5. Discussion
5. DISCUSSION

The detection of biologically active metabolites in a material is one of the strategic approaches in the search for potentially new drugs and plants have been useful in this kind of approach. Evidence for the presence of bioactive metabolites in plant comes from the folklore uses of such plants. Quinine and aspirin for example were discovered when the folkloric use of the plants Cinchona succirubra and Salix purpurea were investigated (Hedner and Everts, 1998). Other sign of useful metabolites come from observations by scientists in the field. It was this method that led to the discovery of the principal antibiotics, ‘penicillin’ by Alexander Fleming. In his laboratory, he observed that a contaminating colony of microorganism prevented the growth of bacteria form the region where it was growing (Harbarth et al., 2003).

Bioactive metabolites can also be identified in plants by searching for a particular type of compound, for example Castanospermine was discovered when the search for alkaloids with glycosidase-inhibiting activity was initiated (Waterman, 1990). Serendipitous discoveries have also been reported (Waterman, 1990); the Madagascaran periwinkle (Catharanthus roseus) from which very potent anticancer agents namely ‘vincristine’ and ‘vinblastine’ were developed was indigenously employed for the management of diabetes mellitus (Murata, 2003). Recently, Curcumin is one of the most studied chemopreventive agents. It is a natural compound extracted from the rhizomes of Curcuma longa L. that allows suppression, retardation or inversion of carcinogenesis. Curcumin has also been shown to possess anti-tumour activity in a variety of in vitro tumour models (cell lines from solid tumours and leukaemia) as well as in tumour animal models. Its particular toxicological profile (doses upto 8000 mg/day are still safe) has
Discussion

allowed the development of a large number of phase II studies (Duvoix et al., 2004; Johnson et al., 2007). As chemopreventive agent, curcumin is currently in phase II studies in colorectal cancer patients (Johnson et al., 2007). Another candidate chemopreventive agent is resveratrol, a polyphenol found in numerous plant species, including mulberries, peanuts and grapes. Its potential as chemopreventive and chemotherapeutic activities have been demonstrated in all three stages of carcinogenesis (initiation, promotion, and progression), in both chemically and UVB-induced skin carcinogenesis in mice, as well as in various murine models of human cancers. Evidence from numerous in vitro and in vivo studies have confirmed its ability to modulate various targets and signalling pathways (Athar et al., 2007). As a chemopreventive agent, resveratrol is currently in phase I studies in colorectal cancer patients and in healthy subjects at high risk of developing melanoma.

There has been extensive work done on biological activities of various Clerodendrum species such as C. trichotomum, C. bungei, C. chinense, C. colebrookianum, C. inerme, C. phlomidis, C. petasites, C. grayi, C. indicum, C. serratum, C. campbellii, C. calamitosum and C. cyrtophyllum that can be used both in conventional therapy or as replacement therapies for the treatment of various diseases (C.f. Shrivastava and Patel, 2007). However, there are no reports available on C. infortunatum even though this plant has been reported to possess beneficial properties against many ailments in traditional medicine. Therefore, the present study is based on the folkloric uses of the plant C. infortunatum that has been undertaken to screen the pharmacological activities, where the phytochemical analysis served as the preliminary step.

5.1. Phytochemical investigations

5.1.1. Soxhlet extraction and phytochemical analysis

In order to understand the factors responsible for the potent pharmacological activities phytochemical analysis was carried out for which crude extracts were isolated through Soxhlet extraction. This method is convenient and widely used for extraction from plant samples because of its continuity in processing, less time and solvent-
consumption than maceration and percolation. The powdered plant was placed in a Soxhlet apparatus, which is on top of a collecting flask beneath a reflux condenser. A suitable solvent was added to the flask and the setup was heated under reflux. The steam of the solvent, which when contacts with the material will dissolve metabolites and brings back to the flask. Extractions can be either ‘selective’ or ‘total’, the initial choice of the most appropriate solvent is based on its selectivity for the substances to be extracted. The plant material was extracted using a solvent of an appropriate polarity following the principles of ‘like dissolves like’ thus non-polar solvents are used to solubilize mostly lipophilic compounds (e.g., alkanes, fatty acids, pigments, waxes, sterols, few terpenoids, alkaloids and coumarins). Medium polarity solvents were used to extract compounds of intermediate polarity (e.g., some alkaloids flavonoids) while more polar solvents were used for more polar compounds (e.g., flavonoid, glucosides, quaternary alkaloids, tannins etc). A selective extraction can also be performed sequentially with solvents of increasing polarity. This has the advantage of allowing preliminary separation of the metabolites present in the material within distinct extracts and simplifies further isolation. By taking this into consideration in this study three different solvents have been used with increasing polarity i.e., petroleum ether, chloroform and ethanol solvents for successive extraction to get the crude extracts of different solvents. After extraction all the extracts were concentrated in vacuum using rotary flash evaporator (Buchi-Flawil, Switzerland). The solvents were removed completely over the water bath and finally desiccator dried. The yields of extracts obtained from C. infortunatum leaf and roots using various solvents are shown in Tables 8 & 8.1. It is clear from these observations that the extraction with ethanol yields the highest amounts of extracts followed by petroleum ether and chloroform. By taking these crude extracts of three different solvents the qualitative chemical analysis was performed. From this phytochemical analysis, presence of glycosides, terpenoids, steroids, carbohydrates, flavonoids and saponins was observed in petroleum ether, chloroform and ethanol extracts of leaf and roots (Tables 9 & 9.1). It is also observed that the terpenoids and steroids content are found in petroleum ether extract of leaf and roots samples. Interestingly, flavonoids and saponins are present only in the ethanol extract. This preliminary phytochemical investigation is in good agreement with the principle of ‘like dissolves like’. Plants are very well known to possess such active
constituents, to mention a few *Ageratum conyzoides* (Asteraceae) possess 0.7-2% essential oil constituents viz., ageratochromene, dimethoxy ageratochromene, cardimine and caryophyllene, alkaloids, saponins, monoterpenes and sesquiterpenes. Similarly, *Boerhaavia diffusa* L. (Nyctaginaceae) contains alkaloids, sisterol, ursolic acid, 5,7-dihydroxy,3,4-dimethoxy, 6, 8-dimethyl flavone, glucose, fructose, sucrose and hypoxanthine-9-1-arabinoside molding hormone, acysone and *Cynodon dactylon* L. (Poaceae) shows sterol, tannin and flavonoids (Siddiqui *et al.*, 2009). Species belonging to the genus *Clerodendrum* (Verbenaceae) are rich source of variety of compounds, e.g., diterpenes (Tian, 1993; Achari, 1992; Linn, 1989), steroids (Akhisha, 1990 & 1988), iridoid monoterpenses (Calis, 1994), triterpenes (Ding, 1999; Ganapathy, 1985) and flavones (Tian, 1999; Gunasgarm, 1993; Barua, 1989). Thus, the preliminary phytochemical analysis of plant *C. infortunatum* also indicated towards a variety of active constituents which suggest the presence of beneficial values.

