6.0 DISCUSSION

One of the problem scientists and medical workers face in the fight against infectious diseases is the development of resistance to the agents used to control them. There has been a remarkable progress in the prevention, control and even eradication of infectious diseases with improved hygiene and development of antimicrobials and vaccines.

However, infectious diseases still remain a leading cause of global disease burden with high morbidity and mortality, especially in the developing world. Furthermore, there have been threats of new diseases during the past three decades due to the evolution and adaptation of microbes and the re-emergence of old diseases due to the development of antimicrobial resistance and the capacity to spread to new geographic areas. The impact of the emerging and re-emerging diseases in India has been tremendous at the socioeconomic and public health levels. Their control requires continuous surveillance, research and training, better diagnostic facilities and improved public health system. Emerging and re-emerging zoonotic diseases, food borne and water borne diseases and diseases caused by multiresistant organisms constitute the major threats in India (Abu et al., 2004; Chugh, 2008).

More than two-thirds of the antibiotics used to treat human diseases are natural products or semisynthetic derivatives of these molecules. Advances in genetics, biochemistry and bioinformatics have transformed the study of antibiotics and other natural products, not just by revealing how
they are synthesized but also by casting them as phenotypes encoded by genes. It has been estimated by the World Health Organization that approximately 80% of the world’s inhabitants rely mainly on traditional medicines for their primary health care (Akinnibosun et al., 2009).

The use of data on traditional medicine provides a very valuable short cut by indicating plants with specific folk medicinal uses, which might be likely sources of biologically active compounds. Recent investigations on medicinal plants used in traditional medicine have led to the discovery of many new drugs and hundreds of pharmacologically active substances for synthetic modifications (Wang et al., 2008). With the advent of modern tools and experimental methodologies, the plant products are subjected to exhaustive screening on various models to find out their potential under various conditions (Balick and Cox, 1996; Akobundu and Agyakwa, 1998).

Sansevieria roxburghiana, commonly known as piles root, Indian bowstring hemp and Jaang Mattai in Tamil (Vernacular) was selected for the study to screen its phytochemical compounds and to investigate its antimicrobial, antioxidant and anticancer activity. The study confirms experimentally the potent bioactive nature of the plant.

6.1 Phytochemical screening

The phytochemical screening of the leaf extracts of S. roxburghiana in various solvents revealed strong presence of various chemical substances such as alkaloids, saponins, proteins and phytosterols. Moderate presence of flavonoids, glycosides, anthocyanin and betacyanin, steroids and
carbohydrates were observed. Tannins and phenols were present in trace amounts.

The rhizome extracts of *S. roxburghiana* revealed strong presence of saponins, moderate presence of various chemical substances such as alkaloids, glycosides, proteins, anthocyanin, betacyanin, phytosterol, steroids and carbohydrates.

The phytochemical biocompounds of *S. roxburghiana* is compared with the available literature of related species such as *Sansevieria trifasciata* and *Sansevieria liberica* (Ogukwe *et al*., 2004; Sunilson *et al*., 2009; Ikewuch *et al*., 2010). The results of the phytochemical screening of the different extracts of *S. roxburghiana* is in accordance with the studies of Sunilson *et al*., (2009) who reported the presence of alkaloids, flavonoids, saponins, glycosides, terpenoids, tannins, proteins and carbohydrate in the leaf extracts of *S. trifasciata*.

### 6.2 Antimicrobial Activity

Gram staining of bacterial strains helps to group and confirm the bacteria as Gram positive and Gram negative (Beveridge, 2001). The result of antibiotic sensitive study against the bacterial strains showed that most of the bacterial strains were sensitive to the standard antibiotic discs whereas *B. cereus* and *S. paratyphi* showed resistance to Ampicillin (25 µg), *S. paratyphi* showed resistance to Norfloxacin (10 µg). *K. pneumoniae* was resistant to Tetracycline (30 µg) and Chloramphenicol (30 µg) and *P. vulgaris* was resistant to Norfloxacin (10 µg).
Norfloxacin and tetracycline were used as a positive control because it inhibits the growth of both Gram-positive and Gram-negative bacteria (Qadri and Johnson, 1989; Chopra and Roberts, 2001). The negative control, dimethylsulphoxide (DMSO), is a colourless liquid and an important polar solvent which dissolves both polar and non-polar compounds from a plant. It is miscible in a wide range of organic solvents including water. It has the distinctive property of penetrating the human skin very readily.

