“Health is dearer than wealth” as quoted by Hamilton (1997); so, the value of medicinal plants is more than what it is in the marketplace, i.e. it can be said to be essentially infinite. Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. As many as 35,000 -70,000 species of plant have been used at one time or another for medicinal purposes (Farnsworth and Soejarto, 1991).

Medicinal plants are of great value in the field of treatment and cure of diseases. It has now been universally accepted that the herbal medicines are far safer than of synthetic medicines for curing of many of complex disease. The traditional system of medicine is so engraved in our culture that, even now 75% of the Indian population depend on this indigenous system for relief and they employ herbs as their primary medicines (Agarwal and Paridhavi, 2007; Yadav et al., 2012).

Plants that have medicinal properties with an optimum active ingredient in some form or another are regarded as medicinal plants. These are invaluable natural resources; they are exhaustible if overused and sustainable if the juxtaposition of present and future needs takes place within the behavioral pattern of various kinds of users. The natural habitats of these medicinal plant resources, mainly natural forests, wild areas and neighbourhoods, have been facing an onslaught since the eighteenth century and consequently a considerable portion of them has already been lost. This has resulted in the loss of wild biodiversity (Rahman, 1999). Factors affecting the loss of biological diversity include: population pressure, natural
Introduction

hazards (such as: cyclones, tidal surges, floods), the overexploitation of biological resources, deforestation, the destruction of habitat, flood control related activities causing the destruction of wetlands.

In the case of most wild medicinal plants, excessive and unregulated exploitation is a common phenomenon, which often jeopardizes their future availability. Most of the local suppliers of the traditional medicine manufacturing companies collect medicinal plants from wild sources and quite often they do it in unplanned and indiscriminate ways which are sometimes so extensive and exhaustive that they solely exploit particular plant species and leave no scope for their regeneration (Ghani, 2003). Thus many plants once found endemic in nature are gradually diminishing.

While this process of gradual loss of medicinal plants is continuing unabated, the demand for medicinal plants and plant-derived drugs is increasing rapidly with the current resurgence of traditional medicines all over the world (Ghani, 2003). There is unprecedented demand for natural medicines, green health products, pharmaceuticals, food supplements, cosmetics, and herbal pesticides, which is bringing about this alarming loss of plant biodiversity. It is estimated that 70-80% of people worldwide rely chiefly on traditional, largely herbal medicine to meet their primary healthcare needs (Shengji, 2001). The global market for herbal medicine is not only large but expanding by 15-20% annually.

Working with traditional medicinal practitioners to identify threatened medicinal plants and to understand important plant
properties, their usage, and potential economic value can significantly benefit the understanding of conservation priorities and resource use. As the very survival and success of the medicinal plant industry lies in the easy and sustainable availability of sufficient quantities of medicinal plants, it is also crucial, for the long term viability of the medicinal plant sub-sector, to find out ways to bridge the gap between the awareness of local communities and user-bodies regarding environmental threats and the potential endangerment of medicinal plants.

1.1 NEED FOR CONSERVATION OF MEDICINAL PLANT

Trends in the use of traditional and complementary medicine are on the increase in many developed and developing countries. The overexploitation of medicinal plants from in-situ sources is certainly posing a threat to natural resources. A combined effort by those concerned with the conservation of medicinal plant species or the healthcare systems dependent on them will be crucial to ensuring the sustainability of the resources and healthcare. Moreover, the loss of traditional methods in resource management and the lack of an appropriate institutional arrangement have had an adverse effect on the people’s (primary producers’) control over resources on which they depend for their sustenance (Jodha, 1991). Sustained and coordinated efforts are needed to transform unsustainable practices of medicinal plant collection from wild sources to more ecologically sustainable, socially acceptable and economically equitable production and utilization systems (Parotta, 2002). The urgency and need to protect this fast disappearing medicinal plant-based traditional knowledge cannot be ignored.
In situ conservation of these resources alone cannot meet the ever increasing demand of the pharmaceutical industry. Advanced biotechnological methods of culturing plant cells and tissues should provide new means of conserving and rapidly propagating valuable, rare and endangered medicinal plants (Nalawade et al., 2003). Plant tissue culture is an alternative method of propagation and is being used widely for the commercial propagation of a large number of plant species (Rout et al., 2000).

