INTRODUCTION

Natural sources represent a virtual untapped reservoir of novel drugs. It has been estimated that only 5-15% of the approximately 2,50,000 species of higher plants have been systematically investigated for the presence of bioactive compounds (Balandrin et al., 1993). There is a dire need to expand the exploration of nature as a source of novel active agents that may serve as the leads efficacious drugs for a multitude of disease indications. Nature is the prime source of novel, active chemotypes as leads for effective drug development.

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic values (Nostro et al., 2000). About 80% of the world population rely on the use of traditional medicine which is predominantly based on plant materials (World Health Organization, WHO, 1993). The World Conservation Monitoring Centre recognized 17 mega diverse countries in 2000. India is one of them and has 17,000 flowering plants. Among the 34 biodiversity hotspots in the world, the Eastern Himalayas and the Western Ghats are the two hotspots in India (Rath, 2005). The Western Ghats, also known as Sahyadri Hills are a chain of hills that run along the western edge of peninsular India. Their proximity to the ocean and through orographic effect, they receive high rainfall. These regions have moist deciduous forest and rain forests. The entire Western Ghats is known for its biodiversity, richness and endemism of different species.

Biogeographically, the hill chain of the Western Ghats constitutes the Malabar province of the Oriental realm, running parallel to the west coast of India from 8° N to 21° N latitudes, 73° E to 77° E longitudes for around 1600 km. Rising up from a relatively narrow strip of coast at its western border, the hills reach up to a height of 2800 m before they merge to the east with the Deccan plateau at an altitude of 500-600 m. The average width of this mountain range is about 100 km. This region is highly species rich and under constant threat due to human pressure. With its complex, heterogeneous landscapes and high levels of biodiversity, it forms an ideal ground for the testing and elaboration. The Western Ghats are very rich in medicinal wealth. The forests and hills of this region is a treasure house of about 700 medicinal
plants, of which, some are used for traditional and folk medicinal practices (www.westernghat.org).

In India, medicinal plants are widely used by all sections of the population with an estimated 7500 species of plants used by several ethnic communities. India also has the second largest tribal population in the world after Africa (Kala, 2005). The state of Karnataka boasts an unparalleled diversity of medicinal plants in the country. It is estimated that, Karnataka is the home to about 4800 species of flowering plants out of which about 2000 species are medicinal (Foundation for Revitalization of Local Health Traditions, FRLHT, 2010). This is quite remarkable, as this number accounts for about 27% of the country’s flora, with just 10% of the geographical area. Karnataka with its unique wild habitats spread across the Western Ghats and the Deccan Peninsula is also the home to several endemic species of commercial importance.

**Medicinal Plants**

The healing power of plants is well known from time immemorial. The WHO recognizes that medicinal plants play an important role in the healthcare of about 80% of world population in developing countries and depend largely on traditional medicines, of which herbal medicines constitute the most prominent part (Farnsworth et al., 1991). Among the rich and varied plants of Indian forests, the medicinal plants constitute an important source.

The most accepted definition of a medicinal plant given by the Agricultural and Natural Resource Development is “Plants that are recognized by people to have reliable and effective medicinal values, are commonly used in treating and preventing specific ailments and diseases, and play an essential role in health care.”

The number of medicinal plants in India, both indigenous and introduced has been estimated to be between 3,000 - 3,500 species of higher plants. The Glossary of Indian Medicinal Plants has listed around 3,000 plants (Asolkar et al., 1992). Two thousand five hundred plants have been reported to be used in ethnomedicine (Jain and Goel, 1995). The number of plants listed in Ayurvedic Materia Nighantu is 560 (Khan, 2001). The Ayurvedic Drug Formulary prepared by the Department of Indian
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System of Medicine, lists 387 plants (Sarin, 1996). The Unani system of medicine describes 440 plants (Said, 1969), of which 360 are common to other systems practiced in the country. The number of plants with confirmed therapeutic properties or yielding a clinically useful chemical compound is around 700 species.

About 10% of the flowering plants have been scientifically examined for their medical application, mostly in a rudimentary way. Many more plant-derived medicinal substances are still awaiting discovery. The information on the medical wealth of the examined 10% of plant species is scattered in different sources.

Out of the large variety of species available in the Western Ghats, about 50 species hold a very high value in the folk and herbal health forms for the treatment of different ailments. The most common plants like *Mimosa pudica* L., *Hibiscus angulosus* Wallich. ex Wight & Arn., *Leucas aspera* Spreng., *Phyllanthus niruri* L., *Calotropis gigantea* R.Br., *Tridax procumbens* L., and *Parthenium hysterophorus* L. are found to have a cure for many major ailments like jaundice, asthma, piles, bronchial and blood disorders. Plants like *Annona squamosa*, *Buchanania lanzan*, *Semecarpus anacardium*, *Dioscorea bulbifera* and *Aphanamixis polystachya* are recommended for various forms of tumor. Frequent doses of medicinal plant extracts of *Rhinacanthus nasuta*, *Momordica dioica*, *Cinnamomum zeylanicum* and *Ophiorhiza mungos* relieves cancer patients (Ved and Goraya, 2008). The spread of knowledge of the natural wealth is more important for our country at a time when the synthetic drugs are stealing the rates of economy.

