The plants used in the phyto-pharmaceutical preparations are obtained mainly from the natural growing areas. Many leading Homoeopathic as well as Ayurvedic pharmaceutical companies in India are dependent on forests for their regular supply of *Cocculus hirsutus* (Oudhia, 2010). Due to the increase in the demand for the crude drugs, the plants are being overexploited, threatening the survival of many rare species. For most of the medicinal plant species no conservation action has been taken. 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 agreeable 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 (Sudhir *et al*., 2010). Hence, it is difficult to restore the population of medicinal plants through conventional propagation methods. In order to rectify the problem by conservation methods using biotechnological tools like multiplication of medicinal plants by *in vitro* regeneration (Micropropagation) methods is the need of the hour.

*Cocculus hirsutus* (L.) Diels (Synonym-*Cocculus villosus*) locally called Jaljamini, belong to the family Menispermaceae, the moon seed family. A family of about 70 genera and more than 450 species, which are almost entirely tropical (Abhimanyu *et al*., 2010). *C.hirsutus* is widely distributed in Sudan, central Asia, China and India (throughout tropical and subtropical regions). The plant grows all over India, especially in dry regions. In India, it is known by various names in different regions viz, Vevati in Gujarat, Huyer in Bengal, Kattukkodi in Tamilnadu and Vasanvel at some places. It is widely distributed throughout the tropical world (Bhavna and Bothara, 2011).
Menispermaceae are rich in alkaloids and herbage. Various species belonging to the family are important plants, being used in the traditional medicines in a number of countries. According to Ayurveda, *C. hirsutus* is known as Patalagarudi in Sanskrit. Root smell is sweetish and pungent, it has been reported to reduce bile and burning sensation and enrich blood. It is used in diseases of urinary system. According to Unani system of medicine, it is antipyretic, tonic, lessens thirsty, good for fractures, and useful in tubercular gland related problems. It is well known herb used as first aid remedy in minor injuries. It alleviates kapha and vata doshas. It is used as depanee, 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. (Govt of India, 2001; Indian drug’s manufacturer’s association, 2002).

The roots and leaves of *C. hirsutus* have great medicinal value and are used both, internally as well as externally for medicinal purpose. Traditionally the plant is praised for its unique property of healing all types of cuts, wounds and boils, swelling in very less time and less pain. It is also used in treatment of gonorrhoea, spermatorrhoea, urinary troubles, diarrhea and hyperglycemia (Kirtikar and Basu, 2002). Juice of the 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. The powdered leaves of this plant are used for the preparation of tea by kani tribes of karayar in Tamilnadu (Kalirajan et al., 2012). Tribals of Jhabua, Khargone and Dhar use the fruit of *C. hirsutus* to cure Jaundice (Samvatsar and Diwanji, 2000).

The term “herbal drug” determines the part/parts of a plant (leaves, flowers, fruits, seeds, roots, barks, stems, etc.) used for preparing medicines (Anonymous, 2007a). Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds.
The medicinal properties of plants could be based on the antioxidant, antimicrobial antipyretic effects of the phytochemical in them (Adesokan et al., 2008). The pharmacologically active principle of many Ayurvedic medicines is being identified and their usefulness in drug therapy is being determined. The presence of radical scavenging potential in plant help us to understand that the plant possess bioactivity and some underlying molecule responsible for it.

Fruits and vegetables are high in flavonoid content; it is estimated that humans consume between a few hundred milligrams and one gram of flavonoids every day (Pietta, 2000). There are over 4000 naturally occurring flavonoids (Harborne and Baxter, 1999). Many pigmented fruits contain high amounts of anthocyanins, which are the largest group of water-soluble flavonoid pigments in the plant kingdom. They attract pollinators and seed dispersers and protect plant tissues from photoinhibition and oxidation resulting from photosynthesis (Gould and Lee, 2002).

Anthocyanins are responsible for the purple, blue, red and orange colours of several fruits and flowers. The anthocyanin content and compositions are different in the pigmented fruits depending on the varieties and origin (Oh et al., 2008). The de-glycosylated forms of anthocyanins are known as anthocyanidins. Mostly six anthocyanidins are present in fruits and vegetables like cyanidin, pelargonidin, malvidin, delphinidin, petunidin and peonidin. These compounds possess beneficial health properties like antioxidant and free-radical scavenging activity, (Prior and Clin, 2003), protective effect against cardiovascular disease (Pascual et al., 2010; Basu et al., 2010), anti-inflammatory and anticarcinogenesis properties (Wang and Stoner, 2008; Stoner et al., 2008).

Antioxidants are defined as molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged (Nidhi and Gaurav, 2012).
Naturally occurring antioxidants are needed to replace the synthetic antioxidants which are restricted due to their inherent carcinogenicity (Abdel Hameed, 2009). The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers.

In recent years antibiotic resistance has become a global concern. This drives the need to screen medicinal plants for novel bioactive compounds as they are biodegradable, safe and have fewer side effects (Prusti et al., 2008).

*In vitro* cytotoxicity is a preliminary screening study for the identification of anticancer potential in a plant parts. There is an emergency to identify new molecules for cure of microbial infections and cancer. Reason being emergence of antibiotic resistance among microorganisms and drug resistance in the case of cancer. Most people lose their life because of different type of cancer among them breast cancer has been reported to be the second in occurrence but if identified earlier can be completely cured. Every year, 75,000 new cases of breast cancer are reported in India. In future, this figure may further increase due to factors like environmental pollution and food habits. The mortality due to cancer is a challenge similar to that posed by HIV/AIDS (Merlin et al., 2010).

Natural products have played an important role throughout the world in the treatment and prevention of human diseases (Chin et al., 2006). Over 60% of the currently used anticancer agents are derived in one way or other from natural sources (Balunas and Kinghorn, 2005; Cragg and David, 2005). Medox®, Black current and Acai tablets are anthocyanin rich antioxidant tablets commonly available in market.
There are no reports on the tissue culture and bioactivity of the plant parts of *C. hirsutus* (L) Diels. Hence, this work is done with the aim of validating the following objectives

- To standardize the protocol for surface sterilization of explants, callus culture, and micropropagation.
- Preliminary phytochemical analysis of *in vivo* and *in vitro* leaves.
- Analysis of enzymatic and non enzymatic antioxidant content of the crude extracts obtained from *in vivo* and *in vitro* leaves.
- Evaluation of antibacterial activity of the different fractions of *in vivo* and *in vitro* leaves.
- Qualitative and quantitative phytochemical analysis of pigmented fruit extract.
- Evaluation of biological activities of crude anthocyanin extract from fruit.
Schematic representation of the present investigation

of *Cocculus hirsutus* (L.) Diels

- Node
- Direct regeneration
- Shoot regeneration
- Rooting
- Hardening
- Phytochemical Analysis
- Antioxidant activity
- Antibacterial activity
- Cytotoxicity
- Cocculus hirsutus
- Leaves
- Callus
- Shoot induction
- Fruits

In vitro Propagation, phytochemical screening and biological activity of *Cocculus hirsutus* (L.) Diels.