Man ever desirous of knowledge has already explored many things and more and greater still remained concealed perhaps reserved for the distant generations who shall prosecute the examination of their Creator’s work and make many discoveries for the pleasure and commerce of life. Plants provide a variety of resources that contribute to the fundamental needs of food, clothing and shelter. Among plants of economic importance Medicinal and Aromatic Plants (MAPs) have played a vital role in alleviating human sufferings (Baquar, 2001). Plants are utilized as therapeutic agents since time immemorial in both organized (Ayurveda, Unani) and unorganized (folk, tribal, native) form (Girach et al. 2003). The healing properties of many herbal medicines have been recognized in many ancient cultures. Early herbalists believed that the plant part resembling any part of the human body was considered useful for the ailment of those parts and there is no part of the body without its corresponding herb, a hypothesis known as the “Doctrine of Signature” (Baquar, 2001). However the relentless exploitation during the transition from traditions to modern and subsistence to market orientation disturbed the ecological balance and adversely affected the economic life of people along with the resource base.

Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization (WHO) estimates that up to 80% of the people still rely mainly on traditional remedies. It is estimated that approximately one quarter of the prescribed drugs contains plant extracts. Thus, medicinal plants (MPs) are under
tremendous pressure all across the globe, especially in India. More than 90% of the
MPs for herbal industries in India and for export is drawn from the natural habitats. It is
estimated that, in 1997 the world market for over-the-counter phyto-medicinal product
was US$ 10 billion, with an annual growth of 6.5% (Soldati, 1997). The WHO
considers phytotherapy in its health programs and suggests basic procedures for the
validation of drugs from plant origin in developing countries (Vulto and Smet, 1988;
OMS, 1991). Eastern countries such as China and India have well established herbal
medicinal industry and Latin American countries have been investing in research
programs in medicinal plants and the standardization and the regulation of
phytomedicinal products, following the examples of European countries, such as,
France and Germany. In Germany 50% of the phytomedicinal products are sold on
medicinal prescription, the cost being refunded by health insurance (Gruenwald, 1997).
In North America where phytomedicinal products are sold as health foods (Calixto
2000), consumers and professionals have struggled to change this by gathering
information about the efficacy and safety of these products, a new guidelines for their
registration are now part of FDA policy (Israelsen, 1997). The potential use of higher
plants as a source of new drugs is still poorly explored. Of the estimated 250,000 to
500,000 plant species, only a small percentage have been investigated phytochemically
(Borris, 1996), and even a small percentage has been properly studied in terms of their
pharmacological properties; in most cases, only pharmacological screening or
preliminary studies have been carried out. It is estimated that 5000 species has been
studied for medicinal use (Payne et al. 1991). Between the years 1957 and 1981 the
NCL (National Cancer institute) screened around 20,000 plant species from Latin
American and Asia for anti-tumor activity, but even these were not screened for other
pharmacological activities (Hamburger and Hostettman, 1991).