Realizing the importance of tissue culture, the present work was taken up with the objective of establishing in vitro procedures for micropropagation of *Cocculus hirsutus* and RAPD analysis of in vitro raised plants to assess their genetic fidelity.

1.2 THE PLANT: *COCCULUS HIRSUTUS* (L.) DIELS

*Cocculus hirsutus* (L.) Diels is an important medicinal plant belonging to the family Menispermaceae. It is commonly known as Jal-jamni (Chopra et al., 1958). Traditionally, the plant was patronized for its unique property of healing all types of cuts, wounds and boils. In recent times, the demand of *Cocculus hirsutus* has increased owing to its medicinal properties. This led to indiscriminate harvesting that threatened its status in the biodiversity and it has been categorized as threatened in Rajasthan (Chaudhuri, 2007). Hence, it has become imperative to establish a suitable protocol for its micropropagation. To our knowledge, there is no report regarding tissue culture studies for conservation of this species. Therefore, this valuable medicinal plant *Cocculus hirsutus* has been selected for the present study. The description of the plant is as follows:-
1.2A Systematic position:-

Kingdom : Plantae
Division : Angiospermae
Class : Dicotyledoneae
Sub-class : Polypetalae
Series : Thalamiflorae
Order : Ranales
Family : Menispermaceae
Genus : Cocculus
Species : hirsutus

In India, some other names are also given to this medicinal plant in different regions, which are shown in Table-1

Table-1

<table>
<thead>
<tr>
<th></th>
<th>Common name</th>
<th></th>
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<tbody>
<tr>
<td>1</td>
<td>Jal jamini</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Local names</td>
<td>Patalgarudi, Jamti-ki-bel</td>
</tr>
<tr>
<td>3</td>
<td>English name</td>
<td>Broom keeper, Ink Berry</td>
</tr>
<tr>
<td>4</td>
<td>Sanskrit name</td>
<td>Patalagaruda, Chilikhinta, Mahamula, Vatsadani</td>
</tr>
</tbody>
</table>

5 Vernacular names

(a) Hindi : Chireta, Farid-buti.
(b) Kannad : Dagadiballi, Dusariballi, Sugadiballi, Yadaniballi, Aadama balli.
(c) Marathi : Vasanvel, Parvel
(d) Malayalam : Patalgarudakkoli, Katukodi
(e) Tamil : Kattukkodi
(f) Telegu : Chipurutige, Dusaritige
(g) Gujerati : Vevati, Vevdi
1.2B Geographical Distribution:

*Cocculus hirsutus* (L.) Diels (Synonym-*Cocculus villosus*) is a climbing scandent shrub with hairy sepals. *C. hirsutus* is widely distributed in India from the foot of the Himalayas to South India, Pakistan, Sudan, Central Asia and China throughout tropical and subtropical regions (Kirtikar and Basu, 2001). The plant grows all over India, especially in dry regions such as, Karnataka, Tamilnadu, Andhra Pradesh, Orissa Gujarat, Madhya Pradesh and Rajasthan.

1.2C Ecology:

*Cocculus hirsutus* occurs in bushland and semi desert scrub vegetation up to 1140 m altitude. It grows on sandy and gravelly soil and can form a dense cover on top of other plants.

1.2D Cultivation:

It is cultivated from seed during monsoon season. It is a rainfed plant and it requires full sunlight during its growth.

1.2E Chemical Contents:

The plant of *Cocculus hirsutus* has been reported to contain essential oil, β- sitosterol, ginnol, glycosides, sterols and alkaloids (Das *et al.*, 1964). Preliminary phytochemical analysis of the leaves showed presence of alkaloids, phenolic compounds, flavonoids, glycosides, and carbohydrates. The phytochemical studies showed the presence of bis-benzyl isoquinoline alkaloids; viz. shaheenine (Rasheed *et al.*, 1991), cohirsinine, hirsutine, jamtinine, jamitine- *N*-oxide, cohirsinine, cohirsitine and haiderine (Viquaruddin *et al.*, 1991),
which are isolated from stem and roots. The alkaloids present in the leaves of *C. hirsutus* are D-trilobine, DL-coclaurine, isotrilobine, (+) syringaresinol and protoquericitol (Jagannadha Rao *et al.*, 1961). Roots are reported to contain D-trilobine and coclaurine, sterols and resins (Viquaruddin and Tahir, 1986).

