The existence of man on the earth has been possible only because of the vital role played by the plants. Rapidly increasing knowledge on nutrition and medicine has dramatically changed the concepts about food, health and agriculture and popularity of herbal medicines, otherwise called complementary and alternative medicine has been increasing over the years. An increased sense of entitlement to quality of life, public access to worldwide health information, concerns about cost, adverse side effects of modern medicines and the popular belief that alternative medicines are safe and healthy have turned the research towards natural products.

Medicinal and aromatic plants play an important role in the spiritual, socio cultural and healthcare needs of tribal and rural people of the emerging countries. A large section of people in developing countries still rely on traditional medicine to meet their health care needs. Now more and more people are turning towards alternative therapies and herbal remedies which results in an increased demand of medicinal plants and their products (Vasisht and Kumar, 2003). Consumers in the developed countries are becoming disillusioned with modern healthcare and are seeking alternatives. In India 90% of the prescriptions contain plant products (Britto and Mahesh, 2007). Many of the effective drugs like taxol, vinblastine, vincristine, camptothecin are of natural origin (Cragg et al., 2006). The Indian system of traditional medicine, Ayurveda uses dry powder or crude extracts of medicinal plants to treat various diseases including cancer (Jagetia and Baliga, 2006). They are non toxic,
having no side effects and easily available at affordable prices (Britto and Mahesh, 2007). Therefore the demand for medicinal plants are increasing in both developing and developed countries.

In fact thousands of plants have been studied for their pharmacological properties (Dahanukar et al., 2000; Rajesh and Latha, 2001). A detailed investigation and documentation of plants used in local health traditions and ethno pharmacological evaluation to verify their efficacy and safety can lead to the development of invaluable herbal drugs or isolation of compounds of great therapeutical value including effective cure for dreadful diseases like cancer and AIDS.

For a medicine to be effective, the exact plant which is having the attributed clinical effectiveness should be used. For the preparation of a plant based drug, one should depend on taxonomy to identify and authenticate the herb and phytochemistry for isolation and identification of the active principle. The inadequate supply of authenticated plant material is the reason for adulteration and substitution. For the accuracy, precision and consistency of formulations along with desired therapeutic effect, quality assurance of raw materials is necessary. A serious limitation encountered in the use and research of traditional medicine is the lack of standardization and quality control of raw materials forming the drug.

In any medicinal formulation, the correct botanical identification of the plant is an important step. Previously taxonomic methodology based on morphological characteristics was employed. Now greater stress is given to phytochemical information which leads to chemotaxonomy.

The literature available reveals that the family Asclepiadaceae consists of several members that are of medicinal importance. Many of them are used in Ayurvedic and traditional medicines. The present investigation
comprises seven medicinal plants viz., *Asclepias curassavica* L., *Calotropis gigantea* (L.) R.Br., *Gymnema sylvestre* (Retz) R.Br., *Holostemma adakodien* Schult., *Pergularia daemia* (Forssk.) Chiov., *Tylophora indica* (Burm.f.) Merr. and *Wattakaka volubilis* (L.f.) Stapf. belonging to seven genera of the family Asclepiadaceae. The focus area of this research is phytochemical analysis and biological activity with a view to explore the chemoprofile.

1.1 Review of literature

1.1.1 Phytochemistry

Phytochemistry deals with the natural distribution, biological function and the chemical structure of all the organic substances accumulated by the plants. It is both a demanding and fast developing field. It deals with the study of secondary metabolites isolated from the plant, their characterization, reactions and biological activities. A systematic search for useful bioactivities from plants is now considered to be the rational approach in nutraceutical and drug research (Wang et al., 2003). Rapid progress in this field was observed in the nineteenth century. The first alkaloid narcotine was isolated in 1803 which was followed by morphine, emetine and strychnine. The studies of the chemical nature of fats and fixed oils were also carried out. By the middle of twentieth century the chemistry of the natural products became the thrust area and isolation and structure determination of a number of compounds were done. Then the attention of natural product chemists turned towards the biosynthetic pathways found in plants using the new techniques of separation and analysis. The study of biosynthetic pathways including stereo-chemical aspects resulted in the origin of comparative phytochemistry (Evans, 2002).
A variety of bioactive compounds and their derivatives have been shown to inhibit carcinogenesis in a number of experimental systems involving initiation, promotion and progression (Huang et al., 1999). Plants contain abundant quantities of substances that have consistently been shown to be associated with a lower risk of cancers at almost every side (Steinmetz and Porter, 1991). The therapeutic effect of plant extracts can be attributed to a particular “active principle” or “chemical entity”. The pharmaceutical industries all over the world are in search of new drugs from natural resources. The most important of the bio-active compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga et al., 2003).

Phytochemical investigation of a plant involves 1) authentication and extraction of the plant material, 2) separation and isolation of the plant constituents, 3) characterization of the isolated compounds, 4) investigation of the biosynthetic pathways to particular compounds and 5) quantitative evaluations (Harborne, 1973). Identification and authentication of the plant material should be done by an acknowledged authority or by a taxonomic expert. The plant material can be ascertained by visiting botanical gardens and matching the botanical profiles with living plants, comparing it with preserved specimens in museums and herbaria. The authenticated plant material can be subjected to different methods of extraction based on the nature of plant material and the components to be isolated (Harborne, 1973). For the isolation and separation of plant constituents the available methodologies are distillation, sublimation, fractional crystallization, chromatography etc. Although many chromatographic methods are presently available, thin layer chromatography (TLC) is widely used for the rapid analysis of drugs and drug preparation especially herbals (Evans, 2002). After isolation and
purification, the plant constituent has to be identified by studying its solubility, Rf properties and its UV spectral, infrared (IR), nuclear magnetic resonance (NMR) and mass spectral (MS) characteristics.

