Any systematic study requires a preliminary review of existing related literature to identify research gap in the area and to provide direction to the researcher. Besides, literature survey helps to enrich research discussion.

In case of present study literature survey contributes to integrate theoretical and practical fund of knowledge that is required for the study of antimicrobial activity and phytochemical analysis in Gymnema sylvestre plant.

Medicinal plants have been curing various disorders in humans from the time immemorial and are considered to be intermittently associated as integral part of the Indian traditional medicinal system, better known as the Ayurvedic system of medicine (Basu, 2002). It is estimated that about 80,000 species of plants are utilized by the different system of Indian medicine (Prajapati et al., 2005). About three quarter of the world’s population relies on plants and their extracts for their healthcare. India represented by rich culture, traditions and natural biodiversity, offers a unique opportunity for drug discovery researchers (Jachak et al., 2007).

Traditional healers and medicine man in India practice and apply few medicinal plants for curing the ailments and are cheap as compared to pharmaceutical drugs (Singh, 2006). The first step towards this goal is the screening of plants used in popular medicine.

Thus antimicrobial research is geared towards the discovery and development of novel antibacterial and antifungal agents. Plant drugs are frequently considered to be less toxic and free from side effects than the synthetic ones (Momin, 1987).
Plants have potent biochemicals and have components of phytomedicine. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites (Lozoya et al., 1989; Karthikeyan et al., 2009). They are grouped as alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrate and essential oils. Every detail of the composition and development of vegetation is dependent on the environmental factors.

Due to limited antimicrobial spectrum, drug resistance in pathogens and serious side-effects of antibiotics, efforts are being directed to identify plant products which have broad spectrum antimicrobial properties and no ill-effects. (Banginwar et al. 2003; Farnsworth, 1998).

**BRIEF HISTORY**

Medicinal plants played an important role in Indian culture since Rigveda (5600 BC) where about 67 medicinal plants were recorded. It is estimated that 80% of about 4 billion population have to rely on traditional medicines due to high cost, lack of availability of required medicines and personal preferences. Out of 2,50,000 higher plants more than 80,000 have medicinal value and India occupies unique position among world’s 12 mega biodiversity centers.

It is identified that about 20,000 plants have good medicinal value and 7500 species are used by traditional communities.

It is estimated that Ayurveda and Unani system of medicine uses about 700 species each, Sidda and Anchi uses 600 species each whereas modern medicine uses only 30 species of medicinal plants. The Bible offers descriptions of approximately 30 healing plants. The fall of ancient civilization forestalled Western advances in the
understanding of medicinal plants, with much of the documentation of plant pharmaceuticals being destroyed or lost (Stockwell, 1998). During the Dark Ages, the Arab world continued to excavate their own older works and to build upon them. Of course, Asian cultures were also busy compiling their own pharmacopoeia. In the West, the Renaissance years saw a revival of ancient medicine, which was built largely on medicinal plant Herbal drugs, in India are also used as household remedy for common ailments since time immemorial. Our ancestors have a profound knowledge of these medicinal plants and they knew innumerable remedies, a fact indicated in the writings of Siddhars of Tamil Nadu. Their expertise if documented properly would help the modern man find more effective prophylactic use of these herbs.

Since time immemorial people have tried to find medications to alleviate pain and cure different illnesses. In every period, every successive century from the development of humankind and advanced civilizations, the healing properties of certain medicinal plants were identified, noted, and conveyed to the successive generations. The benefits of one society were passed on to another, which upgraded the old properties, discovered new ones, till present days. The continuous and perpetual people's interest in medicinal plants has brought about today's modern and sophisticated fashion of their processing and usage.

Mainstream medicine is increasingly repetitive to the use of antimicrobial and other drugs derived from plants, as traditional antibiotics (products of microorganisms or their synthesized derivatives) become ineffective and as new, particularly viral diseases, remain intractable to this type of drug. Another driving factor for the renewed interest in plant antimicrobials in the past 20 years has been the rapid rate of (plant) species extinction (Lewis et al, 1995). There is a feeling among natural products Chemists and Microbiologists alike that the multitude of potentially useful phytochemical structures which could be synthesized chemically is at a risk of being lost irretrievably (Borris, 1996).
There is a scientific discipline known as ethnobotany (or ethnopharmacology), whose goal is to utilize the impressive array of knowledge assembled by indigenous people about the plant and animal products they have used to maintain health (Georges et al., 1949; Rojas et al., 1992; Silva et al., 1996; Vanden Berghe et al., 1986). Lastly, the ascendancy of the human immunodeficiency virus (HIV) has spurred intensive investigation into the plant derivatives which may be effective, especially for use in underdeveloped nations with little access to expensive Western medicines.

The relevance of pharmacognosy in standardization of herbal drugs was long been stressed. Many monographs on pharmacognostic have emerged as an aid in the pharmacognostic investigations (Kalidass et al., 2009a; Edward, 1956). The process of standardization can be achieved by stepwise pharmacognostic studies. These studies help in identification and authentication of the plant material (Ozarkar, 2005).

Since plants may contain hundreds to thousands of metabolites, there is currently a great interest in the medicinal plant research as a possible source of new lead compounds for introduction into therapeutical screening and one such plant is Gymnema sylvestre, which is the important anti-diabetic medicinal plant, there is a growing demand of it in the pharmaceutical trade. Gymnemic acid, the active ingredient of this plant, is extracted from leaves and used widely as anti-diabetic (Shanmugasundaram et al., 1983), anti-sweetner (Kurihara, 1992) and anti- hypercholesterolemia (Bishayee et al., 1994). It also has stomachic, diuretic and cough suppressant property (Kapoor, 1990; Sastri 1956). The plant has been reported to possess antimicrobial and ethnoveterinary medicinal properties (Kalidass et al., 2009b). In addition, it possesses antimicrobial, hepatoprotective, and anti-saccharine activities (Nadkarni et al., 1976; Komalavalli et al., 2000). Hence, because of these properties, Gymnema sylvestre is most important for plant prospecting. Gymnema sylvestre belongs to “Asclepiadaceae” family, Laticiferous genus.
The details of *Gymnema sylvestre* with special reference to its activity for antibacterial, antioxidant, phytochemical and antiulcer have been discussed in the light of available literature as given below.

