1.1 Medicinal Plants and Their Importance

Herbs are staging a comeback and herbal renaissance is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and the environment. Over three-quarters of the total world population depends mainly on plants and plant extracts for health care. As per estimate the World Market for plant derived drugs may account for about Rs.2,00,000 crores; of which the Indian contribution is less than Rs.2000 crores. Of the 2,50,000 higher plant species on earth, more than 80,000 are medicinal.

Traditional herbalists in India use a variety of herbal preparations to treat different kinds of ailments including several microbial infections. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented (Dubey et al., 2004). The demand for plant based medicines, health products and pharmaceuticals is increasing in both developing and developed countries due to the growing recognition that the natural products are non-toxic, have least side effects and easily available at affordable prices (Kalia, 2005; Iniaghe et al., 2009). Plants are a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection. It is estimated approximately that 20% of the plants found in the world alone have been submitted to pharmacological or biological tests (Suffredini et al., 2004).

India is one of the world’s megabiodiversity centers having more than 45,000 plant species. Its diversity is unmatched due to the presence of 16 agroclimatic zones, 10 vegetative zones and 25 biotic provinces (Anonymous, 1998) and 426 biomes (habitats of specific species). Of these 15000 - 20000 plants have good medicinal value. But, only 7000-7500 species are used for their medicinal values by traditional communities. In India, drugs of herbal origin have been used in traditional systems of medicines. The Ayurvedic system of medicine uses about 700 species, Unani 700, Siddha 600, Amchi 600 and modern medicine around 30 species. The drugs are derived either from whole plant or from different organs like leaves, stem, bark, roots, flowers and seeds or from excretory plant product such as gum, resins and latex. The Allopathic system of medicine also has adopted a number of plant derived...
drugs. Some important chemical intermediates needed for manufacturing the modern drugs are also obtained from plants. Eg. diosgenin, solasodine, β-ionone. To put in brief, medicinal plants are the richest bioresource of drugs in traditional systems of medicines, modern medicines, pharmaceutical intermediates, folk medicines and chemical entities for synthetic drugs (Hammer et al., 1999). The present enthusiasm in the search for natural sources of biologically active compounds from plants has all the more enhanced the importance of medicinal plants.

1.2 Present Day Significance of Plant Derived Drugs

Over the last fifty years, expectancy and quality of human life have increased tremendously due to improvements in nutrition and use of antibiotics in treatment of infectious diseases. However, indiscriminate use of synthetic antibiotics resulted in the development of multidrug resistance among the pathogenic bacteria. Recent studies report that common bacterial populations from food and gastrointestinal tract of humans such as lactic acid bacteria and bifidobacteria can act as reservoirs of antibiotic resistant genes. From them resistance can be transferred to human pathogenic bacteria hampering the treatment of infections (Ammor et al., 2007). Similarly, the commonly used food processing bacteria such as Lactobacillus reuteri and Lactobacillus fermentum can also potentially act as reservoirs of antibiotic resistant genes. Acquired resistant genes may be transferred to pathogenic bacteria through food chain or bacterial conjugation in the human gastrointestinal tracts (Egervarn et al., 2007).

Aeromonas species of bacteria are identified as the chief causative organism responsible for the outbreaks of acute gastroenteritis of choleric / dysenteric form or chronic diarrhoea, ulcerative colitis etc. in adults as well as children. They exhibit constitutive resistance to some antibiotics (Balotescu et al., 2003). A study conducted at the University teaching hospital in Yaounde, Cameroon, reported a higher incidence of resistance to amoxicillin, piperacillin and trimethoprin / sulfamethoxazole among pathogenic bacteria from inpatients than from outpatients (Pieboji et al., 2004). The multidrug resistant Acinetobacter infection in patients was found to cause prolonged stay in the hospital and intensive care unit (Sunenshine et al., 2007). In USA the cost of treating hospital acquired drug resistant infection is
estimated to be US $ 4.5 billion annually (McGowan, 1991). In the developing world it is assumed that infections caused by multiple drug resistant strains are by far higher (Gibbons, 1992).