1.1.1.1 Authentication of the plant material

The correct botanical identification of the plant is an important step in any medicinal formulation. For the accuracy, precision and consistency of formulations along with desired therapeutic effect taxonomic identification and authentication is necessary. The detailed taxonomic identification with internationally accepted nomenclature is an integral part of authentication. For a medicine to be effective, the exact plant which is having the attributed clinical effectiveness should be used

1.1.1.2 Physicochemical characterization.

Simple procedures like botanical description, comparison with herbaria, observation with the naked eye and microscopical examination are the widely used methods in drug plant identification. However such methods are inapplicable in the case of crude drugs, drug powders and herbal drug extracts as they do not bear the characteristic morphological and anatomical features of the parent plant. In this context the study of ash values, mineral content and extractive values are useful in the standardization of crude drugs (Kirk and Sawyer, 1997).

Minerals are inorganic elements that come from the soil and water which are absorbed by plants. These are nutrients that our body needs to grow and develop normally. Various functions such as building strong bones, transmitting nerve impulses, regulating body fluids (Khalil and Manan, 1990) and many physiologic and metabolic functions of the nervous system, hormonal secretions, and blood coagulation are controlled by minerals (Close and Menke, 1996). Potassium is essential
for protein synthesis, maintaining water balance for the normal functioning of the neurons and muscles and absorption of glucose and glycogen. Magnesium assists enzymes involved in the synthesis and breakdown of fats, proteins, carbohydrates and synthesis of DNA and RNA (Makkar and Becker, 1997). In many plants the mineral content contribute to its therapeutic activity (Chevallier, 2001). Complementary methods like organoleptic evaluation, chemical tests and UV spectral studies are also helpful to some extent. None of these are complete enough to afford authentic identification. In such situations, depiction of the molecular or biochemical profile of the material using chemical fingerprinting techniques are of great application.

1.1.1.3 Qualitative and chromatographic analysis

A variety of chemical compounds occurring naturally in plants are responsible for the biological significance of the plant. Tracing of the plant constituents can be done using appropriate chemical tests (Wagner and Bladt, 1996).

Thin layer chromatography (TLC) is a technique by which the complex mixture can be resolved into individual compounds. It is widely used for the rapid analysis of herbal drug preparations (Evans, 2002). TLC fingerprinting is preferable due to speed, simplicity, versatality and economy.

High performance thin layer chromatography (HPTLC) is a powerful tool for qualitative and quantitative analysis of the herbal drugs. HPTLC fingerprints of pharmacologically relevant fractions are unique and characteristic of the drugs and they help in their identification. These fingerprints can also be used for the detection of adulterants, differentiation of closely related species, batch comparison of market drug samples and
quality evaluation. HPTLC is used to separate different classes of compounds and the particular substance within the class. The class of compounds can be concluded by studying its response to colour tests, solubility, Rf properties and UV spectral characteristics.

**Pure compound detection**

From the available literature, it is evident that some biologically important compounds like lupeol (Aanjaneyulu *et al.*, 1998; Manju and Alice, 2001), β-sitosterol (Rastogi and Mehrotra, 1991; Manju and Alice, 2001) and quercetin (Jasem *et al.*, 2012) are present in some members of the family Asclepiadaceae. So in the present study an attempt is made to identify their presence in the selected plants using HPTLC.

β-sitosterol is reported to help in curing breast cancer, gynecological disorders, hyperlipidemia, reducing ageing of prostate gland and cholesterol absorption. It acts as an immunomodulator (Best and Duncan, 1956; Becker *et al.*, 1992). The structure of β-sitosterol is shown below.

Lupeol, a naturally occurring triterpene has been shown to possess strong anti-arthritis, anti-inflammatory, anti-mutagenic and anti-malarial activity (Guevara *et al.*, 1996; Geetha and Varalakshmi,
It has been shown to act as a potent inhibitor of protein kinases (Hasmeda et al., 1999; Rajic et al., 2000; Hodges et al., 2003) and inhibits the activity of DNA topoisomerase II, a target for anticancer chemotherapy (Moriarity et al., 1998; Wada et al., 2001). It has also been shown to improve epidermal tissue reconstitution (Nikiema et al., 2001). Recent studies have shown that lupeol induces differentiation and inhibits cell growth of melanoma cells (Hata et al., 2002).

Quercetin is one of the most prominent dietary antioxidant. Its beneficial health effects were due to its ability to scavenge hydroxy radical (Boots et al., 2008). Quercetin increased the genomic stability in the cirrhotic rats (Tieppo et al., 2007), suggesting beneficial effects by its antioxidant properties (Vicente-Sánchez, 2008).

