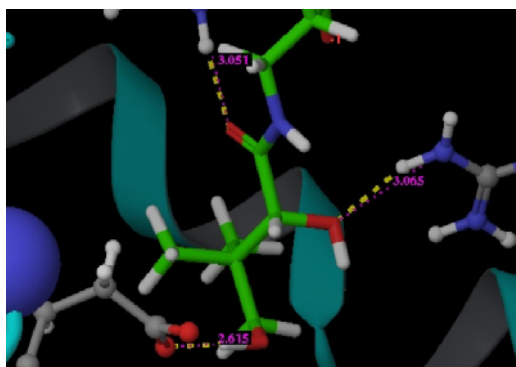
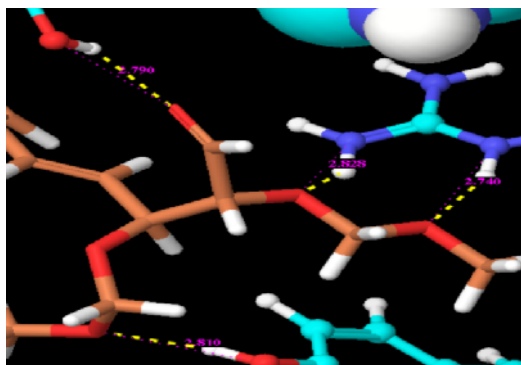


Figure 66

IF docking of compound 5 with Pantothenate Kinase (1SQ5)



4.5.3. Prediction of ADME properties

Nearly 40% of drug candidates fail in clinical trials due to poor ADME properties. These late-stage failures contribute significantly to the skyrocketing cost of new drug development. The ability to detect problematic candidates early will dramatically reduce the amount of wasted time and resources and streamline the overall development process (Modi, 2003).

According to Lipinski *et al.* (1997) “Lipinski’s rule of five” is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity that would make it a likely orally active drug in humans. The rule describes molecular properties important for a drug’s pharmacokinetics in the human body including their Absorption, Distribution, Metabolism and Excretion (ADME). Over the past decade, ADME property evaluation has become one of the most important issues in the process of drug discovery and development. Since *in vivo* and *in vitro* evaluations are costly and laborious, *in silico* techniques had been widely used to estimate ADME properties of chemical compounds. It is gaining acceptance as a useful assessment tool for early identification of likely drug candidate failures (Shen *et al.*, 2010). *In silico* prediction of ADME properties for the two selected plant constituents were performed using the program QikProp and the results are presented in the Table 34.

Here the set of linear descriptors are applied on the highly scored best bioactive plant compounds and tested for Lipinski’s rule of five to evaluate drug likeness. The ADME property prediction on these highly scored compounds (4 and 5) showed that, they do not violate any of the rules for drugs solubility and permeability estimation. The tumorigenic target histone deacetylase (1T69) and tubulin (1Z2B) is a crucial factor in carcinogenesis, as well as a promising target for the development of anticancer drugs and therapies. The rise of multidrug resistance of many human pathogens necessitates the development of new

therapeutic agents against microbial target Deacetoxy C synthase (1W2N) and pantothenate kinase (1SQ5).

Table 34
ADME properties of the bioactive compounds 4 and 5

List of properties	Compound 4	Compound 5
	(Z)-(2S,3S)-2,3 bis [(methoxy -methyl)oxy] -5-(4-methoxyphenyl)pent-4-enol	(E)-(2S,3S)-2,3-bis [(methoxymethyl)oxy]-5-(4-methoxyphenyl) pent-4-enal
Molecular formula	C ₁₆ H ₂₄ O ₆	C ₁₆ H ₂₂ O ₆
Molecular weight (130 to 725)	312	310
Donor HB (0.0 to 6.0)	1.000	0.000
Acceptor HB (2.0 to 20.0)	9.250	9.550
Number of rotatable bonds (0 to 15)	12	11
QPlog _s (-6.5 to 0.5)	-1.236	-0.012
Qplog Po/w (-2.0 to 6.5)	1.704	1.050
QplogBB (-3.0 to 1.2)	-0.514	-0.554
%Human oral absorption (>80%-High <25%-Poor)	100(High)	96(High)
Rule of five	0	0

From the results of ADME properties of the compound 4 [(Z)-(2S,3S)-2,3 bis [(methoxy -methyl)oxy] -5-(4-methoxyphenyl)pent-4-enol] and compound 5 [(E)-(2S,3S)-2,3-bis [(methoxymethyl)oxy]-5-(4-methoxyphenyl) pent-4-enal] may be used as an antitumor and antimicrobial agent to treat the diseases like cancer and microbial infection.

