INTRODUCTION

Medicinal plants constitute an important group of non-wood forest products. Approximately 80% of the world population uses plants as a source of medicine for healthcare (Anonymous, 1998). Of the 250,000 plant species known in the world, the World Health Organization has listed around 21,000 species which are used for medicinal purposes. According to an EXIM Bank report the value of medicinal plants related trade in India is estimated to the order of Rs. 5.5 billion per year, while the world trade is over US$ 60 billion per year, and is growing at the rate of 7% per annum. The annual export of these plants is valued at Rs. 1200 million (http://www.eximbankindia.com/old/press031113.html, 2003).

India is recognized as one of the 12 mega biodiversity regions of the world. It has about 47,000 species of plants, of which 17,500 are angiosperms and of these as many as 4,000 species are of medicinal value (http://www.fao.org/docrep/003/x6900e/x6900e0c.htm). India has been one of the pioneers in the development and practice of well-documented indigenous systems of medicine, particularly Ayurveda, Siddha and Unani. For millennia, the Indian population has depended mainly upon plant based crude drugs for a variety of ailments. This alternative system of medicine is gaining increasing popularity worldwide.

Medicinal plants sector has traditionally occupied an important position in the socio cultural, spiritual and medicinal arena of rural and tribal lives and due to an importance of medical plants, the Government of India established the Department of Indian System of Medicine and Homoeopathy. Recently the Medicinal Plants Board is developed to promote and regulate the sector for maximizing the benefits to the people
as well as to ensure sustainable growth. Medicinal plants have been identified as one of the thrust areas by the Ministry and different programmes have been initiated for conservation of medicinal plants found in the forests and protected areas as well as cultivation of these plants in the degraded forest areas (http://www.keralaagriculture.gov.in/html2aez/mediplant.pdf).

Today the medicinal plants occupies a stable position in modern medicine and the pharmaceutical industries also showing special interest in using or synthesizing natural substances extracted from the plants. Now day’s demands for more and more drugs from plant sources are increasing from the developing countries. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that ‘Green Medicine’ is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects (http://www.sevamandir.org/NLOCT2007/article6.pdf). Traditional medicines are in higher demands in whole world among peoples because it is a source of direct therapeutic agents, affordable by the people, less adverse effects than synthetic drugs, raw material base for the elaboration of more complex semi-synthetic chemical compounds, models for new synthetic compounds, taxonomic markers for the discovery of new compounds, the production, consumption and international trade in medicinal plants and phytomedicines, renewable source (http://precedings.nature.com/documents/1176/version/1/files/npre20071176-1.pdf; Samy and Gopalakrishnakone., 2007).

Plants produce a range of bioactive compounds and are known to harbor many useful compounds. Alkaloids, steroids, flavonoids, dyes, hormones, pigments, pharmaceuticals, drugs, phenols, terpenes and several other compounds have been isolated from plants. The structure and metabolic interactions of these compounds within the cell are apparently different from those involved with the primary growth and metabolism. The various secondary metabolites produced by the plants have some ecological role to play, such as, attractant of pollinator and chemical defense against microorganism, insects and other predator and also some protective action against biotic stresses as UV, temperature, water stress and mineral nutrition. For this reason
plants have been extensively exploited leading to decrease in their availability (Bhojwani and Razdan, 1996).

*Boerhaavia diffusa* (Nyctaginaceae) is an important medicinal plant much used in Ayurveda and Unani medicines and other traditional medicines in many parts of the world. The importance of this plant can be gauzed from the fact that there are almost forty regional names for it the world over ([http://www.raintree.com/ervatostao.htm](http://www.raintree.com/ervatostao.htm)). The plant commonly known in India as Punarnava (and also, Shothagni, Rakta punarnava) in Ayurveda because the top of the plant dies during hot summers and puts forth fresh shoots after rains and is believed to be a rejuvenator. In Brazil *B. diffusa* is known as Erva tostão and is extensively used in traditional medicine ([Hiruma-Lima et al., 2000](http://www.himalayahealthcare.com/herbfinder/h_boerha.htm)). *B. diffusa* is an exclusive or important constituent of several Ayurvedic preparations such as Abana (HeartCare), Bonnisan, Diabecon (GlucoCare), Evecare (MenstriCare), Geriforte (GeriCare / StressCare), Lukol, V-Gel (FemCare Gel), Digyton, Geriforte Aqua, Geriforte Vet, Immunol, Nefrotec Vet, Punarnava, Chyavanaprasha ([http://www.himalayahealthcare.com/herbfinder/h_boerha.htm](http://www.himalayahealthcare.com/herbfinder/h_boerha.htm)).