It is also known to act as an effective antioxidant by chelating metal ions or scavenging free radicals (Terao, 1999). Several studies have demonstrated that quercetin enhanced the antioxidative defence system by upregulating antioxidant enzymes (Alia et al., 2006). It offers protective effect against ethanol induced hepatotoxicity by attenuating lipid peroxidation. This is done by scavenging free radicals and enhancing the activity of antioxidants (Vidhya and Indira, 2009).
1.1.1.4 Amino acid analysis

Amino acids are the basic structural and functional units of proteins. They have immense importance in the herbal medicines. They participate virtually in every process within the cell. The deficiency of enzymes leads to many diseases. It is necessary to include essential amino acids in the diet as they are not synthesised in the body. Non essential amino acids are synthesised in our body. Glutamic and aspartic acids are the only non-essential amino acids, which under certain conditions become contingency nutrient and essential. They are used in higher concentration, to stimulate and stabilize the brain activities (Leon, 1986). The deficiency of these amino acids lead to decreased brain function, including a number of abnormalities, such as impaired memory function, abnormal behavioural pattern, autism, lack of concentration and decreased cognitive ability (Sourkes, 1968; Leon, 1986). *Centella asiatica* and *Herpestis monniera* are used as brain stimulants because of the presence of glutamic acid and aspartic acid in high concentration (Shahnaz et al., 1994).

Amino acids play a major role in regulating multiple processes related to gene expression including modulation of the function of the proteins that mediate messenger RNA (mRNA) translation (Kimball and Jefferson, 2006). Certain amino acids are known to chronically regulate

Amino acids cysteine (Bailey and Sheffer, 1967), tryptophan and phenyl alanine (Davis *et al.*, 1968) possess anti-inflammatory activity. Statistically significant reduction of inflammation in carrageenan induced paw oedema in rats was observed by the use of isoleucine, leucine, methionine (Khanna, 1984). Valine (Khanna and Madan, 1978), glutamine and tryptophan (Madan and Khanna, 1978) were found to suppress acute inflammation. Naik and Sheth (1978) reported that aspartic acid, alanine and glycine possessed promising anti-inflammatory activity.

From the literature survey (Kirthikar and Basu, 1975; Moming, 1987; Wahi *et al.*, 2002), it was known that certain plants under study are used regularly by diabetic patients to reduce the blood sugar and some others have got anti inflammatory property. So in the present study an effort was made for the qualitative and quantitative analysis of amino acids in the selected plants.

1.1.1.5 Quantitative estimation of phytoconstituents

Flavonoids and phenols are ubiquitous secondary products of plants (Harborne, 1977; Witzell *et al.*, 2003). The production of these secondary metabolites shows a wide qualitative and quantitative variation between plant organs and is dependent on plant growth, environmental factors and
stress stimuli such as UV irradiation, drought and high temperatures (Sosa et al., 2005; Witzell et al., 2003). Besides the physiological role of plant flavonoids, they have been recognized as part of the defense strategies being responsible for important ecological functions such as antimicrobial, antifungal, antioxidant and detoxificant of heavy metals. Consequently, they are involved in plant-animal and plant-plant biochemical relationships, as well as in nutrient cycles (Sosa et al., 2005; Simmonds, 2003).

Flavonoids and phenols are responsible for the pharmacological effects of several medicinal plants. As a consequence of their chemical diversity and biological functions, there is an increasing interest in this group of phytochemicals as chemotaxonomic markers, as well as in their ecological role and beneficial health effects in chronic and degenerative diseases. They disclose a wide pharmacological profile including antioxidant, free radical scavenger, lipid peroxidation inhibition, anti-inflammatory, anti-allergic, anticarcinogenic, anti-arthritic and antihypertensive activities (Di Carlo et al., 1997; Robards and Antolovich, 1997). Recent advances in the knowledge on the neuropharmacological and cardiac effects of flavonoids point out to their potential for the management of various psychiatric conditions and cardiac insufficiencies including the treatment of hypertension (Johnson and Beart, 2004; WHO, 2002).

It is well known that the quantitative and qualitative contents of secondary metabolites in a plant show marked variation which are regulated by intrinsic factors (ontogeny and phenology) and also by abiotic (e.g. light, moisture, nutrient availability) and biotic factors such as different physiological and growth stages (Harborne, 1993; Brooks and Feeney, 2000; Sosa et al., 2005; Calixto, 2000). Considering the
medicinal importance of the plants under study, and the lack of information about quantitative variation of phytoconstituents, the seasonal, age wise and habitat wise variation of the total flavonoid and phenolic contents in the leaves are evaluated.

1.1.2 Biological activity

Biological activity or pharmacological activity describes the beneficial or adverse effects of a drug on living matter. This activity is exerted by the substance's active ingredient or pharmacophore but can be modified by the other constituents. Activity is generally dosage-dependent. Further, it is common to have effects ranging from beneficial to adverse for one substance when going from low to high doses.

1.1.2.1 Antibacterial activity

Bacterial resistance to antibiotics represents a serious problem for clinicians and the pharmaceutical industry and great efforts are being made to reverse this trend and one of them is the widespread screening of medicinal plants from the traditional system of medicine hoping to get some newer, safer, and more effective agents that can be used to fight infectious diseases (Natarajan et al., 2003).