**Characterization of Respiratory Tract Infecting Bacteria**

Respiratory tract infections are one of the major public health problems, affecting both children and adults. They are mainly caused by viruses or bacteria that often interact with one another. Although their presence is a prerequisite for subsequent infections, viruses and bacteria may be present in the nasopharynx without causing any respiratory symptoms.

Respiratory tract infections proves to be more serious when located in the lower respiratory tract, when compared to the upper respiratory tract infections (URTIs). It is estimated that 5% of respiratory infections involve the lower respiratory tract (LRT), while the rest are limited to the upper respiratory tract.

In the medical literature it is stated that Gram-positive bacteria are the major culprits causing LRTIs in children (Farha *et al.*, 2005). Children with LRTIs may present life-threatening complications, such as massive parapneumonic or pleural effusion, sepsis, empyema, pericarditis with cardiac tamponade and venous thromboembolism. (Aydemir *et al.*, 2008; Espínola Docio *et al.*, 2008; Pirez *et al.*, 2001; Langley *et al.*, 2008; Al-Sabbagh *et al.*, 2008). Many of these deaths and complications occurring can be prevented by simple inexpensive measures such as early diagnosis and application of appropriate antimicrobial therapy.
A variety of microorganisms can cause lower respiratory tract infections (LRTIs) in children, including bacteria, viruses, parasites, or fungi. *Streptococcus pneumoniae* is by far the most common bacterial cause of pneumonia in young children, while *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* are frequently encountered among older children and adolescents. Group A *streptococcus, Staphylococcus aureus, Haemophilus influenzae* type B and *Moraxella catarrhalis* are less frequently seen.

Upper respiratory infections are common in all parts of the tropics, and are usually caused by viruses and are mostly self-limiting. The combination of poverty, poor sanitation and over-crowding leads to the fast spread of upper respiratory infections. Exposure to many upper respiratory pathogens occurs at a younger age on average in most developed countries. While these infections may not cause significant direct morbidity but, they contribute to a higher prevalence of consequent hearing impairment. It is possible that a higher incidence of acute upper respiratory infection may be part of the reason for the higher prevalence and severity of bacterial pneumonia in urban tropical settings. Bacteria are responsible for the majority of currently treatable infectious disease. More than 200 species of bacteria colonize on upper respiratory tract (Nadel *et al.*, 1999) of which *Staphylococcus aureus, Streptococcus pneumoniae, Neisseria meningitides, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Haemophilus influenzae* were potential pathogens (Todar, 2008). These bacteria are responsible for various respiratory illness of human being.

Treatment of the illness may not be judicious if proper identification of the causal agent is not performed perfectly. Moreover, multidrugs resistant strains are being developed due to indiscriminate use of antibiotics irrespective of the identification of causal agents.

Keryn Christiansen, 1996 described the methods of treating common lower respiratory tract infections with the main focus on pneumonia (*Streptococcus*
pneumoniae, Mycoplasma pneumoniae, Chlamydia pneumoniae or Legionella spp.) and bronchitis (Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis).

Gislene et al., 2000 revealed the importance of plant extracts when associated with antibiotics, to control resistant bacteria like, Staphylococcus aureus, Salmonella choleraesuis, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans, Proteus spp., Klebsiella pneumoniae, Shigella spp., Enterobacter aerogenes, Escherichia coli, which are becoming a threat to human health.

Renders et al., 2001 reviewed the dynamics of the bacterial populations inhabiting the respiratory tract of patients with cystic fibrosis (CF) lung with the main focus on Staphylococcus aureus, Haemophilus influenzae and Pseudomonas aeruginosa with the main focus on the technology used for microbial characterization and also the evolutionary adaptation of pathogens to the CF lung.

Coenye et al., 2002 characterized 51 bacterial isolates recovered from respiratory secretions of cystic fibrosis (CF) patients, using a polyphasic approach (including cellular protein and fatty acid analysis, biochemical characterization, 16S ribosomal DNA sequencing, and DNA-DNA hybridizations) like Acinetobacter sp., Bordetella hinzii, Burkholderia fungorum, Comamonas testosteroni, Chryseobacterium sp., Herbaspirillum sp., Moraxella osloensis, Pandoraea genomospecies 4, Ralstonia gilardii, Ralstonia mannitolilytica, Rhizobiumradiobacter, and Xanthomonas sp.

Steven Myint 2002 reviewed the recent advances (molecular techniques like PCR, RAPD) in the rapid diagnosis of respiratory tract infections, which have applicability across the range of microbial pathogens and mentioned that the essential part of rapid microbiological diagnosis is the collection and transport of the adequate
specimen with in depth knowledge of the basis of new technologies for the correct interpretation of result.

Salvatore et al., 2007 studied the respiratory tract infections with *Mycoplasma pneumoniae* in interleukin-12 knockout mice, evaluated the effects of IL-12 therapy on microbiologic, inflammatory, and pulmonary function indices of *M. pneumoniae* pneumonia in mice. Then concluded that the administration of exogenous IL-12 during *M. pneumoniae* lower respiratory tract infection significantly increases disease severity by both microbiologic and pulmonary parameters.

Michael et al., 2007 described the rapid and sensitive genotyping assay and microarray for upper respiratory tract infections (URTIs) using standard amplification and hybridization techniques, with electrochemical detection (ECD) on a semiconductor-based oligonucleotide microarray and detected four bacterial pathogens (*Bordetella pertussis*, *Streptococcus pyogenes*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*) and 9 viral pathogens (adenovirus 4, coronavirus OC43, 229E and HK, influenza A and B, parainfluenza types 1, 2, and 3 and respiratory syncytial virus.

Henrique Coutinho et al., 2008 described the Cystic fibrosis to be the most common and best known genetic disease with a defect in transepithelial Cl- transport by mutations in the CF gene on chromosome 7, which codes for the cystic fibrosis transmembrane conductance regulator protein (CFTR), caused by the bacterial pathogens *B. cepacia*, *P. aeruginosa* and *S. aureus* with the symptoms in the lungs, augmenting the risk of bacterial infection.