### 1.2F Phytography

It is a wide spread species of the genus (*Cocculus*) growing as climber trees in evergreen forests upto 1140 m.

**Leaves** :- Leaves 4-8 cm long, (2.5-6 cm) broad, variable in shape, that of the leaves in the lower part of the main branches clearly 3-5-lobed, that of the other leaves narrowly to broadly ovate or ovate-oblong, sometimes, base subcordate, cordate, cuneate or truncate, apex obtuse or mucronate, densely tomentose when young, later subglabrous. [Plate-1, Fig. B].

**Petioles** :- Petioles are very short, 0.5-2.5 cm long, dark green, usually sub-auriculate at the base.

**Roots** :- Roots are hairy and dark brown in colour.

**Flowers** :- The flowers are very small, unisexual and green (Plate-2, Fig. A). Male flowers small, in axillary cymose panicles; pedicels slender; bracts minute; subulate, hairy. Sepals oblongovate, hairy outside, the three inner are larger. Petals thinly membranous, obovate, emarginate, embracing the stamens, smaller than the petals of the female flowers. Female flowers in axillary clusters, two-three
together, rarely racemose. Ovaries three, smooth; stigmas terete, thick reflexed. The plant bears flowers from November to April.

**Fruit:** The fruit is drupaceous, green when unripe, dark purple when ripe, 4-8 mm long, 4-5 mm broad, laterally compressed and has a pericarp demarcated into exocarp and endocarp. Endocarp is annular or ribbed with a prominent dorsal crest. The plant bears fruits in April - May. [Plate-2, Fig B; Plate-3, Fig. A].

**Seed:** The seeds are curved around the basal bodies (Plate-3, Fig. B). Testa and tegmen cells are thin-walled and unspecialized. In the ripe seed the inner epidermis of the tegmen persists, whereas the entire testa and the outer layer of the tegmen degenerate.

1.2G Uses

According to Unani system of medicine, *Cocculus hirsutus* is antipyretic, tonic, lessens thirst, good for fractures, and useful in tubercular glands related problems. It is a well known herb used as first aid remedy in minor injuries. It alleviates *kapha* and *vata* doshas. It is used as *deepanee, pachanee* and *raktdoshagni*. It possesses light, oily and slimy attributes. It has a special potency as a detoxifier. It is an aphrodisiac and tonic in properties (Chatterjee, 1996). The juice of the ripe fruits makes a kind of bluish purple ink. The aqueous extract of leaves of *C. hirsutus* decreased the serum glucose level and improved glucose tolerance (Badole *et al.*, 2006). Root smell is sweetish and pungent, lessens bile and burning sensation, enriches blood. It is used in diseases of the urinary system.
1.2H Medicinal Properties

*Cocculus hirsutus* is pungent in taste and has post digestive effect and hot potency. It alleviates ‘kapha’ and ‘vata’ doshas. It possesses light, oily and slimy attributes. It is an aphrodisiac and is used in diseases like arthritis, cystitis and diabetes mellitus. Root is bitter and used as alterative, laxative, diuretic, antiperiodic in fever, in malaria, joint pains, in the treatment of skin diseases, constipation and kidney problems (Chopra et al., 1996). The roots and leaves of *Cocculus hirsutus* have great medicinal value and are used both, internally as well as externally for medicinal purpose. The external application of its paste alleviates the toxins. The leaves are cooling, mucilaginous and are useful in eczema.

