CHAPTER-1
INTRODUCTION

Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Apart from pharmaceutical industries, medicinal and aromatic plants have received much attention in several other fields such as cosmetic, perfumery, liquor and nutritional applications (Manukyan, 2011). India has a rich history of using potent herbs and herbal components as traditional medicines for various ailments. Ancient medicine was not solely based on empiricism and this is evident from the fact that some medicinal plants which were used in ancient times still have their place in modern therapy (Das and Mondal, 2012). One hundred and nineteen secondary plant metabolites derived from plants are used globally as drugs; 15% of all angiosperms have been investigated chemically and of that 74% of pharmacologically active plant derived components were discovered (Samy *et al.*, 2008). The demand of using natural products for medicinal purposes is increasing and researches in relation to medicinal plants use for human consumption are taking place in a big way. Plants genetic improvement, its cultivation for higher productivity of useful metabolites, regulatory processes within cells and to design new therapies are some of the aspects needs to be researched.

Andrographis paniculata (Wall ex.) Nees (Acanthaceae), commonly known as Kalmegh, is an important medicinal plant, occurring wild in India and is used both in Ayurevda and Unani system of medicine (CSIR, 1985). The dried herb is a remedy for a number of ailments related to digestion, hepatoprotection, vermicidal, antiacne, analgesic, anti-inflammatory, antibacterial, antityphoid, antibiotic activities, anti-HIV activity, hypoglycemic, besides immune enhancement (Matsuda *et al.*, 1994; Handa and Sharma, 1990; Singh and Handa, 1995; Otake *et al.*, 1995; Saxena *et al.*, 2000). *A. paniculata* is also used for treating animal diseases, e.g. respiratory infection and diarrhoea, as an alternative to antibiotics (Tipakorn, 2002). The therapeutic activities of this plant are attributed to its major secondary metabolites such as andrographolide and related diterpens, i.e., dexoyandrographolide, 14-deoxy-11, 12- didehydroandrographolide and neo-andrographolide. Modern pharmacological studies indicate that andrographolide protects the liver and gallbladder, and has been found to be slightly more active than Silymann, a known hepatoprotective drug (Saraswat *et al.*, 1995). Neo-andrographolide shows greater activity against malaria (Misra *et al.*, 1992) and is hepatoprotective against carbon tetrachloride (Kapil *et al.*, 1993) whilst 14-deoxy andrographolide producing a more potent hypotensive effect in anaesthetised rats and isolated right atria (Zhang *et al.*, 1998).

Plants produce a vast and diverse assortment of organic compounds, the great majority of which do not appear to participate directly in growth and development. These substances, traditionally referred to as secondary metabolites, often are differentially distributed among limited taxonomic groups within the plant kingdom. The evolving commercial importance of secondary metabolites has in recent years resulted in a great interest in secondary metabolism, particularly in the possibility of altering the production of bioactive plant metabolites (Vanisree *et al.*, 2004). Secondary metabolites are separated into nitrogen compounds (alkaloids, non-protein amino acids, amines, alcamides, cyanogenic glycosides and glucosinolates) and non-nitrogen compounds (monoterpenes, diterpenes, triterpenes, tetraterpenes, sesquiterpenes, saponins, flavonoids, steroids and coumarins). Secondary metabolites play a major role in the adaptation of plants to the changing environment and in overcoming stress constraints. Although secondary metabolites are fundamentally produced by genetic processing but the accumulation of secondary metabolites in plants is a common response to biotic and abiotic stresses (Smetanska, 2008) and their biosynthesis is strongly influenced by environmental factors (Yazdani *et al.*, 2002). Biotic and abiotic elicitors are used to stimulate secondary metabolite product formation in plants, thereby reducing the process time to attain high product concentrations (Barz *et al.*, 1988; Eilert, 1987; DiCosmo and Tallevi, 1985). High levels of stress in medicinal or other plants may suppress secondary metabolite production. Alternatively, the presence of stress in medicinal plants may stimulate production of bioactive compounds in many plant species. Moreover, some research results suggest that abiotic stress may play an important role in triggering plant genes to alter the titers or nature of secondary plant metabolites, although the exact mechanism by which this happens remains unclear (Nasim and Dhir, 2010).

