The relationship between man and plants is in practice from time immemorial but due to the intrusion of modern civilization in the remote countryside most of the people of the globe have abandoned the idea of traditional use of plants in their daily life. Traditional medicines are perceived as efficient, safe and affordable by all masses (Kumar 2008; Sharma et al., 2011 and Sharma and Kumar 2013). Despite convincing progress in synthetic chemistry, plants are the most important sources for preventive and curative medical preparations. Farnasworth (1988) emphasized the role of information derived from various systems of traditional medicine (ethnomedicine) and its utility for drug discovery purposes. They have identified 122 compounds of defined structure, obtained from only 94 species of plants, that are used globally as drugs and demonstrate that 80% of these have an ethnomedical use identical or related to the current use of the active elements of the plant.

Prevalence of lifestyle diseases has again renewed the interest in traditional medicines. Now in the developed countries also people are returning to nature. Use of traditional medicine is the mainstay of primary healthcare, virtually in all developing countries. The reasons for the frequent use of traditional medicine being (i) the strong association of people with local flora and their belief on traditional knowledge regarding plants as medicine, (ii) easy availability of local
medicinal plants, (iii) relatively poor access to allopathic drugs and their high cost and (iv) lower economic profile of the rural people (Parveen et al., 2007). Several studies have been conducted on use of plants in traditional medicine for humans and veterinary (Sharma et al., 2005; Sharma and Kumar, 2007; Sharma and Kumar 2012).

Recent estimates suggest that over 9,000 plants have been known to have medicinal applications in all cultures of various countries (Farnsworth, 1988; Kumar, 2008 and Bhansali et al., 2011). Of the 89 recorded plant species, frequently applied plant species against veterinary and human ailments included: Adhatoda vasica, was the most cited species (43%).

Thar desert of Rajasthan is also highly rich in medicinal plant diversity. Some very important medicinal plants of potent medical value have been discovered through ethnobotanical survey of Thar desert in Rajasthan (Sharma and Kumar 2007; Bopana and Saxena 2007; Sharma and Kumar 2011 and Pareek and Kumar, 2013). With increasing use of traditional medicines globally, attempts are also underway to discover the cure of HIV in the traditional medical system (Kumar, 2000; Kotia and Kumar 2001 and Sharma and Kumar 2012). Modern pharmacopoeia still contains at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants (Sharma et al., 2005).
Much of the recent efforts have focused on finding out the traditional uses of medicines and establish their scientific explanations based on biochemical pharmacological and biotechnological methods. A large number of old remedies have provided basis for the modern medicine. Many plant families have large number of plants used in traditional medicine and order Lamiales has valuable plant families like Lamiaceae and Scrophulariaceae. Many plants of these families are used in traditional systems of medicine. *O. sanctum* has been recommended for the treatment of bronchitis, bronchial asthma, malaria, diarrhoea, dysentery, skin diseases, arthritis, chronic fever and insect bite (Prakash and Gupta, 2005 and Agarwal *et al.*, 2013). *Justicia adhatoda* is used as an herbal remedy for treating cold, cough, whooping cough, chronic bronchitis and asthma, as sedative expectorant, antispasmodic and anthelminthic. The leaves, roots, flowers and bark of *Adhatoda* are all used for medicinal purposes. It is well known for preparation of medicine for bronchitis, asthma and other pulmonary infections. *Glycodin®,* a famous product used for the cure of bronchitis is extracted from the leaves of this plant.

The Acanthaceae is a large (ca, 4000 species in some 230 genera) pantropical family with major concentrations of species in southeastern mainland Asia, insular Malesia, the Indian subcontinent, Madagascar, tropical Africa, Namibia, Botswana, South Africa,
Lesotho, and Switzerland, Brazil, Andean South America, and Mexico-Central America (Daniel, 2000). It is an evergreen shrub growing throughout Indo-Malayan region, Punjab in the North, and Bengal and Manipur in the South-East to Tranvacre of Kerala, at an altitude of 1350 m. The plant is also seen distributed in Sri Lanka, Upper and Lower Myanmar, southern China, Laos, and the Malay-Peninsular and Indonesian Archipelago (Singh et al., 2011).

Knowledge of chromosome numbers among Acanthaceae has proven useful in resolving generic positions of problematic species, reassessing phylogenetic relationships among subfamilial taxa, and understanding morphological variation. Justicia is the largest genus of Acanthaceae with estimates of about 700 species occurring worldwide. Several other genera in the Flora of Southern Africa (i.e., Adhatoda, Aulojusticia, Duvernoia, Siphonoglossa Oerst.) are sometimes included in Justicia (Graham, 1988 and Brummitt, 1992) as well. It has several species like Justicia betonica L., Justicia odorata (Forssk.) Lam., Justicia petiolaris (Nees) T. Anderson. Daniel (2000a, 2000b) discussed the diversity, frequency, and taxonomic distribution of chromosome numbers \( n = 14 \) throughout Justicia and probable basic number of \( x = 7 \) for the genus.