The causes of development of antimicrobial resistance are indiscriminate consumption of antibiotics (Shariff, 2001), the use of broad spectrum antimicrobial agents instead of narrow spectrum ones, consuming antibiotics without expert prescriptions etc. Methicillin resistant Staphylococcus aureus and Staphylococcus epidermidis, Vascomycin resistant beta lactamase producing Enterococcus species etc. have been observed among hospital acquired pathogens (Kaatz et al., 1990). In the case of uropathogenic bacteria like E.coli also, antibiotic resistance is found to develop due to frequent misuse of antibiotics (Tambekar and Khandewal, 2005; Tambekar and Dhanorkar, 2005). Failure in treatment of respiratory infections is also noticed due to the emergence of antibiotic resistant strains among the most common respiratory pathogens (Unasho et al., 2009).

The antibiotics are sometimes associated with adverse effects in the patients, such as hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immuno-suppression and allergic reactions (Idose et al., 1968). Several antibiotics are found to bring about derangement of oral and intestinal bacterial flora (Fitzgerald, 1972). These therapeutic agents generate wider side effects and cross reactivity due to their residual presence in the tissues, cells, body fluids and widely inhibit bio-membrane functioning. Indiscriminate use of synthetic chemicals suppresses the immune resistance of the body to fight against diseases resulting in the failure to control further incidence of microorganisms. Besides, these pathogens are also making immunological identification of antigens/antibiotics and have attained resistance to the antibiotics (Upadhyay et al., 2010).

The resistance to antibiotics acquired by the pathogenic bacteria as well as the adverse side effects to human health due to frequent use of synthetic antibiotics make highly important to explore new sources of more active and potential antibacterial compounds/ components/ extracts of plant origin, which might be safe alternatives of these synthetic drugs. Hence in the present study an attempt is made to screen the
antibacterial activity of the extracts of three species of *Indigofera* and to search for the presence of bioactive phytocompounds responsible for the antibacterial activity.

1.3 Antibacterial Activity of Plants and Plant Products

Traditional medicine was using crude plant extracts for treatment of infectious diseases. *In vitro* screening and evaluation gives a scientific basis to the use of plant extracts for treatment of bacterial diseases.

Several workers throughout the world have carried out antibacterial studies on large number of medicinal plants. Out of the floral population of approximately 10,000 plant species in Kenya, 1200 plant species were reported to have medicinal properties and phytochemical evaluation of some of them have been reported (Midiwo *et al.*, 2005). In many a case the screening for antibacterial activity was done in plants used frequently in popular medicine (Abu-Shanab, 2004). Shahidi Bonjar (2004) studied 195 plant species in 76 families used by the Iranian native people for curing infectious maladies and found that methanolic extracts of 64 samples in 37 families showed antibacterial activity against pathogenic organisms. In the treatment of four different strains of pathogenic bacteria with 36 ethanol extracts from 24 plants currently used in the Peruvian traditional medicine for treatment of several infections, 69% of the extracts were found to show some degree of antimicrobial activity against at least one organism (Rojas *et al.*, 2003). *In vitro* screening of individual plants commonly used in treatment of infectious diseases, against pathogenic organisms is done to confirm its antibacterial activity. The stem and leaf extracts of *Solanum incanum*, a very effective medicinal plant, were found to be highly active against four strains of *E.coli* bacteria (Britto, 2001).

*In vitro* screening experiments conducted with crude as well as purified plant extracts against pathogenic bacteria affirm the specific indication claimed by the traditional healers. 267 extracts of 100 Rwandese medicinal plants of traditional importance on testing for antimicrobial and antiviral properties showed confirmation of the claim of traditional healers (Vlietinck *et al.*, 1995). Further, even in the case of plants having so far no proven antibacterial activity, the tests conducted will make clear whether they are having this property. Identification of antibacterial property in them gives additional source for antibacterial compounds. Confirmation of
antibacterial property in plants leads to isolation and purification of the bioactive principles present in the plant. Hence in the present study antibacterial property of the crude extracts as well as purified fractions of *I.tinctoria*, *Ienneaphylla*, and *I.aspalathoides* on multidrug resistant microorganisms was investigated.

1.4 Phytochemical Products and Their Medicinal Value

Higher plants are valuable sources of industrially important natural products such as flavours, fragrances, essential oils, pigments, sweeteners, antimicrobials, pharmaceuticals etc. These chemical compounds mostly belong to a metabolic group collectively referred to as secondary metabolites. They accumulate in the plant cell in small quantities in specialized cells and detected in lower concentration compared to the primary metabolites. Hence, they are termed as the higher value - lower volume products and their distribution in plant kingdom is restricted (Buchanan et al., 2000).