The traditional medical practitioners use a variety of herbal preparations to treat different kinds of diseases including microbial infections (Mann et al., 2008). *Azadirachta indica* is one such medicinal plant having strong antibacterial activity (Akula et al., 2003). The scientific literature is full of reports of studies on roots, stem bark, seeds, flowers and fruits of higher plants having bioactive substances such as peptides, alkaloids, tannins, phenols, sterols, flavonoids, glycosides amongst others which confer healing properties for their use in medicine (Levin et al., 1979; Benli et al., 2008; El-Mahmood et al., 2008).
Enteric or diarrheal infections are major public health problems in developing countries. Recently, it has been demonstrated that many human pathogenic bacteria have developed resistance against several synthetic drugs (Mojab et al., 2008). Some of the common pathogenic bacteria like *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Salmonella typhymurium* and *Escherichia coli* are selected to understand antibacterial property. There are several reports on antimicrobial activity of crude extracts prepared from plants that inhibit various bacterial pathogens. Combinations of medicinal plants may increase the antimicrobial spectrum and potency of the preparations (Greensfelder, 2000; Patwardhan et al., 2005).

Day by day new dreaded diseases are arising. The rise of antibiotic resistant micro organisms is one of the severe problems in health care systems of the world and infectious diseases are the second most serious cause of death, worldwide (Nascimento et al., 2000; Mojab et al., 2008). Therefore, new drugs have to be found in order to combat such diseases and it is essential to find new compounds that have antimicrobial properties. Flavonoid compounds appear to play vital role in defence against pathogens and predators (Brenda, 1998). Hence an attempt is made to screen the antibacterial potential of these medicinal plants in the control and prevention of bacterial infection.

1.1.2.2 Antioxidant activity

**Oxidative stress**

Pro-oxidants are reactions or chemical compounds capable of generating potential toxic oxygen species and free radicals. On the other hand, antioxidants are reactions or compounds disposing these species or suppressing their formation or scavenging them. There is an appropriate pro oxidants-antioxidant balance in a normal cell. When production of
oxygen species is increased or when levels of antioxidants are diminished this balance can be shifted towards the pro-oxidant. This state is called oxidative stress and if the stress is massive or prolonged, it can result in serious cell damage. Etiopathogenesis of a variety of human diseases are caused by oxidative stress (Frei, 1994).

There has been a major interest in the study for the prevention of uncontrolled oxidative processes during the last two decades. In several studies, oxidative stress has been shown as the cause and progression of diseases like, neurogenerative diseases, atherosclerosis, carcinogenesis, chronic inflammatory diseases, radiation damages, ageing and various other pathological effects (Treitinger et al., 2000).

Free radicals are species with high reactivity, very short half life and damaging activity towards macromolecules like lipids, DNA and proteins. These may be either nitrogen derived (Reactive Nitrogen Species, RNS) or oxygen derived (Reactive Oxygen Species, ROS).

**Role of oxidative stress in human body**

When overall generation of reactive nitrogen species and reactive oxygen species exceeds the total antioxidant activity in the body, it will result in a condition called oxidative stress. This oxidative stress may be mild or severe. Based on various reports (Ghosh and Myers, 1998; Lee and Corry, 1998) the causes and after effects of severe oxidative stresses are shown in the flow chart.
There is a lot of interest in the antioxidant activity of flavonoids and other plant phenolic compounds due to their potential in disease prevention and health promotion (Dillard and German, 2000; Su et al., 2007; Pereira et al., 2009; Parr and Bolwell, 2000). Flavonoids belong to a large group of plant-derived low-molecular-weight polyphenolic compounds biosynthesized from both the shikimic acid and acetic acid pathways (Bruneton, 1999). They deserve attention because of their biological properties, such as vascular protective and venous tonic, anti-inflammatory, diuretic, antispasmodic, hepatoprotective, and hypocholesterolemic effects (Bruneton, 1999; Cazarolli et al., 2008).

Antioxidants are able to scavenge free radicals by various mechanisms (Pereira et al., 2009; Parr and Bolwell, 2000). They contribute to the protection of biologically important cellular components, such as DNA,
proteins, and membrane lipids, from free radical attacks leading to cell damage. This leads to ageing, inflammation, atherosclerosis and cancer (Bruneton, 1999; Su et al., 2007; Stanojevi et al., 2009).

Phytochemicals with antioxidant capacity naturally present in food are of great interest due to their beneficial effects on human health. They offer protection against oxidative deterioration. Antioxidants can protect the human body from free radicals and reactive oxygen species (ROS) effects (Gulcin et al., 2010). Oxidative damages caused by free radicals to living cells mediate the pathogenesis of many chronic diseases, such as Parkinson’s disease, Alzheimer’s disease (Bolton et al., 2000), cancers, ageing, heart ailments, cardiovascular diseases (Sun et al., 2004), atherosclerosis, cataracts and chronic inflammatory diseases, and other degenerative diseases (Ali et al., 2008). Medicinal plants contain many antioxidants such as vitamins, carotenoids, flavonoids, polyphenols, saponins, enzymes and minerals (Ray and Hussan, 2002). Natural antioxidants tend to be safer and also possess anti-viral, anti-inflammatory, anti-cancer, antimutagenic, anti-tumour, and hepatoprotective properties. The source of natural antioxidants may be all or any part of plant (Baravalia et al., 2009; Kaneria et al., 2009; Locatelli et al., 2010). Hence the selected plant extracts were subjected to in-vitro antioxidant activity.

