1.1. Medicinal Plants - an overview

In the beginning of time, people have turned to plants for healing ailments. This form of medicine, the oldest and still the most important in many parts of the world, is considered as an alternative to the synthetic drugs of Western medicine. The plants not only provide potent drugs but also provide the active principles which form the basis of many drugs of today, from aspirin to morphine (Bremness, 1988).

The Ayurvedic System of Hindus and the Siddha System of Dravidian have a long history. In Ayurvedic and Siddha Systems numerous drugs of plant origin have been mentioned. These systems emphasise the use of plant plant medicine for ailments. If drugs from plants failed to cure, then we can use other drugs like parpam, senthooram etc. in which animal parts or metals are added to the plant drugs. There are extensive references to Siddha in ancient Tamil literature also.

The Plant Kingdom is a treasure house of potential drugs and in recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from plants are easily available, less expensive, safe, efficient and rarely have side effects. The compounds from plants are a source of substitutes for chemical drugs. The chemical constituents of plant medicines are a part of the physiological activities of living plants and hence they are believed to have a better compatibility with the human body. These drugs are prepared from renewable resources of raw materials by ecofriendly processes.
The use of medicinal plants has increased in recent years in spite of the advances made in the field of chemotherapy. The reasons described by Magherini (1988) are:

- the use of medicinal plants as materials for the extraction of active pharmacological agents or as precursors for chemico-pharmaceutical hemisynthesis.
- the increased use of medicinal plants by the population without industrial pharmaceutical products, through advertisement by WHO and local governments.
- the increased use of medicinal plants in industrialized countries for Galenic preparations and herbal medicines.

About 80 per cent of the world's population depends on herbal medicines, and the governments of Third World countries, unable to sustain a complete coverage with Western-type drugs, have encouraged the rational development of traditional treatments. Modern research establishments now exist in many countries like India, Pakistan, Saudi Arabia, China and Japan and their work is largely devoted to assess the value of thousands of ethnic remedies along lines acceptable to current medical thinking. Resulting from this type of research the pharmacological effectiveness of many herbal treatments has been vindicated (Evans, 1996). Another feature is that those plants which have been selected for medicinal use over thousands of years constitute the most obvious choice for examining the current search for new therapeutically effective drugs such as anticancer drugs (Dewick, 1996), antimalarial (Phillipson and Wright, 1996) and antihepatotoxic compounds (Evans, 1996).

The World Health Organisation (WHO) has reported that around 21,000 plants have been used for medicinal purpose in the world. About 500 higher species have been thoroughly investigated as potential source of new drugs. Nearly 119 pure chemicals were extracted from 90 plant species (Sara Old Field, 1992). The indigenous system of medicine like Ayurvedic, Siddha and Unani have been meeting the needs of 70 per
cent of population residing in villages. There is a growing tendency all over the world to shift from synthetic to natural based products including medicinal plants. Thus medicinal plants constitute a group of industrially important crop which bring appreciable income to the country by the way of export (Bhattacharjee, 1998).

1.2. *Cichorium intybus* L. (chicory)

*Cichorium intybus* L., a member of Asteraceae, is one of the important medicinal plants cultivated throughout India. It possesses several vernacular names as follows:

<table>
<thead>
<tr>
<th>Common name</th>
<th>chicory</th>
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<tbody>
<tr>
<td>English</td>
<td>chicory, succory, witloof chicory, radichetta, Asparagus chicory, Italian red chicory, Belgian endive etc.</td>
</tr>
<tr>
<td>Tamil</td>
<td>cikkari, kasini</td>
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<tr>
<td>Telugu</td>
<td>cicori, kasini vittulu</td>
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<tr>
<td>Malayalam</td>
<td>cikkari</td>
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<tr>
<td>Kannada</td>
<td>kacani</td>
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<td>Hindi</td>
<td>kasni</td>
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<tr>
<td>Sanskrit</td>
<td>kasani</td>
</tr>
<tr>
<td>Unani</td>
<td>kasni</td>
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Wild chicory is a biennial or perennial plant, while cultivated behaves as a strict biennial and during the first year it shows a vegetative growth with a tap root and a rosette of 30-70 leaves in the first year. At the beginning of the following spring it enters a new period of growth and produces new leaves on an elongated stem. Hartman (1956) and Harrington *et al.* (1959) showed that chicory plants require both vernalization and long days to flower. To induce flowering, chicory must undergo an exposure to low temperature (3 weeks at 4°C) that can be applied to germinating seeds or to the entire plant (Rappaport and Wittwer, 1956). Under natural conditions, chicory plants are vernalized during the winter and subsequently bolt and flower in the second growing season from May until August (Demeulemeester and De Proft, 1999). The rise in temperature and long photoperiod promote stem elongation (bolting), since chicory is an absolute long day plant (Varotto *et al.*, 2000).
The mature plant grows up to 90 cm height with erect stems and a deep perennial tap root. The lower leaves are large and spreading, oblanceolate, 10-20 cm long, pinnatifid or pinnately lobed. The terminal lobe is larger and all the segments are coarsely toothed. The upper leaves are much reduced, sessile, less divided and their bases clasping the stem.