Islam et al., 2010 isolated the bacteria from the respiratory tract of man and identified them based on their morphology, staining, motility, cultural and biochemical properties and subjected to characterize their pathogenicity and antibiotic sensitivity. Among the isolates *Staphylococcus*, *Klebsiella* and *Pseudomonas* were the predominant species.
Avalos-Tellez et al., 2010 reviewed on the information available regarding microbiological aspects of bottlenose dolphins (Tursiops truncatus) describing the pathogenic bacteria associated with stranded dolphins, to comprehend their role in dolphins and people who works in straight contact with them.

Giorgiana Brad et al., 2011 reviewed on the bacterial pathogens (like Pseudomonas aeruginosa, Klebsiella pneumoniae and Enterobacter, Staphylococcus aureus) isolated from sputum, tracheal or bronchial aspirates and pleural effusion, and their antibiotic susceptibility by standard bacteriological techniques of (LRTIs) Lower respiratory tract infections in children.

Fred C. Tenover et al., 2011 compared the Current diagnostic methods which are slow and often of marginal value for patient management if the adequacy of the specimen is not confirmed before culture, with developing Molecular Amplification Methods for Rapid Diagnosis of Respiratory Tract Infections Caused by Bacterial Pathogens, which are highly sensitive, can provide results in hours rather than days but may not distinguish colonization from infection unless a quantification step is included.

Manuel Medell et al., 2012 studied the Characterization and sensitivity of the most prevalent bacteria {Acinetobacter spp. (25.2%), Pseudomonas spp. (18.3%) and Klebsiella spp. (9.4%)} to antibiotics isolated from the lower respiratory tract of ventilated patients admitted at the intensive care units of the hospital and stated that these nosocomial infections are a relevant medical problem.

Sangeeta Raut et al., 2012 studied the Isolation and characterization of protease producing bacteria from upper respiratory tract of wild chicken suffering from influenza and detected the proteolytic activity by sodium dodecyl sulfate polyacrylamide.
gel electrophoresis (SDS-PAGE) and zymogram analysis. The desired protein was precipitated from the crude extract by using ammonium sulfate (60%) followed by dialysis and purified by Ion-exchange chromatography.

Abe Ayotunde et al., 2012 identified and characterized gastrointestinal Pathogens (Salmonella spp., Shigella spp., Escherichia coli), and respiratory tract pathogens (Klebsiella spp., Pseudomonas aeruginosa) a total of five bacterial air pathogens which was carried out in some strategic houses cutting across five zones in Zaria metropolis as it was a great concern to the health of the inhabitants residing in Zaria as the pathogens could be life threatening both in children and adult if not diagnosed properly on time and appropriate antibiotic administered to treat these infections associated with these pathogens.

Astrid Bosch et al., 2013 reviewed the current knowledge regarding specific bacterial–bacterial and viral–bacterial interactions that occur in the upper respiratory niche, and the mechanisms by which these interactions might be mediated. Though the colonization of both, bacteria and viruses of respiratory tract is mostly asymptomatic, synergistic and competitive interspecies but still interactions appear to occur, potentially influencing and disturbing the natural equilibrium of the complex microbiota at the nasopharyngeal niche.

**Plant Material**

A number of plants from different families of angiosperms have been reported to show antimicrobial activity (Palombo et al., 2001; Janagakumari et al., 2005). Traditional medicines have been accepted as an alternative form of health care. The microbial resistance to the available antibiotics has led to investigate the antimicrobial activity of medicinal plants. The increasing failure of chemotherapeutics and antibiotic
resistance exhibited by pathogenic microbial infectious agents has led us to screen several medicinal plants for their potential antimicrobial activity. (Elizabeth, 2005; Kelmanson et al., 2000; Srinivasan et al., 2001).

In the present study, the selection of *Gymnema sylvestre* plant for estimate was based on its traditional usages. Although very small number of works have been done on the antimicrobial activity of this endangered medicinal plant (Devi et al., 2010), it needs further study for verification of its activity against disease-causing microorganisms.

The present review highlights the various folk, ayurvedic uses and pharmacognostical, phytochemical and pharmacological studies conducted on *G. sylvestre*.

Porchezhian et al., 2003 studied an overview on the advances of *Gymnema sylvestre* with respect to its chemistry, pharmacology, patents and reported the use of plant extracts in Japanese, Australian, and Indian folk medicine. Matthew, 2007 provided a systematic review of *Gymnema sylvestre* to give the evidences to support the use of Gymnema extracts so that it can be used for the treatment of diabetes mellitus as the existing treatment options are costly, and have limited, palliative effects.

Shailendra Gurav et al., 2007 reviewed the literature on *Gymnema sylvestre* with respect to its pharmacognostic characters, traditional uses, and chemical constituents, the other aspects like toxicology, precautions and stated its use as a diuretic in Indian proprietary medicines.

Ahalya et al., 2009 assessed and found 20 different fauna out of which 15 were plant feeders, the rest 5 were beneficial, associated with the medicinal herb *G. sylvestre*, in Jaffna, to make awareness to the public especially the diabetic patients who consume raw leaves and for the industries to prepare it in powder form.
Renu Paliwal et al., 2009 studied the effect of gurmar leaf powder intervention on the blood glucose level of 20 non-insulin dependent diabetic women, and concluded that gurmar powder is effective in lowering the fasting as well as postprandial blood glucose levels.

Ankit Saneja et al., 2010 highlighted the various ethnobotanical and traditional uses of *G. sylvestre* plant and mentioned about the phytochemical constituents, revealed some notable pharmacological activities of the plant such as antidiabetic, antimicrobial, antiobesity, anti-inflammatory, hypolipidaemic, free radical scavenging activity. Bharti Sharma et al., 2010 developed, a simple and efficient protocol for direct plant regeneration from apical bud explants of *G. sylvestre* as its extensive use has resulted in overexploitation and the plant is rare in many states of India.

Damini Mahajan et al., 2011 explained the ethnobotanical and pharmacological uses of *Gymnema sylvestre* due to the presence of compounds like Gymnemic acid A, B, C, D, deacyl gymnemic acid, gymnemagenol, to provide better scope for performing the in-vivo experiments and their application in future.

