INTRODUCTION

Plants remain as one of the most important resources of medicines for centuries, and numerous cultures still rely on indigenous medicinal plants for their primary health care needs. Medicinal plants are extensively utilized throughout the world in both traditional and modern systems of medicine. It is estimated that about 80% of the world’s population depends directly on plant based medicine for their health care (WHO, 2003).

In India, references to the curative properties of some herbs in Rig-veda seems to be the earliest records of use of plants in medicines. Our traditional systems of medicines, viz., Ayurveda, Yunani, Siddha and Homeopathy etc. use herbs for treatment. Approximately 3000 plants species are known to have medicinal properties in India (Prakasha et al., 2010). Thousands of specialized metabolites, produced by several plant species collectively called as phytochemicals, present an exciting opportunity for the development of new types of therapeutics. They act as bioenhancers of several physical and biochemical processes in humans.

Research works on medicinal plants and the exchange of information acquired will go a long way in scientific examination of medicinal plants for the benefit of humans. This will in turn decrease the dependence and import of chemically synthesized drugs which have side effects when used for healing various ailments. New approaches, such as metabolomics, metabolic engineering and systems and synthetic biology, are contributing towards the identification, characterization and production of plant-derived medicines.

*Withania somnifera* (L.) Dunal, (Solanaceae), commonly called as Ashwagandha/ Winter cherry/ Indian ginseng, is one of the most reputed holistic herb of India. It is used for curing a range of diseases over 5000 years in ayurveda and other indigenous medicinal systems (Akram et al., 2011). It is an erect, herbaceous, evergreen, tomentose shrub. The leaf is alternate and ovate.
The flowers are yellow and the berry fruit is orange red in colour. The roots have a long and even structure, a strong odour, bitter and acrid taste (Atal et al., 1975). It can be found growing in Africa, the Mediterranean, India, Sri Lanka, Pakistan and Bangladesh.

The roots are the main portions of the plant used therapeutically (Girdhari and Rana, 2007). The biologically active chemical constituents found in abundance are alkaloids (ashwagandhine, cuswhygrine, anahygrine, tropine, etc.), steroidal compounds including ergostane type lactones, withaferine A, withanolides A-Y, withanamides, withasomniferin-A, withanone, sitoindosides (Saponins) and glycowithanolides (Matsuda et al., 2001; Ganzera et al., 2003; Misra et al., 2008, 2012).

Major pharmacologic activities of the plant contributed by withanolides include physiologic and metabolic restoration, antiarthritic, antiaging, nerve tonic, cognitive function improvement in geriatric states, recovery from neurodegenerative disorders like convulsions and tardive dyskinesia and to treat a variety of infectious diseases as well as tremors and inflammation especially osteoarthritis, rheumatoid arthritis, and gout (Bhattacharya et al., 2002; Uddin et al., 2012).

The extracts and also its various isolated bioactive constituents have been proved to hold beneficial properties such as adaptogenic, anticancer, anti-convulsant, immunomodulatory, antioxidative and neurological effects. No/minimal side effects have been reported in its use for all human age groups (Mishra et al., 2000; Gupta and Rana, 2007; Lele, 2010). Its increasing therapeutic benefits continuously attract the attention of pharmacologists for biomedical investigations of its extracts and isolated phytochemicals (Malik et al., 2007a; Mulabagal et al., 2009; Jayapракasam et al., 2010).

The herb has been identified by National Medicinal Plant Board of India as one of the thirty-two selected priority medicinal plants, which are in great demand in the domestic and international markets (Prajapati et al., 2003).
According to an estimate, the annual requirement of the drug is 9127.5 tons while the annual production is 5905.1 tons (Govil et al., 1993; Mishra et al., 1998a, b; Ministry of Health and Family Welfare, 2002), necessitating the increase in its cultivation and higher production.

To meet the increasing demand for this herb, production of large no. of superior chemotypes, without seasonal constraints is desirable. Roots being the major source of desired phytochemicals, their harvest necessitate the uprooting of the entire plant, as field grown plant material has been used for the commercial production of withanolides. Different environmental conditions, pollutants, fungi, bacteria, viruses and insects are some of the several factors that affect the quality of these products and this can lead to heavy loss in yield and alteration in medicinal content of plant. Lack of post harvest storage technology for roots and adequate information on the genetic basis of yield contributing traits (Vitali et al., 1996; Singh and Kumar, 1998) are other unfavorable factors. Biotechnology has offered a solution to address this problem by way of developing in vitro cell and organ cultures.

Culturing of plant cells and organs in vitro is a promising approach which has enormous potential to complement field cultivation and to obtain valuable plant-specific bioactive secondary metabolites at industrial scale. Through manipulation of culture conditions and supplements in media, it is possible to obtain novel metabolites in large quantities using in vitro techniques. Specifically, organ cultures are focused as being differentiated tissues, retain the potential of synthesizing and producing bioactive compounds to levels comparable or even higher than the mother plant.

As roots contain a number of therapeutically applicable withanolides, mass cultivation of roots in vitro will be an effective technique for the production of these secondary metabolites on commercial scale. Adventitious roots are postembryonic roots which arise from the stem, leaves and from nonpericycle tissues in old roots. Adventitious roots induced by in vitro methods are shown to
have high proliferation rate and active secondary metabolism upon phytohormone supplementation.

Even though works on producing adventitious root cultures of *W. somnifera* with higher content of withanolides using different strategies are available, until date not more than one report is available on its mass cultivation. Hence, it is essential to develop an efficient mass cultivation protocol for adventitious roots in a superior variety with potential to be utilized in production of withanolides at large scale applicable for high throughput processing in industry.

A safe and optimal use of herbal medicines requires a full understanding of their ADME profiles, which will aid in extending *W. somnifera* and its constituents as a medicine. Also, chemical modifications and biotransformations can be attempted to identify novel and effective bioactive components.

To bridge the gap existing between reported research findings and the application of modern technological approaches in exploring and extending the potential of *W. somnifera*, the present study was formulated with the following objectives:

1. To develop a reproducible protocol for adventitious root induction and its mass production.
2. To device a methodology to improve the active components present in *W. somnifera* roots.

To meet the objectives the available literature was surveyed extensively and the following experimental approaches were device.

- Identification of an elite cultivar by analyzing secondary metabolite content present in *W. somnifera* roots collected from various locations.
Development of an efficient adventitious root induction protocol for *W. somnifera* and its mass cultivation in bioreactors.

Use of *in silico* tools to identify good drug lead compounds/ bioactive metabolites.

Bioconversion of withanolides using bacterial β-glucosidase isolated from food sources.

The details of the study conducted are presented in the forthcoming chapters.