### 5.1.2. Total phenolic assay

Polyphenols are the major plant compounds by virtue of their antioxidant activity by chelating redox-active metal ions, inactivating lipid free radical chains and preventing hydro peroxide conversion into reactive oxyradicals. Therefore, the total phenolic content was estimated in the crude extracts of *C. infortunatum* by using Folin–Ciocalteu reagent that produced blue colour by reducing yellow heteropolyphosphomolybdate-tungstate anions (Huang *et al.*, 2005). The formation of intense blue coloured complex clearly suggests the presence of large number of hydrogen donating groups in the phenolic compounds. This clearly indicates the presence of polyphenols in the extracts of leaves and roots of the selected plant. Table-10 summarizes the contents of total phenolics, expressed as gallic acid equivalents (GAE). The ethanol extracts of both leaf and root material possesses a maximum quantity of phenolic compounds i.e., 440.6 and 460 mg/gm respectively followed by chloroform extracts. The petroleum ether extract is found to be poor in phenolic content. The results of these experiments strongly suggest that the phenolics are important components of leaf and roots of *C. infortunatum* indicating some of the pharmacological effects could be attributed to the presence of these valuable constituents.
Scientific investigations on some folk remedies leads to bioactive compounds and many of these compounds have been developed into drugs. Examples of these folk remedies are as follows: willow \((Salix purpurea)\), the plant from which the famous analgesic, aspirin was first obtained (Hedner and Everts, 1998), quinine (an antimalarial) from \(Cinchona succirubra\), artemisinin (qinghaosu) which is the antimalarial from a Chinese medicinal herb \((Artemisia 24 annua)\) (Klayman, 1985), Forskolin, which is the antihypertensive agent from \(Coleus forskohlii\) Briq, (Kinghorn, 1987) and the ginkgo tree \((Ginkgo biloba)\), mentioned in Chinese medicinal books used in antiasthmatic and antitussive preparations (Hamburger \textit{et al}, 1991). Folklore medicine seems to have a substantial stock of valuable lead compounds which when scientifically investigated can provide very useful drugs especially in the management of infectious conditions.

The crude extracts from both the leaf and root samples provided the information related to the phytochemical contents. Subsequently, they were used for the pharmacological evaluation by employing various standard parameters. Based on the information obtained from such studies the crude extracts were further subjected for micro analysis which involved the isolation and characterization of the compounds. This fine chemical analysis was carried out by employing the routine IR, Mass s, \(^1\)HNMR, and \(^{13}\)CNMR spectroscopic techniques.

5.2. Characterization of isolated active constituents

5.2.1 Characterization of LP1

Compound LP1 was obtained as white powder from leaf petroleum ether extract and its molecular ion peak appeared at \(m/z\) 412 in El-MS spectrum. The \(^1\)H-NMR spectrum showed signals for two tertiary methyl groups at \(\delta\) 0.69 (3H, s) and 1.02 (3H, s), an allylic methyl group at \(\delta\) 1.58 (3H, s), a secondary methyl group at \(\delta\) 0.92 (3H, d, J= 6.5 Hz), a primary methyl group at \(\delta\) 0.82 (3H, t, J = 7.4 Hz), an oxygenated proton at \(\delta\) 3.53 (1 H, m), terminal methylene protons at \(\delta\) 4.66 (1H, br.d, J=2.3 Hz) and 4.74 (1H, br.dd, J=2.3, 1.4 Hz), and an olefinic proton at \(\delta\) 5.36 (1H, br.d, J = 5.2 Hz). The NMR data of LP1 are in good agreement with the previous data of a chemical compound identified as 'Clerosterol' (Gaspar \textit{et al}., 1996) and hence the compound LP1 is
considered as 'Clerosterol'. Studies on the phytochemical isolation and characterization in several other plant species also suggest the presence of Clerosterol, it has been isolated from various plants from *Teucrium abutilosides, Teucrium betunicum* (Gasper et al., 1996). Research reports on the genus *Clerodendrum* denote that the clerosterol is major class of chemical constituents present in the *Clerodendrum bungei* (Dong et al., 1999; He et al., 1982). *Clerodendrum colebrookianum* (Yang et al., 2000; Goswami et al., 1996). *Clerodendrum cyrtophyllum* (Tian et al., 1993) *Clerodendrum fragrans* (Akihisa et al., 1998).

5.2.2. Characterization of LE1

Compound LE1 was obtained as yellow amorphous from leaf ethanol extract and the IR spectra (cm⁻¹) studies of this compound showed the peak at 3440 for OH, the peak at 1670 indicates aromatic nature of the compound. ¹H NMR δ (ppm) showed the signals at δ 6.92 (1H, d, J=8.5 Hz, H5'), 6.43 (1H, d, J=1.9, H8), 6.22 (1H, d, J=1.9 Hz, H6) and 7.67 (1H, dd, J=2.1 and 8.5 Hz, H6') for five protons namely 6,8,3,12,1 and 6 respectively, the singlet at δ 11.3 is due to OH groups and the molecular ion peak appeared at m/z 303 in El-MS spectrum. The compound LE1 is characterized as 'Quercetin'.

