CHAPTER 3

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As described earlier, the present project marks a step towards establishing the role of the genetic complement in determination of secondary metabolite concentration in plants of therapeutic importance. The first step towards the objective was the selection of appropriate medicinal plant species. The major criteria chosen for the selection were;

- The therapeutic potential of the plant is well established.
- The major chemical constituent responsible for the biological activity of the plant is known.
- The plant has a fairly wide distribution with significant variability known.
- The plant is easily available and accessible for collection purpose.

On the basis of above mentioned criteria two well-known Indian medicinal plants, *Adhatoda vasica* Nees and *Andrographis paniculata* (Burm. f.) Nees were selected for the present study. These plants have been used in traditional herbal preparations since hundreds of years and have proven themselves in the context of effective herbal medicine. The major phytochemicals of these plants that account for biological activity are well known and have been tested and proven on the scale of modern medicine. The detailed description of the plants used in the study is outlined in subsequent sections.

3.1 *Adhatoda vasica*

*Adhatoda vasica*, commonly known as vasaka or malabar nut was the first plant selected for the study (Figure 3A, 3B). The genus which is synonym to *Justicia*, has about 420 species.
3.1.1 Systematic classification

Division  Angiosperms  
Class  Dicotyledonae  
Subclass  Gamopetalae  
Series  Bicarpellatae  
Order  Personales  
Tribe  Justicieae  
Family  Acanthaceae  
Genus  Adhatoda  
Species  vasica

Common names - Vasaka, Malabarnut, Adusa, Ardusi, Adhatodai, Basak

Synonyms - Adhatoda zeylanica Medik, Justicia adhatoda L.

The plant is a 2.5 – 3.5 meter high shrub having large, lanceolate leaves with characteristic odor and bitter taste and with white flowers in a short pedunculate, bracteate spike inflorescence.

3.1.2 Geographical distribution

A. vasica is a small, evergreen, sub-herbaceous plant widely spread throughout the tropical region of south-east Asia. Basically indigenous to India, A. vasica has a very wide distribution and can flourish in a variety of habitats and geographical locations (Raja et al 2008). The leaves make the medicinally important part of the plant as they
principally harbor the therapeutically active principles. The drug is collected from wild resources and there is no systematic cultivation known.

3.1.3 Therapeutic potential

3.1.3.1 Traditional claims

It is one of the highly reputed plant species, utilized in indigenous systems of medicines in India for over 2000 years (Claeson et al 2000). In Ayurvedic medicine, *A. vasica* has been used for a multitude of disorders including bronchitis, leprosy, blood disorders, heart troubles, thirst, asthma, fever, vomiting, loss of memory, leucoderma, jaundice, tumours, mouth troubles, sore-eye, fever, and gonorrhea (Gupta and Tandon 2004). *A. vasica* is useful in treating bronchitis, tuberculosis and other lung and bronchiole disorders (Shrivastava et al 2006, Ahmad et al 2009). A decoction of the leaves of the plant may be used to help with cough and other symptoms of colds (Soni et al 2008). The soothing action helps irritation in the throat and the expectorant will help loosen phlegm deposits in the airway. *A. vasica* exhibits antispasmodic, expectorant and blood purifying qualities (Sampath Kumar et al 2010). The leaves, the roots and flowers of the plant are extensively used in indigenous medicine as remedy for cold, cough, bronchitis and asthma.