The HDAC inhibitors as therapeutic agents to modulate a wide variety of cellular functions, transcriptional activity in cells, cell cycling, angiogenesis, apoptosis and differentiation which are key components of tumor proliferation. HDAC inhibitors may improve the efficacy of existing cancer therapies (Osada

et al., 2001). Histone deacetylase expression in prostate cancer is significantly higher than in benign prostate cell lines and tissues (An, 2007). A novel approach for assessing prostate cancer initiation and disease progression suggest a molecular targets tubulin for therapeutic intervention in prostate cancer and perhaps other forms of cancer by catalyzing the diverse tubulin post translational modifications (McKean *et al.*, 2001).

Deacetoxycephalosporin C synthase (DAOCS) and Pantothenate kinase from *Streptomyces clavuligerus* was first recognized to possess broad substrate specificity and became the major target for research and engineering to develop a drug that prevent the microbial infection (Oster *et al.*, 2004). Saha *et al.* (2010b) reported the molecular docking studies of some novel hydroxamic acid derivatives with human histone deacetylase. Villanueva and Setzer (2010) reported the molecular docking analysis of cembrenoids with tubulin in order to assess the potential of tubulin binding of the cytotoxic agents.

In most bacteria, CoA is synthesized from pantothenic acid (Vitamin B5) in series of 5 steps and phosphorylation of pantothenate by pantothenate kinase is the first reaction. This pathway also exists in eukaryotes. However, in most cases there is no sequence homology between the prokaryotic and eukaryotic CoA biosynthetic enzymes (Liu *et al.*, 2006a). Moreover, these enzymes are essentially required for bacterial survival and/or virulence. Therefore, there is a potential for having highly specific inhibitors of bacterial CoA enzymes. Development of these inhibitors targeting these enzymes is being actively pursued. Among, these, the (N- substituted pantothenamides) have shown the greatest promise as growth inhibitors of both *E.coli* and *S.aureus* (Leonardi *et al.*, 2005a and Virga *et al.*, 2006).

The basic structure of both penicillins and cephalosporins consist of a four member beta-lactam ring. In penicillins this ring is condensed with a five-member sulphur ring (the thiazolidine ring) and in cephalosporins with a six-member ring (the dihydrothiazine ring). Enzymatic conversion by expandase, the deacetoxy cephalosporin C synthetase, converts the five member thiazolidine ring of

penicillins into a larger six member ring, the dihydrothiazine ring (Karchmer *et al.*, 2000).

In Phase V the compounds identified in GC- MS analysis were subjected to SP docking against the tumor target proteins histone deacetylase and tubulin with co-crystal interaction of SHH and VLB and microbial target protein deacetylase C synthase and pantothenate kinase with co-crystal interaction of PN1 and PAU respectively. Out of 24 compounds only seven compounds were found to be successful in SP and XP docking. Induced fit docking showed high score for two compounds namely compound 4 [(Z)-(2S, 3S)-2, 3 bis [(methoxy -methyl) oxy] -5-(4-methoxyphenyl) pent-4-enol] and compound 5 [(E)-(2S, 3S)-2, 3-bis [(methoxymethyl) oxy]-5-(4-methoxyphenyl) pent-4-enal] respectively. ADME properties of these two compounds in QikProp program showed drugable property, solubility and permeability and also obeyed the Lipinski's rule.

The GLIDE docking analysis of 24 bioactive compounds from the medicinal plant - *Ficus racemosa* showed good interaction with all the four targets. The binding sites of the compounds were found to be in close proximity to the binding site of its co-crystal ligands. It was interesting to observe that even though the core structure of all the compounds were same, the degree of interaction and binding site were found to be different. The compounds 4 and 5 identified from methanolic extract of *Ficus racemosa* bark showed highest glide score, glide energy and interactions compared to the other compounds and confirmed their antitumorigenic and antimicrobial role.