Quercetin is a plant derived flavonoid present in a number of plant species *Carrichtera annua* (Khaled et al., 1999). *Tachigalia paniculata* (Giuseppina et al., 2002). *Adina racemosa* (Atsuko et al., 2003). *Triplaris cumingiana* (Ahmed et al., 2005) *Hypericum hircinum* (Franco Chimenti et al., 2006). *Eucalyptus maiden* (Li-Wen Tian, et al., 2009). Earlier literature clearly reveals that the flavonoids are the major class of compounds which are mainly present in *Clerodendrum* speices. Some of the major flavonoids present in this genus are cynaroside, 5-hydroxy-4'-7-dimethoxy methyl flavone, kaempferol, salvigenin, 4-methyl scutellarein, 5,7,4 O-trihydroxyflavone, apigenin, luteolin, acacetin- 7-O-glucuronide, hispidulin, 2'4-4'trihydroxy-6'methyl chalcone, 7-hydroxy flavone, luteolin, naringin-4'-O-α-glucopyranoside, pectolinarigenin, cirsimaritin, cirsimaritin-4'- glucoside, quercetin-3-methyl ether which were isolated from *C. inerme, C. phlomidis, C. petasites, C. trichotomum, C. mandarinorum, and C. infortunatum* (C.f. Shrivastava and Patel, 2007).
5.2.3. Characterization of RPl

The uncharacterized compound RPl was obtained as white powder from root petroleum ether extract and $^1$HNMR spectra showed the typical pattern of pentacyclic triterpene (Mahato and Kundu, 1994). Especially, the $^1$HNMR spectrum was characteristic of the presence of an allylic methyl group at $\delta$ 1.71 (3H, s) and terminal methylene protons at $\delta$ 4.63 (1H, br.s) and 4.75 (1H, br.s). The molecular formula, C$_{30}$H$_{48}$O$_3$ was determined through peak matching of the molecular ion at $m/z$ 456. On the basis of the above evidences, the structure of RPl is considered to be 'betulinic acid'. The NMR and physical properties are in good agreement with the previous data of the compound reported by Mahato and Kundu (1994) which is also reported as 'betulinic acid'. Studies on the phytochemical isolation and characterization in several other plant species also suggest the presence of this compound. Betulinic acid is a triterpene of natural origin isolated from various plants. It has been isolated from *Quisqualis fructus* (Kwon et al., 2003), *Coussarea paniculata* (Prakash et al., 2003), *Caesalpinia paraguariensis* (Woldemichael et al., 2003), *Vitex negundo* (Chandramu et al., 2003), twigs of *Ilex macropoda* (Kim et al., 2002), *Anemone raddeana* (Yamashita et al., 2002), leaves and wood of *Doliocarpus schottianus*, (De Oliveira et al., 2002), *Tovomita krukovii* (Zhang et al., 2002), , *Chaenomeles lagenaria* (Guo et al., 1998), *Berlinia grandiflora* (Enwerem et al., 2001), *Orthosiphon stamineus* (Tezuka et al., 2000).

5.2.4. Characterization of RE1

The IR spectrum of the compound from the root ethanol extract showed a broad absorption band at 3411 cm$^{-1}$ to show the presence of a hydroxyl group. The band at 1637 cm$^{-1}$ indicates the presence of C=C group. The absorption bands at 1431 and 1340 cm$^{-1}$ were to C-H group and the band at 1157 is due to C-O bond. In The $^1$H-NMR Spectrum the signal at $\delta$ 13.00 is due to the presence of aromatic NH proton. The two signals at The IR spectrum showed a broad absorption band at 3440 cm$^{-1}$ to show the presence of a hydroxyl group. The band at 1644 cm$^{-1}$ indicates the presence of C=C group. The absorption bands at 1457 and 1375 cm$^{-1}$ were to C-H group and the band at 1057 is due to C-O bond 7.00 and 6.80 each for one proton shows the presence of two
aromatic protons. The signal at δ 5.00 for one proton indicates a proton at unsaturated carbon atom. The singlet signal at δ 2.50 for three protons suggests the presence of methyl group attached to nitrogen atom. The signal at δ 1.40 and 1.50 each integrating for 6 protons suggests the presence of two isopropyl groups. The signal at 1.65 for six protons indicates that two more methyl groups are attached to an unsaturated carbon atom. The mass spectrum shows a peak at m/z 245 indicating a molecular weight of 245 and a molecular formula of C_{17}H_{27}N. The above data confirms that the structure of the test compound RE1 is “[2,6-Di isopropyl-4-(2-methyl-propenyl)-phenyl]–methyl amine”, this compound appears to novel molecule. Hence, for the sake of convenience we referred it as “cleroamine”.

5.2.5. Characterization of RE2

Mass spectrum of the second compound (RE2) obtained from the root ethanol extract shows molecular ion peak at m/z 469 [M+] (38%). The other peaks appeared at 457.6 (44%), 439.8 (98.7%), 430.5 (52%), 418.2 (28.4%), 382.4 (32%), 372.6 (25%), 318.3 (44.6%), 293.2 (17.6%), 275.5 (24.2%) and 240.3 (45.4%).

The IR spectrum of the compound RE4 showed absorptions bands at 3448 cm⁻¹ and 2943 cm⁻¹ and 1453 cm⁻¹ and 883.34 cm⁻¹ for hydroxyl group and terminal methylene groups respectively. An absorption band at 1644 cm⁻¹ reveals the presence of olefinic C=C group. A sharp Kbr band at 1685.67 cm⁻¹ indicates the presence of carbonyl stretching (C=O).

The ¹H NMR spectrum revealed the presence of terminal methyl groups at δ 0.79 to 1.09, a multiplet at δ 4.56 due to olefinic protons. The multiplet signal at δ 3.00 for one proton suggests the presence of a methylene group in between a carbonyl group and an olefinic group. The rest of the proton signals closely resemble the signals of a cyclopentano phenanthrene nucleus.

The direct insertion probe mass spectrum showed the molecular ion peak at m/z 468.77 corresponding to the molecular formula C_{32}H_{52}O_{2}. Hence the above spectral data of the compound RE2 pin points to the structure of a compound called ‘lupeol acetate’.
Lupeol acetate is found in deertongue leaf (Appleton and Enzell, 1971). *Erythroxylum leaf costae* Chavez et al., 1996, stem-bark of *Artocarpus chaplasha* (Mahato et al., 1971) and *Ficus hispida* (Wang et al., 1975) *P. reticulatus* (Jamal et al., 2008).

From this analysis five active compounds were isolated from both the leaf and root samples, namely ‘Clerosterol’ and ‘Quercetin’ were isolated from the leaf petroleum ether and ethanol extract respectively and three compounds viz., ‘betulinic acid’ ‘cleroamine’ and ‘lupeol acetate’ were obtained from the root petroleum ether and ethyl acetate fractions of the plant *C. infortunatum* respectively. These compounds were confirmed after the spectral studies and structural elucidation.

The characterization study suggests that among the isolated constituents ‘clerosterol’ and ‘quercetin’ from leaf petroleum ether and ethanol extracts belongs to the steroid and flavonoid groups respectively, whereas the other three compounds such as ‘betulinic acid’ ‘cleroamine’ and ‘lupeol acetate’ belongs to triterpenoid group. This study also strengthens the preliminary phytochemical analysis which showed the presence of steroid, flavonoid and triterpenoid groups in the crude extracts of *C. infortunatum*. The most interesting part of this fine analysis is that the compounds ‘betulinic acid’, lupeol acetate and cleroamine appears to be the first report from this plant, as there are no other earlier scientific reports claiming the isolation and characterization of these compounds from the plant under study.