The antimicrobial activity of methanol, acetone, aqueous and ethyl acetate extracts of leaf and rhizome of *S. roxburghiana* was analyzed against fourteen clinically significant organisms using Disc Diffusion method and exhibited varying degree of antibacterial and antifungal activities at a concentration of 100 µg/ml.

The antimicrobial activity of methanol and acetone extracts of leaves *S. roxburghiana* (inhibition zone 9-18 mm) were found to be more pronounced than the ethyl acetate extract (inhibition zone 8-14 mm) against all the organisms tested. The different solvent extracts of leaves proved to be better antimicrobial agents compared to rhizome extracts at the tested concentration. The ethyl acetate extract of rhizome showed better antimicrobial activity compared to other solvent extracts. Whereas the aqueous extract of both leaves and rhizome showed less antimicrobial activity. The poor activity of the aqueous extract against most microbial strains investigated in this study is in accordance with the earlier studies of Koduru *et al.*, (2006). Since water can dissolve polar compounds, due to
the insolubility of the active compounds in water or hot water and denaturation of the active compounds during extraction process could be the reasons for the lower activity of the aqueous extracts (Srinivasan et al., 2001; Girish and Sathish 2008; Igbinosa et al., 2009). Amongst the test microorganisms used, the *M. luteus* was found to be the most sensitive to the different solvent extracts, followed by the *K. pneumoniae, P. vulgaris, S. paratyphi, E. coli, P. fluorescence* and *B. cereus*.

The antimicrobial effect of the plant extracts of *S. roxburghiana* may be due the presence of different phytochemicals such as alkaloids, phytosterols, steroids, glycosides, flavonoids and phenolics extracted by different solvents (Tambekar and Khante, 2010; Thambiraj and Paulsamy, 2011). Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications (Kam and Liew, 2002; Soetan et al., 2006; N’guessan et al., 2007). Karou et al., (2003) have reported that the alkaloids present in *Sida acuta* showed antimicrobial activity against the tested microorganisms. Methanol extract of leaves of *S. roxburghiana* showed strong presence of alkaloids, which could be responsible for its antibacterial activity against *M. luteus, K. pneumoniae,* and *P. vulgaris* (Igbinosa et al., 2009). Acetone extract of leaves also showed strong presence of alkaloids and protein which may contribute to its antibacterial activity against *M. luteus, P. fluorescence* and *E. faecalis* or might be due to synergistic effects of the various bioactive compounds in the extracts.

Steroidal compounds present in *S. roxburghiana* extracts are also of importance and interest because they form part of plants defence system and also due to their relationship with various anabolic hormones including sex hormones (Okwu, 2001; Nazifi et al., 2008). Quinlan et al., (2000)
worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates. Neumann et al., (2004) also confirmed the antiviral property of steroids. Flavonoids, another constituent of *S. roxburghiana* leaf extracts shown to exhibit a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek et al., 2002).

Just et al., (1998) revealed the inhibitory effect of saponins on inflamed cells. Saponin was found to be present in *S. roxburghiana* extracts and has supported the usefulness of this plant in managing inflammation. Ethyl acetate extracts of leaves and rhizome of *S. roxburghiana* showed strong presence of saponin which was absent in all other solvent extracts. *P. vulgaris* showed better growth inhibition with ethyl acetate extract when compared to methanol and acetone extracts of leaves and this could be attributed to the presence of saponins. *C. albicans* also showed very good inhibitory effect with ethyl acetate extract of rhizome (inhibition zone of 15). The present observations are in agreement with the reports of Tatli et al., (2003) who showed that saponin was responsible for significant antifungal activity by weakening the virulence of *C. albicans* and killing fungi by destroying their cell membranes (Zhang et al., 2006). The difference in antimicrobial activity is attributed to the presence of active compounds with insufficient quantities in the crude extracts of respective solvents (Taylor et al., 2001; Sathish et al., 2008; Thambiraj and Paulsamy, 2011).
S. sonnei and P. fluorescence did not show inhibition with any of the solvent extracts of rhizome. As in leaf extracts, amongst the test microorganisms used M. luteus was found to be the most sensitive to all the solvent extracts.