While the general understanding of a medicinal plant is rooted in the use of a plant to cure a disease, it should be noted that more than two third of the total medicinal plant diversity is used for preventive and promotive purposes. In Karnataka, 13 Medicinal Plants Conservation Areas (MPCAs) were established representing all major forest types and different altitude zones of the state (Somashekhar, 2010). They are as below:
Table 1a. Location and forest types of 13 MPCAs in Karnataka

<table>
<thead>
<tr>
<th>MPCA</th>
<th>Area (ha)</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Altitude (msl)</th>
<th>Forest type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biligiri Rangana Hills</td>
<td>150</td>
<td>11° 59’</td>
<td>77° 08’</td>
<td>790-1050</td>
<td>Southern dry mixed deciduous forest</td>
</tr>
<tr>
<td>Talacauvery</td>
<td>80</td>
<td>12° 23’</td>
<td>75° 29’</td>
<td>1000-1355</td>
<td>West coast semi-evergreen forest</td>
</tr>
<tr>
<td>Savandurga</td>
<td>280</td>
<td>12° 55’</td>
<td>77° 20’</td>
<td>800-970</td>
<td>Dry deciduous scrub forest</td>
</tr>
<tr>
<td>Subramanya</td>
<td>200</td>
<td>12° 40’</td>
<td>75° 37’</td>
<td>250-800</td>
<td>West coast semi-evergreen forest</td>
</tr>
<tr>
<td>Charmadi</td>
<td>283</td>
<td>13° 05’</td>
<td>75° 28’</td>
<td>300-1250</td>
<td>West coast semi-evergreen forest</td>
</tr>
<tr>
<td>Devarayadanadurga</td>
<td>178</td>
<td>13° 23’</td>
<td>77° 14’</td>
<td>850-1040</td>
<td>Southern thorn forest</td>
</tr>
<tr>
<td>Kudremukha</td>
<td>110</td>
<td>13° 17’</td>
<td>75° 08’</td>
<td>760-820</td>
<td>Southern hilltop tropical evergreen forest</td>
</tr>
<tr>
<td>Kemmangundi</td>
<td>310</td>
<td>13° 32’</td>
<td>75° 45’</td>
<td>1300-1700</td>
<td>Southern hilltop tropical evergreen forest</td>
</tr>
<tr>
<td>Agumbe</td>
<td>210</td>
<td>13° 29’</td>
<td>75° 07’</td>
<td>600-700</td>
<td>West coast tropical evergreen forest</td>
</tr>
<tr>
<td>Devimane</td>
<td>210</td>
<td>14° 32’</td>
<td>74° 34’</td>
<td>50-500</td>
<td>West coast semi-evergreen forest</td>
</tr>
<tr>
<td>Sandur</td>
<td>350</td>
<td>15° 03’</td>
<td>76° 31’</td>
<td>550-773</td>
<td>Southern dry mixed deciduous forest</td>
</tr>
<tr>
<td>Karpakkapalli</td>
<td>150</td>
<td>17° 37’</td>
<td>77° 26’</td>
<td>600-750</td>
<td>Dry deciduous scrub forest</td>
</tr>
<tr>
<td>Kollur</td>
<td>275</td>
<td>13° 43’</td>
<td>77° 49’</td>
<td>120-240</td>
<td>West coast semi-evergreen forest</td>
</tr>
</tbody>
</table>


Today, there is an increasing desire to unravel the role of ethnobotanical studies in trapping the centuries old traditional folk knowledge as well as in searching newer plant resources for food, drugs etc.
Introduction

Phytochemical investigations of plants

In India, many indigenous plants are used in herbal medicine to cure diseases and heal injuries. The scientific studies available on a number of medicinal plants indicate that promising phytochemicals can be developed for many human health related problems (Dahiru et al., 2005), including diabetes, cancer and infectious diseases. The medicinal values of plants lie in their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body. The phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as a defense system against disease or more accurately, to protect against disease. Phytomedicines play a major role in human health care system. There is a considerable interest in elucidating the mechanism of their action to develop better medicines. Plants contain many free radical scavenging molecules such as phenolic compounds, nitrogenous compounds, vitamins, terpenoids etc. Phenolics are among the most important compounds occurring in plants, comprising at least 8,000 different known structures (Bravo, 1998). These compounds are reported to exhibit anti-carcinogenic, anti-inflammatory, anti-thrombotic, immune modulating and analgesic activities as antioxidants (Vinson et al., 1998). Therefore, the need to develop efficient, safe and inexpensive drugs from plant sources is of great importance.

The past decade has also witnessed an intense interest in herbal medicines in which phytochemical constituents can have long-term health promoting or medicinal qualities. The phytochemicals present in functional foods that are responsible for preventing disease and promoting health have been studied extensively to establish their efficacy and to understand the underlying mechanism of their action. Such studies have included the identification and isolation of the chemical components, establishment of their biological potency both by in vitro and in vivo studies in experimental animals and through epidemiological and clinical-case control studies in man. These phytochemicals are reported to prevent disease mainly through their functions as antioxidants, detoxifiers, neuropharmacological and immunopotentiating agents, and as a source of dietary fibres (nonstarch polysaccharide, NSP).
The active principles differ from plants to plant due to their biodiversity and they produce a definite physiological action on the human body. Calixto (2000) reported that most of the cultivated medicinal and aromatic plants are exported as crude drugs. Ijeh et al. (2004) noted the growing interest in the medicinal properties of a number of common plants. The alkaloids are very important in medicine and constitute most of the valuable drugs. They have marked physiological effect on animals (Edeoga et al., 2005) and alkaloids such as solasodine have been indicated as a starting material in the manufacture of steroidal drugs (Maxwell et al., 1995).