Chemical investigation of genus Justicia resulted in the isolation of flavonoids, alkaloids, triterpenoids and sterols. Compared
with most other classes of natural compounds, alkaloids are characterized by a great structural diversity and there is no uniform classification of alkaloids. First classification methods have historically combined alkaloids by the common natural source, e.g., a certain type of plants. This classification was justified by the lack of knowledge about the chemical structure of alkaloids and is now considered obsolete. Taxonomically the plant is known as *Justicia adhatoda* syn. *Adhatoda vasica*. In local systems it is variously known as Malabar Nut, Adulsa, Arusha, Vasaka, Arusa, Adathodai, Bakash, Adathoda, Adalodakam, Adusoge, Addasaramu, Lion’s Muzzle and Stallion’s Tooth. It is known as Adathoda in Tamil and Adalodakam in Malayalam. It belongs to family *Acanthaceae*. It is a small evergreen and sub-herbaceous bush which is commonly found in the lower Himalayas- up to 1300 meters above sea level, in India, Srilanka, Burma, Malaysia etc. The average height of the plant ranges from 50cm to 90cm. Leaves are broad and lanceolate measuring 10 to 16 cm in length and 5 cm in width.

The dried leaves become greenish brown in colour and bitter in taste. The stem wood is soft and can be used for making charcoal for gunpowder. Flowers are large, fragrant, and attractive with white petals. The filaments are usually free and project beyond the corolla tube. The gynoecium consists of two carpels, syncarpous. Ovary is
superior, bilocular with axile placentation. Ovary is highly elongated and remains situated in a nectar secreting disc. It terminates above in a long narrow style which projects beyond the mouth of the flower, and ends in two small stigmas of various shapes. Fruit is usually bilocular capsule dehiscing loculicidally. Seeds are exalbuminous and usually four in number per fruit. Pollination is mostly brought about through the agency of insects.

More recent classifications are based on similarity of the carbon skeleton (e.g., indole-, isoquinoline-, and pyridine-like) or biogenetic precursor (ornithine, lysine, tyrosine, tryptophan, etc.). However, they require compromises in borderline cases; for example, nicotine contains a pyridine fragment from nicotinamide and pyrrolidine part from ornithine and therefore, can be assigned to both classes.

Secondary metabolites are the compounds produced by plants that are not essential for plant growth and development but can be used as powerful drugs. All plants produce secondary metabolites which are often specific to an individual species or genus during specific environmental conditions making their extraction and purification difficult. As a result, commercially available secondary metabolites (pharmaceuticals, flavours, fragrances and pesticides) are generally considered high value products as compared to primary metabolites and they are considered to be fine chemicals with their
cost ranging from $500 to $10000 per kilogram (Ravishankar and Rao, 2000 and Tanwar et al., 2010). Plants produces a number of secondary metabolites such as alkaloids, flavonoids, phenols, terpenes and quinones which are used in food, pharmaceutical, cosmetic and pesticide industries (Khanuja et al., 1999). They play an important role in providing defense against pest and pathogens, protection against UV radiation and stress and acting as attractive volatile compounds or pigments (Oulu et al., 2003).

Extracts of the leaves of *Adhatoda vasica* are extensively used in cough, asthma, bronchitis, tuberculosis, inflammation and allergy. The plant extract has been reported to contain a number of alkaloids (Chakraborty and Brantner, 2001).

A number of different principles including, alkaloids: vasicine, vasicinone, vasinol, essential oil: betane, vitamins: vitamin C, b-carotene, a non-crystalline steroid: vasakin and a mixture of fatty acids have been identified as contributing to the observed medicinal effects of the plant (Suthar et al., 2009 and Sharma et al., 2014). The extract of its leaves is reported to contain – Quinazoline alkaloids, vasicine, N-oxides of vasicine, deoxyvasicine, oxyvasicine, maiontine and essential oils. The whole plant is reported to contain a peculiar compound named as organic adhatodic acid which is an odorous substance. Roots of *Adhatoda vasica* contain vasicinolone, vasicol,
Discussion

peganine, hydroxyl oxychalcone, glucosyl oxychalcone etc. compounds (Gupta et al., 1977). Flowers of the plant are reported to contain β-sitosterol-D-glucoside, kaempferol, glycosides of kaempferol, and queretin etc. The two main alkaloids vasicine and vasicinone are known to exhibit antiallergic activity. Vasicine is a major alkaloid of vasaka present in the concentration of 1.3%. Minor alkaloids include adhatonine, vasicinol and vasicinolone. Vasicinone formed by oxidation of vasicine at C-8 position, is one of the major alkaloids of J. adhatoda and is known to possess interesting biological activities. This includes respiratory, immunostimulant, bronchodilator and hypotensive activities. The leaves contain several alkaloids (vasicinone, vasicinol, adhatodine, adhatonine, adhavasinone, anisotine and peganine), betaine, steroids, carbohydrates and alkanes. In the flowers triterpines (α-amirine), flavonoids (Apigenin, astragalin, kaempferol, quercetin and vitexin) are found (Laakso et al., 1990 and Suthar et al., 2009).