Najafi et al., 2011 studied the *G. sylvestre* plants growing under various ecological conditions and found histological similarities, but size and thickness of leaves, quantity of gymnemic acids, alkaloids and protein content were different and the analysis of root zone soil indicated the positive correlation between available k and gymnemic acids content of *Gymnema sylvestre*.

Vaidya, 2011 reviewed on the bioactives and some facts and mechanism which make *Gymnema sylvestre* (Ayurvedic herb) as an effective remedy for cure of diabetes. Ashok K. Pandey, 2012 revealed that *G.sylvestre* can be cultivated successfully by seeds as well as by rooted cuttings at a spacing of 50 x 50 cm and showed, that pretreatment of
seeds in cold water for 24 hours, improved the seed germination. For vegetative propagation, hard wood cuttings of 10-15 mm diameter having three nodes were found most suitable. Among hormonal treatments, dipping of cuttings in 500 ppm IBA solution for 30 min was suitable for maximum rooting (52.50%).

Sabitha Rani et al., 2012 reviewed the various morphological, medicinal, chemical, pharmacological activities and biotechnological aspects of *G.sylvestre* and stated its wide use in traditional medicines to treat obesity, diabetes and other major diseases and urged the need to conserve and cultivate this important medicinal plant.

Pratibha Gupta et al., 2012 showed that the tissue culture technique represents an important potential for its propagation over conventional methods for improvement, conservation and large-scale planting of *G.sylvestre* a medicinally important plant and also revealed the maximum seeds germination from immature (green) seeds, incubated longer dark period in comparison to mature or dry seeds.

Magda Abbaker Osman et al., 2013 estimated the level of diversity and genetic relationship among eleven progenies of *G. sylvestre* in relation to their parent, forty random decamer primers were used for RAPD analysis. A total of 56 detectable bands were obtained out of which 31(55.4%) were polymorphic, 6 (10.7%) were unique and 19 (33.9%) were monomorphic.

**Antibacterial Activity**

Rising appearance of resistance to the presently existing antibiotics has necessitated sustained search for new antimicrobial compounds.

Anti-microbial agents are undeniably one of the most important therapeutic discoveries of the 20th century. However, with the ‘antibiotic era’ barely five decades old, mankind is now facing the global problem of emerging resistance in virtually all
REVIEW OF LITERATURE

pathogens (Peterson et al., 2004). During the last decade, the use of traditional medicine has expanded globally and is gaining popularity. Traditional medicines are used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system (Lanfranco, 1999). The herbal medicines serve the health needs of about 80% of the world’s population, especially for millions of people in the vast rural areas of developing countries; more than 65% of the global population uses medicinal plants as a primary health care. (WHO, 2001)

In recent years, many possible sources of natural antibiotics have been in use for several infectious diseases, mostly bacterial and fungal. In view of this, the searches for new anti-microbial agents from medicinal plants are even more urgent in the countries like India where infectious diseases of bacterial origin are not only rampant, but the causative agents are also developing with an increasing resistance against many of the commonly used antibiotics (Abebe et al., 2003). Considering the high costs of the synthetic drugs and their various side effects, the search for alternative products from plants used in folklore medicine is further justified. It is believed that plants which are rich in a wide variety of secondary metabolites belonging to chemical classes such as sterols, alkaloids, glycosides, saponins, flavonoids, tannins, and carbohydrates are generally superior in their anti-microbial activities (Cowan, 1999). Leaves and flower of experimental plants have been used for treating many diseases in traditional medicines.

Nishanta Rajakaruna et al., 2002 screened thirty-two plant species collected from highly stressful serpentine (ultramafic) environments in Sri Lanka against three Gram-positive and two Gram-negative bacteria, a non-acid fast bacterium, and the yeast Candida albicans and found, they having altered antimicrobial activities when compared to their relatives from non-serpentine environments, so urged the need to pay attention to substrate, habitat, etc., when collecting plants to test for antimicrobial properties.
Satdive et al., 2003 demonstrated the antimicrobial activity in ethanolic extract of *Gymnema sylvestre* leaves against *Bacillus pumilis, B. subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus* and inactivity against *Proteus vulgaris* and *Escherichia coli*. Sehgal et al., 2005 showed that the antifungal activity was due to the presence of enzymes and stable cysteine proteases in the latex of *calatropis procera* belonging to Asclepiadaceae family.

Parekh et al., 2007 screened thirty-four Indian medicinal plants belonging to 28 different families for potential antibacterial activity against six bacterial strains belonging to Enterobacteriaceae, viz. *Enterobacter aerogenes* ATCC13048, *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* NCIM2719, *Proteus mirabilis* NCIM 2241, *Proteus vulgaris* NCTC8313, and *Salmonella typhimurium* ATCC23564, using aqueous and alcoholic extracts by Agar disc diffusion method and Agar well diffusion method and found the alcoholic extracts to be more active than aqueous extracts.

Rohini Kiran Kunta et al., 2009 confirmed that the *G. sylvestre* extracts of ethanol, methanol and water had good antimicrobial activity against bacterial pathogens like *Bacillus subtilis, E. coli, Pseudomonas aeruginosa, Proteus vulgaris* and *Staphylococcus aureus*.

Sankar Narayan Sinha et al., 2010 showed that the petroleum ether, chloroform and ethanol solvent extracts of *Gymnema sylvestre* leaf exhibited considerable antibacterial activity against the three Gram positive (*Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus*) and five Gram negative (*Escherichia coli, Vibrio cholerae, Pseudomonas aeruginosa, Shigella dysenteriae* and *Shigella flexneri*) tested bacteria.

Parimala Devi et al., 2010 showed that the methanol extract of *Gymnema sylvestre* had strong antimicrobial activity against *Streptococcus mutans, Staphylococcus*
aureus, Streptococcus mitis, Candida albicans with the zone of inhibition ranging from 12-23mm by Agar well diffusion method.

Shobha S. Borhade, 2011 evaluated the Antibacterial activity of the water extract of Gymnema sylvestre against E. coli, S. aureus at two different concentrations by the diffusion method and found S. aureus to be more active than E. coli.