Phenolics of herbal origin have received considerable attention because of their potential antioxidant activities (Pan et al., 2008). These compounds are known to act as antioxidants due to their ability to donate hydrogen or electrons, and as stable radical intermediates. The literature survey reveals that most antioxidant activities from plant sources are correlated with phenolic compounds (Jain et al., 2008; Huang et al., 2010). Saponins are glycosides of both triterpenes and sterols and have been detected in over seventy families of plants (Basu and Rastogi, 1967). In medicine, they are used as expectorants and emulsifying agents. Tannins are fairly frequently encountered in food products of plant, vegetable origin such as tea and many fruits. The oxidation inhibiting activities of tannins have been known for a long time and it is assumed to be due to the presence of gallic and diagallic acids (Ihekoronye and Ngoddy, 1985).

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties (Milkauskas et al., 2004). The structure antioxidant activity relationship of flavonoid systems has been extensively reported. The antioxidant activity depends upon the number and positions of hydroxyl groups, other substitutes and glycosylation of flavonoid molecules (Bouaziz et al., 2005).

The importance of plants selected for study

**Fabaceae** is the third largest family of flowering plants. Lack of essential oils in the plants of this family can be an advantage in the search for safe and effective medicines.
**Plant description**


Class: Magnoliopsida
Order: Fabales
Family: Fabaceae
Sub family: Faboideae
Tribe: Phaseolae
Sub tribe: Cajaninae
Genus: Cajanus
Species: *Cajanus lineatus* (W. & A.) Maesen
(Source: http:npgsweb.ars.gov).

Erect shrubs, branchlets silky tomentose. Leaves alternate; leaflets 3-4 x 1.5 cm, obovate or rhomboid, apex mucronate, base acute, 3-ribbed, grey tomentose below, subsessile; petiole to 1.5 cm long. Flowers to 1.5 cm long, yellow, in axillary raceme. Calyx tomentose, lobes deltoid, acuminate. Ovary densely villous. Pods to 2 x 0.8 cm, densely hairy; seeds 3. The flowers and fruits appear in June-January which is frequently distributed along the Western Ghats from South Canara and Mysore to Travancore, at 3000 to 5000 ft. (Keshavamurthy, 1994).

*Lamiaceae* is known for its fine medicinal and aromatic herbs like lavender, mint, oregano, sage and thyme and is a rich source of essential oils for the food, pharmaceutical and cosmetic industry.

**Plant description: Leucas ciliata Benth.**

Classification:

Class: Magnoliopsida
Order: Lamiales
Family: Lamiaceae
Genus: Leucas
Species: *Leucas ciliata* Benth.
(Source: www.indiabiodiversity.org)

The plant habit is either herb or under shrubs, usually 30-100 cm high. The stems and branches are obtusely quadrangular and has brownish hairs. The leaves are
ovate or lanceolate in shape, about 3 – 9 cm long and 2.5 - 4 cm wide. The lamina is membranous, sparsely hairy on both sides with an acute apex, a cuneate base and serrate margin. The flowers are white in color with a brown lip and have dense globose whorls. They have slender spinulose bracts equaling calyx. The calyx is tubular, hairy outside, with a ring of hairs at the mouth and measures 1.2 – 1.8 cm in length. The corolla is a long tube annulate inside and measures about 1.8-2.0 cm in length. The nutlets are smooth, oblong-obvoid in shape and brown in color. The flowers and fruits appear in May – August which is frequently distributed along the Western Ghats, the hills of South Canara, Coorg, Mysore to the South- East Wyanaad at 3000 ft., and in the plains along forest edges in India (Keshavamurthy, 1994).

Leucas ciliata commonly known as Burumbi is traditionally used for wound healing and as an antidote for snakebite (Habbu et al., 2007; Hynniewta and Kumar, 2008). In the Chinese medicine, it is used as an antibacterial and antifungal agent (Long and Li, 2004). Preliminary phytochemical analysis of L. ciliata leaves indicated the presence of relatively high levels of flavonoids. Several flavonoids have been reported to possess antioxidant and hepatoprotective properties (Alan and Miller, 1996).

Apocynaceae consists of about 250 genera and 2000 species of tropical trees, shrubs, and vines. In the traditional medicine, species of apocynaceae are used to treat gastrointestinal ailments, fever, malaria, pain, and diabetes (Wiart, 2006). The phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds or secondary metabolites of plants serve as defense mechanism against predation by many microorganisms, insects and herbivores. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids etc. (Hu et al., 2001).