The parts used for medicinal purposes are: the root, leaves or whole flower. The red flower bearing plant is bitter, cooling, astringent to the bowel and useful in biliousness, blood impurity, leucorrhrea, anemia, and inflammation, stomachic and has diaphoretic properties and is also used in jaundice and ascites. Internally, Punarnava is beneficial to treat a wide range of diseases for liver disorders (jaundice, hepatitis, cirrhosis, anemia, flukes, detoxification, chemical injury, etc.); for gallbladder disorders (stones, sluggish function, low bile production, emptying, and detoxification); for kidney and urinary tract disorders (stones, nephritis, urethritis, infections, renal insufficiency/injury, etc.); to tone, balance, and strengthen the adrenals (and for adrenal exhaustion and excess cortisol production), Punarnava is highly beneficial in the treatment of obesity as almost all anti-obesity herbal preparations contain it in one or the other form. It is also beneficial in the treatment of several common ailments; Punarnava increases the secretion and discharge of urine ([http://www.herbalcureindia.com/herbs/boerhaavia-diffusa.htm](http://www.herbalcureindia.com/herbs/boerhaavia-diffusa.htm)).
It is effective in the treatment of dropsy, a disease marked by an excessive collection of a watery fluid in the tissues and cavities or natural hollows of the body. The fresh boiled herb should be given in the treatment of this disease. A liquid extract of the fresh or dry plant can also be given in doses of 4 to 6 gms., herb also useful in the treatment of ascites, a disease characterized by accumulation of fluid inside the peritoneal cavity of the abdomen. It is also beneficial in the treatment of several stomach disorders, particularly intestinal colic as in killing or expelling intestinal worms. Punarnava promotes the removal of catarrhal matter and phlegm from the bronchial tubes. Therefore, it is beneficial in the treatment of asthma. Punarnava is beneficial in the treatment of fevers. It brings down temperature by inducing copious perspiration. Stomach disorders, Asthma, Fevers. Externally punarnava is used for fomentation to alleviate the pain and swelling (http://www.hort.purdue.edu/newcrop/CropFactSheets/punarnav.html).

The fresh juice of its roots instilled into eyes, mitigates the ailments of the eyes like night blindness and conjunctivitis. The paste applied on the wounds, dries up the oozing. The root of the plant is an effective remedy for several skin diseases. A paste of the root can be applied beneficially as a dressing for edematous swellings. A hot poultice of the root can be applied with gratifying results to ulcers, abscesses and similar skin diseases, ascites, congestive cardiac conditions, anaemia, swollen joints in rheumatic disorders etc (http://www.himalayahealthcare.com/aboutayurveda/cahb.htm).

Punarnava works well in combination with guduci, haritaki, devadara and guggulu, the mixture of these powders given with cow’s urine, alleviates anasarca, ascites, anaemia, worm infestation and diabetes. It is also a very good expectorant and used as a cure in jaundice (Anonymous, 2001). The active principle is a body of alkaloid called as punarnavine. The higher active principle of alkaloid is found in dry conditions.

Trichomes are external appendages of the epidermis found on the above ground plant parts exhibiting a great variation in their morphological features and can be categorized into two broad classes: the simple trichomes and the glandular
secreting trichomes. The simple trichomes also referred to as non-glandular are considered to be non-secreting types. The other type of trichomes, the glandular secreting trichome is characterized by the presence of a terminal globular head cell, which also shows variation in size and numbers and accumulates and/or produces an array of metabolites. Depending on the species, a plant may have either only one or both type of trichomes (Fahn, 1982; Wang et al., 2002; Jakoby et al., 2008).