Bhuvaneswari et al., 2011 assessed the antimicrobial activity of G. sylvestre in methanol as the solvent system for the extraction of active principles and showed that the gram positive organisms (B. subtilis, E. faecalis, S. aureus, S. epidermis) and gram negative (E. aerogene, E. cloacae, E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium) were susceptible towards the extracts.

Minal Wani et al., 2012 observed the methanolic extract of the leaves showing activity against all four tested microorganisms i.e, Escherichia coli, Serratia marcescens, Staphylococcus aureus and Candida albicans while aqueous leaf extracts found to be non effective against the four tested microorganisms.

Chinnaperumal Kamaraj et al., 2012 evaluated the antibacterial properties of 21 crude extracts from leaf and flower of Aristolochia indica (A. indica), Cassia angustifolia (C. angustifolia), leaf of Catharanthus roseus (C. roseus), Diospyros melanoxylon (D. melanoxylon), Dolichos biflorus (D. biflorus), Gymnema sylvestre (G. sylvestre) and Justicia procumbens (J. procumbens).

Sudhanshu et al., 2012 studied the antimicrobial properties of G. sylvestre against few bacteria and few fungi by using different solvents like petroleum ether, chloroform, benzene, ethyl acetate, ethanol and distilled water, and showed that Petroleum ether solvent extract of G. sylvestre was less active against all test microorganisms than five other solvent extracts.
Antioxidant Capacity

Medicinal plants contain many antioxidants like vitamins, carotenoids, flavonoids, polyphenols, saponins, enzymes and minerals (Ray et al., 2002). Natural antioxidants tend to be safer and also possess anti-viral, anti-inflammatory, anti-cancer, anti-mutagenic, anti-tumour, and hepatoprotective properties.

A number of reactive molecules such as superoxide radical (O$_2^-$), hydroxyl radical (OH), hydrogen peroxide (H$_2$O$_2$) and nitric oxide (NO) are regarded as reactive oxygen species (ROS) (Aruoma et al., 1989), that are generated by various biological redox reactions and can directly react with biological macromolecules such as proteins, lipids and DNA of healthy human cells and cause cell membrane disintegration, DNA mutation and protein damage, which can further create cancer, atherosclerosis, cardiovascular disease, liver injury, ageing and inflammatory disease (Braca et al., 2002). Almost all human cells are well protected against free radical damage by enzymes such as superoxide dismutase (SOD) and catalase or compounds such as ascorbic acid, tocopherol and glutathione (Niki et al., 1994).

But, sometimes, excessive generation of ROS beyond the capacity of antioxidant defense system leads to a variety of pathological processes such as inflammation, diabetes, genotoxicity and cancer (Gulcin et al., 2002). Antioxidants can interrupt the oxidation process by reacting with free radicals, chelating catalytic metals and also by acting as oxygen scavengers. However antioxidant supplements are important for human body due to their ability to combat oxidative damage.

In the past few years, some synthetic antioxidants like butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT), have been used as antioxidants. They are commonly used to preserve food. Restrictions on the use of these compounds are being
imposed because of their carcinogenicity. Thus, the interest in natural antioxidants has increased considerably (Velioglu et al., 1998). The replacement of synthetic with natural antioxidants (because of implications for human health) may be advantageous. Therefore, the search for natural antioxidants has received much attention to identify and develop more potent antioxidants of natural origin to replace synthetic ones. Different kinds of plant material have already been reported as natural antioxidants (Packer, 1997).

The regular consumption of fruit and vegetables containing natural antioxidants is correlated with decreased risks for diseases such as cancer and cardiovascular diseases (Jang et al., 2010). Epidemiological studies have shown that consumption of plant foods containing antioxidants is beneficial to health because it down-regulates many degenerative processes and can effectively lower the incidence of cancer and cardiovascular diseases (Arabshahi-Delouee et al., 2007).

Anup Srivastava et al., 2006 measured the antioxidant potential of the roots of Decalepis hamiltonii (Dh) by using methanolic and aqueous extracts. Both the aqueous and methanolic extracts inhibited microsomal lipid peroxidation and exhibited strong reducing power and metal chelating activity.

Maryam Zahin et al., 2009 evaluated antioxidant activity in methanolic extracts of Plumbago zeylanica (Root), Acorus calamus (Rhizome), Hemidesmus indicus (Stem) and Holarrhena antidysenterica (Bark), used in Ayurvedic medicines for number of ailments.

Prakash Veeru et al., 2009 found the overall antioxidant activity of D. gangeticum to be the strongest, followed in descending order by A. caudatus, S. nigrum, P. longum, E. alba and O. sanctum. Rhitajit Sarkar et al., 2009 demonstrated that the 70% methanolic extract of G. sylvestre leaves, which contained high amount of
flavonoid and phenolic content, exhibited high antioxidant activity and free radical scavenging activity.

Rachh et al., 2009 observed the *G. sylvestre* extract showing antioxidant activity by inhibiting DPPH, scavenging super oxide as well as hydrogen peroxide and reducing power ability which may be due to presence of flavonoids, phenols, tannins (phenolic compounds) and triterpenoids found in the preliminary phytochemical screening.

Chavda et al., 2010 evaluated the possible Hepatoprotective and Antioxidant potential in the Root Bark of *Calotropis procera* R.Br (Asclepiadaceae). In addition he attributed the hepatoprotective property to the antioxidant principles of the plant. Joshi Amit et al., 2010 investigated the ethanolic extract of *Calotropis gigantea* for its Antioxidant activity, *in vitro* by reducing power, DPPH and nitric oxide method.

Shah et al., 2010 studied Antioxidant activity of hydro-alcoholic extract of leaf of *G. sylvestre* in four in-vitro models, viz., Ferric reducing power, DPPH radical scavenging activity, super oxide free radical scavenging activity, scavenging of hydrogen peroxide which can be attributed due to the presence of phytochemicals.

Chandra Besra et al., 2011 showed the methanolic extract of tubers of *Geodorum laxiflorum* having promising antioxidant and hepatoprotective activity. Hina Fazal et al., 2011 observed significant antioxidant activity of an alcoholic extract of *G. sylvestre* which was found to be due to the presence of acidic compounds, flavonoids, phenols, saponins, tannins and triterpenoids.