It is believed that these compounds have an important role. They can work as pollinators, attractants and as chemical defenses against insects, herbivores and microorganisms (Harborne, 1990).

Secondary metabolites are divided into different categories based on their mechanism of function viz, chemotherapeutic, bacteriostatic and antimicrobial (Purohit and Mathur, 1999). Different groups of compounds are included under secondary metabolites. A major group among them is phenolic derivatives. Important subclasses in this group that showed antimicrobial activity include phenols, phenolic acids, quinones, flavones, flavanoids, flavanols, tannins and coumarins. This group includes metabolites derived from the condensation of acetate units (terpenoids), those produced by the modification of aromatic amino acids (phenylpropanoids and coumarins), flavanoids, isoflavanoids and tannins. The flavones, flavanoids and flavonols have been synthesized in plants in response to microbial infection. Hence, its effectiveness against a wide array of microorganisms as antimicrobials in *in vitro* studies is not at all surprising (Bennet and Wallsgrove, 1994). The flavonoids are reported to have antiallergic, anticancer, antioxidant, antibacterial and antiinflammatory activities (Miller, 1996). *Azadirachta indica* (Ahmad and Beg, 2001; Govindachari et al., 1998) and *Boerhaavia diffusa* (Surange and Pendse, 1972)
are reported to show antibacterial activity due to the flavonoids and terpenoids in them.

The tannins are a group of compounds soluble in water, acetone and alcohol and precipitate when combined with proteins. They are traditionally used for protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, hemorrhides and diarrhoea (Oguleye and Ibitoye, 2003). The coumarins have been found to stimulate the macrophages which could have an indirect negative effect on infection (Cowan, 1999).

A major group of biologically active secondary metabolites are the alkaloids. True alkaloids are derived from amino acids and basic, containing nitrogen in a heterocyclic ring, e.g. nicotine. Common alkaloid ring structures include the pyridines, pyrroles, indoles, phyrrolidines, isoquinolines and piperidines (Bennet and Wallsgrove, 1994). The indoquinoline alkaloids from Cryptolepsis sanguinolenta displayed activity against a number of gram negative bacteria (Silva et al., 1996). Many of the alkaloids act as defense substances against animals and microorganisms (Heldt, 2005).

Alkaloids like isoprenoids and phenylpropanoids act as natural pesticides that protect plants against herbivores and pathogenic microorganisms. Phytoalexins, the defense substances against microorganisms are synthesized in plants in response to infections by fungi.

The terpenes are one of the largest and most diverse groups of plant secondary metabolites. They include sterols and triterpenes, complex compounds that are formed by the cyclization of 2, 3-oxidoqualone. The sterols and triterpenes accumulate as glycoside conjugates in substantial quantities in plants. They are known as saponins. The saponins along with terpenoids and flavonoids are claimed to be responsible for the antibacterial activity of Citrus sinensis (Stange et al., 1993; Nishimura and Satoh, 2006).

Another important subclass of the compounds under the group of terpenes is the essential oils. Monoterpenes, diterpenes and sesquiterpenes are the major compounds in this subclass. The essential oils from Thymus revolutus (Karaman et
Lippia sidoides (Lacoste et al., 1996), Annona squamosa (Chavan et al., 2006), Vitis adanata (Srivastava, 1996) are reported to have antimicrobial activity.

The different types of secondary metabolites are the main components of the phytochemicals that are bioactive, demonstrating antimicrobial, antioxidant, antiinflammatory and antiallergic activities. In the present study also we aim at identifying the secondary metabolites in *I. tinctoria, I. enneaphylla* and *I. aspalathoides* that are responsible for their antibacterial activity.

1.5 Tissue Culture for Clonal Propagation and Production of Secondary Metabolites

Recently, there is an increasing interest for Ayurvedic medicines due to the adverse after effects of synthetic medicines and the emergence of multidrug resistant pathogenic bacteria. The production statistics of the Oushadi, the Government owned pharmaceutical company in Kerala, reported that its total income, had risen from 16 crores in 2006 to 38 crores in 2009. This makes clear the recent increased interest in Ayurvedic medicines. Enhanced use of Ayurvedic medicines increases the need for medicinal plants, giving rise to uncontrolled exploitation of natural resources.