In this study, only five compounds from leaf and root parts of *C. infortunatum* have been isolated and characterized. However, it is presumed that still there is a great scope for further micro analysis as the plant extracts are reservoir of several unknown compounds.

5.3. *In vitro* antioxidant activity

Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects, thus, shifting the attention towards the naturally occurring antioxidants. Numerous plant constituents have proven to possess free radical scavenging or antioxidant activity (Aruoma and Cuppett, 1997). Flavonoids and other phenolic
compounds (hydroxyl cinnamic derivatives, catechines etc) of plant origin have been reported as scavengers and inhibitors of lipid peroxidation (Formica and Regelson, 1995). The medicinal actions of phenolics are mostly credited to their antioxidant capacity, free radical scavenging ability, chelation of redox active metal ions, modulation of gene expression and interaction with the cell signaling pathways. Currently there has been an increased interest globally to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine and the food industry. As plants produce significant amounts of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity. Traditional herbal medicines form an important part of the healthcare system of India. Ayurveda, supposed to be the oldest medical system in the world, provides potential leads to find active and therapeutically useful compounds from plants. Considering the growing interest in assessing the antioxidant capacity of herbal medicine investigations were undertaken to explore the natural antioxidants from petroleum ether, chloroform and ethanol extracts of C. infortunatum leaves and roots by employing free radical scavenging assays and pharmacological methods.

The free radical scavenging activity of C. infortunatum was studied by its ability to bleach the stable radical DPPH. This activity is exhibited by all the three extracts of leaf and root. However, the ethanol extract of leaf and root proved to be strongest exhibiting 92.64 and 94.24% activity at the concentration of 250 µg/mL respectively. This activity is also equivalent in offering protection compared to the standard BHT (Table 11). In case of isolated constituents Table 14, Quercetin showed significant activity 92.42% at the concentration of 150 µg/mL, whilst clerosterol, Betulinic acid and Lupeol acetate showed 12.6, 8.6 and 22.45% respectively. The bleaching of DPPH represents the capacity of extracts to scavenge free radicals independent of enzymatic activity. The present findings have certainly indicated that the extracts have the proton donating ability and the presence of compound Quercetin and cleramine could serve as free radical inhibitors or scavengers acting possibly as primary antioxidants. Most of the authors in their investigations have used quercetin as reference standard for free radical scavenging activity and this confirms the strong antioxidant activity of the plant under
study (Khanduja and Anjana, 2003; Slusarczyk et al., 2009). Therefore, further studies to evaluate quercetin as an antioxidant in *in vivo* system has not been carried out with a presumption that this compound has been already established its potential as an antioxidant and also being used as a standard in the evaluation of antioxidant properties of other unknown compounds.

The hydroxyl radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to mutagenesis, carcinogenesis and cytotoxicity. Hydroxyl radical scavenging activity was estimated by the hydroxyl radical generated by the Fenton reaction in which deoxyribose was used as detector molecule to detect the damage by OH· radicals in the presence or absence of EDTA (Gutteridge, 1987). It is observed that the ethanol extract possess strong abilities in the presence of EDTA, as it is capable to scavenge OH· present in the free solution and thus protecting the degradation of deoxyribose (detector molecule) to thiobarbituric acid reactive material. Experiments carried out to examine the ability of the leaf extract to act as OH· radical scavenging agent by employing the method of Halliwell *et al.* (1987) have prominently indicated the removal of hydroxyl radicals during the reaction. It is believed that the plant extract could prevent damage by removing hydroxyl radicals and thus preventing the degradation of 2-deoxy-2 ribose sugar. Table 11.1 shows 68.58 and 72.65% OH· radical scavenging activity at the concentration of 250µg/mL respectively by ethanol extract of leaf and root.

Superoxide anion is primary radical in most of the biological systems; the radical itself is quite passive compared to other radicals. However, the biological system converts it into more reactive species e.g. OH· radicals (Winterbourn and Kettle, 2003). Ethanol extract of leaf and roots showed 62.06 and 68.57% superoxide anion scavenging activity at a concentration of 250µg/mL (Table 11). The superoxide anion scavenging activity was determined by phenozine methosulphate /NADH-NBT system, wherein O$_2$· derived from dissolved oxygen phenozine methosulphate/NADH coupling reaction reduces NBT. When the plant extract was incubated with above reaction mixture, there was a decrease in the absorbance at 560 nm indicating the consumption of superoxide anion in the reaction mixture. This decrease in absorbance level might be due to the presence of phenolics in the *C. infortunatum* ethanol extract.
The NO radicals play an important role in inducing inflammatory response and their toxicity multiplies only when they react with $O_2^\cdot$ radicals to form peroxynitrite, that damages biomolecules such as, proteins, lipids and nucleic acids (Gulcin et al., 2002). NO radicals were scavenged at the concentration of 250$\mu$g/mL with percentage inhibition of 56.33 and 62.57% respectively by the ethanol extract of both leaf and roots (Table 11.1). Nitric oxide is generated when sodium nitroprusside reacts with oxygen to form nitrite. Incubation with leaf ethanol extract inhibits nitrite formation by competing with oxygen to react with nitric oxide directly. Due to this ability there was a decrease in pink colored chromophore measured at 540 nm against the corresponding blank solutions. In the present results the ethanol extract was active and it might be possessing very potent and novel therapeutic agents for scavenging of NO. These unknown agents may also exert their effects on the regulation of pathological conditions caused by excessive generation of NO and its oxidation product - peroxynitrite.

The reducing power increased with increasing the amount of extract. The reducing capacity of compound may serve as a significant indicator of its potential antioxidant activity (Meir et al., 1995). The absorbance values of the extract at different concentrations were found to be less than that of the reference compound BHT. The value of reference compound is in accordance with the report of Gulcin et al., (2002). The phenolic compounds may contribute directly to anti oxidative action as suggested by Duh et al., (1999).

Such analysis was not been shown in the plant C. infortunatum by earlier workers. Nevertheless, similar investigations have also been reported by several workers in various plants showing the presence of potent antioxidant activity for ex., Cytisus scoparius Link -A natural antioxidant (Raja Sundararajan et al., 2006), Halleria lucida (Adedapo et al., 2008); Chukrasia tabularis (Kaur et al., 2008); Artocarpus communis and Artocarpus elasticus (Kai-Wei Lin et al., 2009); Lycopus lucidus Turcz (Sylwester et al., 2009); Turbinaria ornata (Brown Alga) (Ananthi et al., 2009).