Alkaloids are often divided into the following major groups: (1) "True alkaloids", which contain nitrogen in the heterocycle and originate from amino acids. Their characteristic examples are atropine, nicotine, and morphine. This group also includes some alkaloids that besides nitrogen heterocycle, contain terpene (e.g., evonine) or peptide fragments (e.g. ergotamine). This group also
includes piperidine alkaloids coniine and coniceine although they do not originate from amino acids (2) "Protoalkaloids", which contain nitrogen and also originate from amino acids. Examples include mescaline, adrenaline and andephedrine (3) Polyamine alkaloids – derivatives of putrescine, spermidine, and spermine (4) Peptide and cyclopeptide alkaloids (5) Pseudalkaloids – alkaloid-like compounds that do not originate from amino acids. This group includes, terpene-like and steroid-like alkaloids, as well as purine-like alkaloids such as caffeine, theobromine, theacrine and theophylline. Some authors classify as pseudoalkaloids such compounds such as ephedrine and cathinone. Those originate from the amino acid phenylalanine, but acquire their nitrogen atom not from the amino acid but through transamination.

Some alkaloids do not have the carbon skeleton characteristic of their group. So, galantamine and homoaporphines do not contain isoquinoline fragment, but are, in general, attributed to isoquinoline alkaloids. Plants produce near about 50,000 types of secondary metabolites, in which 12000 types of alkaloids, 60 types of cynogenic glycosides, 15000 types of terpenes, 4000 types of flavonoids and 100 types of glucosinolates have already identified. Natural products obtained from plants; alkaloids, flavonoids, saponins, anthraquinones, tanins, glycosides, cardiac glycoside, phenolic acids, steroids which
are secondary metabolites and their derivatives are used for the discovery and production of drugs. In plants, the malonate and shikimate pathway are extensively involved in production of secondary metabolites and linked with primary metabolism via AcetylCoA and pentose phosphate pathway.

An extract containing the alkaloid vascinol and 20% vasicine (Paliwa, 2000) inhibited ovalbumin-induced allergic reactions by about 37% at a concentration of 5 mg. Vasicinone has been shown to be a potent anti-allergen in tests on mice, rats and guinea pigs as studied by Wagner (1989). Also, the methanolic extract from the entire plant has been shown to possess anti-allergic and antiasthmatic activities in the guinea-pig after inhalation or intragastric administration at doses of 6 mg per animal or 2.5 gm/kg, respectively (Mullar 1993).

*Justicia adhatoda* contains valuable saponins. Acanthaceae belongs to order Lamiales which also has family Lamiaceae and is rich in saponins. Saponins are a structurally diverse class of compounds occurring in many plant species, which are characterized by a skeleton derived of the 30-carbon precursor oxidosqualene to which glycosyl residues are attached. Traditionally, they are subdivided into triterpenoid and steroid glycosides, or into triterpenoid, spirostanol, and furostanol saponins. In this study, the
structures of saponins are reviewed and classified based on their
carbon skeletons, the formation of which follows the main pathways
for the biosynthesis of triterpenes and steroids. In this way, 11 main
classes of saponins were distinguished: dammaranes, tirucallanes,
lupanes, hopanes, oleananes, taraxas-teranes, ursanes, cycloartanes,
lanostanes, cucurbitanes, and steroids. The dammaranes, lupanes,
hopanes, oleananes, ursanes, and steroids are further divided into 16
subclasses, because their carbon skeletons are subjected to
fragmentation, homologation, and degradation reactions. With this
systematic classification, the relationship between the type of skeleton
and the plant origin was investigated (Vincken et al., 2007).

All parts of the plant have medicinal value. Vasaka is a bitter
quinazoline alkaloid, the major alkaloids are vasicine and vasicinone
which is present in all parts of the plant. Vasicine, an abundantly
available quinazoline alkaloid from the leaves of Adhatoda vasica, has
been successfully employed for metal- and base-free reduction of
nitroarenes to the corresponding anilines in water. The method is
chemoselective and tolerates a wide range of reducible functional
groups, such as ketones, nitriles, esters, halogens, and heterocyclic
rings. Dinitroarenes reduced selectively to the corresponding
nitroanilines under the present reaction conditions.

The leaves contain several alkaloids (vasicinone, vasicinol,
adhatodine, adhatonine, adhavasinone, anisotine and peganine),
betaine, steroids, carbohydrate and alkanes. In the flowers triterpines
(α-amirine), flavonoids (Apigenin, astragalin, kaempferol, quercetin,
vitexin) have been found. (Kokate *et al.*, 2003). The extract of
*Adhatoda* is found to have anti-ulcer activity against ulcers induced by
the consumption of ethanol, pylorus and aspirin. The leaf powder of
*adhatoda* showed a considerable degree of anti-ulcer activity in
experimental rats when compared with control. Research has shown
that syrup of *adhatoda* improves the symptoms of dyspepsia.