Demand and supply at present is mismatching. At present 90 percent of the supply of medicinal plants is from forest and only 10 percent by way of cultivation. Traditionally, the tribes and local communities living in and around the forest were allowed to collect minor forest products and there were only 80 medicinal plants in the list of minor medicinal forest products. Due to unscientific, unsustainable and discriminative collection practices followed, availability of medicinal plants in its natural home has been depleted over the years. Some of the species even became scarce / endangered due to over exploitation. Rapid expansion of area under food crops and commercial crops, conversion of non forest areas for other alternate land use, degradation of forest through fire and grazing etc. have reduced availability of valuable medicinal plants. This has further led to the extinction of many valuable medicinal plants. Many others have become endangered (Ajithkumar and Seeni, 1998). Two remedies for this alarming situation are the reintroduction of medicinal plants through cultivation and the utilization of an alternate source like *in vitro* culture developed callus for extraction and isolation of secondary metabolites.
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Major problem faced in large scale cultivation of medicinal plants is the scarcity of planting materials. *In vitro* propagation is the viable solution to this problem. Clonal propagation of several medicinal plants like *Terminalia bellerica* (Ramesh et al., 2005), *Holostemma ada-kodien* (Martin, 2002) *Aloe polyphylla* (Abrie and Staden, 2001), *Macuna puriens* Linn. (Vibha et al., 2009) are some of the reports in this regard. This fact is all the more important in the case of endangered plants like *Entada pursaetha* DC. (Vidhya, 2005), *Curculigo orchioides* Gaertn. (Thomas and Jacob, 2004) etc.

Plant cell cultures can also be used as resources of flavors, aromas, bioactive compounds *etc.* The production of secondary metabolites in culture has the following advantages over and above the natural plant resources.

- Production can be more reliable, simpler and predictable.
- Isolation of phytochemicals can be rapid, efficient and simple as compared to extraction from complex whole plants.
- Compounds produced *in vitro* can be directly parallel to compounds in the whole plant.
- Interfering compounds that occur in the field grown plant can be avoided in cell cultures.
- Cell cultures can be a source of defined standard phytochemicals in large volumes.
- Cell cultures are a superb model to test elicitation.
- Cell cultures can be radiolabelled, such that the accumulated secondary products when provided as feed to laboratory animals can be traced metabolically.
- Secondary products in plant cell cultures can be generated on continuous year-round basis, without any seasonal constraints.

While some research has succeeded in producing a wide range of valuable secondary phytochemicals in unorganized callus or suspension cultures, in other cases, production requires differentiated microplant or organ cultures (Dorenenberg and Knorr, 1997). Even though in some cases the callus cultures are a good source of all the secondary metabolites produced in the mother plant, in other cases only one or
few phytochemicals are produced in the in vitro culture. For example, in the case of Opium poppy (Papaver somnifera L.) the mother plant contains a number of pharmaceutically important alkaloids of the benzylquinoline type including morphine, codeine, papaverine and sanguinarine in high concentration, only the phytoalexin sanguinarine has been found at significant level in Opium poppy cell cultures (Facchini and Bird, 1998). Conversely, the extracts of in vitro induced callus of Alternanthera maritima showed bioactivity against the same strains in which adult plant extracts showed bioactivity. But the intensity is quite low in the callus extracts due to lesser concentration of antibacterial compounds in the callus (Salvador et al., 2003). To obtain callus as a continuous source of bioactive compounds non-browning callus stock cultures should be maintained from which new callus cultures can be formed. Such static and suspension callus cultures conducive for production of secondary metabolites were reported in Artemisia annua (Basile et al., 1993).

Apart from callus and suspension cultures, the in vitro developed hairy roots are another source of secondary metabolites. Hairy root cultures have many advantages over cell cultures due to their genetic and biochemical stability. They can be grown in hormone free medium for the economic production of plant secondary metabolites on a commercial scale in a biofermenter. Production of artemesinin from hairy root cultures of Artemisia annua (Liu et al., 1997), indigo from hairy roots of Polygonum tinctorium (Chae et al., 2000) and sesquiterpenes from hairy root cultures of Solanum tuberosum (Komaraiah et al., 2003) are typical examples. In the present study also experiments were designed and conducted in order to develop a standardized protocol for micro propagation of the three selected Indigofera sps, to induce a proliferating callus from various explants and to assess the presence of secondary metabolites in the callus. Attempts were also made to develop roots from the clumps of cells in the suspension.

