CHAPTER - V

DISCUSSION
Since ancient times, plants have been model source of medicines as they are reservoir of chemical agents with therapeutic properties. The general population is increasingly using herbal medicines as dietary supplements to relieve and treat many different human disorders.

In India, the use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times. In modern medicine, plants occupy a very significant place as raw material for some important drugs, although synthetic drugs and antibiotics brought about a revolution in controlling different diseases. 80% of the world’s population depends on traditional medicine for primary health care. Medicinal plants in the form of whole plant extracts or active ingredients make up the major part of traditional medicines.

So the present investigation mainly dealt with the study of the effect of antibacterial activity of various extracts of *Gymnema sylvestre* plant against different pathogens isolated from clinical samples from the patients suffering from respiratory tract infections, the Antioxidant capacity, Phytochemical constituents, LC-MS analysis and Antiulcer activity.

In the present investigation, human pathogens from various clinical samples like sputum, throat and nasal swabs were collected from the patients suffering from respiratory tract infections as the nasopharynx is reported to be the chief carrier of common respiratory pathogens (Belliveau 1973).

The human pathogens identified were *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, based on the colony appearance, pigmentation on selective media and the characterization by Gram staining, Motility Test and Biochemical Tests.
The virulence factors in the isolates were studied by Cell Surface Hydrophobicity, Protease Enzyme Production Test, β Lactamase Test, Slime (Biofilm) Activity Test. Antibiotic sensitivity tests was also conducted for the bacterial isolates, where in, the following results were obtained.

Cell Surface Hydrophobicity: *Pseudomonas aeruginosa* showed the maximum Cell Surface Hydrophobicity.

Protease enzyme production: Sputum sample isolates showed highest percentage of protease activity where as throat samples isolates showed lowest activity.

β lactamase production: The good amount of activity was observed in *Pseudomonas aeruginosa, E.coli* and *Klebsiella pneumoniae* and less activity in *Staphylococcus aureus*.

Slime production (Biofilm): *E.coli* had highest activity and lowest was observed in *Klebsiella pneumoniae*.
Slime production has been reported in strains of all *Staphylococcus* spp. associated with the infection of biomedical devices (Kloos et al., 199; Gara et al., 2001).

Antibiotic Sensitivity Test: The respiratory samples isolates were subsequently tested for antibacterial drug resistance based on Kirby-Bauer disk diffusion method against the antibiotics like Tetracycline, Ciprofloxacin, Co-trimaxozole, Ampicillin Nitrofurantoin, Nalidixic acid, Norfloxacin, Penicillin, Tobramycin and Kanamycin. Almost all the isolates were resistant to one or more antibiotics.
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Similar test was conducted on *Pseudomonas aeruginosa* collected from clinical and environmental samples against various antibiotics and the common resistance pattern of cefotaxime, gentamicin and tetracycline (21.6%) was reported. (Ndip et al., 2005)

The plant material used in the present study was *Gymnema Sylvestre* plant belonging to Asclepediaceae family which was collected from G.K.V.K, Bangalore. It was authenticated by Dr. Sathyanarayana Prof. & Head, (dated 9/5/13, Ref no. Hort./45/2013-14), Department of Horticulture (Medicinal and Aromatic Plants Section), University of Agricultural Sciences, G.K.V.K Bangalore-65. The leaves were processed and subjected to proximate analysis test.

*Gymnema Sylvestre* showed the presence of following contents in the proximate analysis test: Moisture (9.06%), Protein (26.28%), Crude Fat (3.18%), Crude Fibre (15.05%), Total Ash (12.02%), Carbohydrate (33.71%) and Energy (386.09%). This plant is used for treatment of many problems like hyperglycemia, obesity, high cholesterol levels, anemia and digestion, constipation, jaundice, asthma, bronchitis, conjunctivitis, leucoderma. (Chopra et al., 1992; Vaidyaratnam 1995). According to the Sushruta of the Ayurveda it helps to treat Madhumeha i.e. glycosuria. It is also reported that traditionally these leaves are used to control the diabetes mellitus and stomach ache and even used as diuretic agent. (Reddy et al., 2004).

In this study, Antibacterial activity of *G. sylvestre* Leaf extracts was studied by using different solvent extracts such as Petroleum ether, Chloroform, Acetone, Methanol and Ethanol by Agar well diffusion method.

Among the five extracts, methanol and ethanol extract were more effective against all the four bacterial pathogens i.e. *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, than Petroleum ether,
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Chloroform, Acetone extracts. The zone of inhibition could be matched with that of standard antibiotics like tetracycline.