Magnoliopsida is the class of dicotyledons, whereas Liliopsida
is the class of monocotyledons. Both classes belong to the sub-
kingdom, Tracheobionta, which are all vascular plants, and the
division, Magnoliophyta, which are the flowering plants or the
angiosperms. Within the class of Magnoliopsida, there are six sub-
classes, namely, Asteridae, Caryophyllidae, Dilleniidae, Hamamelidae,
Magnoliidae and Rosidae. Within the class of Liliopsida, there are
three sub-classes, namely, the Commelinidae, Liliidae and
Zingiberidae. Each sub-class contains 1 or more orders. For instance,
Asteridae and Rosidae consist of 8 and 9 orders, respectively.

Phylogenetic tree showing the plant subclasses from which
saponins have been isolated and characterised.
Infectious diseases of fungal origin are major health hazards all over the world and specially in tropical regions. In addition, these infectious diseases have increased markedly during the last decade (Meis and Verweji, 2001). Because of the dramatic rise in fungal infections and the current trend towards increasing awareness in traditional medicine, plant derived antifungal compounds are attracting much interest as natural alternatives owing to their versatile applications. Antibiotic resistance is increasing worldwide in both outpatients as well as hospitalized patients. It varies according to
geographic locales and is directly proportional to the use and misuse of antibiotics. Despite newer antibiotic, continued selective antibiotic pressure and bacterial adaptation have resulted in a problem that can no longer be ignored. Resistance can now be demonstrated against all available classes of antibiotics. Despite the introduction of improved antifungal drugs for treatment and prophylaxis, invasive fungal infections remain a significant clinical problem. Infections caused by eukaryotic organisms like yeasts generally present more difficult therapeutic problems than do bacterial infections. There are no treatments available for infections by many of the antibiotic-resistant bacteria, and resistance to commonly used antibiotics is steadily increasing. Multiple drug-resistant organisms used in the current study are becoming common causes of infections in the acute and long-term care units in hospitals. *P. aeruginosa* is responsible for 16% of nosocomial pneumonia cases, 12% of hospital acquired urinary tract infections, 8% of surgical wound infections, and 10% of bloodstream infections (Nandgopal *et al.*, 2011).

Plants are known to produce an enormous variety of small-molecule (MW 500) antibiotics—generally classified as 'phytoalexins'. Their structural space is diverse having terpenoids, glycoSteroids, flavonoids and polyphenols. Be that as it may, it is interesting to note that most of these small molecules have weak antibiotic acitivity-
several orders of magnitudes less than that of common antibiotics produced by bacteria and fungi. In spite of the fact that plant-derived antibacterials are less potent, plants fight infections successfully. Hence, it becomes apparent that plants adopt a different paradigm-"synergy"- to combat infections (Hamaiswarya et al., 2008).

The recent developments in genomics, proteomics and metabolomics have created a new platform to distinguish the synergistic efficacy of phytoextracts and for the determination of their mode of action. By the application of the "-omic" technologies it should be possible to detect the mechanism of action as the gene/protein expression profiles of the combination of drugs can be entirely different from the ones induced by the single drugs. This may lead to new phytobased paradigms towards the use of complex plant mixtures in medicines (Ulrich-Merzenich et al., 2007).

During the present investigation, the antimicrobial activities of methanol and leaf extracts of *Justicia adhatoda* were tested on *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Proteus vulgaris, Aspergillus niger, Aspergillus flavus, Fusarium oxysporum* and *Penicillium digitatum*.

The antibacterial activity of the extracts of *J. adhatoda* methanolic and leaf; solvent and antibiotic was determined in terms of inhibition zone. Methanol and leaf extracts of *J. adhatoda* were most
effective against *Staphylococcus aureus* (22 mm and 24 mm) followed by *Proteus vulgaris* (18 mm and 20 mm), *Bacillus subtilis* (17 mm and 20 mm) and *Pseudomonas aeruginosa* (16 mm and 18 mm inhibition zone respectively). Lowering the concentration of methanolic and leaf extracts had reducing antibacterial activity accordingly.

Leaf extract of *Justicia adhatoda* showed maximum Activity Index (0.85) against *Fusarium oxysporum* followed by *Penicillium digitatum* (0.81), *Aspergillus niger* (0.67) and *Aspergillus flavus* (0.64).

Plant-derived flavonoids are a large group of naturally occurring phenylchromones found in fruits, vegetables, tea, and wine. They have been shown to have a wide range of biological activities, including antiallergic, antibacterial, antidiabetic, anti-inflammatory, antiviral, antiproliferative, antimutagenic, antithrombotic, anticarcinogenic, hepatoprotective, oestrogenic, insecticidal and antioxidant activities. Additionally, some flavonoids are formed as antimicrobial barriers in plants in response to microbial infection. Recently, there has been an enormous increase in the number of studies on flavonoids as potential agents (Orhan et al., 2010).

Bacteria have evolved numerous defenses against antimicrobial agents, and drug-resistant pathogens are on the rise. In the recent years, incidence of multidrug resistance in pathogenic and opportunistic bacteria has been increasingly documented (Jones et al., 2004). These
multidrug resistant bacteria have also created immense clinical problems in cancer and immune compromised patients.