1.6 Importance of Isolation and Identification of Antibacterial Compounds:

Even though from ancient times onwards plant extracts were used for treatment of infectious diseases, the chemical basis of the effects of these plant extracts were not subjected to a detailed study. The crude plant extracts contain several groups of compounds like alkaloids, terpenoids, flavonoids, tannins, saponins etc. The type of
bioactive compounds present as well as the chemical nature of the individual compounds has not been traced out. Identification and detailed study of the active principles will enable their use as natural drugs. Also, several compounds are found inside the plant in the precursor/intermediate form, which can be isolated and modified as drugs. Further, several derivatives have been semi-synthesized to enhance their bioactivity than original compounds, suggesting a potential for drug development. Typical example for this is the studies conducted in *Andrographis paniculata*. Active compounds extracted with ethanol or methanol from the whole plant, leaf and stem include over 20 diterpenoids and over ten flavonoids. Andrographolide (C$_{20}$H$_{30}$O$_5$) was the major diterpenoid identified and the structure of which was also elucidated (Cheung *et al.*, 2001; Pholphana *et al.*, 2004). The structures of other nine andrographolides were also traced out. Similarly the structures of four flavones were also identified (Chao and Lin, 2010). The andrographolide, isolated and purified, exhibited multiple pharmacological properties and is a potential chemotherapeutic agent (Verma *et al.*, 2009). The intact plant/crude plant extracts are used in traditional medicine. The crude plant extracts contain several compounds like alkaloids, terpenoids, flavonoids, tannins, saponins etc. But there is no possibility for attributing the efficacy to any specific compound. Hence, in the present study an attempt is made to isolate, purify and characterize the active principle/principles from the three species of *Indigofera* after ascertaining their antibacterial property. The active principle responsible for antibacterial activity can be isolated using chromatographic methods and characterized using spectroscopic studies.

### 1.7 Importance of the Antibacterial Study in *Indigofera* Species.

Several species of the genus *Indigofera* like *tinctoria*, *enneaphylla*, *aspalathoides*, *colutea*, *macrocalyx*, *nigritana*, *pulchra*, *daleoides*, *spicata*, *trita*, *truxillensis*, *suffruticosa* have been subjected to studies related to anticancer, antioxidant and hepatoprotective activities. Of them *I.tinctoria*, *I.ennephylla* and *I.aspalathoides* are three species commonly available in Kerala and Tamil Nadu. These three species are used in folklore and traditional medicines for treatment of several diseases in Kerala and Tamil Nadu. Practically there are no antibacterial activity studies in *I.ennephylla* and *I.aspalathoides*. With regard to *I.tinctoria*, there
are only very few indirect reports like using it for urinary complaint, bronchitis etc. Hence *I. tinctoria*, *I. enneaphylla* and *L. aspalathoides* are selected for *in vitro* screening against the eight strains of pathogenic multidrug resistant bacteria to detect the antibacterial activity and to identify the antibacterial principles in them. The identifying characteristics of the *Indigofera* genus and the three species *enneaphylla*, *tinctoria* and *aspalathoides* are given below. Also their common medicinal properties and the phytochemical compounds identified from them are listed.

### 1.8 Botany of *Indigofera* Genus:

The genus belongs to the sub family *Papilionaceae* under the family *Leguminosae*. With the following characteristics the genus is identified:

Herbs or under shrubs or shrubs, with appressed laterally attached hairs, sometimes mixed with basifixed hairs, frequently silvery-canescant. Leaves simple, trifoliate or imparipinnate, the side leaflets usually opposite, but sometimes alternate; stipules usually small, shortly adnate to the petiole. Flowers generally very small, usually reddish or purple in axillary recemes or spikes, rarely solitary, rarely panicled, each flower pedicelled in the axil of a caducous bract; bracteoles 0. Flowers Zygomorphic, calyx minute, campanulate, teeth subequal or the lowest longest; corolla papilionaceous, standard ovate or orbicular, sessile or slightly clawed; wings oblong, slightly adherent to the keel, keel petals erect, obtuse, with downward spur on each side near the base. Stamens diadelphous, vexillary stamen free, the others with connate filaments, anthers uniform, apiculate. Ovary sessile or sub sessile, 1-2 or many ovulate, style glabrous, stigma capitate, sometimes pedicellate. Pod usually linear, cylindric, rarely oblong or globose straight or curved, septate within between seeds. Seeds, globose, cylindric and truncate (Gamble, 1997).