*Pseudomonas aeruginosa* showed maximum zone of inhibition in methanol, ethanol, Chloroform and Petroleum ether extract where as *E.coli* showed maximum zone of inhibition in acetone extract. These results indicate that *Gymnema sylvestre* is a broad spectrum antibacterial agent which proved the finding of Segarajah Ahalya et al., (2011) in the study of the inhibition action of bacterial pathogens by *G. sylvestre* leaf extract.

The saponin fractions of *G.sylvestre also have shown* significant antibacterial and antifungal activity against *P. aeruginosa, E. coli, S. typhi, K. pneumoniae, P. mirabilis, S. aureus* and for fungal pathogens as reported by Venkatesan Gopiesh khanna 2008.

The antibacterial activity was reported to be good in organic solvents extracts of *G. Kollimalayanum* when compared with that of aqueous extract against eight Gram-negative (*Klebsiella pneumoniae, Proteus vulgaris, Salmonella paratyphi, Shigella boydi, S. brunci, S.dysentriae, Vibrio parahaemolyticus, Yersinia enterocolitica*) and two Gram-positive (*Cornybacterium diptheriae and Enterococus faecalis*) bacterial strains by disc diffusion method (Ramachandran et al., 2010).

In the present research, the leaf powder was prepared, extracted with Methanol, Ethanol, Petroleum ether, Chloroform, Acetone. Various concentrations of each solvent extract were assessed by DPPH Method for its Antioxidant activity.

Among five solvent extracts used, the Methanol extract displayed highest antioxidant activity (67.5%). Highest antioxidant activity in methanolic extract was also reported by Laxmi Ahirwal et al., 2013 in the *in vitro* Antioxidant studies of *G. sylvestre*. 
The results suggest that *G. sylvestre* can be used as a source of antioxidants for pharmacological preparations and it might be due to the presence of the phytochemicals like alkaloids, tannins, saponins, flavonoids, glycosides and carbohydrates.

The results of *in vitro* antioxidant activity of essential oil isolated from leaves of *Gymnema sylvestre* by DPPH method was in a dose dependent manner (Dattatraya *et al.*, 2011).

In the present investigation, the screening of various phytoconstituents was done in different solvent extracts of *G.sylvestre* leaves by various Qualitative Chemical Tests. The following phytochemicals i.e. Alkaloids, Flavanoids, saponins, Steroids, Tannins, Glycosides, Terpenoids, Triterpenoids, Phenolic compounds were found to be present. The presence of these phytochemicals is an indicator that the plant can serve as a potential source of precursors in the development of the drugs.

The findings about the phytochemical constituents obtained here are similar to the findings obtained by Yasa Swetha (2012) in Phytochemical studies of *Gymnema sylvestre*.

Shafi Thompson *et al.*, 2011 reported the presence of steroids/ terpenoids and coumarins in the Hexane and Chloroform extract of *Gymnema sylvestre*.

Quantitation of Gymnemagenin from *Gymnema sylvestre* using HPTLC (Puratchimani *et al.*, 2004; Valvirathi *et al.*, 2006) HPLC, RP-LC method has been reported earlier. A very recent method i.e., LC/MS Method has been used in the present investigation. As the technique used had high sensitivity and great selectivity and it proved to be highly applicable and extraordinarily powerful analytical tool. It enables effective analysis of complex matrices.
Therefore, the present research included the isolation of the compound Gymnemagenin (Saponin), which was accomplished by LC/MS Analysis. Identification and peak assignment of the isolated compound was done by comparing its Retention time with corresponding Standards. The LC–MS analysis provided full fingerprints of the principal compound gymnemagenin in the medicinal plant *Gymnema sylvestre*.

In the present study, the Ehanolic Leaf Extract (ELE) of *G. sylvestre* was investigated for antiulcer activities in Wistar rats against Ethanol, Pyloric ligation and Indomethacin induced ulcer.

The antiulcer activity of ELE was evidenced by the significant attenuation of gastric volume, pH, free acidity & total acidity in the gastric juice in a dose dependant manner. The findings indicate that ELE of *G. sylvestre* possess active principle (S) that protects gastric mucosa against induced gastric changes possibly due to its antioxidant effect. Thus the present study confirms the statement of Salim *et al.*, 2012 who found the protective effect of of *G. sylvestre* leaves ethanol extract on gastric mucosal injury in rats, exhibited by mucous production, increase in nucleic acid and NP-SH levels, which might be mediated through its free radical scavenging ability.