3.1.3.2 *In vitro, in vivo* biological activity

The traditional claims regarding the uses of *A. vasica* in various ailments like cough, chronic fever, asthma, bronchitis, leucorrhoea, prostrate enlargement, bleeding piles etc. (Samy et al 2008), has led to the scientific evaluation of the plant to prove its therapeutic importance. Alcoholic and aqueous extracts of the plant have shown significant antibacterial activities against various gram positive and gram negative bacteria (Dey et al 2010, Kartikeyan et al 2010). The ethanolic extract of *A. vasica* exhibited potent radioprotective effect on testis tissue architecture and various cell populations (Kumar et al 2007). Anthelmintic activity of *A. vasica* roots and anticestodal activity of leaf extracts was reported by Lateef et al (2003) and Yadav and Tangpu (2008). Ethanolic extract of roots were shown to possess remarkable antiinflammatory and analgesic activities (Mulla et al 2010). Plant also showed hepatoprotective effects against carbon tetrachloride...
induced hepatotoxicity (Pandit et al 2004). A polyherbal combination DCBT4567-Astha-15 with vasaka as the main ingredient was found as efficacious as salbutamol (12 mg/day) or salbutamol (12 mg/day) in combination with theophylline (200 mg/day) in the treatment of reversible asthmatics (Murali et al 2006). The plant has also shown credible antitussive activity as comparable to codeine, bronchodilator activity in both in vivo and in vitro studies, antihyperglycaemic activity, antiviral activity, antitumour activity and immunostimulant activity in various studies (Ahmad et al 2009, Alam et al 2010, Sampath Kumar et al 2010).

3.1.3.3 Herbal preparations

The plant has been used in the indigenous system of medicine in worldwide as herbal remedy for treating various disorders as stated earlier. 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. A. vasica is an important ingredient of many Ayurvedic formulations, some of which are Vasavaleha, Vasarishta, Vasavarasa, Vasadigutika, Vasaghrita, Vasakhanda, Chyawanprash, Maharasnadhi quthar etc. (Mulla et al 2010, Parle and Bansal 2006, Soni et al 2008). A few examples of branded herbal products are ‘Herbal vasaka cough syrup’ by Vyas Pharmaceuticals, ‘Vasavaleha by’ Dabur, ‘Vasaka’ by Himalaya Healthcare, ‘Vasarishtam’ by Arya Vaidya Pharmacy etc.

3.1.4 Toxicological risks

The literature does not report any serious side effects of the drug (Ahmad et al 2009). However, uterotonic and abortifacient effects of A. vasica extracts has been reported (Ayyanar and Ignacimuthu 2008). Vasicine as the major active compound was considered responsible for this activity. The animal and clinical data does not support the theory of either abortifacient or teratogenic effects of vasicine (Claeson et al 2000). There is no scientifically reliable evidence that A. vasica extract given orally to pregnant rats would induce embryo or foetotoxicity leading to complete resorptions. On the contrary there are studies which support the view that A. vasica extract given to rats and mice during gestation does not interfere with the outcome of pregnancy (Burgos et al 1997).
3.1.5 Chemical composition

The therapeutic efficacy of the plant is attributed to the presence of a wide range of bioactive secondary metabolites which largely include alkaloids, flavonoids, phytosterols and triterpenes. Crude extracts of the leaves obtained using solvents of varied polarity, indicated the presence of phenols, tannins, alkaloids, anthroquinones, saponins, flavonoids, amino acids and reducing sugars (Karthikeyan et al 2009). The alkaloids especially the quinazoline alkaloids namely vasicine (peganine) (Figure 4) and vasicinone have been found to be particularly responsible for the bronchodilatory effects of the plant (Alam et al 2010). Other alkaloids include vasicinol, vasicinine, vasicoline, vasicolinone, adhatodine and anistonine. The leaves of vasaka are rich in vitamin C, carotene and an essential oil betane which is also an important phytochemical found in the plant (Sampath Kumar et al 2010).

3.1.6 Vasicine

Vasicine (peganine) with the molecular formula of $C_{11}H_{12}N_{2}O$ is the major bioactive constituent found in $A.~vasica$. Pharmacological investigations of vasicine have shown bronchodilatory activity (comparable to theophylline) both in vitro and in vivo; whereas vasicinone showed bronchodilatory activity in vitro only (Avula et al 2008). Chemically designated as (1, 2, 3, 9-tetrahydro-pyrrolo-(2-1b)-quinazoline-3-ol), vasicine has been shown to possess bronchodilatory, hypotensive, uterotonic, antileishmanial and potent respiratory activities (Atal et al 1980, Claeson et al 2000, Khaliq et al 2009). The widely used mucolytics ambroxol and bromhexine are derivatives of vasicine (Bhatia et al 2008) and these have also shown potent antitubercular activities in lung tissues (Grange and Snell 1996). Thus, vasicine is an important chemical entity with attractive scaffolds for drug discovery (Patwardhan et al 2005).