The development of antibiotic resistance can be natural (Intrinsic) or acquired and this can be transmitted within same or different species of bacteria. Natural resistance is achieved by spontaneous gene mutation and the acquired resistance is through the transfer of DNA fragments like transposons from one bacterium to another. Chemical modifications in the antibiotic target may result in reduced affinity of the antibiotic to its binding site. This is a mechanism employed by a number of pathogenic bacteria in evading the effect of antibiotics. Modifications are usually mediated by constitutive and inducible enzymes. Bacteria gain antibiotic resistance due to three reasons namely: (i) modification of active site of the target resulting in reduction in the efficiency of binding of the drug, (ii) direct destruction or modification of the antibiotic by enzymes produced by the organism or, (iii) efflux of antibiotic from the cell. β-Lactamases are one such family of enzymes that cleave the β-lactam ring of the cephalosporins and penicillins. One strategy employed to overcome these resistance mechanisms is the use of combination of drugs. Inhibitors of β-lactamases have been long known and they are administered with antibiotics as co-drugs. AmpC β-lactamases are intrinsic cephalosporinases found on the chromosomal DNA of many
Gram-negative bacteria, which confer resistance to penicillins, 2\textsuperscript{nd} -and 3\textsuperscript{rd} - generation cephalosporins including β-lactam/inhibitor combinations, cefamycins (cefoxitin), but usually not to 4\textsuperscript{th} -generation cephalosporins (Cefepime, cefquinome) and carbapenems; a growing number of these AmpC enzymes are now plasmid-borne. In the recent years, there has been an increased incidence of plasmid-mediated Extended Spectrum β-lactamase (ESβLs) that hydrolyse and cause resistance to oxyimine-cephalosporins and aztreonam among Gram-negative bacteria. Such enzymes are mainly plasmid encoded in \textit{E. coli}, \textit{Klebsiella pneumoniae}, \textit{Enterobacter}, \textit{Pseudomonas} and \textit{Shigella}. Being plasmid mediated, these enzymes spread fast amongst the bacterial population and impacted on chemotherapy. Therefore, search for new antimicrobials to combat infectious diseases caused by multidrug-resistant bacteria including fast-spreading ESβLs-producing enteric bacteria is urgently needed. Novel antibacterial actions of plant extracts or phytocompounds have been documented which include inhibition of MDR-efflux pump, β-lactamase activity, anti-antibiotic resistance properties (Lee, 2006) and R-plasmid elimination (Beg and Ahmad, 2000). Similarly, few plant extracts and phytocompounds exhibited synergistic interaction with antibiotics against Gram-positive bacteria (Aqil and Ahmad, 2007 and Ahmad and Aqil, 2007). Such compounds or active fractions may not necessarily have strong
antibacterial activity but may enhance the activity of classical antibiotics synergistically be above-known or novel mode of action. Screening of crude extracts for synergistic interaction with antibiotics is expected to provide bioactive compounds to be used in combinational therapy.

Emergence of such resistance raises question about the future of these drugs in chemotherapy, as the transmission of such resistant plasmid to other bacteria will help in the fast dissemination of resistance genes (Hopkins et al., 2005). However, β-lactamases continue to be the leading cause of resistance to β-lactam antibiotics in Gram-negative bacteria (Bradford, 2001).

These studies support our observation that Justicia adhatoda leaf extract has capacity to control microbial growth and prevent disease spread.

Phytochemical analysis of plant extracts indicates that the presence of one or more groups of phytoconstituents like tannins, flavonoids, glycoside, phenols, saponin, etc. are responsible for antibacterial activity alone or in combinations. However, further studies on isolation of active compounds and their mode of action/target site on ESβLs-producing enteric bacteria as well as in vivo stability and efficacy are needed to exploit them in the
management of infectious diseases caused by such multidrug resistant bacteria (Ahmad and Aqil, 2007).

Diseases transmitted by blood-feeding mosquitoes, such as dengue fever, dengue haemorrhagic fever, Japanese encephalitis, malaria and filariasis are increasing in prevalence, particularly in tropical and subtropical zones. To control mosquitoes and mosquito-borne diseases, which have a worldwide health and economic impacts, synthetic insecticide based interventions are still necessary, particularly in situations of epidemic outbreak and sudden increases of adult mosquitoes. Each year, larger quantities of synthetic insecticides are applied, leading to increased dangers for humans and other organisms and progressively greater environmental damage. The indiscriminate use of conventional insecticides is fostering multifarious problems like widespread development of insecticide resistant, toxic hazards to mammals, undesirable effects on non-target organisms, and environmental pollution (Yang et al., 2002; Kiran et al., 2006 and Senthikumar et al., 2008).

Even though plants and their preparations were the only pest management agents available before the advent of synthetic organic chemicals, only a few insecticides of plant origin are now commercially available. Furthermore, most of them have lower and shorter-lived efficacy than the synthetic substances. Due to the high
degree of biodegradation, however, plant – derived bioproducts are currently attractive as replacements for synthetic insecticides or for use in integrated management programs to minimize human health hazards and reduce the accumulation of harmful residues in the environment.