5.4. Hepatoprotective activity and in vivo antioxidant activity

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as
metabolism, secretion and storage. It has great capacity to detoxify toxic substances and synthesize useful principles. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences (Shahani, 1999; Subramoniam and Pushpangadan, 1999). Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages (Recknagel, 1983; Wendel et al., 1987; Dianzani et al., 1991). In spite of tremendous advances in modern medicine, there are no effective drugs available that can stimulate liver function, offer protection to the liver from damage or help to regenerate hepatic cells (Chattopadhyay, 2003). In the absence of reliable liver-protective drugs in modern medicine, there are a number of medicinal preparations in ayurveda recommended for the treatment of liver disorders and their usage is in vogue since centuries and are quite often claimed to offer significant relief (Chatterjee, 2000).

The CCl₄ induced hepatotoxicity assay is trusted in vivo model of oxidative stress. The metabolites of CCl₄ viz. Trichloromethyl radical (CCl₃) and the trichloromethyl peroxy radical (CCl₃O₂), are involved in the liver pathogenesis (Singh et al., 1999). CCl₄ damage to liver raises the serum level of enzymes such as ALT, AST, ALP and LDH by releasing them into the blood stream (Asha, 2001). The present experimental results shows that the rats treated with CCl₄ have shown elevated levels of all these enzymes significantly \( P < 0.001 \), indicating a severe hepatic cell necrosis. Massive generation of free radicals in the CCl₄ liver induced damage provokes a sharp depletion of hepatic glutathione and elevation of lipid peroxidation in liver (Brien et al., 2000). The major effect of free radical on the mean liver detoxificant enzymes (such as, catalase, superoxide dismutase and peroxidase) has shown reduction in the enzyme activity due to enzyme inactivation during the catalytic cycle. However, results shown in Tables 12, 12.1, 13 and 13.1. From this study indicate the hepatoprotective effect against CCl₄-induced liver damage in a dose-dependent manner by normalizing the elevated levels of the hepatic enzymes when treated with the ethanol extract of leaf and root of C. infortunatum. Histopathological observations (Figs.15.2 and 16.2) also support the hepatoprotective potential of leaf and root ethanol extracts, which could be attributed to the presence of polyphenols and flavanoids (Tables 9, 9.1 & 10) as discussed previously, that they are known potent free radical scavengers.
Flavonoids like quercetin, myricetin and kaempferol induce a concentration-dependent decrease of both the nuclear glutathione (GSH) content and glutathione S-transferase (GST) activity in a model system of isolated rat liver nuclei, which could lead to oxidative DNA damage (Sahu and Gray, 1996). Most of the authors used quercetin, myricetin and kaempferol as reference standard for free radical scavenging activity (Khanduja and Anjana, 2003; Slusarczyk et al., 2009). It is well established that flavonoids have been described as health-promoting, disease-preventing dietary supplements, and have activity as cancer preventive agents. Additionally, they are extremely safe and associated with low toxicity, making them excellent candidates as chemopreventive agents.

5.5. Wound healing activity

The concept of developing drugs from plants used in indigenous medical system is much older, while in some cases direct links between a local and biomedical use exists, in other cases the relationship is much more complex (Heinrich and Gibbons, 2001). Wounds and particularly chronic wounds are major concerns for the patients and clinicians alike; chronic wounds affect a large number of patients and seriously reduce their quality of life. Research on wound healing agents is one of the developing areas in modern biomedical sciences. Many traditional practitioners across the world particularly in countries like India and China with age old traditional practices have valuable information of many lesser-known hitherto unknown wild plants used by the traditional healers for treating wounds and burns. Several drugs of plant, mineral and animal origin are described in the traditional texts of Indian systems of medicine like Ayurveda for their healing properties under the term ‘Vranaropaka’. Besides the classical systems of Indian Medicine, the folk and the tribal medicine also employ a number of plants and animal products for the treatment of cuts, wounds and burns. Some of the plants have been screened scientifically for the evaluation of their wound healing activity in different pharmacological models and human subjects, but the potential of most of the plants remain unexplored. To validate the ethnotherepeutical claim of C. infortunatum plant in wound healing, an attempt has been made by employing the topical treatment of extracts on the excised wounds. In the present experiments we have clearly observed an enhanced
wound contraction induced by the petroleum ether and ethanol extracts of leaf (Table 15 and Figure 17). Similar results have been obtained in the chloroform and ethanol extracts of root (Table 16 and Figure 19). This enhanced wound contraction could be attributed to the enhanced contractile property of myofibroblasts resulting in an increase of epithelialization. Thiem and Goslinska (2004) have made similar observations in *Rubus chamaemorus* that the topical application of compounds with free radical scavenging properties in patients have shown to improve wound healing significantly and protect tissues from oxidative damage. The presence of phenolics in *C. infortunatum* (Table 10) also supports these results that phenolic compounds are known for free radical scavenging property.

In the incision repair model the breaking strength of the wounds after topical application of the extracts has been measured (Table 16.1 and 17.1). The breaking strength is the ability of healing wound which is measured experimentally by the amount of force required to disrupt it. In the initial stages wound will be having little breaking strength because the clot alone will be holding the edges together (Kumara Swamy *et al.*, 2007). Thereafter breaking strength increases rapidly as collagen deposition increases and cross-linkages are formed between the collagen fibers. In this study as well we have observed an increased breaking strength of skin. In another model of wound healing to evaluate breaking strength, dry weight and histological changes experiments were conducted by employing oral treatment of extracts to the animals. The results are more encouraging exhibiting an increase in the breaking strength, dry weight of the granulation tissue developed over the inserted pith. Similar results have been obtained by Shirwaikar *et al.* (2003) and Singh *et al.* (2005). Several other workers have also reported the enhancement in the wound healing process by various plant extracts and isolated compounds in animal models. However, the exact mechanism involved in the wound healing process is still not clear. Further, it is a fact that there are number of parameters which are involved in the healing of wound including epithelialization, antioxidant defense and biochemical changes. Hence, majority of the researchers restrict the screening of plants to simple healing of wounds and do not go deep into the details of the healing process (C.f. Kumara *et al.*, 2007).
The percentage of wound contraction and mean period of epithelialization in animals treated with the constituents isolated from leaf and roots of *C. infortunatum* i.e., clerosterol, betulinic acid and lupeol acetate are shown in Table 17. Among the tested isolated constituents, clerosterol and lupeol acetate showed significant reduction in the wound area i.e., upto 92.00±0.58 and 91.88±1.00% respectively. The scar area and time required in days for complete epithelialization of excision wound was also significant for clerosterol and lupeol acetate (19.4±0.58 and 18.26±0.64 respectively). In other wound incision model significant increase in skin breaking strength was noticed in the animals treated with lupeol acetate (486.36±8.40) followed by clerosterol (477.27±5.08). The animals treated with standard reference drug nitrofurazone showed significant increase in the tensile strength of the incision wound (548.33±9.08). Among the isolated constituents, clerosterol and lupeol acetate acid treated animals demonstrated excellent wound healing property. The increased dry weight of the granuloma tissue (68.83±3.08 and 62.83±1.16) also increased tissue breaking strength (348.93±5.87 and 336.01±5.87 respectively). However, the betulinic acid did not show any significant activity on excision, incision and dead space wound model as it can be clearly seen in Table 17 and 17.1).