The use of herbs and medicinal plant as the first medicines is a universal phenomenon
and the medicinal plants have been used since ancient times and they play a vital role
for the development of new and potent drugs. Every culture on the earth, through
written or oral tradition, has relied on the vast variety of natural chemistries found in
plants for their therapeutic properties. All drugs from the plant are substances with a
particular therapeutic action extracted from plants [Serrentino, 1991]. Evidence of
using these natural resources (herbal remedies) in Iran goes back to the history itself and there are lots of scientific documents in this area. Ibn Sina has wrote many books on a wide range of topics but he is perhaps most famous for his Laws of Medicines which contains sections on the formulation of medicine, general medicine and other subjects that discuss the herbal medicines in details [Lothfipour et al. 2008]. During 1950-1970 approximately 100 plants based new drugs were introduced in the USA drug market including deserpaine, reseinnamine, reserpine, vinblastine and vincristine which are derived from higher plants. From 1971 to 1990 new drugs such as ectoposide, E-guggulsterone, teniposide, nabilone, plaunotol, Z-guggulsterone, lectinan, artemisinin and ginkgolides appeared all over the world. 2% of drugs were introduced from 1991 to 1995 including paciltaxel, toptecan, gomishin, irinotecan etc. Plant based drugs provide outstanding contribution to modern therapeutics; for example, serpentine isolated from the root of Indian plant Rauwolfia serpentina in 1953 was a revolutionary event in the treatment of hypertension and lowering of blood pressure. Vinblastine isolated from the Catharanthus rosuesus (Farnsworth and Blowster, 1967) is used for the treatment of Hodgkins, choriocarcinoma, non-hodgkins lymphomas, leukemia in children, testicular and neck cancer. Vincristine is recommended for acute lymphocytic leukemia in childhood advanced stages of hodgkins, lymmphosarcoma, small cell lung, cervical and breast cancer (Farnsworth and Bingel, 1977). Podophyllotoxin is a constituent of Podophyllum emodi currently used against testicular, small cell lung cancer and lymphomas. Indian indigenous tree of Nothapodytes nimmoniana (Mappia foetida) are mostly used in Japan for the treatment of cervical cancer. Plant derived drugs are used to cure mental illness, skin diseases, tuberculosis, diabetes, jaundice, hypertension and cancer. Plant derived drugs came into use in the modern medicine through the uses of plant material as indigenous cure in folklore or traditional systems of medicine. More than 100 plants have been found to possess significant antibacterial and antidiabetic properties (Arcamone et al. 1980). Chatterjee et al. 2004 reported that an active compound from the Strychnus nux vomica seed extract inhibited viper venom induced lipid peroxidation in experimental animals. The mechanism of action of the plant derived micromolecules induced venom neutralization need further attention for the development of plant-derived therapeutic antagonist against snakebite for the
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community. However, the toxicity of plants has known for a long period of time, and the history of these toxic plants side by side with medicinal ones are very old and popular worldwide and they considered the major natural source of folk medication and toxication even after arising of recent chemical synthesis of the active constituents contained by these plants (Heinrich, 2000; Pfister et al. 2002).

India in general and Kashmir in particular are endowed with a rich wealth of medicinal plants. These have made a good contribution to the development of ancient Indian materia medica. One of the earliest treatises on Indian medicine, the Charak Samhita (1000 B.C), records the use of over 340 drugs of vegetable origin. Most of these continue to be gathered from wild to meet the demand of the medical profession. Thus, despite the rich heritage of knowledge on the use of drugs, little attention had been paid to grow them as in the country till the latter part of the nineteenth century. During the past seven or eight decades, there has been a rapid extension of the allopathic system of medical treatment in India. It generated a commercial demand for pharmacopoeial drugs and products in the country. Thus efforts were made to introduce many of these drug into Indian agriculture, and studies on the cultivation practices were undertaken for those which were found suitable and remunerative for commercial cultivation. In general, agronomic practices for growing poppy, isabgol, senna, cinchona, ipecac, belladonna, ergot and a few others have been developed and there is now localized cultivation of these medicinal commercially. The average annual foreign trade in crude drugs and their Phytochemicals is between 60 and 80 million rupees and this account for a little over 0.5 per cent of the world trade in these commodities. During the last two decades, the pharmaceutical industry has made massive investments on pharmacological, clinical and chemical researches all over the world in an effort to discover and still more potent drugs; in fact, a few new drug have successfully passed the tests of commercial screening. However, benefits of this labour would reach the masses when the corresponding support for agricultural studies for commercial cultivation is provided. In fact, agricultural studies on medicinal plants, by its very nature, demand an equally large investment and higher priority. India, in particular, has a big scope for the development of the pharmaceutical and phytochemical industry. However, it should be
stated in all fairness that our knowledge of the genetic and physiological make-up of most of the medicinal plants is poor and we know still less about the biosynthetic pathways leading to the formation of active constituents for which these plants are valued and multidisciplinary approach with good cooperation between botanists, phytochemists and pharmacologists is required. More appropriate assay requirements are to be used in plant screening. Isolation of bioactive molecules still remains empirical and demands experience and patience. The need of the hour is development of better and cost effective, superlative science and straight forward spectroscopic techniques.