Flower heads are numerous, placed in the axils of the stem-leaves in clusters of 3-4. When fully expanded, the blooms are rather large, about 3 cm broad and of a delicate tint of blue which is said to specially appealing the humble bee. In the early afternoon every bloom is closed, its petals drawing together. Linnaeus used the chicory as one of the flowers in his floral clock at Upsala, because of its regularity in opening at 5 a.m. and closing at 10 a.m. in that latitude. Here it opens between 6 and 7 a.m. in the morning and after 5 hours it closes in the noon. Chicory is a summer active plant that is dormant in winter but responds quickly to warm temperatures in the spring (Li et al., 1997).

1.2.1. Cultivation

Chicory is cultivated for the roots. The dried root is used as a coffee substitute or adulterant (Jung et al., 1996). Initially chicory was listed in the Weed List in 1939 (Hill, 1983), but because of its value as forage for cattle, farmers grow it as forage plant. In recent years this plant is cultivated for its medicinal, culinary and other uses.

Chicory is a hardy perennial plant and grows in almost any type of soil. The seeds are sown in May or June in drills about 1 inch deep and about 12 inches apart in rows. The fresh root is bitter, with a milky juice. To obtain root of a large size, the ground must be rich, light and well manured. Under cultivation the root becomes large and fleshy with a thick rind and is used extensively after roasting and grinding for blending with coffee.
1.2.2. Medicinal and other uses of chicory

The whole plant (root, leaf, seed) is used medicinally, because of the presence of inulin, a major reserve carbohydrate, bitter sesquiterpene lactones, coumarins, flavonoids and vitamins. It is used as antihepatotoxic, antiulcerogenic, antiinflammatory, appetiser, digestive, stomachic, liver tonic, cholagogue, cardiotonic, depurative, diuretic, emmenagogue, febrifuge, alexetic and tonic. It is useful in vitiated conditions of kapha and pitta, cephalalgia, hepatomegaly, inflammations, anorexia, dyspepsia, flatulence, colic, gout, burning sensation, allergic conditions of the skin, insomnia, jaundice, splenomegaly, hyperdipsia, skin diseases, leprosy, strangury, amenorrhoea, dysmenorrhoea, chronic and bilious fevers, ophthalmia, pharyngitis, vomiting, diarrhoea, arthralgia, lumbago, asthma and general debility. (Nadkami, 1976; Rastogi and Mehrotra, 1994). This plant is also used to treat AIDS, cancer, diabetes, dismenorrhoea, impotence, insomnia, splenitis, tachycardia, etc. (Duke, 1983).

Studies on antifeedent (Rodriguez et al., 1976; Rees and Harborne, 1985; Takasugi et al., 1985; Tahara et al., 1988), antifungal (Monde et al., 1990), postcoital contraceptive (Keshri et al., 1998), antiulcerogenic (Ahmad et al., 1998), antiinflammatory (Ki et al., 1999), antihepatotoxic (Gadgoli and Mishra, 1997; Mitra et al., 1998) and anticancer (Hughes and Rowland, 2001) properties of C. intybus revealed that inulin and secondary metabolites produced in this plant play a key role in pharmacology. Inulin is used to replace fat or sugar and reduce the calories of food. Due to the non-digestibility it is suitable for consumption by diabetics (Niness, 1999) and is used in inulin clearance test to measure glomerular filtration rate - GFR (Vasudevan and Sreekumari, 1998).

The roots, leaves, flowers and chicons are used in culinary in the form of salads, pickle buds, sauce etc. The major quantity of root is rich in alkaloids which forms an ingredient or adulterant to coffee. The deep purple flower heads yield blue dye (Bremness, 1988).
1.3. Plant Tissue Culture

Micropropagation is a suitable method for obtaining a large quantity of genetically homogenous and healthy plant material which can be used for planting (Pierik, 1987). The tissue culture is an alternative method of propagation as there is an increase in the propagation rate of plants, availability of plants throughout the year, protection of plants against pests and pathogens under controlled conditions and the availability of uniform clones and uniform production of secondary metabolites (Bajaj et al., 1988).