The flavonoid natural products exert a wide range of biochemical and pharmacological precise properties (Canivenc-Lavier *et al*., 1996; Shih *et al*., 2000). It is interesting to mention that most of the species from the genus *Clerodendrum* contain similar types of compounds (Jacke and Rimpler 1983; Akihisa *et al*., 1989). Therefore, it can be deduced that the biological property exhibited by the petroleum ether and ethanol extracts of leaves, chloroform and ethanol extracts of roots of *C. infortunatum* might be due to the presence of compounds like flavonoids, triterpenoids and other chemical constituents. There are reports that the plants having antioxidant property would also enhance wound healing activity (Shirwaikar, *et al*., 2003). Triterpenoids (Scortichini and Pia Rossi, 1991) are also known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialization. However, in this study it has been observed that antioxidant property of isolated constituents by using DPPH free radical scavenging assay, the isolated constituent betulinic acid is very poor in scavenging DPPH free radicals where as lupeol
acetate and clerosterol showed moderate antioxidant activity compared to betulinic acid. Similar observations have been made by Perumal Yogeeswari & Dharmarajan Sriram. (2005) and Kumiko et al., (2007). On the other hand, Khadeer Ahamed et al. (2007) reported that the lupeol exhibited significant antimicrobial activity whilst betulinic acid did not show any significant antimicrobial activity. Recently, Donatus and Nnamdi (2009) reported that clerosterol exhibits significant antimicrobial activity. Hence, in the present study wound healing activity exhibited by lupeol acetate and clerosterol might be due their antioxidant and antimicrobial activity, where as betulinic acid is poor in demonstrating antioxidant and antimicrobial activity this could be the reason for insignificant wound healing activity of betulinic acid. Thus, this study may help pharmacologists to consider the exact part of the plant and its precise use (cuts, burns or wounds) in the traditional system of medicine. This study strengthens the ethnopharmacological claims and help in building the global acceptance of the wound healing agents of plant origin.

5.6. Antitumor activity

Cancer continues to be one of the major causes of death worldwide and only modest progress has been made in reducing the morbidity and mortality of this dreadful disease. Extensive preclinical and clinical research has led to substantial progress in understanding the multistep nature of the prolonged tumorigenesis process. This understanding has led to the realization that most human malignancies should be fought on multiple fronts. Thus, in addition to cancer therapy, cancer prevention has become an important approach to control cancer (Hail, 2005; Sun et al., 2004). Common prevention strategies include avoiding exposure to known cancer-causing agents, enhancement of host defense mechanisms against cancer, lifestyle modifications and chemoprevention. Recent pharmacological studies revolve around the urgency to evolve suitable chemotherapeutic agents for the treatment of tumours (benign and malignant) with out having toxic effects, the indigenous system of medicine has several medicinal plants with versatile antitumor properties that need detailed research for the development of antitumor drugs. The contribution of ethanomedicinal plants in discovering new drugs has been enormous for the development of novel drugs from natural resources which may help in treating diseases like cancer, hypertension, diabetes, etc.
In the light of traditional claim of the plant *C. infortunatum* in tumor prevention and cure, in the present study for the first time the *in vitro* and *in vivo* antitumoral effects of petroleum ether, chloroform, ethanol extract and ethyl acetate fractions from (ethanol extracts) of leaf and roots of the plant were evaluated. Selective cytotoxicity is a desired feature of a new candidate anticancer agent. Most of the clinically used antitumor agents possess significant cytotoxic activity in cell culture system (Ajith and Janardhanan, 2003). Among the cytotoxic assays available, Trypan blue exclusion test permits evaluation of the structural integrity of the cell membrane. This is a simple and most convenient assay.

In the current investigation by using the trypan blue exclusion test, different concentrations of petroleum ether, chloroform, ethanol extracts and ethyl acetate fraction of leaf and roots used have exhibited the inhibition of viability of Ehrlich ascites tumor (EAT) cells. The data in Table 18 clearly reveals that among the treated extracts / fractions, petroleum ether extract and ethyl acetate fraction of roots showed significant inhibition of viability of cells amounting to 56.24 and 88.66% respectively at the concentration of 100\(\mu\)g/mL after three hours of exposure. Whereas, ethanol extract of leaf and root showed moderate cytotoxicity of 22.66 and 28.42% respectively at 1mg/mL.

In the *in vivo* study, petroleum ether, ethyl acetate fraction and ethanol extracts of leaf and roots of *C. infortunatum* were evaluated for its antitumor efficiency on one of the most commonly used experimental tumor model Ehrlich ascites tumor (EAT) which is a rapidly growing carcinoma with very aggressive behavior (Segura et al., 2000). It is able to grow in almost all mice strains suggesting that the recognition and immune responses to this tumor are independent of MHC (Chen and Watikins, 1970). In EAT-bearing mice, a regular rapid increase in ascitic tumor volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells (Weber and Osborn, 1969). The daily treatment with petroleum ether extract, ethyl acetate fraction of roots of *C. infortunatum* in the animals bearing EAT cells showed a significant decrease in the total EAT cell count and also inhibited the tumor volume, viable tumor cell count, and increased the life span of the tumor-bearing mice in comparison with the EAT control.
and ethanol extracts of leaf and roots (Table 18.1). The reliable criteria for judging the value of any anticancer drug are the prolongation of the life span of animals (Tsai and Frasch. 1982). Daily treatment with petroleum ether extracts and ethyl acetate fraction restored total and differential WBC cell count to normal near values (Table 18.2). This indicates that extracts/fraction possess protective action on the haematopoietic system.