Most of the botanical, chemical, physical and microbial techniques employed in pharmacognosy are applicable to the analysis of drugs and therefore, used by public analysts, forensic scientists and quality control chemists associated with industries (Agarwal et al., 2013). Pharmacognosy is basically divided into conventional and modern pharmacognosy. Conventional pharmacognostical study is based on macroscopic, microscopic and quantitative microscopy. Macroscopic characters include shape, size, colour and texture of the drug in crude or powdered form. Microscopic characters include the anatomical details of drug producing plant as seen in transverse and longitudinal sections, maceration study and the size measurement of various type of cells. Quantitative microscopy includes the vein islet number, palisade ratio, stomatal number and stomatal indices and so restricted to leaf drug only. The modern pharmacognosy utilizes characteristics of analytical, phytochemical and certain physical constant values over the traditional science of taxonomy in plant systematics (Sharma et al., 2005; Sharma and Kumar 2012 and Sharma et al., 2012).
The different fields within today's pharmacognosy include: (i) Ethnobotany or ethnopharmacology; study of the traditional use of plants in the society. Ethnobotany refers to any use of the plants, whereas ethnopharmacology refers more specifically to the medical use of the plants. (ii) Phytochemistry, or natural product chemistry; a field closely related to organic chemistry, studying the chemical composition of living organisms. It is also closely connected to the process of finding new drug candidates from natural sources. (iii) Phytotherapy study of crude drugs, i.e. extracts from natural sources in medical use.

Natural products are the important source of bioactive compounds and have potential for the development of novel therapeutic agents. Over the last decade there has been a growing interest in drugs of plant origin (Castello et al., 2002). A wide range of products can be obtained from plants i.e., perfumes, insecticides, flavouring agents, coloring agents, medicines etc. Among these, pharmaceuticals form an important class of plant products useful for disease control. Many of the secondary metabolites from plants have tremendous potential for use in treatment of various diseases. Some of the most important drugs used today are from plants viz., steroids from diosgenin, codeine, atropine, glycosides etc. The use of natural drugs is of great interest in human pathology from time to time.
(Bandow et al., 2003). Preparations from plants have achieved reputations as cures for various diseases.

The leaves are rich in Vitamin C and carotene and yield an essential oil. Chemical compounds found in leaves and roots of this plant includes essential oils, fats, resins, sugar, gum, amino acids, proteins and vitamin C etc. The leaves also contain a very small amount of an essential oil and a crystalline acid. Extracts of the leaves of *Adhatoda vasica* are extensively used in cough, asthma, bronchitis, tuberculosis, inflammation and allergy. Both vasicine and vasicinone, the primary alkaloid constituents of *Adhatoda* are well-known for their therapeutical respiratory agents. Vasaka is mentioned as an herbal remedy for treating cold, cough, whooping cough and chronic bronchitis and asthma, as sedative expectorant, tuberculosis, Inflammation, anti-allergic, antispasmodic and anthelmintic. The drug is employed in different forms such as fresh juice, decoction, infusion and powder; also given as alcoholic extract and liquid extract or syrup. Vasicine and vasicinone are reported to have bronchodilatory and respiratory stimulation effects and hence regarded as biological markers for standardization of *A. vasica* extract. The two main alkaloids vasicine and vasicinone are known to exhibit antiallergic activity. A combination of both the alkaloids showed a bronchodilatory activity both *in vitro* and *in vivo* (Singh et al., 2010).
Several active constituents have also been isolated from different parts of *Adhatoda vasica* though the plant is traditionally used in the treatment of jaundice in Bengal, more evidence is needed to substantiate its pharmacological effects. The plant has been included in the WHO manual (WHO 1990). The Use of Traditional Medicine in Primary Health Care, which aims to profit health workers in South-East Asia to keep them informed of the therapeutic utility of their surrounding flora (WHO 1990).

This is not to say that it always cures all these diseases but it does give immediate relief.

Being a very good expectorant, it draws out all kapha (phlegm) accumulated in the lungs. In many cases where bronchitis is due to lack of appetite and poor digestion, the juice of Vasaka is mixed with the juice of ginger and honey and given in the early morning on an empty stomach. Given in the early stages of tuberculosis, the juice of Vasaka, thrice a day, helps a patient who is prone to incessant coughing. In many of the cough syrups that are available, Vasaka has been used either as a base or as an ingredient. Boiled and put on the bladder region, the leaves produce a diuretic action, reduce the swelling of the kidney and lead to clear urination. Persons suffering from bleeding piles or diarrhoea, accompanied by bleeding, or women suffering from menorrhagia can take the juice of Vasaka 2 to 3 times a
day to great advantage. Dried and powdered leaves also form a remedy for bronchitis in the dose of 40 grains twice a day.

The medicinal value of plants lies in some bioactive compounds such as alkaloids, flavonoids, tannins and phenolic compounds that produce physiological action on the human body (Edeoga et al., 2005).