The Indian medicinal plant Rauwolfia serpentina have been analyzed for their chemical composition, vitamins and mineral by Harisaranraj et al. (2009) the results revealed the presence of bioactive constituents comprising alkaloids, saponins, flavonoids, phenols and tannins. The plant contained ascorbic acid, riboflavin,
thiamine and niacin. The importance of these chemical constituents is discussed with respect to the role of these herbs in ethnomedicine in India.

**Plant description: Rauvolfia densiflora (Wall) Benth. Ex. Hook.f**

**Classification**

Class: Magnoliopsida  
Order: Gentianales  
Family: Apocynaceae  
Genus: Rauvolfia  

(Source: [www.indiabiodiversity.org](http://www.indiabiodiversity.org))

*R. densiflora* (Wall) Benth. ex Hook. f. syn. *R. verticillata* F., is known to the Kanikkar tribes as “Paarisirunila /Sirumarapatchilai”. It is commonly known as Dense-Flowered Snake Root in English. It is a large shrub 3-6 m high with milky juice and obovate or ob lanceolate leaves, rose-red or white flowers, obliquely ellipsoid, brownish or purple drupes, compressed pyrenes pointed at the tip, found in Dajipur, Hassane, Patagaon, Tambyachiwadi, Khandala, Mahabaleshwar, Matheran, Lonavala, Maharas tra, the Himalayas, Khasi and Aka hills, Annamalai hills, Hills of Tinnevelly and Travancore and the Western and the Eastern Ghats (Gamble, 1928).

**Plant description: Gomphostemma heyneanum Wall. Ex. Benth.**

**Classification**

Class: Magnoliopsida  
Order: Lamiales  
Family: Lamiaceae  
Genus: Gomphostemma  

(Source: [www.indiabiodiversity.org](http://www.indiabiodiversity.org))

The plant is tall, shrubby and herbaceous. Stem obtusely quadrangular, densely covered with stellate hairs. Leaves 25 x 15 cm, broadly elliptic, acute at both ends, thickly stellate hairy below, sparsely hairy above, crenate, nerves 5-7 pairs; petiole 4-6 cm long. Racemes 18 x 2.5 cm. Flowers yellow, 10 to 20 together, densely packed, bracts elliptic, acute. Calyx 12 mm long, lobed to the middle, lobes lanceolate; corolla 15 mm long, tube 7 mm broad, cylindrical, midlobe of lower lip
obtuse, emarginate; filaments unequal, glabrous. Nutlets glabrous. Flowers appear in September-January. Plants are distributed along the southern Western Ghats, Southeast Wyanaad at 300 ft., Mudumalai forest and Annamalai hills, evergreen forests of Travancore, Hills of Tinnevelly (Gamble, 1928).

By studying the presence of phytochemicals in this plant, the uses of this plant in the traditional treatment can be explained scientifically.

**Endophytes**

Microbial natural products have played a major role as sources of drug lead compounds during the last century. Their biological activity, rich structural diversity and complexity has prompted synthetic chemists to produce them in the laboratory, for therapeutic applications. Many drugs used today are natural products or natural-product derivatives. These natural products are produced by all microorganisms; but are also known from plants, insects, fungi, algae and prokaryotes. Williams *et al.* (1989) proposed that all secondary metabolites serve the producing microorganism by improving their survival fitness—“by acting on specific receptors in competing organisms”.

The endophytes are microbes that colonize the living, internal tissues of plants without causing any immediate, overt negative effects (Bacon and White, 2000). The endophytes (fungi, bacteria and yeasts) represent a huge diversity of microbial adaptation that have developed and sequestered environment and their diversity and specialized habitation makes them an exciting field of study in the search for newer therapeutics. Many scientists are of the opinion that plants growing in lush tropical rainforests where competition for light and nutrients are severe are most likely to host the greatest number of bioactive endophytes. The survey of plants for the endophytes started about two decades ago and indicated the ubiquitous colonization of land plants by these organisms. The endophytes have developed the biochemical ability to produce compounds similar or identical to those produced by their host plants as a result of recombination during the evolutionary process (Bascom-Slack *et al.*, 2009).
**Actinomycetes**

The actinomycetes belong to the subdivision Actinomycetales of the Prokaryotae (Lechevalier, 1989). They form a distinct phylogenetic line in the 16S rDNA tree and have been of major scientific interest in the past decades, with the discovery a large number of metabolites produced by its diverse genera. They are of special biotechnological interest since they are known to produce chemically diverse compounds from anti-cancer agents to various alkaloids with a wide range of biological activity from antibiotics to enzyme inhibitors. The Indian subcontinent has immense biological diversity and it is increasingly recognized that a large number of novel chemical entities exist as metabolites in the microflora. The actinomycetes have evolved as a group with greatest genomic and metabolic diversity. Efforts should be directed towards exploring actinomycetes as a source in the discovery of novel secondary metabolites.

In India, actinomycete research in both marine and terrestrial ecosystems has prospered significantly in past few decades. This valuable class contains a large number of genera and demands more attention for exploration. Though substantial work in this field has been carried out, the diversity from the extreme environments in the Indian Peninsula remain unexplored. Marine actinomycete research has been restricted to the coastal ecosystems, while the deep sea oceanic floors remain untapped. Bioprospecting of actinomycetes for bioactive molecules has not been explored in the extremophilic environments in India and the molecular mechanisms for the production of various bioactive compounds are yet to be reported.