Abiotic stress (salinity, drought, heat/cold, light and other hostile conditions) leads to the overproduction of reactive oxygen species (ROS), which stimulates formation of highly active signaling compounds capable of triggering production of bioactive compounds (secondary metabolites) that enhances the medicinal value of the plant. In fact, a common denominator in all these adverse conditions is the over production of the reactive oxygen species (ROS) within different cellular compartment of the plant cell (Pinheiro and Chaves, 2011). In chloroplasts, photosystem I and II (PSI and PSII) are the major sites for the production of $^1O_2$ and O_2. In mitochondria, complex I, ubiquinone and complex III of electron transport chain (ETC) are the major sites for the generation of O_2 (Gill and Tuteja, 2010). ROS formation is initiated by the univalent reduction of molecular oxygen using either one, two or three electrons generating superoxide, hydrogen peroxide (H_2O_2) or hydroxyl radical ($OH^•$) respectively or by the formation of singlet oxygen ($^1O_2$) by transfer of excess excitation energy to O_2 (Alscher *et al.*, 2002; Collakova and DellaPenna, 2003; Wu *et al.*, 2008). ROS are divided in two main classes consisting of non-radical species (H_2O_2) or free radical forms (O_2^-; $OH^•$; $OH_2^•$). Accumulation of high concentrations of ROS is potentially detrimental to plant cells causing damage to valuable biomolecules like DNA, proteins, lipids, chlorophyll, membrane etc. (Blokhina *et al.,* 2003). Generated at low amounts, ROS are involved as early messenger molecules in signaling cascades of reaction activated by several external and development stimuli and ROS serve also in defense reactions in apoplast (Asada and Takahashi, 1987; Abbasi *et al.,* 2007). It is well documented that the overproduction of reactive oxygen species (ROS) in plants are highly reactive and toxic and ultimately results in oxidative stress (Gill and Tuteja, 2010). The antioxidant defense machinery protect plants against oxidative stress damages. Plants possess an efficient enzymatic (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione peroxidase, GPX; guaicol peroxidase, GOPX and glutathione-S-transferase, GST) and non-enzymatic (ascorbic acid, AA; glutathione, GSH; phenolic compounds, alkaloids, non-protein amino acids and α-tocopherols) antioxidant defense systems which work in concert to control the cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging of ROS (Smirnoff, 1995; Ali *et al.*, 2008; Gill and Tuteja, 2010). Therefore, the plants with the ability to scavenge or control the level of cellular ROS may be useful in future to withstand harsh environmental conditions.

Drought is one of the most important abiotic stress factor (Dash and Mohanty, 2001), affecting plant growth and productivity and leaf photosynthesis (Ludlow and Muchow, 1990; Flexas *et al.*, 2004) and altering physiological and biochemical properties of plants (Zobayed *et al.*, 2007). Some plants have a set of physiological adaptations that allow them to tolerate water stress conditions. The degree of adaptations to the decrease of water potential caused by drought may vary considerably among species (Save *et al.*, 1995). Plant response to water stress include morphological and biochemical changes and later results in functional damage and loss of plant parts (Sangtarash, 2010). Researchers linked various physiological responses of plant to drought with their tolerance mechanisms, such as: pigment content and stability and high relative water content (Clarke and McCaig, 1982). Drought stress is also known to increase the secondary metabolite production in a variety of medicinal plants, like artemisinin in leaves of *Artemisia annua* (Charles *et al.*, 1993), hyperforin in *Hypericum perforatum* leaf tissues (Zobayed *et al.*, 2007) and ajmalicine in *Catharanthus roseus* roots (Jaleel *et al.*, 2008e). Cultivation of a medicinal plant like *Trachyspermum ammi* in water-deficient areas increased its defense system and the level of active compounds (Azhar *et al.,* 2011). Understanding of physiological mechanisms that enable plants to adapt to water deficit and maintain growth and productivity during stress period could help in screening and selection of tolerant genotypes and using these traits in breeding programs (Zaharieva *et al.*, 2001).

Another abiotic stress due to soil salinity is detrimental to plant growth and productivity as it causes nutritional imbalances by altering the uptake of nutrients (nitrogen, phosphorus, potassium, calcium, etc.) and interferes with the cellular metabolism by causing ion toxicity and osmotic perturbations (Joshi *et al.*, 2011). Under salinity the predominant cause of ion toxicity is the replacement of K^+ with Na^+ and non-covalent interactions of Na^+ and Cl^- ions with amino acids of proteins and enzymes. The effect of this ionic imbalance manifests at the cellular as well as at the whole-plant level. Massive changes in ionic and water balance cause molecular damage and arrest growth (Jaleel *et al.* 2008a). As a consequence of salinity stress-induced ion imbalance and hyper-osmotic effects, the functioning of important cellular organelles like mitochondria and chloroplast is adversely affected. Production of toxic reactive oxygen species (ROS) intermediates is the primary cause of oxidative stress. Reactive oxygen species severely hamper normal cellular functioning through oxidative damage to lipids, proteins, and nucleic acids (Demiral and Turkan, 2005; Mandhania *et al.*, 2006). However, in spite of having a potential antioxidant defense system, plants do suffer extensive damage on exposure to salinity stress. This indicates that the plant's defense system has a limited capacity to detoxify reactive oxygen species produced as a result of exposure to salinity stress. There is evidence to suggest that the alleviation of oxidative damage and increased resistance to salinity stress is correlated with an efficient antioxidant defense system (Acar *et al.* 2001; Shigeoka *et al.* 2002). Salt stressed plants also showed significant higher concentration of tropane alkaloids (Brachet and Cosson, 1986) and flavanoids (Ali and Abbas, 2003). Environmental factors like salinity stress strongly influence the biosynthesis of secondary metabolites (Yazdani *et al.*, 2002). Therefore, need is to understand physiology of *Andrographis paniculata* when grown in salt stress conditions.