The anti-tubercular activity of *Adhatoda vasica* was studied by Barry et al. (1955). Bromohexine and ambroxol- two widely-used mucolytics, semi-synthetic derivatives of vasicine from *Adhatoda vasica* have growth inhibitory effect on *Mycobacterium tuberculosis* (Grange and Snell, 1996). *Mycobacterium tuberculosis* is the causative agent of tuberculosis. The recent emergence of multidrug-resistant strains of *M. tuberculosis* poses a serious threat to tuberculosis control programmes worldwide and estimates around 3.3 % of all new TB cases. Recently resistance to the anti-tuberculosis drugs has been reported and the discovery of new drug targets are necessary for the treatment of tuberculosis (Kremer and Besra, 1999). Jha et al. (2012) reported that six different alkaloids from *J. adhatoda* leaf can be used as anti-tubercular agents. These alkaloids inhibit the activity of mtFabH there by preventing the initial step of fatty acid biosynthesis and can be effective against *M. tuberculosis*. They investigated these novel inhibitors by bioinformatics approach. The six alkaloids of *J. adhatoda* have potency as inhibitors of fatty acid biosynthesis in *M.*
tuberculosis and if developed and tested can be a good arsenal to arrest the infection at their initial stage (Jha et al., 2012).

The low permeability of the \textit{M. tuberculosis} membrane (that plays an important role in its intracellular survival) and its resistance to many chemotherapeutic agents are conferred by the long-chain \(\alpha\)-alkyl-\(\beta\)-hydroxy fatty acids (mycolic acids) that constitute approximately 50\% of the weight of the lipid-rich cell envelope of the bacterium. Among the enzymes involved in mycolate biosynthesis, the beta-ketoacyl-[acyl-carrier-protein] synthase III enzyme of \textit{M. tuberculosis} (mtFabH) plays a crucial link between the fatty acid synthase-I and fatty acid synthase-II. Fatty acid synthase-I (FAS-I) is involved in the synthesis of C16 and C26 fatty acids. The C16 acyl-CoA product acts as a substrate for the synthesis of mero-mycolic acid with the help of fatty acid synthase-II (FAS-II), and the C26 fatty acid comprises the alpha branch of the final mycolic acid. mtFabH has been proposed to be the link between FAS-I and FAS-II by converting C14–CoA generated by FAS-I to C16–(AcpM), which is channelled into the FAS-II cycle. In addition, the length and complexity of current TB treatment regimens results in poor patient compliance, a major contributing factor in the emergence of multidrug-resistant TB and extensively drug-resistant TB (Grange and Snell, 1996).
Bioprospecting has been widely used to assess the economic potential of different plant species and their value-addition. Our folklore with embedded cultural heritage has tremendous possibilities and potential for bioprospecting. The World Resource Institute (WRI) defined bioprospecting as ‘Exploration of commercially valuable genetic and biochemical resources’. It is felt that the term bioprospecting should include the cultivation of traditional concepts with contemporary tools, techniques and prospective. It should cover the unconditional welfare of biodiversity itself, with the communities where knowledge taken through bioprospecting (Sharma and Kumar, 2011 and Upadhyay et al., 2010 and 2011).

Recent developments in plant cell and tissue culture technique have provided an alternative to whole plant cultivation for the production of plant derived chemicals i.e. secondary metabolites (Staba, 1980; Bhojwani and Razdan, 1983; Kumar and Shekhawat, 2009 and Sharma et al., 2013). The application of different elicitors, cell immobilization as well as genetic transformation results in significant production of secondary metabolites from plant tissue cultures. There is a considerable demand of plants in India and this demand is met from the natural habitat. This leads to rapid depletion of plant material due to over exploitation of important plants. Plant tissue and cell culture system are being exploited for the accumulation
of the variety of natural products. In view of interesting biological activities associated with vasicine despite the extensive use of vasaka, tissue culture studies for production of vasicine have been limited. The present study was therefore, focused on initiation and establishment of tissue cultures of *A. vasica* to compare the potential of *in vitro* cultures for production of secondary metabolites. The large variation obtained using different analytical methods in vasicine content of different samples created havoc for herbal drug industries and even for government agencies in export of *A. vasica* and its formulations. Hence, it urgently needs a highly sensitive, advanced, and high throughput analytical method for correct quantification and revalidation of vasicine content among different locational samples of the Indian subcontinent, which are used by herbal drug industries.

*In vitro* tissue culture also offers an alternative method for rapid multiplication of plants referred as micro-propagation and has proved to be the most reliable method for large scale production of officinal plants. It is generally found that in plants high concentrations of secondary metabolites tend to accumulate in specific cell types at specific developmental stages, therefore, tissue cultured cells from these plants typically accumulate large amounts of secondary compounds under specific conditions. The plant as a source for important drug molecules is witnessed through the discoveries of
various bioactive molecules such as Taxol, Vincristine, Vinblastine, Metformin, Morphine etc. The synthetic aspirin used by the modern world today is a derivative of a plant-based drug (Raskin and Ripoll, 2004). Many new plant-based drugs such as Prostratin, CAPSOROLS, CCS and over 60 anti-cancer compounds are under active preclinical trials along with the introduction of herbals in the form of nutraceuticals and dietary supplements (Saklani and Kutty, 2008). The various factors such as physical and chemicals affect the in vitro cultured tissues and leads to a wide variety of responses including callus formation, somatic embryogenesis and shoot or root regeneration.