A direct relationship has been reported between the levels of phenolic compounds and antioxidant potential of plants (Robards et al., 1999). Flavonoids do possess antioxidant activity due to the presence of phenolic ring in the moiety (Mikamo et al., 2000). Hence the total phenol and flavonoid content of the tested materials and its correlation with antioxidant activity is analyzed.
1.1.3 General description of the family

The family Asclepiadaceae is named after Asclepius- the god of medicine and healing from Greek mythology. It is also called milkweed family as most of the plants contain milky latex. Members of this family are quite interesting, because they are medicinal as well as toxic to human. Some are taken in as medicine while some others are used externally for ailments. Medicinally important plants such as Asclepias curassavica L., Calotropis gigantea (L.) R.Br., Gymnema sylvestre (Retz.) R.Br., Holostemma ada-kodien Schult., Pergularia daemia (Forssk.) Chiov., Tylophora indica (Burm.f.) Merr. and Wattakaka volubilis (L.f.) Stapf. belonging to the family Asclepiadaceae are selected for the present study. These are used in many of the crude as well as purified drugs in both traditional and modern systems of medicines.

The family Asclepiadaceae includes herbs or shrubs, usually twining, the stems usually woody, sometimes succulent. Leaves opposite or whorled, rarely alternate, stipules 0. Flowers hermaphrodite, regular, solitary or few or many together, usually in axillary, sometimes terminal, umbelliform cymes; bracts usually small or wanting. Calyx inferior, usually deeply 5- lobed, the lobes imbricate, usually with glandular scales at the base within. Corolla various, gamopetalous, 5-lobed; tube usually short, often furnished within with processes forming a coralline corona; lobes imbricate, contorted or valvate, often recurved, sometimes erect and connate at their tips. Stamens 5, inserted at or near the base of the corolla and alternating with its lobes; filaments free or more or less connate in a staminal-column with its apex united to the dilated part of the style; usually with more or less fleshy processes on the back forming a staminal corona; anthers 2-celled, the cell dehiscing by longitudinal or transverse slits, the tips often produced into inflexed membranes; pollen contents of
each anther-cell granular or united into one or two pollen masses; when granular each granule formed of about 4 grains loosely united and contained in a spoon – or trowel-like appendicle, attached by a caudicle to the pollen-carrier on the style-apex; when united, the pollen masses of two adjacent but different anther cells either sessile on or connected by arm-like caudicles to one of the 5 usually horny pollen-carriers which rest on the angles of the style-apex. Ovary of 2 or 1 celled many-ovuled carpels, the styles united above and dilated in the style-apex bearing the pollen-carriers on its angles and beneath them the 5 stigmatic cavities. Fruit of 2 (or 1 by suppression) follicular mericarps, dehiscing by the ventral suture and usually liberating the placenta. Seeds usually flat, sometimes ovoid or cylindric often marginal, crowned with a coma of long silky hairs; testa thin or thick, albumen usually thin; embryo large; cotyledons flat; radical superior, usually cylindric (Gamble, 1956).

1.1.3.1 Chemical and therapeutic importance

*Asclepias curassavica* L.

The phytochemical investigations on this plant have revealed the presence of the cardenolide glycosides, doubly linked cardenolide glycosides, pregnane steroids and triterpenoids (Abe *et al.*, 1991 and 1992; Michael *et al.*, 2005). Calotropin isolated from this plant has been reported as a potent cytotoxic agent against human carcinoma cells (Kiuchi *et al.*, 1998). Six new steroidal glycosides were obtained from the aerial parts along with two oxypregnanes (Liu *et al.*, 2008).

The root is regarded as purgative, subsequently astringent and also a remedy in piles and gonorrhoea. It has emetic and cathartic properties. The juice of the leaves has been strongly recommended as anthelmintic and is useful in arresting haemorrhages. The latex is applied to corns, the powdered leaves and flowers are used for treating sores and wounds.
(Kirthikar and Basu, 1975). The plant is used for cancer treatment in traditional medical practice, but only limited research has been carried out concerning the cytotoxic constituents (Kiuchi et al., 1998).

**Calotropis gigantea (L.) R.Br.**

The latex contain calotropin, calotoxin, uscharin, voruscharin, uschridin, uzarigenin, syriogenin, calotonic acid, proceroside, calotropin DI and DII (Sengupta et al., 1984; Pal and Sinha, 1980) and a cysteine proteinases calotropin FI and FII (Abraham and Joshi, 1979). Apart from these, latex also contains constituents like α-amyrin, β-amyrin, taraxasterol and β-sitosterol (Rastogi and Mehrotra, 1991).

Root bark is tonic, anti-spasmodic, expectorant, anthelmintic and laxative. In syphilitic infection it is regarded as a great remedy so much so that it is called as vegetable mercury. The mixture of the powder of root bark with black pepper twice a day is also used to cure jaundice (Nadkarni, 1954). As per Ayurveda the flowers are bitter, digestive, astringent, stomachic, anthelmintic tonic and analgesic, normally used to cure inflammation, tumors, kapha, loss of appetite and ascites (Kirthikar and Basu, 1975; Nadkarni, 1954; Chopra et al., 1956; Agharkar, 1991; Warrier et al., 1995). According to Unani the flowers are stomachic and good for the liver, dried flowers in 1 to 2 grains doses with sugar is given in leprosy, secondary syphilis and gonorrhoea with milk diet (Kirthikar and Basu, 1975; Nadkarni, 1954). According to Unani the leaves are useful in the treatment of paralysed parts, the oil in which leaves have been boiled, is also applied to paralyzed parts. The leaves are also used in the treatment of arthralgia, swelling and intermittent fever (Kirthikar and Basu, 1975). The powder of the dried leaves is dusted upon wounds and ulcer to prevent excessive granulation and promote healthy action (Nadkarni, 1954).
Gymnema sylvestre (Retz.) R.Br.