The actinomycetes are ubiquitously distributed in terrestrial, freshwater and marine environments and are involved in the breakdown of organic matter and xenobiotic compounds. The Indian peninsula harbours its own diverse habitats which support the growth of various actinomycete communities in specific microbial niches. Hence, in India, the actinomycete diversity has been an important source for natural product discovery. Over the years, novel species of actinomycetes have been discovered from diverse habitats of India: Novel species of actinomycetes viz., *Rhodococcus kroppenstedtii, Planococcus stackebrandtii, Agrococcus lahulensis* and *Kocuria himachalensis* have been reported from Himalayas (Mayilraj *et al.*, 2005;
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Mayilraj et al., 2006a, b and c). Two novel species of actinomycetes were discovered from coal mine viz., Agrococcus carbonis and Yaniella fodinae (Dhanjal et al., 2010 and 2011). Syed et al. (2008, 2009a and b) have reported two new species, Streptomyces gulbargensis and Saccharomonospora saliphila from the muddy soils of Gulbarga, Karnataka producing substantial amounts of α-amylase and keratinase. Rhodococcus canchipurensis sp. nov. has been discovered by Nimaichand et al. (2012) from the limestone deposit site of Manipur. Streptomyces sundarbensensis sp. nov. which produced 2-allyloxyphenol was reported from the mangrove forest of Sundarbans by Arumugam et al. (2011). Recently Malviya et al. (2011) and Tripathi et al. (2011) reported the diversity of Streptomyces sp., in the wheat fields of the Indo-Gangetic plains and from pulp and paper mill effluent-treated crop fields respectively, depicting diverse actinomycete populations and metabolic profiles.

It is generally accepted that the Streptomycetes have a particular capacity to produce various bioactive compounds that have wide spectrum activity (Zin et al. 2007). 45% of the presently known bioactive microbial metabolites are still isolated from various actinomycetales species, and the Streptomyces species produces 7600 compounds (74% of all actinomycetales), while the rare actinomycetes represent 26%, altogether 2500 compounds (Berdy, 2005). It appears that the endophytes are relatively untapped sources of novel natural products, and Streptomyces also exist as endophytes within the living tissues of higher plants. Moreover, it has been suggested that they serve as sources of novel bioactive products (Ryan et al., 2008).

Screening for bioactive compounds

Plants continue to provide new chemical bioactive lead molecules for drug development against various pharmacological targets such as cancer, Human Immunodeficiency Virus (HIV)/ Acquired Immuno Deficiency Syndrome (AIDS), diabetes, malaria, Alzheimer’s disease and pain. Several natural products, drugs of plant origin are available and to name a few are taxol, paclitaxel, camptothecin-derived analogues, gacrinol, hydroxycitric acid, arteether, galanthamine, tiotropium etc. (Jachak and Saklani, 2007).
Antioxidant activity

It is currently believed that reactive oxygen species (ROS) have an important role in the etiology of several non-communicable diseases. Oxidants and free radicals such as singlet molecular oxygen, superoxide, hydroxyl peroxide and lipid peroxides are known to cause tissue damage. Such free radicals also include nitrous oxide radicals that are generated in the gastrointestinal tract. Tissue damage caused by free radicals, when it becomes cumulative, is considered to play an important role in the pathogenesis of several degenerative diseases, for example, cancer, cataract, coronary heart disease, dementia, diabetes mellitus, rheumatic arthritis, muscular degeneration, pulmonary dysfunction and radiation sickness. Superoxide dismutase converts the $O_2$ radical to hydrogen peroxide, which can be further destroyed by catalase. Glutathione reductase, for which riboflavin is the coenzyme, is also a part of the in vivo defense system against oxidation damage in the cells. Selenium too plays a role in this system through glutathione peroxidase. This in vivo system of detoxifying oxygen free radicals may not be capable of neutralizing all of the free radicals produced in the body as well as those derived from the environment, and there is a need for an external source of antioxidants to neutralize the free radical load in the body.

Oxidative stress induced by oxygen radical is believed to be a primary factor in various degenerative diseases such as cancer, atherosclerosis, gastric ulcer and other conditions. Antioxidants protect other molecules from oxidation when they are exposed to free radicals and reactive oxygen species, which have been implicated in the aetiology of many diseases and in food deterioration and spoilage (Koleva et al., 2000). Synthetic antioxidants such as butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA) and propyl gallate (PG) have been widely used as antioxidants in the food industry (Nawar, 1996). However the safety of these synthetic antioxidants has been questioned. BHA has shown to be carcinogenic and BHT has been related to internal and external hemorrhaging at high doses in rats and Guinea pigs (Ito et al., 1983). These findings together with consumer interest in natural food additives have reinforced the need for effective antioxidants from natural resources as an alternative to prevent deterioration of food during processing and storage. Natural antioxidants protect the human body from free radicals and retard the progress of
many chronic diseases as well as related lipid oxidative rancidity in food, cosmetics, and pharmaceutical materials (Lai et al., 2001).