It is also observed from the results that the methanol and acetone leaf extracts had wide antibacterial activity against both Gram positive and Gram negative bacteria as well as the fungal strains. This may be due to the capability of methanol and acetone to dissolve both polar and non-polar compounds. The activity of the extracts against the Gram negative bacteria is noteworthy as these bacteria are known to exhibit high degree of resistance to conventional antibiotics (Vlietinck et al., 1995; Aliero et al., 2008; Girish and Sathish, 2008).

As leaf extracts showed significantly better antimicrobial activity compared to the rhizome extracts, minimum inhibitory concentrations of different solvents extracts of leaves were assessed using Agar Dilution method. The results showed that the methanol extract required less concentration to inhibit the growth of microorganisms among the different solvent extracts. Methanol extract of leaf showed MIC of 1.0 mg/ml against M. luteus and P. vulgaris whereas E. coli and S. aureus was inhibited at 8.0 mg/ml. Methanol leaf extract showed inhibited growth of all the tested bacteria from 1.0 to 8.0 mg/ml.

Most of the bacterial pathogens showed inhibited growth in acetone extract up to 8.0 mg/ml. But some of the pathogens like S. sonnei, C.
*neoforans*, and *C. albican* were not inhibited up to 8.0 mg/ml in acetone extract. So their values were assumed to be >16.0 mg/ml. Ethyl acetate extract also showed MIC of 1.0 mg/ml to 8.0 mg/ml against some bacteria. *M. luteus*, *B. cereus* and *S. typhi* showed inhibited growth at 2.0 mg/ml and 4.0 mg/ml respectively. Whereas *S. aureus*, *E. coli*, *P. aeruginosa*, *S. sonnei*, *P. vulgaris*, *K. pneumoniae*, *S. paratyphi*, *C. albicans* and *C. neoformans* showed varying degree of inhibition in disc diffusion testing (9-14 mm) but did not show MIC up to 8.0 mg/ml, so were assumed to be >16.0 mg/ml.

MIC results obtained in the present study shows that methanol and acetone leaf extracts of *S. roxburghiana* had better antimicrobial activity compared to ethyl acetate and aqueous extracts. In acetone extract most of the pathogens inhibited growth at higher concentration whereas methanol extract proved to be better antimicrobial agent against most of the microorganisms by showing MIC effect less than ≤ 4.0 mg/ml. The results of this study are in accordance with the findings of Aliero et al., (2008) on *S. hyacinthoides*.

The results of this study reflect the presence of potent phytochemicals in solvent extracts of leaves and rhizomes of this plant which could be responsible for its antimicrobial activity. (Jimenez and Riguera, 1994; Reynolds and Dweck, 1999; Ashrafu et al., 2008; Pande et al., 2009. Therefore in view of these results, the ability of the extracts to inhibit the growth of several bacterial and fungal species is an indication of
the broad spectrum antimicrobial potential of *S. roxburghiana* which makes the plant a candidate for bio-prospecting for antimicrobial drugs.

The few variations in results between the disc diffusion and MIC results could be due to the different susceptibility of the bacterium to the plant extract, the rate of growth of bacteria, solvents used to extract the plant compounds and the rate of plant extract diffusion (Ntombeziningi, 2009).

### 6.3 Thin Layer Chromatography

The wide range of Rf values from 0.1 to 0.8 signifies the presence of different secondary metabolites in this plant. The results showed that the antimicrobial activity of this plant extract could be attributed to the compounds observed at the various Rf values on the TLC separation (Talukdar *et al.*, 2010).

#### 6.3.1 Antibacterial activity of TLC separated fractions

The three prominent fractions F₁, F₂ and F₃ from methanol extract of leaves were investigated for its antibacterial activity against six pathogenic bacteria. All the tested organisms showed better zone of inhibition against F₁ and F₂ fractions except *P. vulgaris* that showed inhibition only against F₁ fraction. None of the bacteria showed inhibition against F₃ fraction.

Antibacterial activity of the TLC separated fractions of the methanol leaf extract was found to be more pronounced than the crude solvent extract. Crude extracts showed pronounced antibacterial activity at
concentrations of 100 µg/disc whereas the TLC separated fractions showed appreciable activity at a concentration of 5 µg/disc.

From the observed results it is clear that the antibacterial activity of the fractions were more pronounced with Gram negative bacteria *E. coli*, *K. pneumoniae*, *P. vulgaris*. The results of antimicrobial activity of crude plant extracts and partially purified fraction of methanol extract of leaves of *S. roxburghiana* showed that the compounds responsible for the inhibition of microorganisms are more than one in each extract which is supported by the zones of inhibition on the plates. This is in agreement with the studies by Reynolds and Dweck, (1999). Taylor et al., (1995) showed that the larger the variety of compounds in an extract the better the chances of inhibition.