*Arabidopsis thaliana*, the model plant, has only unicellular, branched or unbranched simple trichomes (Szymanski et al., 2000) that have been extensively studied for understanding cell fate and differentiation (Wagner, 1991; Breuer et al., 2009; Marks et al., 2009). Genes expressing for secondary compounds such as anthocyanins, flavonoids, and glucosinolates involved in defence have been detected in the simple trichomes (Lange et al. 2000). Simple non-glandular trichomes (e.g. those of *Arabidopsis thaliana* and cotton) currently represent useful systems for studying cellular differentiation and development at the molecular level (Hulskamp, 2004).

Secretory glandular trichomes (SGTs) are found on the surfaces of above ground plant tissues of approximately one third of all vascular plant species. Humans have been familiar with these structures because they contain the essential oils that give cooking herbs their distinctive smell and taste. In addition to making foods taste better, these uni- and multi-cellular appendages are proposed to play a critical role in plant protection against various environmental stresses including herbivore attack, pathogen infection, extreme temperature, excessive light and as pollination attractants (Peter and Shanower, 1998; Larkin et al., 2003; Wagner et al. 2004; Martin and Glover, 2007; Dai et al., 2009).

One of the most remarkable features of trichomes is their capacity to synthesize, store and sometimes secrete large amounts of varied types of specialized metabolites. These include various classes of terpenes (Gershenzon et al., 1992; Hallahan, 2000; van der Hoeven et al., 2000), as well as phenylpropanoid derivatives (Gang et al., 2001), acyl sugars (Kroumova and Wagner, 2003; Li and Steffens, 2000), methylketones (Fridman et al., 2005) and flavonoids (Voirin et al., 1993). Many trichome borne compounds have significant commercial value as pharmaceuticals,
fragrances, food additives and natural pesticides (Duke et al., 2000; Wagner, 1991; Wagner et al., 2004; Kang et al., 2010). Trichomes may also be important for sequestration of toxic metals (Choi et al., 2001).

The multifarious functions of trichomes make them natural targets for genetic manipulation strategies to improve plant disease or pest resistance by introduction of constitutively produced antimicrobial or insecticidal compound or heavy metal tolerance by introduction of metal chelating proteins or peptides (Aziz et al., 2005). The trichome-borne artemisinin from *Artemisia annua* is still the most effective drug against malaria, and the early steps of its biosynthetic pathway have been extensively studied (Duke et al., 1994; Lommen et al., 2006; Liu et al., 2006; Arsenault et al., 2008). The small procumbent glandular secreting trichomes of *Nicotiana tabacum* (Shepherd et al., 2005) and sunflower (Kroumova et al., 2007) accumulate antimicrobial proteins. The anti-nausea agent tetrahydrocannabinol is produced by the trichomes of *Cannabis sativa* (Sirikantaramas et al., 2005). Alkaloids, though not common in the glandular trichomes, have been reported in *Sigesbeckia jorullensis* (Heinrich et al., 2002) and *Salvia officinalis* (Corsi and Bottega, 1999).

It is noteworthy that many trichome-borne compounds have significant commercial value as pharmaceuticals, fragrances, food additives, and natural pesticides. For this reason, the prospect of exploiting SG Ts as “biofactories” to produce high-value plant products has recently captured the attention of plant biochemists and biotechnologists alike. Realization of this goal will be facilitated by genome-scale research focused on the identification of genes that control the development and biochemical functions of SG Ts (Duke et al., 2000). Trichomes being epidermal appendages, their contents can be sampled relatively easily, facilitating analysis of small molecules, proteins and mRNAs (Fridman et al., 2005; Gang et al., 2001; Gershenzon et al., 1992). cDNA libraries from glandular trichomes have been prepared from several species and this has permitted the identification of biosynthetic enzymes for a variety of pathways (Lange et al. 2000; Gang et al. 2001; Hoelscher et al. 2003; Ranger et al., 2005; Fridman et al. 2005; Bertea et al. 2006; Falara et al.
Trichomes on the members of the family Solanaceae have received extensive attention. An integrated approach to lay the foundation for a longer-term ‘systems biology’ understanding of the entire network of genes and proteins involved in the development of each of the different types of SGT found in Solanum plants and the genes and enzymes responsible for their biosynthetic capacity is being developed by the Solanum Trichome Project (http://www.trichome.msu.edu/ about/overview_continued.html).