The high cost of synthetic modern drugs and their bio incompatibility with human body (harmful side effects) have lead to increasing global interests and consumer demand for herbal products. Thus, demands for seeds, flowers, roots, rhizomes, leaves as well as whole plants are very high, causing unscientific and unplanned exploitation of these high value medicinal plants from their natural or wild habitats. Several valuable medicinal plants which were once in abundance in Himalaya are now becoming endangered. Depletion of naturally occurring genetic resources and germplasm pool, further limits the ability to develop new pharmaceutical and other useful products (Plucknett et al. 1983). Thus, not to speak of their availability for mankind to use as medicines even germplasm of such natural resources is being under the threat of extinction. Although methods like protection of habitats, field gene and seed banks are recommended for their conservation, but apprehensions is there that these may prove insufficient to prevent them from extinction because of ineffective legislation and over exploitation. This has raised global concern for the conservation of germplasm. Most of the plant raised through seeds are highly heterozygous and show great variations in growth, habit and yield and may have to be discarded because of poor quality of products for their commercial release. Likewise, majority of the plants are not amenable to vegetative propagation through cutting and grafting, thus limiting multiplication of desired cultivars. Moreover many plants propagated by vegetative means contain systemic bacteria, fungi and viruses which may affect the quality and appearance of selected items. Of the different conservation and large-scale multiplication techniques presently being employed, tissue culture has proved to be a
very useful tool. This technology has been successfully used for commercial production of pathogen free plants (Debergh and Maene, 1981) and to conserve the germplasm of rare and endangered species (Fay, 1992) and to obtain genetically pure elite populations under in vitro conditions rather than have indifferent populations. (Dodds and Roberts, 1985) Tissue culture has now become a well established technique for culturing and studying the physiological behavior of isolated plant organs, tissues, cells, protoplasts and even cell organelles under precisely controlled physical and chemical conditions. Most of the medicinal plants either do not produce seeds or seeds are too small and do not germinate in soils. Thus mass multiplication of disease free planting material is a general problem. In this regard, the micropropagation holds significant promise for true to type, rapid and mass multiplication and conservation of valuable genotype under disease free conditions. (Sen and Sharma, 1991; Sudhir et al. 2010). Cultivation of plant tissue in synthetic media offers an alternative as a way of producing metabolites of interest to the traditional cultivation in fields or greenhouses (Dornenburgh and Knorr, 1995; Stockigt et al. 1995; Bourgaud et al. 2001; Ramachandra and Ravishankar, 2002). Traditionally, most of this work has been concerned with undifferentiated cells, but also differentiated cells, such as in hairy root tissue, have been cultivated. In successful cases, cell suspension cultures can offer a repeatable method to produce secondary metabolites from elite mother plants with easily controlled conditions and with a continuous supply of material. An increasing number of compounds are being isolated from cell cultures, including at the moment compounds from at least ten groups of phenylpropanoids, ten groups of alkaloids, five groups of terpenoids, and three groups of quinones (Stockigt et al. 1995; Ramachandra and Ravishankar, 2002). The production of secondary metabolites can be enhanced using bioreactors. Bioreactors offer a great hope for the large-scale synthesis of therapeutically active compounds in medicinal plants. Since the biosynthetic efficiency of populations varies, a high yielding variety is recommended as a starting material. Genetic transformation may provide increased and efficient system for in vitro production of secondary metabolites. The improved in vitro plant cell culture systems have potential for commercial exploitation of secondary metabolites. Tissue culture
protocols have been developed for several plants but there are many other species, which are over exploited in pharmaceutical industries and need conservation.