### 6.4 Antioxidant potential of *S. roxburghiana*

#### 6.4.1 DPPH radical scavenging activity

The free radical scavenging activity of methanol, acetone and ethyl acetate extracts of leaves of *S. roxburghiana* was evaluated based on its ability to scavenge the synthetic DPPH. This assay provided useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons. DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet color) (Husain et al., 1987, Visioli et al., 2000, Parr and Bolwell, 2004; Solai Raj et al., 2010). As the electron is paired in the presence of free radical scavenging, the absorption vanishes and the resulting discoloration stochiometrically coincides with respect to the number of electrons taken up. The bleaching of DPPH
absorption is representative of the capacity of the methanol, acetone and ethyl acetate extracts to scavenge free radicals independently (Montalleb et al., 2005; Arokiyaraj et al., 2008; Muthukumaran et al., 2011).

The methanol, acetone and ethyl acetate extracts of leaves of S. roxburghiana were screened for antioxidant activity using DPPH radical scavenging activity and has shown that the scavenging effects on the DPPH radical increases sharply with the increasing concentration of the samples (100-1000 μg/ml) and standard (BHT) to a certain extent and hence are said to be strongly dependent on the extract concentration.

In presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (Koleva II et al., 2002; Vinayakak et al., 2010). Though the DPPH radical scavenging abilities of the extracts were less effective than the commercial available synthetic like BHT, the study showed that the extracts have the ability of donating a proton and could serve as free radical inhibitor or scavenger, acting as primary antioxidants. Unlike BHT the plant extracts are quite safe and their toxicity is a not a problem of concern. Therefore the plant S. roxburghiana could be exploited as an antioxidant additive.

However, the antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging (Diplock, 1997; Vinayakak et al., 2010).
Methanol extract rendered a better antioxidant potential than acetone and ethyl acetate extract by exhibiting a high scavenging activity. The percentage of inhibition increased from concentrations (100 μg/ml to 1000 μg/ml) with a scavenging activity of 47.15 ± 3.27 to 85.68 ± 3.47.

6.4.2 Nitric Oxide Scavenging activity

The methanol, acetone and ethyl acetate extracts of leaves of *S. roxburghiana* showed increased percentage of nitric oxide scavenging in a dose dependent manner. The reducing properties are generally associated with breaking of the free radical chain by donating a hydrogen atom which has been shown to exert antioxidant action. Nitric oxide is an essential gas required for several normal physiological processes like neural signal transmission, immune response and control of blood pressure. However the elevation of nitric oxide level was found in several pathological conditions including cardio vascular disease and diabetes (Rees *et al.*, 1989; Bredt and Snyder, 1990; Gold *et al.*, 1990). Sodium nitroprusside (SNP) serves as a chief source of free radicals. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine is used as a marker for nitric oxide scavenging activity (Palmer *et al.*, 1987; Javanmardi *et al.*, 2003).

Suppression of released NO may be partially attributed to direct NO scavenging, as the extracts of *S. roxburghiana* decreased the amount of nitrite generated from the decomposition of SNP *in vitro*. Methanol leaf
extract seem to be a better antioxidant when compared to acetone and ethyl acetate extracts.

Methanol leaf extract at 1000 µg/ml exhibited about 86.07 ± 2.18 of NO scavenging activity which found to be comparable to BHT that showed a scavenging activity of 89.12 ± 2.39.