Free radicals or reactive oxygen species are formed in our body as a result of biological oxidation. The over production of free radicals such as hydroxyl radical, super oxide anion radical, hydrogen peroxide can cause damage to the body and contribute to oxidative stress (Diplock, 1994; Thomson, 1995). Oxidative damage of proteins, DNA and lipids are associated with chronic degenerative diseases including cancer, coronary artery disease, hypertension, diabetes etc. (Lee et al., 2000) and compounds that can scavenge free radicals have great potential in ameliorating these disease processes (Behera et al., 2006). Most of the reactive oxygen species are scavenged by endogenous defense systems such as catalase, superoxide dismutase and peroxidase-glutathione system (Rice-Evans and Bourdon, 1993). But these systems may not be completely efficient, requiring them to depend on exogenous antioxidants from natural sources.

Generally, antioxidants have been identified as major health beneficial compounds reported from varieties of medicinal plants and are sources for alternative medicines (Daniel, 2006). There have been several recent epidemiological studies that implicate dietary antioxidant phytochemicals like carotenoids, phenolic compounds and flavonoids as protective agents against cancer and cardiovascular disease (Zeigler et al., 1992). The flavonoids act by scavenging superoxide anions, singlet oxygens and lipid peroxyradicals, and through sequestering metal ions that promote oxyradical formation. Quercetin, the major flavonoid, inhibits oxidation and cytotoxicity of low-density lipoprotein. Flavonoids also inhibit cyclooxygenase, leading to lower platelet aggregation and reduced thrombotic tendencies.

The human body system has a number of mechanisms to eliminate the free radicals formed. When the normal levels of antioxidant defense mechanism is not sufficient for the eradicate of free radical-induced injury, the administration of the antioxidant will have a protective role to play (Kumar and Kuttan, 2009). To protect biological targets from oxidative damage, the ROS should be scavenged by antioxidants before they react with other cellular components. Natural antioxidants from plant extracts are safe and do not have any side effects. Plant sources have
provided a number of drugs. Most of the modern clinical drugs are of natural product origin. The secondary metabolites generally accounts for less than 10% of the total plant metabolites, its products are the main plant constituents with pharmaceutical properties (Ali, 2010).

**Enzyme inhibitors from plants**

The enzyme inhibitors are molecules that combine with the enzyme in such a manner as to prevent the normal substrate-enzyme combination and the catalytic reaction there by it affects the activity of enzymes. The control of enzyme activity through the use of inhibitors has a vast variety of uses. A large number of commonly used drugs are enzyme inhibitors; these include drugs to treat HIV, cancer and heart disease. The enzyme inhibitors are used in agriculture as pesticides and herbicides. The enzyme inhibitors are naturally occurring, but many have also been designed and created synthetically. There are several different mechanisms of enzyme inhibition all of which have their own characteristic effect on the kinetics of the enzyme (Bogoyevitch et al., 2006).

Many drug molecules are enzyme inhibitors, so their discovery and improvement is an active area of research in biochemistry and pharmacology. A medicinal enzyme inhibitor is often judged by its specificity (its lack of binding to other proteins) and its potency (its dissociation, which indicates the concentration needed to inhibit the enzyme). A high specificity and potency ensure that a drug will have few side effects and thus low toxicity.

Many plants are known to contain the drug bases as alkaloids, saponins, tannins, cardiac glycosides, and polyphenols and more than 30% of commercial drugs are being extracted from plants (Barrett and Udani, 2011).

Phytochemicals have shown alpha amylase inhibitory activity. Phaseolamin (Barrett and Udani, 2011), quercetrin (Lo Piparo et al., 2008), rutin (Kim et al., 2000), kaempferol (Lo Piparo et al., 2008) belong to flavonol, catechin hydrate- a Proanthocyanidin (Silva Pinto et al., 2009), strictinin- a tannin (Jullian et al., 2007), Squalene -a terpene (Conforti et al., 2005) possess inhibition of α-amylase activity.
α-amylase inhibitors

The inhibition of α-amylase, the enzyme that plays a role in digestion of starch and glycogen, is considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes and obesity, as well as, dental caries and periodontal diseases. Plants are an important source of chemical constituents with potential for inhibition of α-amylase and can be used as therapeutic or functional food sources.

Disorders of carbohydrate uptake may cause severe health problems such as diabetes (Van de Laar, 2008), obesity (Yanovski and Yanovski, 2002), and oral diseases (Touger-Decker and Loveren, 2003). This could be done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolysing enzymes, α-glucosidase and α-amylase, present in the small intestinal border that are responsible for the breakdown of oligosaccharides and disaccharides into monosaccharides suitable for absorption (Van de Laar, 2008). Inhibitors of these enzymes, like Acarbose™, delay the carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise (Cheng and Fantus, 2005).

Due to their purported ability to prevent starch breakdown and absorption, α-amylase inhibitors have been used for weight loss in humans (Bailey, 2003). Acarbose™ and Voglibose™ are currently used as α-amylase and α-glucosidase inhibitors, but also induce side effects such as abdotension, bloating, flatulence and diarrhoea (Chakrabarti and Rajagopalan, 2002). It has been suggested that such adverse effects might be caused by the excessive inhibition of pancreatic α–amylase resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon (Bischcoff, 1994). Therefore, natural α-amylase inhibitors from the dietary plants can be used as an effective therapy for post prandial hyperglycemia with minimal side effects (Kwon et al., 2006).