Figure 4- Structure of vasicine
3.2  *Andrographis paniculata*

The second plant selected for the study is *Andrographis paniculata* (Figure 5A, 5B). The genus *Andrographis* consists of 28 species of small annual shrubs essentially distributed in tropical Asia. Only a few species are medicinal, of which *A. paniculata* is the most renowned.

![Figure 5A- A. paniculata plant](image1)

![Figure 5B- A flowering twig.](image2)

### 3.2.1 Systematic classification

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<th>Division</th>
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Common names - Kalmegh, Bhoonimba, Kirayat, Mahatita

**Synonyms**  *Justicia latebrosa* Russ., *J. paniculata* Burm. f., *J. stricta* Lam. ex Steud.
A. paniculata is a small, erect, annual herb with a height of ½ to 1 meter. The plant is extremely bitter in taste having quadrangular dark green stem with longitudinal furrows, lanceolate leaves and small solitary white flowers with rose-purple spots on the petals (Jarukamjorn and Nimoto 2008).

3.2.2 Geographical distribution

It is widely distributed in tropical Asian countries which include India, China, Thailand and Sri Lanka (Sabu et al 2001). It grows wildly in a variety of habitats viz. plains, hill slopes, waste lands, farms, dry or wet lands and native populations of A. paniculata are spread throughout south India and Sri Lanka which perhaps represent the centre of origin and diversity of the species. For medicinal purposes, the drug is extensively collected from the wild resources in India, whereas it has been cultivated in China and Thailand. Whole plant is used as the drug.

3.2.3 Therapeutic potential

3.2.3.1 Traditional claims

A. paniculata has been a wonder drug in traditional Ayurveda and Siddha systems. It has the reputation of ‘a medicinal herb with several benefits’. It is the ‘kalmegh’ of ayurveda, well-known for its extremely bitter properties (often called king of bitters) and is used traditionally as a remedy against common cold, dysentery, fever, tonsillitis, diarrhoea, liver diseases, inflammation, herpes, etc. (Pannosian et al 2002, Patarapanich et al 2007). The herb is reported to possess astringent, anodyne, tonic and alexipharmic properties (Anonymous 1, 1985). A decoction of the plant is a blood purifier. It is used as a cure for torpid liver and jaundice. There are also reports of the plant used as a treatment for snake bites, scorpion sting, intestinal worms and other poisonous bites (Gupta and Tandon 2004).

3.2.3.2 In vitro, in vivo biological activity

The plant has been subjected to a number of pharmacological investigations due to its remarkable range of biological activities. The plant has shown hypoglycaemic and antihyperglycaemic potential in diabetes type I and type II animal models (Rao 2006, Reyes et al 2006, Subramanian et al 2008, Zhang and Tan 2000). The traditional use of
*A. paniculata* against common cold, diarrhoea, fever, etc. marks it as a plant with strong antiinfective potential. It has shown toxic activities against pathogenic bacteria, fungi, malarial parasites and even viruses. Various extracts of the plant (aqueous, ethanolic, methanolic etc.) have shown potent antibacterial activity against many gram positive and gram negative bacteria (Radhika and Lakshmi 2010, Sule *et al* 2010, Xu *et al* 2006, Zaidan *et al* 2005). The plant even exhibits antityphoid activity, antifungal activity, antimalarial activity and schizontocidal activity (Anonymous 1985, Mishra *et al* 2009, WHO 2002).