Patil Prakash et al., 2011 demonstrated superoxide scavenging activity in *calotropis procera* (Asclepiadaceae) and inferred that antioxidant property of the plant may prevent formation of free radical and so inhibit the lipid peroxidation and offers the hepatoprotection against CCl₄ toxicity.
Mohammad Ahmad Khasawneh et al., 2011 showed the alcoholic extract partition fractions of *Calotropis procera* to yield promising antioxidant, anti-inflammatory and anti-cancer properties. Sinchan Biswas et al., 2010 studied the antioxidant activity of the aqueous extracts of leaves of six medicinal plants including *Gymnema sylvestre* and revealed that it is due to the presence of phenolic compounds.

Meena Thomas Irimpan et al., 2012 characterized seven medicinal plants of the family Asclepiadaceae for their Antioxidant activity by DPPH (1, 1-diphenyl-2-picryl-hydrazyl) assay method and found direct correlation between polyphenol content and antioxidant activity (correlation coefficient $R^2 = 0.6557$).

**Phytochemical Activity**

Indian Systems of Medicine covers the system which originated in India or which originated outside and got adapted in the course of time (Sharma, 1995). Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Geissman, 1963). Most are secondary metabolites, of which only 12,000 have been isolated, which are estimated to be less than 10% of the total (Schultes, 1978). Of these, only small percentage has been investigated phytochemically and the fraction submitted to biological or Pharmacological screening is even lower.

Numerous studies have identified compounds within herbal plants that are effective antibiotics (Basile et al., 2000). Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics (Okpekon et al., 2004). Some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria (Kone et al., 2004). The results of this indicate the need for further research into traditional health systems.
(Romero et al., 2005). It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity (Ebana et al., 1991; Manna and Abalaka, 2000).

The chemical compounds synthesized by plants can be classified by their chemical class, bio synthetic origin and functional groups into primary & secondary metabolites. Primary metabolites comprise common sugars, amino acids, proteins and chlorophyll while secondary metabolites consist of alkaloids, flavonoids, tannins and so on (Deb et al., 2005).

The screening of plant extracts of plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). Chemically constituents may be therapeutically active or inactive. The ones which are active are called active constituents and the inactive ones are called inert chemical constituents (Iyengar, 1995).

The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyan et al., 2006).

These natural compounds derived from plants formed the base of modern drugs that we are using today (Adome et al., 2003; Ayoola et al., 2008; Hindumathy2011). “Phyto” is the Greek word for plant. There are many families of phytochemicals and they help the human body in a variety of ways. Phytochemicals may protect human from various diseases. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties.

The potential of the phytochemicals have large scale pharmacological and biological activities such as antioxidant constituents (hydrolysable tannins, phenolic acid
and flavonoids) of the plant materials for the care of health and protection from coronary heart diseases, cancer, anti-carcinogenic and anti-mutagenic effects (Loliger, 1991).

Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds (Criagg et al., 2001). Knowledge of the chemical constituents of plants is desirable because such information will be valuable for synthesis of complex chemical substances (Mojab et al., 2003).

The amount and type of phytochemical compounds vary from plant to plant. In Gymnema species a number of phytochemical constituents have been reported. Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects (Liu, 2003; Manach, 1996). Saponins possess hypocholesterolemic and antidiabetic properties (Shinde et al., 2003). The terpenoids have also been shown to decrease blood sugar level in animal studies. The steroids and saponins are responsible for central nervous system activities (Sapan et al., 2006). More recently, drug discovery techniques have been applied to the standardization of herbal medicines to elucidate analytical marker compounds (Balunas, 2005).

Danmalam et al., 2007 reported the presence of the phytochemicals like saponins, flavonoids, cardiac glycosides, terpenes and/or steroids in the ethanol extract of the root-bark of Calotropis procera (Asclepiadaceae). Herve Zabri et al., 2008 determined the amount of flavonoids (anthocyan) in the methanol extract of leaves and stems of Secamone afzelii (Asclepiadaceae), and found the abundance of it in the leaves (336 mg/100 g) than in the stems (180 mg/100 g).

Khandelwal et al., 2010 revealed the presence of many secondary metabolites in the root part of Jatropha curcas like-glycosides, alkaloids, flavonoids, phenolic compounds/tannins, terpenoids etc by phytochemical analysis of the extracts.
Kalidass et al., 2010 showed that the ethanolic extracts of the *G. sylvestre* contained the alkaloids, terpenoids, coumarin, tannin, saponin, flavonoids, phenols, anthraquinones, quinones, carbohydrate and glycosides. Shanthi et al., 2010 confirmed the presence of saponins, tannins, resins, essential oils, sterols and glycosides as Preliminary phytochemicals in *Hemidesmus indicus* (Linn.) R.Br. Family: Asclepiadaceae.

Hiren Doshi et al., 2011 screened the phytochemicals in *Calotropis Procera*. R. Br. (Asclepiadaceae) like alkaloids, carbohydrates, glycosides, saponins, proteins, fixed oils, starch, triterpenoid, phenolics and tannins. Sukesh et al., 2011 revealed the presence of steroids/terpenoids and coumarins in the Hexane and Chloroform (99%) extracts of *Gymnema sylvestre* (Retz) and *Andrographis paniculata*.

Vaghasiya et al., 2011 investigated total phenolic content by Folin-Ciocalteus reagent method and flavonoid content by Aluminium chloride Colorimetric method in 53 traditionally used medicinal plants of Western region of India which included *G. sylvestre* also.

Dheepakamalini Thangavelu et al., 2012 screened the phytochemicals and revealed that there is some variation in the concentration of active compounds in *Gymnema sylvestre* hairy species on correlation with normal species. Koona Saradha Jyothi et al., 2012 analysed the phytochemicals in *G. sylvestre* by following Harborne method and revealed the presence of secondary metabolites like steroids, alkaloids, phenols, flavonoids, coumarins, saponins, tannins and triterpenoids.