Tannins and saponins are the chief chemical constituents present in Gymnema sylvestre (Retz.) R.Br. and are known to possess anti-arthritic activity (Kokate, 1999). Leaves contain triterpene saponins belonging to oleanane and dammarane classes. Oleanane saponins are gymnemic acids and gymnemasaponins, while dammarene saponins are gymnemasides. Besides this, other plant constituents are flavones, anthraquinones, α and β-chlorophylls, phytin, resins, tartaric acid, formic acid, butyric acid, lupeol, β-amyrin related glycosides and stigmasterol. The plant extract also tests positive for alkaloids. Leaves of this species yield acidic glycosides and anthroquinones and their derivatives (Dateo and Long, 1973). A single phytococonstituent gymnemic acid could be used to combat diabetes and obesity simultaneously (Rekha et al., 2007).

It is a potent antidiabetic plant and used in folk, ayurvedic and homeopathic systems of medicine. Leaves of the plant is anti-diabetic (Anonymous, 1988) astringent, bitter, acid, thermogenic, anti-inflammatory, anodyne, digestive and liver tonic (Malik et al., 2008). It is also used in the treatment of asthma, eye complaints, inflammations and snake bite. In addition it possesses antimicrobial, antihypercholesterolemic, hepatoprotective and sweet suppressing activities. It also prevents dental caries caused by Streptococcus mutans and used in skin cosmetics (Komalavalli and Rao, 2000). In Japan, there are teas being made from Gymnema sylvestre (Retz.) R.Br. leaves and are being promoted as a natural method for controlling obesity and diabetes (Nakamura et al., 1999). It possesses anti-arthritic activity (Kokate, 1999).

Holostemma ada-kodien Schult.

The terpenoid sugars and amino acids present in the root tubers of the plant are responsible for the medicinal properties (Ramiah et al.,

The plant is used as antidiabetic (Moming, 1987), rejuvenative, aphrodisiac, expectorant, galactogogue, stimulant, and in ophthalmic disorders and the leaves, flowers and fruits are eaten as vegetable. They are useful in orchitis, cough, burning sensation, stomachalgia, fever and thridosha. (Warrier et al., 1995). There is huge demand for this plant; more than 150 tonnes is required every year in South Indian pharmacies (Karmarkar et al., 2001). The plant is used for maintaining youthful vigour, strength and vitality (Gupta, 1997). It also cures ulcers, biliousness, diseases of the blood, worms, itching, gonorrhoea and orchitis (Manju and Alice, 2001).

Pergularia daemia (Forssk.) Chiov.

The most commonly found phytochemicals from the leaves are flavonoids, alkaloids, terpenoids, tannins, steroids and carbohydrates (Karthishwaran et al., 2010). The presence of cardenolides, triterpenes, saponins are also reported (Aanjaneyulu et al., 1998). The seeds of Pergularia daemia contain calactin, calotropin and other cardenolides and a bitter resin pergularin (Patel and Rowson, 1964; Rowson, 1965).

The plant Pergularia daemia (Forssk.) Chiov. is used as anthelmintic, laxative and antipyretic. It cures asthma, ulcers, eye troubles, uterine complaints and inflammations (Kirtikar and Basu, 1975). Aerial parts of this plant were reported to have the various pharmacological activities like hepatoprotective (Sureshkumar and Mishra, 2006) and anti-diabetic (Wahi, et al., 2002). The plant is used as folk medicine for liver disorders in Chittoor district of Andhra Pradesh. The plant is described as expectorant, anti-diarrheal and anti-malarial (Kirtikar and Basu, 1975).
has analgesic and anti-inflammatory activity (Sathish et al., 1998). Ethanol and Butanol extract of leaf exhibited significant anti-inflammatory activity (Hukkeri et al., 2001).

*Tylophora indica* (Burm.f.) Merr.

The important bioactive constituents are alkaloids, tannin, flavonoid and phenolic compound (Edeoga et al., 2005) The plant has been reported to contain major alkaloids like tylophorine, tylophorinine (Philipson et al., 1974), tylophorinidine (Govindachary et al., 1973), non alkaloidal compounds like α and β amyrins, kaempferol, sigma sterol, wax, pigments etc. (Gupta, 2003).

The leaves of *Tylophora indica* (Burm.f.) Merr. have been used traditionally as a folk medicine. The dried leaves are emetic, diaphoretic, expectorant and useful in dysentery (Kirtikar and Basu, 1975). The leaves and roots are useful in respiratory problems such as asthma, allergies, bronchitis, diarrhoea, hydrophobia, ulcers, gout (Warrier et al., 1995) and have cathartic, laxative, and purgative properties and other reported activities include immunomodulatory (Gopalakrishnan et al., 1980) and antiamoebic (Bhutani et al., 1987) activity. The root and leaf powder is used in diarrhoea, dysentery and intermittent fever (Rao, 1987). The plant has been used traditionally for the treatment of bronchial asthma, jaundice and inflammation (Chopra et al., 1956). The antitumor (Huang et al., 2004), immunomodulatory (Ganguly et al., 2001; Ganguly and Sainis, 2001; Gopalakrishnan et al., 1980), antioxidant (Jagetia and Baliga, 2004), antiasthmatic (Huntley and Ernst, 2000), antihistaminic, hypotensive and antirheumatic (Gopalakrishnan et al., 1979) activities of *Tylophora indica* (Burm.f.) Merr. is scientifically proven. In Ayurveda the plant has been used in the treatment of asthma, dermatitis and rheumatism (Chopra et al., 1956). The other reported activities include
cytotoxic effect (Huang et al., 2004) and anticancer activity (Ganguly and Sainis, 2001).