Growing populations and resultant food scarcity is a compelling and rising issue and plant biotechnology could offer quick and reliable method of plant breeding (Kumar and Roy, 2006). Genetic transformation has represented a more precise and predictable method for producing plants with new and desirable traits (Basu et al., 2010, 2011). Plant tissue culture has invariably been used to obtain genetically transformed plants in direct gene transfer (Neumann et al., 2009; Kumar 2010; Kumar and Sopory, 2008, 2010 and Kumar and Roy, 2011). One of the most important emergent research platforms under the realm of plant biotechnology is “Molecular farming” and
Discussion

has been reported to synthesize several value-added products like neutraceuticals and pharmaceuticals (Acharya et al., 2010).

Dependence of success of in vitro morphogenesis on explants, physiological status of explants, media factors and different growth regulators is well recognized (Wang et al., 2010). Concentrations of different growth regulators in the media are crucial to regulate the in vitro growth and morphogenesis (Neumann et al., 2009 and Kumar and Sopory, 2010). The role of growth regulators for shoot regeneration from callus is well documented in several plants Valeriana edulis (Castello et al., 2000), Pelargonium species (Saxena et al., 2000), Lathyrus sativus (Zambre et al., 2002) etc.

Primary metabolites like carbohydrates, proteins, lipids and nucleotides are the raw material of the plants. These are essential for the growth and development in plants and have a key role in metabolic processes such as photosynthesis, respiration and nutrient assimilation. Carbohydrates play a key role in living organism. As the main respiratory substrate, they provide nearly all the cellular energy and act as the starting point for biosynthesis of all cellular molecules. Their comparative distribution in the plant kingdom has been well studied by Pigman (1957) and Bell (1962). Several studies have been carried out to determine the reducing sugars, sucrose and starch from various plant parts using different methods. Plant sugars can be used as artificial sweetener and they can even help in diabetes by
supporting the body in its rebuilding (Freeze 1998). Many workers have reported the composition of primary metabolites from various plants such as *Nerium indicum, Gloriosa superba, Ricinus communis* and *Euphorbia hirta* (Kumar and Vijayvergia, 2007; Rishi and Sarin, 2009 and Vijayvergia *et al.*, 2009).

Free radicals are implicated in a number of diseases that can have serious effects on the cardiovascular system, either through lipid peroxidation or vaco-constriction. Plants are the potential source of natural antioxidants which can augment cellular defense and help to prevent oxidative damage to cellular components. Besides playing an important role in physiological systems, antioxidants have been used in the food industry to prolong the shelf life of foods, especially those rich in polyunsaturated fats.

Plants are an alternative source for naturally occurring antioxidants. An antioxidant is a compound that inhibits or delays the oxidation of substrate even if the compound is present in lower concentration than the oxidized substrate (Halliwell and Gutteridge, 1981). Antioxidant properties of plants have been studied (Barman *et al.*, 2013). The antioxidant activity of plant secondary metabolites has been investigated by a number of workers (Misawa, 1994; Wang *et al.*, 1999; Mau *et al.*, 2002; Singh *et al.*, 2004; Singh *et al.*, 2005 and Dung *et al.*, 2008).
Plants are an alternative source for stored products protection because they constitute a rich source of bioactive chemicals and are sometimes used as natural drugs and flavouring agents for food and beverages and nutraceutical value (Pareek and Kumar, 2014 and Rani and Kumar, 2014).

The plant *J. adhatoda* pantropical in distribution is widely present in Rajasthan and has a number of traditional uses.

The results of phytochemical screening of *Justicia adhatoda* leaves revealed the presence of major alkaloids, vasicine and vasicinone, flavonoids, triterpenoids, tannin, sterol and glycosides, confirming its antibiotic properties and usefulness by the traditional medicinal practitioners. Flavonoids are also known to have a wide array of therapeutic activities as antihypertensive, antirheumatism, antimicrobial diuretic and antioxidants.

The inhibition capacities of the extracts were considerably close to the inhibition ability on the positive controls suggesting its antimicrobial potential specially with respect to *Mycobacterium tuberculosis*.

There is considerable demand of this plant in India and this demand is met from the natural habitat. This plant shows low seed germination and conventional propagation through cuttings. This leads to rapid depletion of plant material due to over exploitation.
Micropropagation technique will help in mass propagation fulfilling the increasing demand of this plant.

Pharmacognostical studies will help in establishing structure, activity relationship and promote agroindustries based on natural products by providing correct identification.

Margaret Chan, Director-General, World Health Organization, emphasized that the traditional medicine holds great potential to improve people's health and wellness. It is an important, yet often underestimated, part of health care (Chan, 2014).

Subjecting *Justicia adhatoda* to these studies could advance our knowledge of the indigenous ethnopharmacopoeias and at the same time result in higher hit role for promising pharmacological effects.