The present study showed that the leaf extracts of *S. roxburghiana* had a strong antioxidative power on DPPH radicals and NO radicals and this could be attributed to the different phytochemical compounds present in these extracts (Naruthapata and Supaporn, 2009). The role of flavonoids and phenolic compounds as antioxidants has been well established and there have been numerous reports on structure-activity relationships in the last decade (Abbas *et al.*, 2006; Balasundram *et al.*, 2006; Michalak, 2006; Rastija and Saric, 2008). In the present study only methanol leaf extract of *S. roxburghiana* showed the presence of phenols and flavonoids. The pronounced antioxidant potential of the methanol extracts could be attributed to the presence of phenols and flavonoids (Ferguson, 2001; Ferguson *et al.*, 2004). Apart from phenols and flavonoids, methanol extract of leaves showed the strong presence of alkaloids, glycosides, phytosterol and steroids. Therefore, it is yet to be ascertained whether the antioxidant potential reported can be attributed to the flavonoids and phenols alone, or to any of the other phytochemicals, or to a synergistic effect of the compounds present (Garcia *et al.*, 2004; Miliauskas *et al.*, 2004; Ali *et al.*, 2008).
Literature survey shows a strong relationship between alkaloid content and antioxidant activity (Quezada et al., 2004; Maiza et al., 2007). As alkaloids also have a significant protective effect against H\textsubscript{2}O\textsubscript{2} and they have the ability to scavenge hydroxyl radicals which contributes to their antioxidant and antimutagenic effects (Moura et al., 2007).

The present study shows that DPPH and NO radical scavenging revealed the antioxidant potential of methanol extract to be more pronounced than acetone and ethyl acetate extracts and this could be attributed to the presence of high content of alkaloids, sterols, flavonoids and saponins.

Concentration of a sample at which the inhibition percentage reaches 50% is the IC\textsubscript{50}. Lower the IC\textsubscript{50} higher the antioxidant potential. Methanol extract was found to show better antioxidant potential when compared to acetone and ethyl acetate leaf extracts using DPPH (228.06 ± 4.82 μg/ml), and NO scavenging activity method (77.81 ± 4.32 μg/ml).

6.5 Anticancer activity of *S. roxburghiana*

Based on the antimicrobial and antioxidant activity, methanol leaf extract of *S. roxburghiana* was tested for its anticancer activity. In the present study, MTT (3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay, caspase and DNA fragmentation were used to detect the anti-proliferative activity of *S. roxburghiana*.

MTT assay was used to determine the non toxic dose of the methanol extract of leaves of *S. roxburghiana* on normal 3T3 cell line and cell
viability was quantified. Cell viability was found to be 92.2% at a concentration of 125 μg/ml but decreased with increase in concentration. This result shows that *S. roxburghiana* is safe and non toxic to normal cells at the tested concentration. Results of morphological changes of extract treated 3T3 cells and its comparison with the untreated cells shows that, as the concentration increases from 125 μg/ml to 500 μg/ml, the percentage of viable cells decreased. 3T3 cells treated with the standard cyclophosphamide showed more dead cells than cells treated with 500 μg/ml of leaf extract.

To evaluate the effect of extract on cell proliferation, the effects of methanol extracts of *S. roxburghiana* on the growth and morphology of human liver cancer HepG2 cell line was investigated. Cells were exposed to increasing doses of methanol extract and cell viability was determined by the MTT assay. Cell viability markedly decreased from 125 μg/ml to 500 μg/ml in a dose-dependent manner.

The methanol leaf extracts of *S. roxburghiana* showed a potent cytotoxic activity against HepG2 liver cancer cells. The concentration of leaf extract at 500 μg/ml showed an inhibition of 81.6 %, which was comparable to the positive control cyclophosphamide that showed a cytotoxicity of 85%. Leaf extracts at 250 μg /ml and 125 μg/ml showed cytotoxic activity of 70.8% and 57.3% respectively.

The morphological changes of extract treated HepG2 cells when compared with the untreated cells showed, that as the concentration of leaf
extract increased from 125 µg/ml to 500 µg/ml the number of dead cells also increased in the treated cells. The number of dead cells in 500 µg/ml of methanol extract was comparable to the positive control cyclophosphamide. These observations of morphological changes may be due to the presence of active chemical compounds like flavonoids, phenols, phytosterols and alkaloids (Fotsis et al., 1997; Hudson et al., 2004; Ogukwe et al., 2004; Moura et al., 2007; Jones et al., 2009; Ikewuch et al., 2010; Mazumder, 2010; Dai and Mumper, 2010).

Therefore the minimum effective concentration of methanol extract of leaves that was non-toxic to 3T3 cells but toxic to 50% HepG2 cells were recorded (IC₅₀) at a concentration lesser than 100 µg/ml of the leaf extract. It was observed that the extract of S. roxburghiana caused marked cell growth inhibition in the human liver cancer cell line. The antitumour (Haldar et al., 2010) as well as anticancer (Fouche et al., 2008) activities of rhizome of S. roxburghiana have been studied. There are no previous reports available on the anticancer activity of leaves of S. roxburghiana. Therefore the present study contributes to identify the metabolites of S. roxburghiana as potent anticancer agents.