Internally, it is useful in the treatment of various diseases. Leaves and stem are used for treating eye disease especially conjunctivitis. According to Ayurveda, the decoction of its roots, dried ginger and pippali (*Piper longum*) is given along with milk in rheumatic disorders. To alleviate the abdominal pain, the combination of its roots and latakaranja (*Caesalpinia crista*) seed, matted in water is given orally. In gonorrhoea, the juice of leaves with cumin seed powder and sugar, works well. As a general tonic, the powder of its leaves is recommended along with milk and rock candy. *Cocculus hirsutus* has mild laxative digesting and appetite stimulant properties. It is used in anorexia, with great benefit. It also works well in asthma, cough and cold. In premature ejaculation, *Cocculus hirsutus* has extremely good results in delaying the ejaculation. Aerial parts of the plant reported to be used as a diuretic, laxative (Ganapathy et al.,
2002) and root extract showed analgesic and anti-inflammatory effect (Nayak and Singhai, 1993). Leaf juice of this plant is used in the treatment of eczema (Masilamani et al., 1981). Kalirajan et al., 2012 reported the antimicrobial and wound healing activity of the methanolic extracts of *Cocculus hirsutus*. The hypoglycemic and antimalarial activities of this medicinal plant are well known. A recent study has reported upon the elaboration of anti-tumor metabolites by *C. hirsutus* in tissue culture and high alkaloid producing cell lines have been established (Tevari et al., 1992). The bioactive phytochemicals present in the extracts of the plant that have been directly related to health benefits have been identified as alkaloids. Due to the presence of phenolic compounds in the plant, it is used as antimicrobial, preventive in infection and it enhances healing. Tannins and flavanoids are present in glycosidic combination. Tannins have stringent and healing properties. Hence it is used in the treatment of wounds, burns and ulcers (Chatterjee, 1996). Some commercial drugs obtained from *Cocculus hirsutus* are Swasthi, Aravindh gold pills, Stressnil capsule etc. shown in (Plate-4, Figs. A-E).

### 1.2I Medicinal Importance

**Part used** – Whole plant.

**Leaves property and uses:** Juice of leaves coagulates in water and forms mucilage which is used externally as cooling medicine in eye problems and soothing application in prurigo, eczema, impetigo and dyspepsia. When juice is sweetened with sugar, it is given in acute
gonorrhea. Aerial parts of the plant have been reported to be used as diuretic and laxative (Ganapathy et al., 2002).

**Stem property and uses:** It has sedative, hypotensive and cardiotonic properties. Due to the presence of phenolic compounds in the plant, it is used as antimicrobial, preventive in infection and enhances healing (Guhabakshi, 1984).

**Root property and uses:** The roots are useful in serpent bites; also, the roots have digestive, carminative, demulcent, depurative, emollient, tonic and antipyretic properties. Decoction of the root mixed with *Piper longum* is used in chronic rheumatism and syphilitic cachexia (Chadha, 1950; Nandkarni, 1986). They are also useful in leprosy, skin diseases, pruritus, dyspepsia, colic flatulence, bronchitis, cough, gout, intermittent fever, tubercular glands, hypertension and general debility. Roots rubbed with bonduc nuts in water are given for stomach problems especially in children (Caius, 1986).

**Fruit property and uses:** The juice of the ripe fruits makes a kind of bluish purple ink. The water soluble fraction of ammonical extract has sedative, hypotensive, bradycardiac, cardiotonic, spasmolytic and slight anti-convulsant actions (Marya and Bothara, 2011).

### 1.3 PLANT CELL TISSUE AND ORGAN CULTURE

Plant tissue culture has emerged as a potential tool and forms the backbone of plant biotechnology. Tissue culture techniques are widely applied for the improvement of field crops, forests, horticulture and plantation crops for increased agricultural and forestry production. This technique has been commercialized globally and has contributed
significantly towards the enhanced production of high quality planting material.

Clonal propagation of selected phenotypes is an essential step in most of the plant breeding programmes. It is a faster method of asexual reproduction in comparison to propagation through seeds. Plants raised through seeds are highly heterozygous and one has to select plants from a wide population which have the best qualities. Owing to heterozygosity, the seed raised plants show high variation in growth, habit and yield and they may have to be discarded because of the poor quality of their flowers and fruits for commercial release. Likewise, majority of the plants propagated by vegetative means contain systemic bacteria, fungi and viruses which affect the yield, quality and appearance of selected plants. Moreover majority of plants are not amenable to vegetative propagation through cuttings, budding and grafting, thus limiting multiplication of desired cultivars. In recent years, tissue culture has emerged as a promising technique to obtain genetically pure elite populations under *in vitro* conditions.

