7.0 SUMMARY

Plants are the basic source of knowledge of modern medicine. Almost all the parts of the plant, namely leaves, flowers, fruits, bark, roots, stem and seeds are known to have various medicinal properties. The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries and for the presence of antimicrobials, anticancer agents and antioxidants. Higher plants have been shown to be a potential source for new antimicrobial compounds. As plants are commended as potent biochemists, man is able to obtain from them a wondrous assortment of industrial chemicals.

In the present study the herb, Sansevieria roxburghiana was selected to evaluate its antibacterial, antioxidant and anticancer properties. *S. roxburghiana* is used in the treatment of ear infection, earache, toothache, hemorrhoids, ulcers and intestinal worms and are strongly implicated in traditional medical practices. Even though the leaves and rhizomes are used in traditional medicine against several disorders, especially those against microbial infections, no systematic study on the nature of the antimicrobial action and the phytochemicals responsible for this action has been reported. The medicinal properties of *S. roxburghiana* are due to the presence of secondary metabolites in it. In the present study the medicinal properties of this plant is explored to identify its bioefficacy.
Phytochemical screening revealed the presence of alkaloids, saponins, proteins and phytosterols, flavonoids, glycosides, anthocyanin, betacyanin, steroids, tannins and phenols in leaves of *S. roxburghiana*. 

In rhizome extracts of *S. roxburghiana*, presence of saponins, alkaloids, glycosides, proteins, anthocyanin, betacyanin, phytosterol, steroids and carbohydrates were revealed. Tannins, phenols and flavonoids were not present in different solvent system of rhizome extracts.

Susceptibility testing by disc diffusion assay exhibited broad spectrum of antimicrobial activity of methanol and acetone extracts of leaves of *S. roxburghiana* against Gram-positive bacteria such as *M. luteus*, *B. cereus*, *E. faecalis*, *S. aureus*, Gram-negative bacteria such as *P. vulgaris*, *P. aeruginosa*, *S. typhi*, *P. fluorescens*, *S. paratyphi*, *K. pneumoniae*, *S. sonnei*, *E. coli* and fungal strains *C. neoformans* and *C. albicans*.

The intensity of the antimicrobial action varied depending on the microorganism. Ethyl acetate extract of rhizomes only exhibited appreciable antimicrobial activity against most of the pathogens tested. The aqueous extract of leaves and rhizomes showed less antimicrobial activity.

Minimum Inhibitory Concentration (MIC) of the methanol extract by Agar Dilution method ranged from 1.0 to 8.0 mg/ml against the tested pathogens. Acetone and ethyl acetate extracts required high concentrations than methanol extract to show the inhibitory effect against microorganisms. As rhizome extracts showed less pronounced antimicrobial activity.
compared to leaf extracts, further studies were carried out with leaf extracts only.

As methanol and acetone extracts showed the presence of most of the phytochemicals tested and due to their higher antimicrobial activity, methanol and acetone extracts were subjected to TLC to isolate the fractions in two different solvent medium. The result showed wide range of Rf values from 0.1 to 0.8 which signifies the presence of different secondary metabolites in this plant.

The antibacterial activity of chromatographically separated fractions of leaves of *S. roxburghiana* was investigated. Two fractions out of the three showed pronounced activity at 1mg/ml against Gram positive and Gram negative bacteria.

*In-vitro* antioxidant potential of methanol, acetone and ethyl acetate extracts of *S. roxburghiana* of leaves using DPPH and NO radical scavenging activity method, showed significant percentage of inhibition in a dose dependent manner with BHT (butylated hydroxytoluene) as a standard reducing agent. It is evident from the results that the different solvent extracts of *S. roxburghiana* exhibited good antioxidant effect and strong free radical scavenging effects on a battery of free radicals and oxidants (DPPH, NO).

Methanol extracts of *S. roxburghiana* showed pronounced antioxidant activity than acetone and ethyl acetate extracts which could be attributed to the presence of alkaloids, steroids, flavonoids and saponins.
All the solvent extracts studied showed appreciable antioxidant activity which might be due to the synergistic effect of the compounds present.

As methanol extract of *S. roxburghiana* leaves showed better antimicrobial and antioxidant activity compared to other solvent extracts, the anticancer activity of *S. roxburghiana* was tested using the methanol extract on HepG2 cancer cell line and it was compared with normal 3T3 cell line to find out the non toxic dose of methanol extract. Normal 3T3 cell line using MTT assay showed cell viability of 92.2% at 125 µg/ml which decreased with increase in concentration of leaf extract.

Anticancer activity of leaf extracts of *S. roxburghiana* on HepG2 cancer cell line showed potent cytotoxic activity. The inhibition with regard to cytotoxicity was found to be 81% at 500 µg/ml which was comparable to positive control cyclophosphamide, that showed a cytotoxicity of 85%.

Therefore the minimum effective concentration of methanol extract of leaves that was non-toxic to 3T3 cells but toxic to HepG2 cells (IC$_{50}$) was recorded at a concentration lesser than 100 µg/ml of the extract.

The results of this study showed that the methanol leaf extract of *S. roxburgiana* at 125 µg/ml was non-toxic to normal cells and also had both anticancer and anti-proliferative activities against cancerous cell. This study points to the probable anticancer potentials of methanol extract of *S.roxburghiana* leaves.
The leaf extract of *S. roxburghiana* has been shown to contain glycosides, alkaloids, steroids and saponins which might be responsible for strong antioxidant and anticancer activity.

The mechanism of anticancer activity was studied to determine whether the methanol extract of this plant exerted an inhibitory effect on cancer cell proliferation and caused cell death. The results of this study suggest that methanol extract of *S. roxburghiana* possess moderate cytotoxic effects on liver cancer cells.

In the present study, it was found that the methanol extract of *S. roxburghiana* was cytotoxic and induced apoptosis in HepG2 cells by caspase-3 and caspase-9 activity only whereas caspase-8 showed no activity suggesting, extrinsic apoptotic pathway is probably not involved in this process.

The cancer cells exposed to methanol extract of *S. roxburghiana* exhibited morphological and biochemical changes that characterize 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, the results of caspase activity and DNA fragmentation, confirms the potential of *S. roxburghiana* as an agent of chemotherapeutic and cytostatic activity against human liver cancer cells.

Since the TLC results of methanol and acetone fractions showed similar Rf value, GC-MS analysis was carried with only methanol fraction. GC-MS spectra strongly indicated the presence of 16 bioactive
phytochemical compounds present in methanol extract. These compounds could also be responsible for the antimicrobial, antioxidant and anticancer properties of this plant.

Thus, the present study strongly establishes the medicinal properties of the plant, *Sansevieria roxburghiana*, and scientifically validates folkloric use of this plant as a remedy for various infections.

The results obtained from phytochemical screening, antioxidant, antimicrobial and anticancer activity of *S. roxburghiana*, indicate this plant as a “natural herbal source” which can be used in pharmaceutical industry.