**Wattakaka volubilis (L.f.) Stapf.**

An unusual novel triterpenoid ether from *Wattakaka volubilis* (L.f.) Stapf. has been reported (Niranjan et al., 2002) Three novel polyoxypregnane glycosides, volubiloside A, B and C were isolated from the flowers of *Dregea volubilis* (Shau et al., 2002). Protection against selenite cataract in rat lens by drevogenin D, a triterpenoid aglucone from *Dregea volubilis* was reported (Biju et al., 2007).

This plant is widely used in Indian traditional medicines. The leaf paste is used to treat rheumatic pain, cough, fever and severe cold (Muthu et al., 2006; Rajadurai et al., 2009). It is taken along with pepper to treat dyspepsia (Pandikumar et al., 2007). The bark paste mixed with hot milk is used internally to treat urinary troubles (Silija et al., 2008) and the leaf powder taken orally along with cow’s milk has anti diabetic activity (Ayyanar et al., 2008). The root is applied as an antidote in snake bite and also used in native medicine for fever in children. The root is applied to snake bites and given to women to cure headache after child birth. It is emetic, diaphoretic and diuretic (Agarwal, 1986). The antidiabetic properties of plants (*Andrographis lineate, Andrographis paniculata, Costus speciosus, Wattakaka volubilis* (L.f.) Stapf.) used by two major tribal groups in South Tamil Nadu, India, has been reported (Ayyanar et al., 2008). *Wattakaka volubilis* (L.f.) Stapf. leaf juice mixed with a little lime can cure sprains (Sanyasi et al., 2008).

1.1.4 **Biochemical systematics or chemotaxonomy**

Traditionally taxonomic methodology was much based on morphological characteristics. The modern trend in plant classification
uses the data from comparative anatomy, embryology, cytogenetics, phytochemistry (chemotaxonomy), palynology, molecular biology etc. Biochemical systematics or chemotaxonomy is the most rapidly developing field in Phytochemistry. Phytochemical characters have attracted the plant taxonomist from 1960. The newly developed advanced analytical techniques have facilitated the screening of large number of taxa in a very short time. The phytochemical characters of taxonomic significance are grouped into three viz. i) primary constituents like protein, nucleic acid, chlorophyll and polysaccharides; ii) secondary constituents like alkaloids, flavonoids, phenols, sterols etc. and iii) miscellaneous substances. These phytochemical data are used in Chemotaxonomy (Waterman, 1998).

The family Asclepiadaceae is a family in dispute. Different authors classified the members of the family in different methods and placed them under different orders and tribes. The infrafamilial classification of Asclepiadaceae proposed by Brown (1810) divided the family into three groups of genera 1) Periploceae, characterised by granular pollen collected in solitary cornucopia – shaped pollen carriers, 2) Asclepiadeae Verae (true asclepiads), characterised by waxy pollinia and 3) an unnamed group with a single genus, Secamone R.Br. Bentham and Hooker in 1876 elevated Asclepiadaceae to the rank of an order comprising seven tribes namely 1) Periploceae 2) Secamoneae 3) Cynancheae 4) Gonolobeae 5) Marsdenieae 6) Ceropegieae and 7) Stapelieae. Later Hooker (1883-1885) classified the order Asclepiadaceae into five tribes 1) Periploceae 2) Secamoneae 3) Cynancheae 4) Marsdenieae and 5) Ceropegieae. The tribe Gonolobeae was absent in his classification because he studied only the Indian elements. In the last two classification, three of the plants under study Asclepias curassavica L., Calotropis gigantea (L.) R.Br. and Holostemma ada-kodien Schult. come
under the tribe Cynancheae and the rest four *Gymnema sylvestre* (Retz.) R.Br., *Pergularia daemia* (Forssk.) Chiov., *Tylophora indica* (Burm.f.) Merr. and *Wattakaka volubilis* (L.f.) Stapf. come under the tribe Marsdenieae.

A modified classification divided the family Asclepiadaceae into three tribes – Secamoneae, Asclepiadeae and Stapelieae (Swarupanandan *et al.*, 1996). According to them *Asclepias curassavica* L., *Calotropis gigantea* (L.) R.Br., *Holostemma ada-kodien* Schult. and *Tylophora indica* (Burm.f.) Merr. come under the tribe Asclepiadaeae and *Gymnema sylvestre* (Retz.) R.Br., *Pergularia daemia* (Forssk.) Chiov. and *Wattakaka volubilis* (L.f.) Stapf. come under the tribe Stapelieae.