There are a plethora of reports on the antioxidant potential of *A. paniculata*. Methanolic extract of the plant inhibited formation of oxygen derived free radicals in mice (Batkhuu *et al* 2002, Sheeja *et al* 2006, Verma and Vinayak 2007). *A. paniculata* has a strong antiinflammatory potential (Chiou *et al* 2000 and Chiou *et al* 1998) which possibly arises from its ability to prevent the production of Reactive Oxygen Species (ROS). The plant exhibits a strong immunomodulatory potential. It can stimulate both antigen specific and antigen non-specific immune response in the body and shows effectiveness against variety of infectious and oncogenic agents (Puri *et al* 1993, Sagrawat and Khan 2007). It has been explored for its anticancer potential in the recent times (Varma *et al* 2011). In addition to showing toxicity against cancerous cells and disease causing organisms, *A. paniculata* exerts a shielding effect towards vital organs against chemotoxicity. It is used extensively in traditional medicines as a hepatoprotectant and hepatostimulant (Chaturvedi *et al* 1983, Tripathi and Kamat 2007, Trivedi and Rawal 2000). There are also reports on renoprotective, cardioprotective and neuroprotective effects of *A. paniculata* (Chan *et al* 2010, Singh *et al* 2009, Zhao and Fang 1990).

### 3.2.3.3 Herbal preparations

*A. paniculata* is a predominant constituent of no less than 26 Ayurvedic formulations (Mishra *et al* 2007), of which a dozen are used in form of hepatoprotectant (Lattoo *et al* 2008). It forms the major constituent of the Ayurvedic drug SG-1 Switradilepa, which is effective in treating vitiligo – a dermatological disease. It is reported as a cold property herb in TCM and is used to get rid of body heat and to expel toxins. Many other polyherbal formulations which have *A. paniculata* as an ingredient have shown efficacy against skin disorders, arthritis, diabetes, fever, hepatotoxicity, etc. (Anand Kumar and

### 3.2.4 Toxicological risks

*A. paniculata* has generally been regarded as a safe herb with little or no toxic effects reported in most of the studies. However, there have been a few reports that emphasize on toxic effects of the plant on human body. High doses of *A. paniculata* in various forms orally may cause gastric discomfort, anorexia and loss of appetite (WHO 2002). Also due to potential anaphylactic reactions, crude extracts of *A. paniculata* are not recommended as injectables (Yin et al 1991). The herb is generally well tolerated in clinical trials as discussed above with minor cases of headache, nausea, dizziness, drowsiness etc. reported in very few cases (Coon and Ernst 2004). However, the major concern in administration of this herb is the reported reproductive toxicity (Mishra et al 2007). *A. paniculata* is suggested to be avoided during pregnancy. There have been certain reports based on animal studies as described in the earlier section but still there is no clinical evidence of foetal malformation or abortions after oral intake of *A. paniculata* or its chemical constituents (Sakila et al 2009). There are also no teratogenic effects reported. The results related to male reproductive toxicity are also conflicting (Akbarsha et al 2000, Sattayasai et al 2010). Thus, there exists a lot of contradiction with respect to reproductive toxicity of *A. paniculata*. In the nutshell, it can be postulated that *A. paniculata* is a generally safe herb but can be avoided during pregnancy as per traditional recommendations.

### 3.2.5 Chemical composition

Phytochemical analysis of the plant extracts has revealed the presence of numerous active principles, which mainly include diterpenoid lactones, flavonoids, and polyphenols (Rao et al 2004). More than 20 diterpenes and over 10 flavonoids from this plant have been
reported in last three decades (Li et al 2007) with many more getting added to the compendium. The crystalline, colourless, extremely bitter diterpenoid lactone andrographolide ($C_{10}H_{20}O_{5}$) is the most abundant and most important phytochemical found in *A. paniculata* (Figure 6). The therapeutic potential of the plant is generally attributed to the presence of andrographolide. The plant also contains other important diterpenes, flavonoids, polyphenols etc. which include 14-deoxy-11,12-didehydroandrographolide, 14-deoxyandrographolide, 14-deoxy-11-oxoandrographolide, the non bitter neoandrographolide, homoandrographolide, andrographosterol, andrographone, andrographosterin, andrograpanin, $\alpha$-sitosterol, stigmatosterol, andrographin, dihydroxy-di-methoxyflavone, panicolin, andrographiside, 14-deoxyandrographiside, andropaniculoside A, andrographin, isoandrographolide and skullcaflavone to name a few (Kulyal et al 2010, Niranjan et al 2010, Rastogi and Mehrotra 1999). Isolation and characterization of bioactive constituents of *A. paniculata* has been excellently reviewed in a recent publication (Chao and Lin 2010).