The current research is focused on secretory glandular trichomes (SGTs) to understand and exploit their ability to secrete phytochemicals that might improve resistance to insects, microbes and herbivores, modify gland metabolism towards improving properties of exudates (e.g. Flavor and aroma in herbs) and allow commercial production of useful compounds (molecular farming). Plant sources are increasingly being exploited for new drug development, and there is increased interest in validating traditional medicines and herbal remedies. Many of the phytochemical principals involved are surface secretions (Verpoorte, 2000; Duke et al., 2000; Mahmoud and Croteau, 2002; Wagner et al., 2004).

*B. diffusa* is richly covered with trichomes. Since this plant has so far not been studied for the type of trichomes and their metabolite contents it warrants a thorough study of this aspect.

Medicinal plants are the most important source of life saving drugs for the majority of the world’s population. The biotechnological tools are important to select, multiply and conserve the critical genotypes of medicinal plants. *In vitro* regeneration and genetic transformations holds tremendous potential for the production of high-quality plant-based medicine. With the micropropagation, the multiplication rate is greatly increased. It also permits the production of pathogen-free material. Micropropagation of various plant species, including many medicinal plants, has been reported. Propagation from existing meristems yields plants that are genetically identical with the donor plants. Plant regeneration from shoot and stem meristems has
yielded encouraging results in medicinal plants like *Catharanthus roseus*, *Cinchona ledgeriana* and *Digitalis* spp, *Rehmannia glutinosa*, *Rauvolfia serpentina*, *Isoplexis canariensis* (Perez-Bermudez et al., 2004).

Since many years man has been dependent on plants. Habitat destruction, overexploitation and climate change have markedly increased the rate of loss of medicinal plants from their local niches. It is anticipated that global warming and climate change may alter the production of secondary metabolites, which constitute active medicinal principles in these plants. Regrettably only a few medicinal plants are being cultivated on a commercial scale with a majority still being collected from the wild. There is, therefore, an urgent requirement for large-scale cultivation of authentic plant material whose yield and potency of active principles is known. Large-scale cultivation of medicinal plants would require a thorough understanding of their cytological, embryological, phytochemical and agronomical aspects. Further, conservation and commercial cultivation of medicinal plants would require quality planting material on a large scale, so *in vitro* responses of the plant require to be worked out. Secondary metabolite production in cell suspension cultures and bioreactors, where feasible, require to be developed as a conservation strategy.

*In vitro* somatic embryogenesis in tissue culture is common in woody plants, including angiosperms and gymnosperms, and reported from almost all the plant families (Jain et al., 1994). The major advantage of somatic embryogenesis is that it provides a powerful tool for economical clonal propagation for largescale production of plants of commercial interest (Jain et al. 1994). Somatic embryos usually originate from “induced embryogenic cells” within callus tissue (Sharp et al. 1980). Somatic embryos can also develop directly from “pre-embryonic determined cells” that are programmed for embryonic differentiation. Thus, somatic embryogenesis could be induced in two distinctly different media, one with auxin for initiation of embryonic cells in callus tissue and the second without auxin for subsequent cytodifferentiation of embryoids. Over the last two decades, an increasing number of studies have dealt with seaweed tissue culture, following the techniques used in higher plants. Callus induction and thallus regeneration have been reported from a wide variety of