The development of improved and powerful analytical techniques contributed much in the field of phytochemistry. Studies confirmed that phytochemical characters show a high degree of correlation with other plant characters (Van Beek *et al.*, 1984). The taxonomical studies in relation to chemistry involve the study of the distribution of chemical compounds in related plants of the same family and plants of the related families. The biosynthetic pathways responsible for these compounds also differ from one taxonomic group to another. The chemical data can provide informations in situations where other forms of data are insufficient (Singh, 2000). The characters more often studied in chemotaxonomy are secondary metabolites of pharmaceutical significance such as alkaloids, glycosides, flavonoids etc (Kokate *et al.*, 2004). The ratio between flavone and flavonol can be used as an indicator of the evolutionary trends for a taxon (Harborne, 1977). Analysis of the flavonoid profiles solved the taxonomic controversy among the five plant taxa of *Solanum nigrum* complex (Ayesha *et al.*, 2009). Hence the data from the phytochemical investigation of these seven plants are used to
analyse the taxonomic relationship between the species. Much work has not been reported in the above mentioned criteria in the selected members of the family Asclepiadaceae viz., *Asclepias curassavica* L., *Calotropis gigantea* (L.) R.Br., *Gymnema sylvestre* (Retz.) R.Br., *Holostemma adakodien* Schult., *Pergularia daemia* (Forssk.) Chiov., *Tylophora indica* (Burm.f.) Merr. and *Wattakaka volubilis* (L.f.) Stapf.

### 1.1.5 Study of pollinial morphology

Members of the family Asclepiadaceae are unique due to the association of pollen grains into definite sac-like structures called pollinia, the product of one anther and it is transferred during pollination as a single unit. Pollinial morphology is taxonomically significant like the pollen morphology of other Angiosperms (Sanjit and Amal, 2011; Sajith and Sreedevi, 2005). Nature of the corpusculum, caudicle, pollinial sac, germ furrow are the important features for distinguishing different taxa of the family.

Woodson (1941) considered the excavated or depressed (concave) surface of pollinia as a criterion for distinguishing the sub families of family Asclepiadaceae. Translator of pollinia had been treated as a tool by Brown (1810), for segregating Asclepiadaceae from Periplocaceae. Within the Asclepiadaceae, absence of caudicles distinguishes the Secamoneae and Fockeeae from the rest (Swarupanandan *et al.*, 1996). Orientation of pollinia has been used as one of the important characters in the segregation of tribes within Asclepiadaceae (Don, 1838; Decaisne, 1844). Brown (1810) used the orientation of pollinia as a character for separating groups of genera.

The study of pollinial morphology as an effective aid to plant taxonomy and phlogeny has been amply demonstrated through a large
volume of publications. Hence in the present investigation along with the floral morphological and phytochemical evidences, pollinial morphology is taken as valid taxonomic criteria for distinguishing different genera of the plants under study viz., *Asclepias curassavica* L., *Calotropis gigantea* (L.) R.Br., *Gymnema sylvestre* (Retz.) R.Br., *Holostemma ada-kodien* Schult., *Pergularia daemia* (Forssk.) Chiov., *Tylophora indica* (Burm.f.) Merr. and *Wattakaka volubilis* (L.f.) Stapf.

**1.2 Significance of the study**

In this globalized world, alternative systems of medicine like Ayurveda, Siddha, Unani, Neuropathy and Homeopathy are still in existence and efficacy, safety and quality control of these plant drugs are important. To achieve precision, accuracy and consistency of formulation along with desired therapeutic effect, quality assurance of raw material is necessary. The available literature reveals that the family Asclepiadaceae consists of several members that are of medicinal importance and many of them are used in the preparation of drugs. Hence there is a need for preparing a protocol for checking the exactness of the herb. Many of the species have not been studied in this aspect. The present investigation comprises seven medicinal species belonging to the seven genera of the family Asclepiadaceae. The focus area of this research is phytochemistry of seven plants with a view to provide the correct botanical identity and to explore the chemoprofile.

All the parameters considered in the present study, would help to identify and authenticate the plant at source and to check the purity and quality of the plant drug. The fingerprint profiles can be used to detect the presence of adulterants and substitutes which are very common in the raw drug industry.
Identification of the presence of various biologically active compounds in these plants and the results of anti bacterial and anti oxidant study will provide scientific validity for ethno pharmacological uses of these plants in the treatment and prevention of various diseases and disorders. The analysis of mineral content and amino acid will surely emphasize the nutritional and medicinal aspects of the drugs.

The quantitative estimation of polyphenols and flavonoids will reveal the correlation between the biological activity and phytochemical content as well as the variation in the quantity of the phytochemical content based on season, habitat and age of the plant. The phytochemical data gathered from the present study can be used in biochemical systematics or chemotaxonomy.

1.3 Objectives of the present investigation

The main objectives of the present investigation are:

- To identify and authenticate the seven plants of the family Asclepiadaceae.
- To determine the ash value, concentration of selected inorganic elements and extractive value.
- To analyse and estimate the polyphenols and flavonoids of the plants and the preparation of chemoprofile and detection of pure compounds using HPTLC.
- To study the amino acid composition using HPLC technique.
- To study the antimicrobial and antioxidant activity and find the correlation between antioxidant capacity and estimated phytoconstituents.
▪ To compare the age-wise, habitat-wise and seasonal variations of secondary metabolite concentration.

▪ To study the pollinial morphology of the selected members.

▪ To prepare dendrogram by cluster analysis using the phytochemical data and thereby analyse the relationship between the species studied.