### 3.2.6 Andrographolide

Diterpenoid lactone andrographolide is the principle marker compound found in *A. paniculata*, which is mainly concentrated in leaves and can be easily isolated from the crude plant extracts as crystalline solid (Lomlim et al 2003, Rajani et al 2000). Chemically designated as (3-[2-[decahydro-6-hydroxy-5-(hydroxymethyl)-5, 8-adimethyl-2-methylene-1-napthalenyl] ethylidene] dihydro-4-hydroxy-2(3H)-furanone), andrographolide exhibits extraordinarily vast range of biological activities. Andrographolide and its conjugate with lipoic acid have shown glucose lowering and insulinitropic effects as a promising antidiabetic agent (Yu et al 2003, Zhang et al 2009). As an antiinfective, andrographolide was found to recover the susceptibility of multidrug resistant strain of *Psuedomonas aeruginosa* to certain antibiotics and this recovery was attributed to the inhibition of transcription expression of antibiotic efflux pumps like mexAB-OprM (Wu et al 2008). There are also reports on antiviral activity of andrographolide against human immunodeficiency virus (Calabrese et al 2002, Reddy et al 2005). An andrographolide conjugate with lipoic acid was found to be highly active against various influenza viruses including the deadly H5N1 strain (Chen et al 2009).
Exploration of antineoplastic potential of andrographolide is an interesting endeavor in light of significant anticancer activity shown by the plant (Jada et al 2007, Jiang et al 2007, Matsuda et al 1994, Zhou et al 2008). It has been demonstrated that andrographolide can activate extrinsic death receptor pathway in various human cancer cell types (Kim et al 2005, Rajagopal et al 2003, Sukardiman et al 2007, Zhou et al 2006) inducing cell-cycle arrest in various kinds of cell lines at G0/G1 stage (Cheung et al 2005, Geethangili et al 2008). The antineoplastic potential of the compound has been discussed in detail in one of our publications (Varma et al 2011). Handa and Sharma, (1990) demonstrated the hepatoprotective activity of andrographolide in CCl₄, galactosamine as well as paracetamol intoxicated rats. The compound could reverse an experimental liver carcinogenic condition in mice to normal (Trivedi et al 2009). The compound exhibited very strong antiinflammatory potential in a large number of studies. Andrographolide inhibits Erk1/2 and Akt signaling thus restraining the chemotactic migration of macrophages on inflammation site (Tsai et al 2004). It has also been reported to suppress interleukin-2 production and T-cell proliferation in a mixed lymphocyte reaction and to inhibit dendritic cell maturation and antigen presentation (Iruretagoyena et al 2005). In addition, andrographolide inhibited lipopolysaccharide induced nitric oxide (NO) production and inducible NO synthase (iNOS) expression in murine macrophage-like cell line RAW264.7 (Chiou et al 2000) and in primary rat microglia cells (Wang et al 2004).

Figure 6- Structure of andrographolide
Thus, two highly reputed medicinal plants species (*Adhatoda vasica* and *Andrographis paniculata*) were selected for the study owing to their wide geographical distribution, potential therapeutic application, known bioactive components and reported intra-specific phytochemical variation. Vasicine from *A. vasica* and andrographolide from *A. paniculata* were selected as the marker compounds as these are principally responsible for majority of biological activities of *A. vasica* and *A. paniculata* respectively. Thus, to evaluate the hypothesis of genetic component playing a part in determination of concentration of bioactive constituents, the marker compounds were estimated in individual plants followed by the genetic analysis to identify any correlation between the two factors.