### 6.6 Apoptosis of HepG2 cells

Apoptosis is characterized by chromatin condensation and DNA fragmentation, and is mediated by caspases (Hengartner, 2000; Elmore, 2007). The family of caspases regulates apoptosis. Caspases are normally present in the cell as proenzymes that require limited proteolysis to activate enzymatic activity (Nunez et al., 1998). Once activated, caspases cleave a
variety of intracellular polypeptides, including major structural elements of the cytoplasm and nucleus, components of the DNA repair machinery, and a number of protein kinases. Collectively, these divisions disrupt the survival pathways and disassemble important architectural components of the cell, which contribute to the stereotypic morphological and biochemical changes that characterize apoptosis. Among the caspases, caspase-3 is most commonly activated in the apoptotic process (Janicke et al., 1998). Caspase-3 is a key executioner of apoptosis, whose activation is mediated by the initiator caspases such as caspase-9 that cleave a number of substrates which act in response to DNA strand breaks leading to apoptosis (Nicholson and Thornberry, 1997; Mancini et al., 1998; Soldani and Scovassi, 2002). This biochemical and morphological changes in apoptotic cells are cell shrinkage, chromatin condensation, DNA fragmentation, and plasma membrane blebbing (Boe et al., 1991; Vaculova and Zhivotovsky, 2008).

Effect of methanol extract of leaves of S. roxburghiana on the activities of caspase-3, caspase-8, and caspase-9 were studied in HepG2 cells. Treatment with methanol extracts resulted in a significant increase in apoptotic cell death in the HepG2 cells and also resulted in a strong dose dependent proteolytic cleavage of caspase-3 and caspase-9. Caspase-8 was not activated in this assay suggesting, extrinsic apoptotic pathway is probably not involved in this process. Therefore elevation in activities of caspase-3 and caspase-9 suggests that methanol extract of leaves of S. roxburghiana can probably induce apoptosis through activation of these
proteases, particularly caspase-3 and caspase-9. (Alnemri et al., 1996; Salvesen and Dixit, 1997; Stennicke and Salvesen, 2000.)

The DNA fragmentation technique is well studied hallmark of apoptotic cell death (Wyllie, 1980). Gel electrophoresis results revealed fragmentation in cells treated with 500 μg/ml of methanol extract of S. roxburghiana, while DNA fragments were absent in the control cells. Since the methanol extract of S. roxburghiana showed clear fragmentation it provides further support for its apoptotic activity (Haefena et al., 2002; McKeague et al., 2003; Akbar et al., 2011).

To examine whether the apoptotic pathway was involved, DAPI staining was performed on HepG2 cells treated with 500 μg/ml of methanol extract of S. roxburghiana. After 48 h treatment with the plant extract, chromatin condensation, membrane blebbing, cell shrinkage, increased number of nuclear body fragments and irregular edges around the nucleus were observed in treated HepG2 cells, while round, clear edged, uniformly stained cell nuclei were noted in the untreated control cells.

The goal of the investigation was to determine whether the methanol leaf extract of this plant exerted an inhibitory effect on cancer cell proliferation and caused cell death. The results of the studies suggest that methanol extract of S. roxburghiana possess moderate cytotoxic effects on liver cancer cells.

Current studies on the development of effective cancer preventive approaches have focused mainly on the utilization of natural bioactive
agents that can induce selective apoptosis in cancer cells (Mukherjee et al., 2001). Methanol extract have been screened for anticancer properties because traditional practitioners believed that most of the polar compounds were responsible for their claimed anticancer potential (Marjorie 1999; Magesh et al., 2009). In this study, the methanol extract of *S. roxburghiana* showed significant cytotoxic activity on HepG2 cells.

The activities of this plant may be due to the presence of highly complex glycosides, flavonoids, alkaloids, phytosterols and saponins (Tan et al., 2005; Magesh et al., 2009). In the present study, it was found that the methanol extract of *S. roxburghiana* was cytotoxic and induced apoptosis in HepG2 cells by the activation of caspase-3 and or caspase-9.

The HepG2 cells exposed to methanol extract of *S. roxburghiana* exhibited morphological and biochemical changes that characterized apoptosis as shown by loss of cell viability and DNA fragmentation. Since apoptosis is regarded as a new target in the discovery of anticancer drugs, these results confirm the potential of *S. roxburghiana* as an agent of chemotherapeutic and cytostatic activity against human liver cancer cells.