### 1.4 MICROPROPAGATION OF MEDICINAL PLANTS

Micropropagation is a relatively new technology and application of innovative methods has served to overcome the barriers to progress, in the multiplication of plant species. According to Chirangini *et al.*, (2005), for rapid multiplication of slow propagating species, tissue culture technique remains an indispensable tool.

Micropropagation is a good choice for propagation of medicinal plants which are difficult to propagate from seed or by conventional
vegetative multiplication. The rate of vegetative propagation is too slow to supply the plants that could be necessary for the drug companies. Micropropagation is a method by which identical plants can be obtained within a short span of time usually in a limited space. It is of great value in the process of economically valuable, rare and endangered plants. *In vitro* methods also help in conservation of germplasm of the medicinal plants that are over-exploited. It is also used for the long term preservation and conservation of plant genetic resources (Ali *et al*., 2012; Yadav *et al*., 2012).

Plants raised through micropropagation are of uniform quality, pathogen free, can be produced much more rapidly as new cultivars could become commercially available within two to three years from development rather than 5 to 10 years needed using conventional propagation, produce uniformly superior seeds and show improved vigor and quality. Recently, emphasis has been laid on genetic transformation, especially for (1) increased production of secondary metabolites, (2) production of alkaloids, pharmaceuticals, nematocidal compounds, and also some novel compounds not found in the whole plant, (3) regeneration of plant resistant to herbicides, diseases, and pests, (4) scale up of cultures in bioreactors, (5) plants with different morphological traits, and (6) transgenic plants for the production of vaccines etc. These developments have far-reaching implications in the improvement of medicinal plants as well (Bajaj, 1998).

The micropropagation of elite or selected plants have shown good results, which benefit agriculture, horticulture and forestry (Drew, 1997). Worldwide there is much interest to promote the
development of an *in vitro* technology that permits the propagation and breeding of commercial valuable woody, semi woody, ornamental, basic food, industrial and medicinal plants. Species which are in the danger of extinction should receive a priority in terms of germplasm conservation (Deberg and Zimmerman, 1990).

In our country, various research groups at different places are engaged in micropropagation of medicinally important plants (Sharma and Modgil, 2003). The work of tissue culture of medicinal plants has been reviewed by a number of workers in our country. Some medicinally important micropropagated plants are *Acacia catechu* (Jain *et al*., 2009), *Cadaba heterotricha* (Abbas and Qaiser, 2010), *Citrullus colocynthis* (Meena *et al*., 2010), *Commiphora wightii* (Kant *et al*., 2010), *Bacopa monnieri* L. (Rout *et al*., 2011), *Ceropegia juncea* (Krishnareddy *et al*., 2011), *Woodfordia fruticosa* (Grover and Patni, 2011), *Solidago virgaurea* L. (Paul *et al*., 2012), Pigeon pea (Kaur *et al*., 2012), *Cocculus hirsutus* (Meena *et al*., 2012) and *Ananas comosus* (Ibrahim *et al*., 2013).

1.5 BIOCHEMICAL STUDIES

Nature has been recognized as a rich source of medicinal compounds for thousands of years. It is estimated that about one third of the currently marked drugs are related to natural products (Grabley and Thiericke, 1999). Analysis of various cellular metabolites (primary as well as secondary), enzyme activities, isozyme profiles during *in vivo* and *in vitro* experimentation provide a reasonable
approach towards understanding the biochemical basis of medicinal values of the plant species (Singh, 2005).

A characteristic feature of higher plants is the formation of a wide variety of natural products, the so-called secondary metabolites. These compounds exhibit several interesting biological or therapeutical activities that are useful to mankind and thus are economically important.

Secondary metabolites have high economical and pharmaceutical importance and the industries are deeply interested in a large variety of chemical substances being produced by plants due to their lesser toxicity. In the search for alternatives to the production of desirable medicinal compounds from plants, biotechnological approaches, specifically, plant tissue culture are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites (Rao and Ravishankar, 2002).