Protein inhibitors of α-amylase occur widely in plants. The inhibitors are believed to make plants less palatable, even lethal to insects, thus contributing some selective advantage to the plants (Sasikiran et al., 2002). Amylase inhibitors are known as starch blockers because they prevent dietary starches from being digested and absorbed by the body. This could be useful for treating obesity and diabetes.
mellitus - a metabolic disorder characterized by chronic hyperglycemia resulting from 
defects in insulin secretion (Ali et al., 2006).

Two proteins (A-1 and B-2) with α-amylase inhibitory activity were extracted and partially purified from Colocasia esculenta tubers. The inhibitors inactivated α-amylases of animal origin, but had no effect on the fungal amylase. Inhibitor A-1 also exhibited activity towards plant amylases, while inhibitor B-2 has no activity on plant amylases. Inhibitor A-1 was the most active against human salivary amylase (McEwan et al., 2010).

Inhibitors of α-amylase from plants

Plants have been reported to show α-amylase inhibitory activity and so may be relevant to the treatment of type 2 diabetes. About 800 plant species have been reported to possess anti-diabetic properties. A wide range of plant-derived principles belonging to compounds, mainly alkaloids, glycosides, galactomannan gum, polysaccharides, hypoglycans, peptidoglycans, guanidine, steroids, glycopeptides and terpenoids, have demonstrated bioactivity against hyperglycemia (Mentreddy, 2007).

Several plant species of the angiosperm families and their extracts have proven to exhibit α-amylase inhibitory activity (Kumar et al., 2008; Funke and Melzing, 2006). A wide array of plant derived chemical compounds have demonstrated activity consistent with their possible use in the treatment of diabetes. Research on new bioactive compounds from medicinal plants have led to the isolation and structure elucidation of a number of exciting new pharmacophores. The oligosaccharide inhibitors of the trestatin family that contain the acarviosine moiety (e.g., Acarbose), proteinaceous inhibitors isolated from microbial sources and plant tissues (Svenssund and Sinervo, 2004) and molecules present in plants comprise the natural inhibitors of α-amylase (Lo Piparo et al., 2008).

Phenolic compounds are a large group of structurally diverse naturally occurring compounds that possess at least a phenolic moiety in their structures. Most of these compounds possess various degrees of antioxidant or free radical scavenging properties as well as medicinal properties and have long been used as drugs. Flavonoids are an abundant class of natural phenolic compounds with several
biological activities. Also, there is a need for novel agents, therapeutic strategies or designing functional foods that could act on the physiological regulation of sugar uptake, blood sugar levels, and prevention of oral diseases.

**Inhibitors of α-amylase from the actinomycetes**

When compared to the enzyme inhibitors from animals and plants, the microbial inhibitors are low molecular weight compounds derived from the hydrolysis of macromolecular substances (Imada, 2004). The α-amylase enzyme inhibitor-producing marine actinomycete, *S. corichorusii* subsp. *rhodomarinus* subsp. nov., is the only enzyme inhibitor reported from marine actinobacteria (Imada, 2005). Considering the importance of α-amylase enzyme inhibitors, especially of microbial origin, the present study was attempted to evaluate the α-amylase enzyme inhibitory activity of the endophytic actinomycetes.

Acarbose™, a well known drug widely used for clinical treatment of diabetes mellitus, is a pseudotetrasaccharide, produced by the fermentation of *Actinoplanes* sp., consisting of a polyhydroxylated aminocyclohexene derivative (valienamine) linked via its nitrogen atom to a 6-deoxyglucose, which is α-1,4-linked to a maltose moiety. It is a competitive inhibitor of α-amylase and the mechanism of inhibition is due to the unsaturated cyclohexene ring and the glycosidic nitrogen linkage that mimics the transition state for the cleavage enzymatic state of glycosidic linkages (Yoon and Robyt, 2003).

Many researchers have reported the isolation of actinomycetes from various sources such as soil, marine, plants exhibiting α-amylase inhibition activity. *Streptomyces corchorusii*, *S. calvus*, *S. hygroscopicus-limoneus*, *S. dimorphogenes*, *S. corichorusii* subsp. *rhodomarinus* subsp. nov., *Actinoplanes utahensis* are marine actinomycetes with potential α-amylase inhibitory activity (Arai et al., 1985; Yokose et al., 1984; De Melo et al., 2006).

*Streptomyces olivochromogenes* isolated as an endophyte of *Tinospora crispa* (Pujiyanto et al., 2012) and *Streptomyces* sp. *loyola UGC* from the root fragments of *Datura stramonium* L. (Christhudas et al., 2013) have shown the ability to inhibit the α-glucosidase activity.
Characterization of bioactive compounds

A compound or a substance having biological activity, if it has a direct effect on a living organism. These effects may be positive or negative depending on the substance, the dose or the bioavailability (Aksel, 2010). The medicinal value of these bioactive compounds lies in its physiological action on the humans and other animals (Raj et al., 2012). In most of the cases, these compounds were found to include secondary metabolites like antibiotics, mycotoxins, alkaloids, sterols, carotenoids, food grade pigments, plant growth factors and phenolic compounds (Kris-Etherton et al., 2002).