The major biological activities reported from this genus *Clerodendrum* are antihypertensive, antidiabetic, antihyperlipidemic, and larvicidal including antitumor activities. Recently Huey-Hwa Cheng et al. (2004) reported that a new phenylethanoid glycoside, two new cyclohexylethanoids, one new phenolic glycoside, and a new farnesane-type sesquiterpenoid, namely 2-phenylethyl 3-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside (I), 6"-O-[(E)-caffeoyl] rengyoside B (II), clerodenone A (III), 2-{6-O-[(4-hydroxy-3-methoxyphenyl)carbonyl]-β-D-glucopyranosyl}oxy)-2-methylbutanoic acid (IV), 2-{(2S,5R)-5-[(1E)-4-hydroxy-4-methyl hexa-1,5-dien-1-yl]-5-methyltetrahydrofuran-2-yl}propan-2-yl β-D-glucopyranoside (V), together with 16 known compounds, were isolated from the roots of *Clerodendrum bungei*. The new compounds showed modest *in vitro* inhibition of the proliferation of the HeLa human cervical carcinoma cell line (CCL-2), with IC50 values in the range of 3.5-8.7 μM. Shi et al., (1993) also reported the *C. bungei* showed antitumor activity in hepatic cells of mice at a dose of 100 g/kg. Two compounds, isoacteoside and jionoside D isolated from *C. trichotomum* also reduced the levels of apoptotic cells induced by the action of hydrogen peroxide (Chae et al., 2004, 2005). Here the phytochemical analysis of the petroleum ether extract and ethyl acetate fraction indicate the presence of triterpenoids. Triterpenoids are the major constituents of some medicinal herbs and are widely present in all parts of a variety of plants (Connolly and Hill, 2005). Thousands of structures have been reported with hundreds of new derivatives being described each year (Connolly and Hill, 2007). The biological activities of triterpenoids have recently attracted more attention in the biochemical and medical fields because of their immunomodulatory and antitumor effects (Dzubak et al., 2006). From the present results of antitumor activity it may be opined that the presence of triterpenoids in the petroleum ether and ethyl acetate fractions of roots of *C. infortunatum* might be responsible for exerting anti-tumour activities in the models used. Furthermore, it can also be presumed
that there could be more compounds present in very small quantities which are also exerting a synergistic response with other major compounds. These compounds could be belonging to the major groups of bioactive constituents like triterpenoids and phenolics.

In the case of isolated constituents the cytotoxicity exhibited by betulinic acid and lupeol acetate was more potent (100 and 92.16 % respectively) at the concentration of 100µg/mL where as clerosterol failed to exhibit cytotoxicity up to a concentration 1mg/mL. Among the isolated constituents the treatment with betulinic acid and lupeol acetate demonstrated significant inhibitory effect when administered intraperitonially on Ehrlich tumor growth. Daily treatment of Ehrlich tumor-bearing mice with betulinic acid and lupeol acetate separately for 10 days resulted in a significant reduction in ascitic volume, total and differential cell count of blood. This also decreased the weight of tumor-bearing mice, and significantly increased mean survival time and the life span of tumor bearing animals (%ILS, 121.6 and 76.56) compare to control group (Table 19.2) Betulinic acid and lupeol acetate were more effective because in the EAT-bearing mice, cells are present in the peritoneal cavity and the intraperitoneal treatment with compounds inhibits the tumor growth. Tumor inhibition might be due to the direct effect of the compounds on the tumor cells. It is also interesting to mention that betulinic acid and lupeol acetate are first to be reported in C. infortunatum. It is well recognised that Betulinic acid is a widely distributed pentacyclic lupane-type triterpene in the plant kingdom and has been shown to exhibit a variety of biological activities including inhibition of human immunodeficiency virus (HIV), antibacterial, antimalarial, antiinflammatory, anthelmintic and antioxidant properties. Betulinic acid is also identified as a highly selective growth inhibitor of human melanoma, neuroectodermal and malignant tumor cells and is shown to induce apoptosis in these cells (Fischer, 1994). Another constituent Lupeol acetate also exerted cytotoxic effect on EAT cells. Lupeol acetate is a derivative of lupeol; it is also known that lupeol is a pentacyclic triterpene. This triterpene, a common secondary metabolite of plant, has been isolated from many plant species. It is present in Salvia harmonium (Ulubelen et al., 1977) Berbveris vulgaris (Said and Begum, 2004) and other species (Das and Mahato, 1983; Jassbi, 2006). Diverse ranges of biological activities triggered by lupeol have been reported. The stimulation of programmed cell death in human leukemia cell line (HL-60) by lupeol was observed and
reported by Aratanechemuge et al. (2003). Lupeol and some related compounds have demonstrated antitumor activities in several cancer cell lines (Cf. Margareth et al., 2009). Lupeol acetate was documented to exert antiarthritic and antiurolithiatic activities (Argay et al., 1997) including inhibition against stress induced ulcers in rats (Mallavadhani et al., 1998). It was hypothesized that the activity of this pentacyclic triterpene may be due to an immunosuppressive action and the inhibition of cell migration to the inflammation site as well as to a reduction in the release of pro-inflammatory chemotactic factors (Fernandez et al., 2001). It is clear from the earlier reported studies on betulinic acid and lupeol acetate that they induce apoptosis which is characterized by prominent morphological features such as membrane blebbing, nuclear condensation and DNA fragmentation. In this study also it has been observed that the morphological alterations of untreated and betulinic acid and lupeol acetate treated EAT cells were monitored by light, phase contrast microscope. The data revealed that betulinic acid and lupeol acetate treated cells exhibited membrane blebbing and apoptotic bodies in contrast to control cells. Therefore, it is very clear from the results that antitumor activity of said plant is due to the presence of triterpenoids and other bioactive constituents. Numerous studies over the last few years aimed at elucidating the molecular mechanisms of betulinic acid mediated antitumor activity. One of the characteristic features of betulinic acid’s cytotoxicity is its ability to trigger the mitochondrial pathway of apoptosis in cancer cells (cf. Simone Fulda, 2008).