### 6.7 GC-MS Analysis

The characterization of the methanol fractions of leaves of *S. roxburghiana* were carried out by GC-MS. The results of GC-MS revealed the presence of 16 bioactive phytochemical compounds in the methanol fractions of *S. roxburghiana*. The GC-MS retention time (RT) and percentage peak of the individual compounds were used to interpret the
mass spectrum of GC-MS which was done using the database of National Institute of Standards and Technology (NIST).

The identification of phytochemical compounds were based on the peak area, molecular weight and molecular formula as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (Woerdenbag et al., 1993; Pala et al., 1999; Derwich et al., 2009). It is evident from this table that all fractions have a complex chemical composition.

The major phytoconstituents present in the GC-MS profile of methanol fractions of leaves of *S. roxburghiana* were 3,4-Dimethoxybenzoic anhydride (32.73%), 1,2-Benzenedicarboxylic Acid, BIS(2-Ethylhexyl) ester (17.30%), Palmitaldehyde, Diallyl Acetal (16.08), 1-Butyl 2-(8-Methylnonyl) Phthalate (15.78%), Delta.-Undecalactone (14.23%), n-Hexadecanoic acid (10.15%), 6,10,14-trimethyl-2-Pentadecanone (7.08%), Dodecanoic acid (6.58%), 2,5-Dimethoxy benzhydrazide (6.35%), Palmitic acid methyl ester (5.34%), Diisobutyl phthalate (3.40%), 2(4H)-Benzofuranone (3.10%), 3,3-Dimethylhexanal (2.69%), Diethyl Phthalate (2.17%) and 6-Methyl-1-octanol (1.03%).

The GC-MS spectrum showed the presence of more long chain hydrocarbons. When the number of carbon atoms increases in the molecule, hydrophilicity is reduced and the lipophilicity is increased. Higher the lipophilicity of a drug higher is its distribution, because once the drug is in systemic circulation, it distribute to all the tissues at a particular rate
depending on its physicochemical characteristics such as lipophilicity and charge. (Wils et al., 1994; Panchagnula and Thomas, 2000; Parasarman et al., 2009).

Various aliphatic acids, aromatic compounds and ketones were also identified in the studied fractions. Compounds identified by GC-MS analysis possess various pharmaceutical applications. 3,4-Dimethoxybenzoic acid and its derivatives are known for their anti-inflammatory and anti-oxidative properties and also as one of the principal anti-microbial preservative used in food and beverages (Kroes et al., 1991; Ivanova et al., 2002; Ivanova et al., 2010).

The compound diethyl phthalate is used medicinally for the preparation of about 67 consumer formulations including bath preparations (oils, tablets, and salts), eye shadow, toilet waters, perfumes, fragrance preparations, skin care preparations and as a component in insecticide sprays, mosquito repellents and camphor substitute (Jayaraman et al., 2011).

Delta-undecalactone, Palmitic acid methyl ester, Dodecanoic acid, n-Hexadecanoic acid and 6,10,14-trimethyl-2-Pentadecanone possess potent antioxidant, anticancer and antimicrobial properties (Bodoprost and Rosemeyer, 2007; Jiang et al., 2008; Preethi et al., 2010). It could be reasonably argued that the presence of these compounds in the fractions must also be responsible for the antimicrobial, antioxidant and anticancer activity of S. roxburghiana. Jayaraman et al., (2011) has reported that
phytochemicals like diethyl phthalate in the plant extracts could be used for the treatment of various infections including skin transmitted infections.

In conclusion, the study has revealed the presence of important separable phytochemicals such as 3, 4-Dimethoxybenzoic Acid, Palmitaldehyde, 1, 2-Benzenedicarboxylic Acid, Delta-Undecalactone etc., by GC-MS analysis in the leaves of S. roxburghiana showing potent antimicrobial, antioxidant and anticancer activity.

The above results obtained indicate a potent and powerful antioxidant, antimicrobial and anticancer activity of S. roxburghiana. Hence the plant S. roxburghiana can be recommended as a more impressive and promising “natural herbal” source to be used in pharmaceutical industry. The results also lend credence to the folkloric use of this plant in treating microbial infection and shows that S. roxburghiana could be exploited for the production of new potent antimicrobial agents.