However tissue culture techniques, combined with a mutagenesis treatment speed up the breeding program. Genetic variability is fundamental to successful breeding programs in vegetatively and sexually propagated plants. This variation can occur naturally or can be induced through mutations, using physical, biological or chemical mutagens and has attracted the interest of plant breeders for many decades. Mutation breeding in crop plants is an effective tool in plant breeders especially in crops as they have narrow genetic base. Mutagenesis has been popular over past decades because it is simple, cheap to perform, applicable to all plant species and usable at small or large scale. By varying mutagen dose, the frequency of induced mutations can be regulated and saturation can be readily achieved (Robbie et al. 2006). Mutations have been used to produce many cultivars with improved economic value (Broertjes and Van Harten, 1988; IARA & FAO, 1995) and study of genetics and plant developmental phenomena (VanDen-Bulk et al. 1990; Bertagne-Sagnard et al. 1996). The in vitro conditions help exposure of many varieties to mutagens easily as they can be exposed to mutagens in a relatively small space for reliable screening against mutations in M₁ generation. Appropriate selection pressure can be applied to the culture to select mutants, which can save time, money and space compared to growing thousands of plants in the greenhouse or field. Mutagens have been applied to suspension, callus and embryo cultures in many species including barley, soybean, carrot, maize, Kalanchoe, banana and morning glory (Blixt, 1965 a,b, 1967 a,b; Broertjes and Lefferring, 1972; Kleinhofs et al. 1978a,b, Bhagwat and Duncan, 1998; Bhate, 2001). However, most of these studies have been performed in 1960s and 1970s. Successful use of mutagens requires optimum conditions to retain maximum germination capacity of seeds or adventitious shoot regeneration capacity of explants. Besides, the timing and dose of mutagen application are very critical and must be determined empirically. In vitro culture not only provides relatively uniform and large populations of cell and tissues in a disease free situation for irradiation, but also, because of miniature size of micro-propagules, it is possible to irradiate very large numbers, and also to further separate the desired mutated sectors from the other ones in short time. Beside it, mutagenesis in cell cultures
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has allowed the isolation of mutants, deficient in metabolic compounds or resistant to toxic chemicals. This can be achieved by successive culture of buds or regeneration of shoots and somatic embryos from cell suspension and callus culture derived from irradiated tissues and explants. Induction of mutation in in vitro cultured material and subsequently in vivo multiplication for two or more cycles is helpful in separating mutated sectors from chimeric tissues. Irradiation in combination with in vitro culture has proven to be a valuable method of producing desired variation and rapid propagation. Mutations are the tools used by the geneticist to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops (Adamu et al. 2004). The main advantage of mutation breeding is the possibility of improving one or two characters without changing the rest of the genotype. Induced mutations have great potentials and served as a complimentary approach in genetic improvement of crops (Mahandjiev et al. 2001). Induced mutations have been used to improve major crops such as wheat, rice, barley, cotton, peanut and cowpea, which are seed propagated. A number of workers (Coe and Neuf, 1977; Mashenkov, 1986; Ricardo and Ando, 1998) have reported the role of chemical mutagens in enhancing genetic variability in higher plants. Genetic variability is the fundamental to successful breeding programs in vegetatively and sexually propagated plants. The mutants so produced facilitate the isolation, identification and cloning of genes used in designing crops with improved yields, increase stress tolerance, longer shelf life and reduced agronomic characters. The general procedures for using induced mutations are rather simple and have a strong basis in the laws of genetics (FAO/IAEA, 1977). About 30 years ago the plant breeding and genetic section of the joint FAO/IAEA Division began to collect information related to newly released crop varieties developed directly after mutagenic treatments or by crosses involving mutant lines (Sigurbjornsson and Micke, 1974). The first mutant variety was released by Stadler (1930) after the fourth year’s publication of mutagenesis in plants. Today the FAO/IAEA Mutant variety Database has 1737 accessions (Maluszynski et al. 1991). Mutations can be induced by chemical, physical, and biological means. The latter technique, where genes are disrupted by insertion of DNA, originating from primary transformation events. The most commonly used
physical mutagens are ionizing radiation, such as X-rays, gamma-rays, UV rays and laser beam (Jain, 2002b). Each type of radiation produces deletions at a high frequency. Most commonly used mutagens (alkylating agents) are Ethyl methane sulphonate (EMS), hydrazine hydrate, N-methyl-N-nitrosourea (NMU), sodium azide (NaN₃), Ethynitroso-urea (ENH), Methylnitroso-urea (MNH) and colchicines.