#### 1.8.1. The *tinctoria* species is marked by the following characters:

An erect procumbent herb. Branches covered with soft, silky hairs. Leaves alternate, imparipinnately compound. Leaflets 9-13 oblong, ovate flowers red, sessile, many flowered in axillary receme, shorter than leaves. Pedicels very short, Fruit pod, refluxed or straight 1-2 inches long, about 10 seeded (Retnam and Martin, 2006). The morphology of the plant is given in Fig.1.1.
1.8.2. The following features characterize *enneaphylla* species:

Subshrubs or herbs with appressed- biramous hairs, sometimes mixed with other indumentum. Leaves 3, many foliate, odd-pinnate, leaflets opposite chartaceous, sometimes gland dotted, stipules small, often setaceous, flowers in axillary recemes, rarely in panicles or heads, bracts caducous, bracteoles 0. Calyx tube campionate, lobes subequal, lower one longest. Corolla usually exceeding calyx; petals usually clawed; standard orbicular to ovate, gradually narrowed to base, glabrous, wings oblong slightly cohering with keels; keels erect, obtuse, basally with a downward spur an each side. Stamens 9+1, vexillary stamen free, anthers uniform, usually apiculate, ovary sessile, ovules α or 1 or 2; style penicillate, pod usually oblong, linear, terete or 4–gonous, sometimes curved, torulose or not, septate usually dehiscent, endocarp often spotted, seeds globose, ovoid, 4 gonial or terete estrophiolate (Mathew, 1999). Few plant twigs are given in Fig.1.2.

1.8.3 The *aspalathoides* species is identified by the following characters:

Spreading herb or subshrub. Leaves digitately compound, 3- 5 foliate, leaflets linear to oblanceolate, membranous, appressed, pubescent, base cunate, apex obtuse, epetiolate, stipules linear. Flowers small, red, axillary, solitary or few in short receme. Fruit pod small, 1.5 cm long straight terete or subangular, 6-8 seeded. Seeds small about 1 mm in diameter, cuboid and smooth (Retnam and Martin, 2006). Fig.1.3 demonstrates the morphology of the plant.

1.9 Medical Use of the Three Selected Species

1.9.1 *Indigofera tinctoria*:

The *tinctoria* species has been extensively used in folklore and traditional medicine systems for treatment of several disorders. In Ayurveda and Siddha it is used for Tikita Rasam, Katu rasam, Ushna veeryam, Katu, Vipaka etc. The plant demonstrates anthelmintic, analgesic and antiperiodic properties. Roots are used for anti poison, giddiness, colic and gonorrhoea and as a hair tonic. Leaves are used for jaundice, vatha, fever etc. Decoction of the leaves is used in blennorrhagia and treatment of hydrophobia. Roots are used for treatment of urinary complaints and hepatitis. It is also used as a sedative and for treatment of piles and ulcers (Nadkarni, 1926). According to Bhavaprakasha, nili is purgative in action, bitter, hot, cures
giddiness, abdominal enlargement, vatarakta, gout and intestinal obstruction. The decoction or powder of the plant is used in whooping cough, bronchitis, palpitation of heart, enlargement of the liver and spleen, dropsy, diseases of lungs and kidney, epilepsy and nervous disorders (Prajapati et al., 2004). A poultice of the leaves is recommended for skin diseases and haemorrhoids. Root decoction is given in calculus diseases and used as an antidote to arsenic poisoning. The seed of the plant powdered and steeped in arrack or rum yield a tincture and it is sprayed for destroying lice. Indigo, the dye extracted from the leaves is a soothing balm for burns and scalds, insect stings and animal bites. The use of indigo (dye extracted from the plant) and its constituents, indirubin and indigotin prevents allergic contact dermatitis.

1.9.2. \textit{Lenneaphylla}

The juice of \textit{enneaphylla} plant is having antiscorbutic, diuretic, antidiarrhoeal and alternative properties. The plant is boiled with oil and applied to burns. A decoction is given in epilepsy and insanity. The plant is reported to have wound healing activity (Anonymous, 1991; Hemalatha et al., 2001). The extract of the plant is believed to be effective in venereal diseases (Kirtikar and Basu, 1975).