Biosynthesis of secondary metabolites from intact plants, plant parts and tissue culture has gained increasing attention over the years. Advances in the field of plant, tissue and cell culture technology have proved to be of immense value for the biosynthesis of secondary products in plants (Mulabagal and Tsay, 2004).

Nowadays, research is more focused on isolating target compounds rather than isolating all compounds in the extract. These compounds may belong to certain chemical classes, having certain physical and biological activities. Different bioassays are available to
check the biological significance of the compound which includes antimicrobial, anti fungal and antioxidant assay.

1.6 ANTIOXIDANT ASSAY

Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities. However, if free radicals are produced in excess amount they can be destructive leading to inflammation, ischemia, lung damage and other degenerative diseases. Free radical reactions, especially with the participation of oxidative radicals, have been shown to be involved in many biological processes that cause damage to lipids, proteins, membranes and nucleic acids, thus giving rise to a variety of diseases.

Medicinal plants possess many bioactive compounds including phenolic and polyphenolic compounds which play a key role in the detoxification of stress induced by free radicals and exhibit antimicrobial activities (Hara-Kudo et al., 2004). All living organisms contain antioxidant metabolites and enzymes which ameliorate various free radical induced damages. Researchers have found a correlation between oxidative damage and the occurrence of diseases (Halliwell and Gutteridge, 1999).
Introduction

Synthetic oxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have restricted use in foods as they are suspected to be carcinogenic. Natural antioxidants, especially phenolic and flavonoids are safe and also bioactive. Therefore, in recent years, considerable attention has been directed towards identification of plants with antioxidant ability that may be used for human consumption (Jain et al., 2009). The researchers have focused on natural antioxidants and numerous crude extracts and pure natural compounds have been reported to possess antioxidant properties. The use of natural antioxidants symbolizes safety in contrast to the synthetic products (Patel et al., 2010).

1.7 ANTIMICROBIAL ASSAY

Medicinal plants are potential sources of new compounds of therapeutic value and are sources of lead compounds in drug development (Kumar et al., 2006). In recent years, the indiscriminate use of commercial antimicrobial drugs/chemical to treat infectious diseases has resulted in the development of multiple drug resistance in both human and plant pathogens (Kumar et al., 2006). One alternative approach to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents. This situation has forced scientists to search new antimicrobial substances in various sources like medicinal plants (Edeoga et al., 2005). Medicinal plants such as Bixa spp. and Bidens spp. have been claimed more efficient to treat infectious diseases than synthetic antibiotics by traditional healers (Rojas et al., 2006). So it becomes necessary to evaluate the scientific base for the potential use
of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases (Shah, 2005). They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant.

Antimicrobial activity of plants can be detected by observing the growth response of various microorganisms to plant tissue or extracts which are in contact with them. Many medicinal plants have been evaluated for their antimicrobial activities. Antimicrobial activity of various plant parts has also been observed by several workers viz. Ripa et al., 2009; Chahal et al., 2010; Pandey and Singh, 2011; Singh, 2011; Kaur et al., 2012; Maragathavalli et al., 2012; Deepa et al., 2012 and Kang et al., 2013. Several studies revealed that phenolics are the predominant active chemical in these plants, especially against gram positive bacteria which are most sensitive against these compounds (Mishra and Mishra, 2011; Nunez et al., 2012).

Even though hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not yet been evaluated (Balandrin, 1985).

1.8 RAPD ANALYSIS

Maintaining genetic fidelity is one of the major concerns of tissue culture studies because variations within the progeny can result in serious losses to the end-users. Several approaches, such as karyotyping and isoenzyme profiling, can be used to assess the genetic fidelity of the in vitro derived clones, but most of these methods have
their own limitations. Karyotyping does not reveal the alterations in specific genes or small chromosomal rearrangements (Isabel et al., 1993) whereas isoenzyme markers are subject to ontogenic variations.