The results of the study support to a certain degree, the traditional medicinal uses of the plants evaluated both for human and animal disease therapy and reinforce the concept that the ethnobotanical approach to screening plants as potential sources of antimicrobial substances is successful.
CHAPTER VI

SUMMARY & CONCLUSION
SUMMARY AND CONCLUSION

The present study includes,

- The effect of antibacterial activity of various extracts of *Gymnema sylvestre* plant against different pathogens isolated from clinical samples from the patients suffering from respiratory tract infections.
- Determination of Antioxidant capacity in different solvent extracts of *Gymnema sylvestre*.
- Screening the preliminary phytochemical constituents followed by secondary metabolites in leaf extracts of *Gymnema sylvestre* using different solvents.
- LC-MS analysis.
- Antiulcer activity of *Gymnema sylvestre* Leaf extracts.

1) The *Gymnema sylvestre* leaf extract was prepared and Proximate analysis was performed. It contained 9.06% of Moisture, 26.28% of Protein, 3.18% of Crude Fat, 15.05% of Crude Fibre, 12.02% of Total Ash, 33.71% of Carbohydrate and 386.09% of Gross Energy in the leaf.

2) Petroleum ether, Acetone, Chloroform, Ethanol and Methanol extracts were prepared using *G. sylvestre* by soxhlet apparatus. All the extracts were concentrated under reduced pressure by Rotary Vacuum Evaporator.

3) Antibacterial activity of Acetone, Chloroform, Ethanol, Methanol and Petroleum ether extract were evaluated against various bacterial pathogens (obtained from clinical samples) i.e. *Pseudomonas aeruginosa, Staphylococcus aureus, E.coli, Klebsiella pneumoniae* by Agar well diffusion method. The susceptibility of microorganisms to plant extracts was compared with each other and with standard Antibiotic like Tetracycline.
4) All the solvent extracts of *Gymnema sylvestre* leaf inhibited the growth of all the four Bacterial species tested in a dose dependent manner. Among the five types of solvent extracts Methanol and Ethanol extract showed the highest Antibacterial Activity.

5) Antioxidant activity was determined with various concentrations of the solvent extracts like Petroleum ether, Acetone, Chloroform, Ethanol and Methanol by DPPH Method and found the highest antioxidant activity in Methanolic extract.

6) Preliminary Phytochemical Screening was done in different extracts of *G. sylvestre* leaves for the analysis of phytochemical constituents by performing various Qualitative Chemical Tests. It revealed the presence of Alkaloids, Flavanoids, saponins, Steroids, Tannins, Glycosides, Terpenoids, Triterpenoids, Phenolic compounds.

7) The compound Gymnemagenin (Saponin) was isolated by LC-MS analysis. Identification and peak assignment of the isolated compound was based on comparison of its Retention time with corresponding Standards.

8) The Ehanolic Leaf Extract (ELE) of *G. sylvestre* was investigated for antiulcer activities in Wistar rats against Ethanol, Pyloric ligation and Indomethacin induced ulcer. The antiulcer activity of ELE was evidenced by the significant attenuation of gastric volume, pH, free acidity & total acidity in the gastric juice in a dose dependant manner. The findings indicated that ELE of *G. sylvestre* possess active principle (S) that protect gastric mucosa against induced gastric changes possibly due to its antioxidant effect.
Conclusion:

We have a firm belief that the study of medicinal plants as antimicrobial agents is necessary for gaining insight into medicinal flora and their real value, but the use of a standard methodology for investigation is very important. Likewise, the concentrations or dilutions used must be appropriate. Moreover, research in this area should be continued until the agent responsible for the activity or the most active fraction has been determined. Hence different kinds of studies on the mechanisms of action, interactions with antibiotics or other medicinal plants or compounds, and the pharmacokinetic profile of the extracts should be given high priority.

Today most of the pathogenic organisms are becoming resistant to antibiotics. As medicinal plants are safe and economic, it can safeguard us, to a certain extent against our natural enemies like bacterial pathogens i.e. they can be used as an alternative to treat infectious diseases. The exploration of secondary metabolites from plant sources seems to be an excellent choice for the development of new age antimicrobials, given the vast biodiversity in the subcontinent.

Therefore this alarming problem can be overcome by the discovery of novel active compounds against new targets as it is a serious matter of urgency. The same is shown in the present investigation. The different solvent extracts of *Gymnema sylvestre* have displayed good activity against pathogenic organisms and its protective effect on gastric mucosal injury of rats, exhibiting its potential in the development of new phytopharmaceuticals. As these drugs are plant based, they can be considered safe for human consumption.

Thus, the study of plants and its applications ascertains its value used in Ayurveda, which could be of considerable interest to the development of new drugs.