According to the WHO, nearly 20,000 medicinal plants exist in 91 countries, including 12 mega biodiversity countries. The premier steps to utilize the biologically active compound from plant resources are the extraction, pharmacological screening, isolation and characterization of bioactive compounds, toxicological and clinical evaluation (Fig. 1a). The bioactive compounds from plant extracts are characterized with the common phytochemical screening assay, chromatographic techniques, such as High Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Mass Spectrometry (FTMS).

Gas chromatography and Mass Spectrometry (GC-MS)

Chromatographic techniques for the detection and identification of metabolites in plant materials have undergone major changes in recent years due to improvements of analysis time, detection limit and separation characteristics. Gas chromatography (GC) in particular is characterized by sensitivity and reliability of separations and detection of complex sample mixtures coupling with mass spectrometry (MS), it provides highly robust analysis platforms compared to liquid chromatography (LC-MS) and allows for the identification of compounds based on the use of commercially or publicly available MS libraries and resources in combination with the retention time index (RI) data.

GC-MS analysis is the first step towards understanding the nature of active principles in the medicinal plants. GC-MS method is a direct and fast analytical approach for the identification of flavonoids, terpenoids and steroids and only a few
grams of plant material is required. The importance of the study is due to the biological activity of some of these compounds.

**Figure 1a. General approaches in the extraction, isolation and characterization of bioactive compound from plant extracts (Source: Sasidharan et al., 2011)**

**Molecular docking studies**

Natural products represent a rich source of biologically active compounds and are an example of molecular diversity, with recognized potential in drug discovery and development. Despite changing strategies in the natural product research, so the rate of discovery of truly novel natural product drugs is also increased. The advent of bioinformatics reduced the cost and time of drug screening process. In order to reduce the cost of developing new medicines and their time to market, pharmaceutical companies have attempted to streamline the drug discovery process using computational methods. Today, every drug company has adopted a computational methodology in most stages of the design process (Tramontano, 2006). Many computational methods complement one another and may be combined to help rationalize the drug discovery process. In cases where it is possible to determine the
3-dimensional (3D) structure of the biomolecular target, molecular docking (Sousa et al., 2006) becomes possible and allows structure-based hit identification and/or lead optimization (Kitchen et al., 2004).

Drug design is the inventive process of finding new medications based on the knowledge of a biological target (Madsen et al., 2002). The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as, a protein, which in turn results in a therapeutic benefit to the patient. In the most basic sense, drug design involves the design of molecules that are complementary in shape and charge to the biomolecular target with which they interact and therefore will bind to it. Drug designing relies on computer modeling techniques. This type of modeling is often referred to as computer-aided drug design. The drug design that relies on the knowledge of the three-dimensional structure of the biomolecular target is known as structure-based drug design (Reynolds et al., 2010).

The most fundamental goal in drug design is to predict whether a given molecule will bind to a target. Molecular mechanics or molecular dynamics are most often used to predict the conformation of the small molecule and to model the conformational changes in the biological target that may occur when the small molecule binds to it.

There are two major types of drug design. The first is referred to as ligand-based drug design and the second, structure-based drug design (Reynolds et al., 2010). Ligand-based drug design (or indirect drug design) relies on knowledge of other molecules that bind to the biological target of interest. Structure-based drug design (or direct drug design) relies on knowledge of the three dimensional structure of the biological target obtained through methods such as X-ray crystallography or Nuclear Magnetic Resonance (NMR) spectroscopy (Leach et al., 2007). Current methods for structure-based drug design can be divided roughly into three main categories (Klebe, 2000). The first method is the identification of new ligands for a given receptor by searching large databases of 3-D structures of small molecules to find those fitting the binding pocket of the receptor using fast approximate docking programs. This method is known as virtual screening. A second category is de novo design of new ligands. In this method, the ligand molecules are
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built up within the constraints of the binding pocket by assembling small pieces in a stepwise manner. These pieces can be either individual atoms or molecular fragments. The key advantage of such a method is that novel structures, not contained in any database, can be suggested (Schneider and Fechner, 2005). A third method is the optimization of known ligands by evaluating proposed analogs within the binding cavity (Klebe, 2000).

Purpose of the present study

According to the literature survey, the phytochemical constituents as well as the endophyte profile of the selected medicinal plants are limited. The isolation and characterization studies of actinomycetes from India are limited. But, these findings are restricted to the isolation, identification and their maintenance, while few studies have focused on the antagonistic properties against microbial pathogens. The chemical diversity of these organisms suggests that they are the potential sources of novel bioactive compounds. The antioxidant activity of the above mentioned plants may throw some light on the related pharmacological properties. The convergent nature of our strategy will facilitate to isolate novel compounds from plant extracts and the endophytic actinomycetes. The study helps in the scientific validation of the plants as drug targets. The biologically active secondary metabolites will be useful for pharmaceutical purposes. The extended significance of the productivity of endophytes for some important phytochemicals provides an alternative strategy for easing the impact on the growing population of plants, which are needed for the preservation of biological diversity and ecosystem. The inhibitors of α-amylase is considered as drug target for the treatment of diseases such as diabetes, obesity and hyperlipaemia. Screening for these inhibitors from both plants and microbes has received much attention and are promising sources of unusual chemical structures with potent inhibitory activity.