1.9.3 \textit{Laspalathoides}:

In traditional medicinal system, the leaves, flowers and tender shoots are said to be cooling and demulcent, stimulant and alternative. The leaf decoction is used against leprosy and cancerous affections (Kirtikar and Basu, 1975). The leaves are applied to abscesses and boils. The whole plant is used for edema syphilis and its ashes are used in preparations for dandruff (CSIR, 1959). Root is chewed to treat tooth ache (Retnam and Martin, 2006) and used in aphthae. Stem extract demonstrates antiarthritic effect (Rajkapoor \textit{et al.}, 2009). It is also used for treatment of elephantiasis. The plant is reported to be useful in the treatment of various skin ailments by Siddhas in Tamil Nadu with remarkable healing potential.
1.10 Medicinal Properties of *I.tinctoria, I.enneaphylla* and *I.aspalathoides*

The medicinal properties of the three species selected for study is due to the bioactive principles present in it. Several compounds have already been identified and isolated. The compounds isolated and identified are given below:

1.10.1 *Indigofera tinctoria:*

Indicine (5-15 mg/g, dry basis), flavonoids, epigenin, kampferol, luteolin and quercetin are present in various plant parts, maximum in the leaves and minimum in the roots. The presence of coumarins, cardiac glycosides, saponins, tannins, galactomannan composed of galactose and mannose and conjugated indoxyl (indican) are also reported. The rotenoid content is decreased with age; among the parts maximum content is found in leaves and minimum in roots. Six rotenoids (deguelin, dehydrodeguelin, rotenol, rotenone, tephrosin and sumatrol) were isolated and identified from this species (Kamal and Mangla, 1993). Indingtone, an active principle isolated and identified from the leaves possess hepatoprotective activity (Singh *et al.*, 2004). Indirubin, the phytochemical compound isolated from the leaves is a promising anticancer drug (Han, 1994).

1.10.2 *Indigofera enneaphylla:*

It is regarded by Indian medical practitioners as alternative in old venereal infections. The juice of the plant is used as an antiscorbutic and diuretic. Chatterji and Dutt (1937) reported two unsaturated hydrocarbons, named ‘Indigoferin’ and ‘Enneaphyllin’ in it.

1.10.3 *Indigofera aspalathoides:*

It contains flavonoids, tannins and alkaloids (Hirano *et al.,* 1989). A flavonoid glycoside with molecular formula C_{23}H_{24}O_{11} which shows cytotoxic activity against tumour cell lines was isolated from it (Balasubramanian *et al.,* 2007).

The details given above bring out some of the bioactive phytochemical compounds present in the three species of *Indigofera*. Identification of many more compounds can be expected in future. Detection of medicinally important
phytocompounds from them will surely increase the requirement for the natural plant resources. Such a situation will bring about uncontrolled exploitation of the plant resources leading to dwindling of the population of these plants. Solution to this problem is to resort to large scale cultivation of the species, for which large number of saplings are required. Application of micropropagation technology will ensure enough and more number of planting materials. An alternate method to bypass the situation is to develop callus as well as suspension cultures and maintain them so that bioactive compounds can be extracted from them.

Hence, this study was conducted to screen for the detection of antimicrobial principles in *I. tinctoria*, *I. enneaphylla* and *I. aspalathoides* and to isolate and identify some of the bioactive compounds from them. Study was also envisaged to formulate a standardized technology for micropropagation of the three species and to induce a proliferating callus from various explants of them. In the circumstances stated above this study was conducted with the following objectives.

1.11. Objectives of the Study

I. To screen in *in vitro* condition for the antibacterial activity of the three species of *Indigofera* namely, *I. tinctoria*, *I. enneaphylla* and *I. aspalathoides* by testing against the following eight strains of pathogenic bacteria: *Escherichia coli*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Enterobacter aerogens*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus vulgaris* using the extracts of leaf, stem and root in Petroleum ether, Chloroform, Acetone, Ethyl acetate, Methanol and Water.

II. To develop a standardized protocol for micropropagation of *I. tinctoria*, *I. enneaphylla* and *I. aspalathoides* in order to supply plantlets for large scale cultivation.

III. To determine the presence of antibacterial principles in the *in vitro* induced callus from various explants so that the callus can be used for the isolation of antibacterial principles instead of the intact plant.
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IV. To compare the antibacterial activity of the extracts of the intact plant part to that of the callus.

V. To isolate the important antibacterial bioactive compound/compounds from the plant extracts, identify and characterize the structures using standard analytical methods.
Fig. 1.1 *Indigofera tinctoria*  
Fig. 1.2 *Indigofera enneaphylla*  
Fig. 1.3 *Indigofera aspalathoides*