Murugan et al., 2012 screened two medicinal plants namely *Gymnema sylvestre* and *Morinda pubescens* for phytochemical analysis and found the presence of alkaloids, flavonoids, phenols, tannins, and terpenoids. Gopinath et al., 2012 confirmed the presence of various phytochemicals such as alkaloids, flavonoids, tannins, saponins,
terpenoids and Quinone in *Gymnema sylvestre, Phyllanthus amarus, Phyllanthus reticulatus* leaves.

Gajendiran *et al.*, 2012 observed the presence of alkaloids, anthraquinones, flavonoids, carbohydrates and certain aminoacids in the aqueous extracts of *Cocculus hirsutus* and *Gymnema sylvestre*.

**LC-MS Analysis**

Medicinal plant extracts and herbal preparations are complex mixtures of active and ballast substances which may contain up to several hundreds of different constituents with not exactly defined structures.

Hence chromatography is undoubtedly fundamental to overcome the challenges of phytoanalytics. The usual high performance liquid chromatography (HPLC) associated detectors, such as ultra violet (UV), fluorescent, electrochemical and refractive index (RI), are not as selective and sensitive as a modern detector of today should, and required to be. Mass spectrometry (MS) offers great selectivity and sensitivity and by coupling to high performance liquid chromatography (LC-MS) it enables effective analysis of complex matrices and is also proven to be the most powerful analytical tool for pharmacokinetics and drug discovery (Papac and Shahrokh, 2001; Tolonen *et al.*, 2009).

LC-MS represents a well-established, rapid, powerful and robust technique, available with a wide range of ionization modes for the analysis of non volatile polar, semipolar and in some extent apolar compounds.

Since typical and potential drug molecules are rather polar and water soluble, LC-MS has a greater significance than GC-MS. Nuclear magnetic resonance
spectroscopy (NMR) serves as a complement analytical tool for LC-MS systems in unambiguous structure elucidation. Today LC-NMR-MS is perhaps the most promising hyphenation technique, but it still needs a few years time to be put in routine, not to talk about its stratospherical price, while LC-MS rapidly becomes a routine technique for the fast and powerful analysis of almost any complex matrix. LC/MS/MS technologies are extremely important for characterization and quantitation of herbal medicines because full characterization of these products is a desirable goal (Wang Xue et al., 1997; Wang Sakuma et al., 1999).

Liquid chromatography (LC) combined with mass spectrometry (MS) is a powerful tool in, inter-alia, pharmaceutical and plant metabolism analytics, and the use of this hyphenated technique is now commonplace in bioanalytical laboratories (Cuyckens et al., 2004; Korfmacher, 2005). This is a result of recent developments in separation sciences and instrumentations in general, and leads to the use of modern ultra-performance liquid chromatography (UPLC) together with the high selectivity and performance of mass spectrometry.

Xiaomin Wang et al., 2000 studied the characterization and quantification of the cardiac glycosides oleandrin, odoroside, neritaloside and the aglycone oleandrigenin, all in a patented-hot-water extract of Nerium oleander L (Anvirzel), by HPLC/MS/MS method. Ana et al., 2004 confirmed the presence of maitenin and pristimerin in detectable amounts in hydroalcoholic extracts of Maytenus aquifolium leaves by liquid chromatography coupled to mass spectrometry (LC-MS) Method.

Blazics et al., 2008 identified and structurally characterized 17 simple phenolic acids and flavonoid glycosides in Euphrasia rostkoviana Hayne by LC-MS/MS, and concluded it to be a universal analytical tool for the qualitative and quantitative analysis of phenolics from simple phenolic acids and salicylates towards more complex structures, like flavonoid glycosides and macrocyclic phenolics.
Madhurima et al., 2009 studied the Phytochemical analysis, including quantification of gymnemagenin using HPTLC method in Gymnema sylvestre. Farzana Chowdhary et al., 2010 isolated and characterized gymnemic acid from indigenous Gymnema sylvestre leaves, and showed that the yield was maximum in 95% ethanolic extract (6.15% m.f.b.) and minimum in aqueous extract (1.66% m.f.b.) and further purified by preparative chromatographic methods.

Parikshit et al., 2010 described a rapid, sensitive and accurate liquid chromatographic tandem mass spectrometric method for the determination of Mimosine in Mimosa pudica Linn whole plant powder. Priti et al., 2011 showed that G. sylvestre leaf powder contained gymnemagenin in the concentration range of 0.3 – 0.34 % w/w on a dry weight basis in extract by a simple liquid chromatographic method.

Qiao et al., 2011 summarized in his review that LC/MS not only allows fast and accurate structural analysis of flavonoids in complicated mixtures, but also provides a rapid and sensitive technique for the quantitative detection of flavonoids in herbal extracts and even in biological matrices.

Yu-Tse Wu et al., 2011 reviewed the definition, causes and evaluation of matrix effects in liquid chromatography-mass spectrometry (LC/MS) analysis and described it as a sensitive analytical tool to determine trace amount of analytes in plasma, which is beneficial for researchers to observe the detailed pharmacokinetic profiles of drugs.

Balamurali krishna et al., 2012 isolated and characterized the Gymnemic acid, from seventeen ecotypes of Gymnema sylvestre leaves with different solvent systems like petroleum ether, benzene, and methanol and purified the Gymnemic acid by preparative chromatographic methods i.e., TLC and HPTLC where HPTLC showed 30% purity of Gymnemic acid.
Kang et al., 2012 detected the presence of the anti-hyperglycemic compounds gymnemagenin and gymnemic acids in *G. sylvestre* extract by LC/MS analysis. Hanisa et al., 2012 found the presence of curcuminoids mainly curcumin, demethoxycurcumin, bisdemethoxycurcumin, and dihydrocurcumin in *Curcuma longa* L and madecassic acid in *Centella asiatica* L and verbascoside in *Strobilanthes crispus* L, by liquid chromatography-mass spectrometry-electrospray ionization Method.

Wei-Jun Kong et al., 2012 analysed the contamination levels of zearalenone (ZON) and its metabolite α-zearalenol (α-ZOL) in 100 widely consumed foods and medicinal plants in China by LC-MS-MS Method.