In order to diffuse cultivation of medicinal plants, the first step is the production of high quantities of healthy genetically homogeneous plant material, which can be propagated at low cost. If the propagated material does not meet demand, even a suitable crop and an optimal environment will not bring economic success (Magherini, 1988).

Though the conventional breeding techniques have considerably increased the productivity of modern crops, the application of biotechnology could speed up further crop improvement. It overcomes the barriers in conventional vegetative propagation and fulfills the demand for large-scale cultivation in a short period by rapid mass multiplication. To date we can speed up the production rate of the average plant by approximately 10,000 times and a large number of productive plants can be multiplied routinely through tissue culture (Rao et al., 1996).

1.4. Pharmacognosy

Pharmacognosy is the study of those natural products, principally plants, that find use in medicine and one of the essential aspects to determine the botanical identity of a drug and to establish its purity and genuineness. It is closely related to both botany and plant chemistry and indeed, both originated from the earlier scientific studies on medicinal plants. In the beginning, the subject had developed mainly on the botanical side, being particularly concerned with the description and identification of drugs, both
in the whole state and in powder, and with their history, commerce, collection, preparation and storage. Such branches of pharmacognosy are still of fundamental importance, but rapid development in phytochemistry and pharmacology have enormously expanded the subject. As a result it is now possible to approach the study of medicinal plants from the Botanical, Phytochemical and Pharmacological viewpoints.

The use of modern isolation techniques and pharmacological testing procedures means that new plant drugs usually find their way into medicine as purified substances rather than in the form of galenical preparations. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value which have yet to be discovered. Large number of plants are constantly being screened for their possible pharmacological value particularly for their anti-inflammatory, hypotensive, hypoglycaemic, amoebicidal, antifertility, cytotoxic, antibiotic and anti-parkinsonism properties. The wealth of uninvestigated material available is illustrated by the fact that in 1985, it was reported that natural product research elicited some 3500 new chemical structures of which more than 2600 were from higher plants (Evans, 1996).

Chicory contains a variety of bioactive compounds which play a key role in medicinal and dietary properties. The most abundant ones are inulin, sesquiterpene lactones and coumarins. Several sesquiterpene lactones have been isolated from chicory roots and leaves - lactucin, lactucopicrin, 8-desoxylactucin, 11β,13-dihydrolactucin, 11β,13-dihydrolactucopicrin, 11β,13-dihyro-8-desoxylactucin (Schenck and Graf, 1939, Pyrek, 1985; Rees and Harborne, 1985; Seto et al., 1988; Van Beek et al., 1990). Monde et al. (1990) isolated an antifungal compound, cichoralexin, a guaianolide phytoalexin from C. intybus. Among coumarins, esculin, esculetin, cichoriin and umbeliferone have been reported in chicory (Evans, 1996; Bais et al., 1999, 2000, 2001a; Rehman, 2003).

Most of the microbes are getting resistance against synthetic drugs because of the repeated usage. By using crude extracts the efficacy of the antimicrobial activity
can be tested and justified against synthetic drugs. Since most of the killer diseases are of microbiological origin, research and development of antimicrobial therapeutics from plant origin could be invaluable (Gundidza et al., 1993). So an attempt was also made to test the efficacy of the alcoholic extract of root and leaf of both in vivo and in vitro plants of chicory against some pathogenic microorganisms.

1.5. Objectives of this study

This plant possesses multiple uses and significances and attracted the attention for commercial utilization. Recently this plant has got an important place in herbal medicine for the treatment of most common diseases like diabetes, inflammation, ulcer, cancer, etc. The conventional propagation is beset with problems of poor seed viability and slow germination. Being a long day plant, it enters the reproductive cycle in the second season with long photoperiod. Hence there is a need to apply the non-conventional propagation method for conservation and improvement of this species. This plant also contains many potential drugs to cure many diseases mentioned above. So, there is a need to evaluate the herbal drugs for their purity and to test the efficacy. Based on these facts, the present study has been undertaken with the following objectives:

1. To standardize a protocol for direct regeneration of plantlets.
2. To standardize a protocol for callus induction and regeneration of plantlets from callus.
3. To induce in vitro flowering and thus reducing the breeding cycle.
4. To induce auxin induced lateral roots from callus.
5. To determine the botanical identity of the phytodrug through pharmacognostic studies (exomorphic, microscopic and phytochemical studies) and to compare the in vivo and in vitro plant parts.
6. To test the efficacy of the alcoholic extract of root and leaf of in vivo and in vitro plants against some pathogenic microorganisms.