Similarly, heavy metals, like cadmium cause stressful conditions for the plants growing in its presence. Cadmium is a widespread, non-essential, toxic metal and is a cumulative toxin (Goering *et al.*, 1994). It releases in to the soil through thermal power station, waste incinerator, mining, electroplating industries and urban traffic. The annual anthropogenic emission of Cd is 29.19×10^{3} tonnes/year (Sanita di Toppi and Gabbrielli, 1999) and its level in soil is increasing overtime (Jones and Johnston, 1989; Jones *et al.*, 1992). It is considered to be highly mutagenic and designated as a human carcinogen by International Agency for Research on Cancer (IARC, 1993; Filipic and Hei, 2004). Although plants do not require Cd for their growth and reproduction, but the bioaccumulation index of Cd in green plants exceeds to other elements (Kabata-Pendias and Pendias, 1992). It is retained for many years in the human body and consumption of material high in Cd may induce chronic toxicity (Jackson and Alloway, 1992; FAO/WHO, 1995). WHO has set a maximum permissible Cd limit of 0.3 ppm for medicinal plants due to its high toxicity. Several workers studied the effect of Cd on different physiological and biochemical parameters of various plants (Shaw, 1995; Gallego *et al.*, 1996; Chaoui *et al.*, 1997; Sanita di Toppi and Gabbrielli, 1999; Dixit *et al.*, 2001), but very little attention has been paid on therapeutically active constituents and ultra-morphological variations in different parts of the medicinal plants. The plant systems which were studied to investigate Cd stress are: *Allium sativum* (Liu and Kottke, 2003), *Hordeum vulgare* (Finkemeier *et al.,* 2003), *Cannabis sativa* (Linger *et al.,* 2005), *Colocassia esculentum* (Patel *et al.,* 2005), *Triticum aestivum* (Khan *et al.,* 2007), *Arabidopsis thaliana* (Smeets *et al.,* 2008), *Lactuca sativa* (Cornu *et al.,* 2008), *Nicotiana attenuata* (Wang *et al.,* 2008), *Solanum lycopersicon* (Chamseddine *et al.,* 2008), *Sorghum bicolor* (Kuriakose and Prasad, 2008), *Glycine max* (Luan *et al.,* 2008; Shamsi *et al.,* 2008), *Vigna mungo* (Molina *et al.,* 2008), *Zea mays* (Kumar *et al.,* 2008), etc. However, studies in relation to Cd stress in *Andrographis paniculata* needs to be taken up.

It is clear that plant growth, productivity and production of secondary metabolites are adversely affected due to abiotic stress factors. Oxidative stress induced by abiotic stress triggers signaling pathways that affect production of specific plant metabolites. In particular, reactive oxygen species (ROS), generated during abiotic stress, may cause lipid peroxidation that stimulates formation of highly active signaling compounds capable of triggering production of bioactive compounds (secondary metabolites) that enhances the medicinal value of the plant. Keeping these facts in mind, present study has been undertaken to see the relative tolerance of *Andrographis paniculata* to abiotic stress factors.

1.1 AIMS AND OBJECTIVES

The aim of the present study is to evaluate the relative tolerance of *Andrographis paniculata* to abiotic stress factors in terms of growth, morphological, physiological and biochemical characters, antioxidant defense potential and the concentration of active constituents i.e., andrographolide. To achieve the aim, following objectives were set:

i) To examine the influence of water, salinity and cadmium stress on germination and growth of *Andrographis paniculata*
ii) To evaluate the biochemical assays and osmolyte concentration under abiotic stress
iii) To evaluate enzymatic antioxidants, non-enzymatic antioxidants and radical scavenging effects in the leaves of *A. paniculata*
iv) To determine quantitatively the secondary metabolites at different growth stages and under various stresses.