5.7. *In vivo* anti mutagenic activity

Mutations are the cause of innate metabolic defects in cellular system, triggering the morbidity and mortality in living organisms. A plethora of synthetic and natural substances, apart from various genotoxic physical and biological agents are known to act as mutagenic, co- carcinogenic and / or carcinogenic agents (Mitscher et al., 1986). Since the mutagens are involved in the initiation and promotion of several human diseases including cancer, the significance of novel bioactive phytocompounds in counteracting the promutagenic and carcinogenic effects are gaining credence. Such chemicals that reduce the mutagenicity of physical and chemical mutagens are called antimutagens. Many naturally occurring compounds have been reported to possess cancer preventive
effects (De Stefani et al., 2004). It is also established that chromosome damage is always associated with cancer cells. There are several reports indicating the antimutagenic and anticarcinogenic properties of medicinal plants. A systematic screening of plant extracts, food supplements or dietary products for their potential as chemo-preventive and antimutagenic against chemical carcinogens is essentially needed. Based on this idea and non availability of reports on the antimutagenic potential of *C. infortunatum* thus far, the present investigations were undertaken to evaluate the antimutagenic property in *in vivo* animal system. In view of certain advantages like smaller size, easy maintenance, well established karyotype and low frequency spontaneous aberrations, mouse as *in vivo* system has been used in the present investigations. Chromosomal aberrations have been selected as a parameter to evaluate antimutagenic abilities. In the preliminary experiments from the root samples an active extract (petroleum ether) and a fraction from ethanol extract (ethyl acetate) were selected based on their performance as anti-tumour in nature against EAT cells to evaluate the anti-mutagenic potential. Subsequently, similar experiments were also carried out to check this property of some of the isolated constituents namely, betulinic acid and lupeol acetate. The result as shown in the table... demonstrates that the frequency of aberrations produced by normal control (0.1% DMSO) and after the treatment with petroleum ether and ethyl acetate fraction alone at the doses of 250 and 150 mg/kg.b.w. showed no significant increase in chromosomal aberrations in the bone marrow cells of tested animal groups. Hence, these results clearly suggesting that the petroleum ether and ethyl acetate fractions of roots of *C. infortunatum* do not contain phytochemical constituents with mutagenic property. Whereas, EMS being a known mutagen induced maximum frequency of chromosomal aberrations. The results are in conformity with the earlier reports of Mahmood and Vasudev (1993) have shown the induction of chromosomal aberrations at 80 mg/kg body weight EMS in bone marrow cells. However, the combined treatments of petroleum ether extract + EMS and ethyl acetate fraction + EMS yielded 3.06 and 4.17 frequencies of chromosomal aberrations respectively, whereas, the isolated constituents with EMS, betulinic acid + EMS and lupeol acetate + EMS produced 7.81% and 6.93% respectively, which is significantly lesser than the frequency of 12.09% obtained after EMS alone (Tables 20-20.1). Thus, it is a clear indication that the extract and fraction used exerts their protective effects
against EMS induced chromosomal aberrations. Furthermore, betulinic acid and lupeol acetate also exhibited similar protective effects against EMS induced chromosomal aberrations.

The phytochemical screening of *C. infortunatum* showed the presence of triterpenoids, flavonoids and saponins, these results are in agreement with earlier reported results of Roy *et al.* (1996) and Dilipkumar *et al.* (2009) who have also observed the presence of flavonoids and saponins. The flavonoids are known for their ability to give a hydrogen atom to free radicals, thus neutralizing cations capable to produce the hydroxyl radical (Jovanovic *et al.*, 1998; Merken and Beecher, 2000). These compounds can also modulate a wide range of mammalian enzyme activities, such as cytochrome P<sub>450</sub> and several antioxidant enzymes (Ferguson, 2001). Thus, it is probable that the antioxidant activities elicited by the extracts as well as the antimutagenic effect against EMS induced chromosomal damage in mice may be due to the antioxidant action of the flavonoids present in the plant that are acting against the genotoxic effects of the mutagen. There are large number of reports on the protective effects of polyphenols and flavonoids. Weisburger *et al.* (1996) examined the effects of specific tea phenols against a number of genotoxic carcinogens. Anticlastogenic and antimutagenic effects of black tea polyphenols have been studied by Halder *et al.* (2005). Thus, it may be presumed that the extracts may contain certain bioactive compounds which could be the possible reason for exerting inhibitory effects on the induction of *in vivo* chromosomal damage by the EMS.

Lupeol treatment of mice before benzo[a]pyrene- induced clastogenicity reduced aberrant cells, micronuclei presence and cytotoxicity in the bone marrow cells as well as caused an increase in the mitotic index, revealing the lupeol’s antigenotoxic potential (Prasad *et al.*, 2008). Lupeol was also investigated for its ability to provide protection against metal toxicity which can lead to cancer. Rats exposed to cadmium when treated with lupeol showed an antioxidant enzyme levels and peroxidative status (Nagaraj *et al.*, 2000). Betulinic acid is a naturally occurring pentacyclic triterpenoid and has been shown to exhibit a variety of biological activities including inhibition of human immunodeficiency virus (HIV) (cf. Perumal Yogeeswari and Dharmarajan Sriram, 2005). Since lupeol acetate is a derivative of lupeol, betulinic acid and lupeol belongs to
pentacyclic terpenoids. Hence, it is presumed that betulinic acid and lupeol acetate isolated from petroleum ether and ethyl acetate fraction respectively exerted antimutagenic activity against EMS induced chromosomal aberrations.

Thus, the overall results of this study have evidently confirmed that, the *C. infortunatum* is a medicinally important plant. The preliminary phytochemical analysis of the extracts has shown the presence of several active constituents which are pharmacologically very important, viz., phenolic compounds, flavonoids, alkaloids, terpinoides, saponins etc., Further, the antioxidant and pharmacological studies (hepatoprotective, wound healing, antitumour and antimutagenic activities) have contributed extensively on the medicinal information of *C. infortunatum*. The active extracts were further subjected for fine phytochemical analysis by isolating and characterizing the active components using spectroscopic techniques such as, IR, $^1$H NMR, $^{13}$C NMR and Mass (FAB$^+$ and EIS) spectral studies. The characterized compounds were found to be clerosterol, quercetin, betulinic acid cleramine and lapel acetate. This plant is scientifically proved to possess medicinal properties including anti-tumor activities, which have been validated by the observations made in different pharmacological investigations. Therefore, in the light of these observations and the earlier reports it is evident that *Clerodendrum infortunatum* is not only responsible for the cure of several diseases but its active principles can also be exploited for the treatment of tumours. It is hoped that the outcome of this scientific investigation will aid in the proper utilization of this plant as a better economical and abundant bioresource of phenolics and flavonoids in the cosmetics and pharmaceutical industry. It may also open new avenues for further investigations leading to the detection and isolation of specific compounds that may become invaluable drugs in future for the treatment of hepatitis, wound healing and tumors.