Keeping in view all these aspects, present study will be taken for a valuable plant of our valley namely, Cichorium intybus L. It belongs to asteraceae family, is an erect perennial herb 80-90cm in height with a fleshy taproot up to 75cm in length. The genus Cichorium consists of six species with major distribution areas in Europe and Asia (Table 1). It is also known as common chicory, bluesailor's succory and witloof. It is cultivated in countries such as the UK, Belgium, France, Netherlands, Germany, South Africa, the USA and India. It is native to the temperate parts of the Old World and is found wild in India in the Punjab and Andhra Pradesh regions. It is also cultivated in Bihar, Gujarat, Himachal Pradesh and Tamil Nadu (Anonymous, 1992a). Chicory has been successfully cultivated in India since 1918 at Coimbatore and subsequently Nilgiris in Tamil Nadu and at Broach, Amalsad and Jamnagar in Gujarat (Muthuswami et al. 1980). Chicory forms owering shoots and seeds after overwintering. Initially, chicory seed was imported into India, but nowadays it is successfully produced locally (Anonymous, 1992a). Commercial seed production is undertaken in Jammu and Kashmir, temperate areas of Himachal Pradesh and some hilly regions of Uttar Pradesh (Anonymous, 1992a). The agroclimatic conditions of these dry temperate areas are conducive to quality seed production (Arya, 1980; Arya, 1982). During medieval period, chicory was regularly prescribed as diuretic, laxative and tonic, especially “as a strengthener of weak and feeble stomach”, other internal uses were for ague (fever), inflamed eyes, pain in lactating breasts “passions of heart,” loss of appetite, gout, dropsy, headache in children and as a liver tonics. Externally, it was used for swelling and various skin diseases (Le Strange, 1977), roots are used as tonic (Le Strange, 1977) laxative and diuretic (Le Strange, 1977; Foster and Duke, 1990; Grive, 1998) and to treat skin eruptions and fever (Foster and Duke, 1990). Root decoctions were once taken to mitigate jaundice, gout, rheumatic complaints dyspepsia and cancer (Le Strange, 1977). Studies have shown that chicory complements coffee, when it is used as
a supplement due to its lactucin and lactucopicrin and may serve to counteract stimulating effects of caffeine. It has also sedative action on Central Nervous System.

Raftilin, inulin, raftilose and oligofructose are fibres extracted from its roots that can’t be digested from small intestine. Some animal researches have been done on chicory inulin, which appears to enhance calcium absorption in rats (Roberfoid, 1993).

**Chicory as a vegetable**

Cultivars of chicory developed for use in salads have more and larger leaves than other cultivars. Salad leaves are often blanched in the field to reduce possible bitterness. Young and tender roots can be boiled and eaten as a vegetable. Chicory extracts are used in alcoholic and non-alcoholic beverages (Simon et al. 1984). Related vegetables of commercial importance include lettuce (Lactuca sativa L, Cichorium tribe), the leafy salad vegetables referred to as endive and escarole (C. endivia L), radicchio (C. intybus L) and chicory grown for use as a coffee substitute (C.intybus L). In the USA the term endive usually refers to endive or escarole. While the common name Belgian endive helps to distinguish it from the crops in C. endivia, it is not as descriptive as the common name witloof chicory, which translated means `white leaf' chicory. Some operations have combined the two common names and use the name witloof endive for the purpose of making this market distinction. Hereafter the name witloof chicory will be used (Corey et al. 1990).

**Table. 1. Geographical distribution of wild species of the genus Cichorium.**

<table>
<thead>
<tr>
<th>Species name</th>
<th>Geographical Distribution</th>
<th>Highest altitude above sea level (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. intybus L.</td>
<td>Northern and Central Europe, Siberia, Turkey, Afghanistan, North and Central China, South America, South Africa, Ethiopia, Madagascar, India, Australia New Zealand</td>
<td>3000</td>
</tr>
<tr>
<td>C. endivia L.</td>
<td>Southern Europe, Turkey, Egypt, Tunisia</td>
<td>1500</td>
</tr>
</tbody>
</table>
It is in this context that the present study aims at standardizing a protocol for in vitro culture of \textit{C.intybus}. Besides, the study also endeavors to see the effect of mutagen on biochemical parameters and secondary metabolite production under in vitro conditions.

The main objectives of the present research work are given under following subheadings:

- Establishment of media and hormonal requirement for Callus culture.
- Direct/indirect regeneration.
- Effect of mutagen in culture.
- Root induction.
- Transplantation.
- Biochemical (protein, proline, chlorophyll, carotenoid content).
- Study of active compounds by HPLC.

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{C. spinosum} L.</td>
<td>Eastern Mediterranean, Italy</td>
<td>300</td>
</tr>
<tr>
<td>\textit{C.glandulosum}</td>
<td>Boiss Syria, Turkey, Armenia, Iran, Iraq</td>
<td>1000</td>
</tr>
<tr>
<td>\textit{C. bottae D.}</td>
<td>Yemen</td>
<td>5000</td>
</tr>
<tr>
<td>\textit{C.calvum} Schultz-Bip</td>
<td>Ethiopia, Afghanistan, Pakistan</td>
<td>1200</td>
</tr>
</tbody>
</table>