Micropropagation coupled with modern methods like DNA markers can be used to study the plant at the gene or molecular level. Molecular markers have come up as the most desirable tool for establishing genetic uniformity of the micropropagated plantlets. This is apparent in studies conducted to screen somaclonal variations produced in tissue cultured plants such as in tea (Singh et al., 2004), soyabean (Hofmann et al., 2004), Date palms (Saker et al., 2006), Swertia chirayta (Joshi and Dhawan, 2007), leguminous trees (Hussain et al., 2008), sugarcane (Lal et al., 2008), Spondias mangifera (Tripathi and Kumari, 2010), Guadua angustifolia (Nadha et al., 2011), Citrus jambhiri Lush (Savita et al., 2012) Picrorrhiza kurroa (Rawat et al., 2013). Within DNA markers, the RAPD technique is suitable to study the frequency of polymorphism. Molecular studies of the in vitro calli clones can be carried out using RAPD marker to study the extent of genetic variations (William et al., 1990). PCR based molecular markers such as RAPD, SSR, ISSR and AFLP have several advantages over the other methods and have been used for genotyping and detection of polymorphism/variability in plants. A better analysis of genetic stability of plantlets can be made by using a combination of two types of markers that amplify different regions of the genome (Martins et al., 2004). Polymerase chain reaction (PCR) techniques using random amplified polymorphic DNA (RAPD) markers are considered to be sensitive enough to detect the
variations or genetic relationship among individuals between and within species (Tripathi et al., 2007). RAPD markers have been successfully used to assess genetic stability and quality among micropropagated plants, thus ensuring the quality of tissue cultured plantlets.

The RAPD technique, being simple and cost effective, has been used to assess the genetic variations of tissue cultured plants in numerous studies (Salvi et al., 2001; Martins et al., 2004; Joshi and Dhawan, 2007). Additionally this technique has advantages such as low development cost as per say (Karp et al., 1997). RAPD’s can be used to device conservation strategies for an endangered plant by studying the genetic variability and geographical spread of remaining species of that genus.

1.9 PRESENT STATUS OF RESEARCH ON THE PLANT

From the review of literature, it was found that no detailed work has been made on micropropagation and RAPD analysis of Cocculus hirsutus. Some biochemical studies have been done on C. hirsutus. Ahmad (1986) studied the chemical constituents of Cocculus hirsutus. Ahmed et al., (1987) isolated a triterpenoid from whole plant of Cocculus hirsutus. Ahmad et al., (1991) isolated cohirsinine, an alkaloid from the leaves of Cocculus hirsutus. Ahmad et al., (1991) isolated cohirsinine, an alkaloid from the leaves of Cocculus hirsutus. Ahmad and Iqbal (1992) isolated cohirsitinine, a new isoquinoline alkaloid from aerial parts of Cocculus hirsutus. Ahmad and Iqbal (1993) isolated a new alkaloid jamtinine from whole plant of Cocculus hirsutus and its structure was assigned on the basis of chemical and spectral studies.
1.10 OBJECTIVES OF THE PRESENT WORK

Keeping in view, the medicinal value and availability of this plant in wild sectors, the present study was carried out to develop a highly efficient, reliable and reproducible protocol for the micropropagation of *Cocculus hirsutus*. RAPD analysis was carried out to analyze the plant at the molecular level as there are instances of patenting of developing country plants, by developed countries emphasizing the need to generate databases which can be used for future reference. In this context, the present study was undertaken with the following major objectives:-

- Establishment of tissue cultures from various adult plant parts to obtain multiple shoots through axillary and lateral bud proliferation and also through callus organogenesis.
- Multiple shoot formation from seedling explants viz. cotyledonary node and hypocotyl.
- Study of physical and chemical factors affecting regeneration from various explants directly or via callus.
- Induction of roots in the *in vitro* developed shoots in order to obtain complete plantlets and hardening and pot transfer of these plantlets.
- Analysis of various secondary metabolites in *in vivo* and *in vitro* tissues and isolation of the active principles(s) by standard biochemical technique.
- Antioxidant and antimicrobial assay of methanolic extracts of different plant parts and callus.
- RAPD analysis of *in vitro* raised plants to assess variability if any, between original and *in vitro* raised plants.