Wenquan Lu et al., 2011 analysed the eight components (rosmarinic acid, lithospermic acid B, L-phenylalanine, t-cinnamic acid, 4-coumaric acid, L-tyrosine, 4-hydroxyphenyl pyruvic acid and homogentisic acid) involved in lithospermic acid B biosynthesis pathway in *Salvia miltiorrhiza* hairy root cultures by liquid chromatographic–tandem mass spectrometric method.

**Antiulcer Activity**

Medicinal plants are among the most attractive sources of new drugs, and have been shown to produce promising results for the treatment of gastric ulcer (Borrelli and Izzo, 2000).

Gastric and duodenal ulcers are illnesses that affect a considerable number of people in the world. Stress, smoking, nutritional deficiencies and ingestion of nonsteroidal anti-inflammatory drugs grow the gastric ulcer lesions incidences (Belaiche et al., 2002).
In the market for the treatment of gastric ulcers, including antacids, proton pump inhibitors, anticholinergics and histamine $\mathrm{H}_2$ antagonists, most of these drugs produce several adverse reactions, such as hypersensitivity, arrhythmia, impotence, gynecomastia and hematopoietic changes (Chan and Leung, 2002; Duran et al., 2003; Scholl et al., 2005). Thus, there is a need for more effective and less toxic antiulcer agents.

Peptic ulcer is one of the major gastro-intestinal disorders, which occur due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors. Some other factors such as inadequate dietary habits, excessive ingestion of non-steroidal anti-inflammatory agents, stress, hereditary predisposition and infection by Helicobacter pylori, may be responsible for the development of peptic ulcer. Consequently, reduction of gastric acid production as well as reinforcement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease. (Ali et al., 2008)

Hiruma-Lima et al., 2006 suggested that the efficacy of Qualea grandiflora in preventing and healing ulcers is based on its ability to stimulate mucus synthesis (an important feature in the gastroprotection) as well as on the stimulation of an antisecretory effect.

Muralidharan et al., 2009 evaluated the anti ulcerogenic activity of Morinda citrifolia Linn (Rubiaceae) by employing aspirin and alcohol induced ulcerations in rats and found that the plant extract had increased the mucous and decreased the acid volume, free and total acid contents in rats.

Thirunavukkarasu et al., 2009 showed that pretreatment with the leaf extract (both hot water and cold water) of E. agallocha caused a beneficial effect on NSAID-induced gastric ulcer in rats as evidenced by the reduction in the ulcer score. The
E. agallocha was able to decrease the acidity and increase the mucosal defense in the gastric areas, thereby justifying its use as an antiulcerogenic agent.

Ghodekar et al., 2010 showed an antiulcer activity against histamine and naproxen induced gastric ulceration in rats by using different doses of methanolic extract of Tylophora indica (T. indica, Family Asclepiadaceae). The antiulcer activity shown was probably due to decrease in lipid peroxide level and by blocking H2 receptor respectively.

Kalimuthu et al., 2010 evaluated the anti-ulcer activity in pylorus ligature and swim stressed induced ulcer in Wistar rats by using methanolic extract of Acalypha indica (MEAI), which showed significant reduction of gastric volume secretion, acidity and ulceration in pylorus legated and swim stressed rats at p < 0.001.

Vinothapooshan et al., 2010 evaluated the anti-ulcer activity in rats by using three models, i.e. Aspirin, Alcohol and pyloric ligation models experimentally induced gastric ulcer, by using Methanol, chloroform and diethyl ether extracts of Mimosa pudica and indicated that the alcoholic extract significantly (P < 0.001) decreased the volume of gastric acid secretion, pH, free acidity, total acidity and ulcer index with respect to control.

Khaja Zeeyauddin et al., 2011 evaluated aspirin induced ulceration (200mg/kg) in albino rats by using bark extracts of Boswellia serrata (Family Burseraceae) and showed, the petroleum ether (250mg/kg) and aqueous extracts (250mg/kg) to be having significant antiulcer activity.

Rupesh Kumar et al., 2011 found Various plants like Amomum subulatum, Scopariadulcis, Jasminum grandiflorum, Davilla rugosa, Kielmeyera coriacea, Larrea
divaricata, Qualegrandiflora, Mammea Americana, Anacardium occidentale, Ocimum sanctum, Azadirachtaindica to be active in antiulcer therapy.

Srinivas et al., 2011 found that the ethanolic extract of Ixora pavetta significantly decreased the gastric secretion, free acidity as well as gastric ulcers in the aspirin induced and pylorus ligated rats and the effects were compared with Omeprazole.

Ashok Kumar et al., 2012 investigated the anti-ulcer activity of ethanolic extract of Annona squamosa leaves on aspirin induced ulcer models and pylorus ligation in wistar rats. In both models the common parameter determined was ulcer-index. Ethanolic extract of dosage 50, 100 mg/kg p.o produced significant inhibition of gastric lesions induced by pylorus ligation and aspirin induced ulcers.

Chandaka Madhu et al., 2012 studied the anti-ulcer activity of aqueous extract of Aeglemarmelos leaves on indomethacin induced ulcer models in wistar rats and determined ulcer-index. Aqueous extract of dosage 175,350 mg/kg p.o produced significant inhibition of gastric lesions induced by indomethacin induced ulcers. Dhanaraj et al., 2012 investigated the Anti-ulcer activity of crude aqueous extract of leaves of Mukia maderasapatana against stress induced in rats, by using the biochemical markers of ulcer like MDA, GSH, gastric juice, volume and pH and recorded a significant alteration under stress condition in rats.

Elamaran Tamil Jothi et al., 2012 validated the anti-ulcer potential of the ethanolic and ethyl acetate extracts leaves of Tecomaria capensis against in vivo Aspirin induced method. The extracts (100 and 200 mg/kg) significantly reduced the ulcer index and significantly increased the pH of gastric acid while at the same time reduced the volume of gastric juice and total acidities.
Urivish B. Patel et al., 2012 showed that methanolic extract of *Gymnema sylvestre* at a concentration of 100 mg/kg, 200 mg/kg, 400 mg/kg, had ulcer protective activity comparable with standard drugs Sucralfate 400mg/kg (cytoprotective agent) and Ranitidine 50mg/kg in Albino wistar female rats.