Many of these natural products have been used as a source of large number of industrial products, including agricultural chemicals, pharmaceuticals, dyes, fragrances, and flavoring agents. Alkaloids, for example are important pharmaceutical compounds of high economic value. There is a revival of interest in the use of natural products in pharmacy, both from the pharmaceutical industry and as a source of new lead molecules (Bhojwani and Razdan, 1996). Wide ranges of bioassay system are used to test, extract and guide the isolation of new molecules. Novel compounds often serve as lead molecules in development of new drugs (Bapat and Ganpati, 2005). In recent times a large number of novel compounds useful for pharmaceutical industry have been isolated. Some examples are, (i) Antifungal indole alkaloid from *Schizzygia coffaeoides*, (ii) Flavone-C-glycosides from Chinese *Viola yedoensis*, and (iii) Novel cathedulins from khat, *Catha edulis*.

In recent times plant cell cultures have been widely used for the production as well as for understanding the basic mechanism involved in the synthesis of secondary metabolites. The advantages of using the cell culture techniques for secondary metabolites production.

The herb and roots are rich in proteins and fats. The herb contains 15 amino acids, including 6 essential amino acids, while the root contains 14 amino acids, including 7 essential amino acids. Seth et al. (1986) isolated a new antifibrinolytic compound ‘punarnavoside’ from the roots of *B. diffusa*. Phytochemical screening of the roots from garden-grown *in vivo* plants of *B. diffusa* of different ages revealed that the maximum alkaloid content (2 %) accumulated in the roots of 3-year old mature plants. The *Boerhaavia diffusa* plant contains a large number of such compounds as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins, and glycoproteins. Punarnavine C_{17}H_{22}N_{2}O mp 236–237 °C (Surange and Pendse, 1972), boeravinone A.F (Lami et al., 1990; 1992), hypoxanthine 9-L-
arabinofuranoside (Ahmad and Hossain, 1968), ursolic acid (Mishra and Tiwari, 1971), punarnavoside (Jain and Khanna, 1989), liirodendrin (Aftab et al., 1996), and a glycoprotein having a molecular weight of 16–20 kDa (Verma et al., 1979) have been isolated and studied in detail for their biological activity. Chopra et al. (1923) reported that the plant contained large quantities of potassium nitrate, besides punarnavine. The herb and roots are rich in proteins and fats.

Punarnava contains  β-Sitosterol, α-2-sitosterol, palmitic acid, ester of β-sitosterol, tetracosanoic, hexacosanoic, stearic, arachidic acid, urosilic acid, Hentriacontane, β-Ecdysone, triacontanol etc. The roots contain alkaloids (0.05 %), triacontanol hentriacontane, β-sitosterol, ursolic acid, 5,7-dihydroxy-3,4-dimethoxy-1,8-dimethyl flavone, and an unidentified ketone (m p 86°). The roots also contain the rotenoid boeravinones AI, BI, C2, D, E and F besides the new dihydroisofurenoxanthin, borhavine and an antifibrinolytic agent, punarnavoside. Two lignans, liriodendrin and syringaresinol mono-β-D-glucoside, have also been reported in the roots (Agarwal and Dutt, 1936; Basu et al., 1947; Surange and Pendse, 1972; Kadota et al., 1989; Lami et al., 1990; 1992; Ahmad and Hossain, 1968; Mishra and Tiwari, 1971; Jain and Khanna, 1989; Aftab et al., 1996)

Boerhaavia diffusa is such an important medicinal plant that almost 2000 Metric tonnes of the whole plant or its roots are collected annually (Anonymous, 2000). So much is the collection pressure on this plant that it has almost become extinct from the campus of Banaras Hindu University, Varanasi and the authorities had to put a total ban on its collection (Singh, 2007).
Objectives of the Present Work: Keeping in view of the tremendous importance *Boerhaavia diffusa* has been chosen for the present Doctoral work. The objective was to develop a holistic understanding of the plant. With these views the following objectives were chosen:

1. Investigate various aspect of reproductive biology with special reference to pollen-pistil interaction, fruit set.
2. Trichomes, their morphology, histochemistry and at molecular biology.
3. *In vitro* regeneration studies using embryo and other explants.
4. Investigate secondary metabolites produced in cell cultures.