REVIEW OF LITERATURE
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The history of medicine in India can be traced back to the Vedic period. The ‘Rig-Veda’ perhaps the oldest repository of human knowledge having been written about 4500-1600 B.C. claims about the uses of 99 medicinal plants. The ‘Yajur veda’ deals with 288 plants almost all having medicinal ingredients and were used to cure deadly diseases. As per the Vedas, the Kalpsutras describe some about 519 plants.

The ‘Ayurveda’, science of Indian medicine (about 2500 B.C.) contains a more detailed account of many drugs and their uses. Ayurveda in fact is the foundation stone of the ancient medicinal science of life and art of healing. Charaka samhita is another earlier treaties of Ayurveda (600 B.C.) which lists a total of 341 plants and plant products for use in health management. Susruta Samhita also deals with plants related to medicine. Subsequent authors of later treaties have extended the list of ayurvedic single plant drugs to the 600 species of plants.

Bhiksu Atreya, a well known Professor of the University of Taxila, his student named Juaka, who later became the physician of Bimbisara of Magadh Kingdom. Dhanvantari and Nagarjuna were the well-known persons, whose works deal primarily with the characteristics of the medicinal plants and medicines.

Nagarjuna, a learned person in Hindu chemistry, was the inventor of Kajli (a compound of Sulphur and Mercury) and art of calcination (Bhasma). He was not only a renowned Vaidya but was an authority on Astronomy, Chemistry and Magic. Bhoja Prabandha, a treatise written about 980 A.D. contains a reference to inhalation of medicaments before surgical operations and an anaesthetic called Sammohini is aid to have been used in the time of Buddha.
A worldwide shift towards herbal preparations over synthetic pharmaceuticals has resulted in over exploitation of a number of wild plants of medicinal value. The practice of plant tissue culture has changed the way some nurserymen approach plant propagation. In the recent past, the applicability of this technology to the propagation of medicinal plants has been documented. Some firms have established tissue culture facilities on commercial scale operations are presently in operation for the mass propagation of valuable medicinal species. The intent of this research update is to briefly examine "what is being done" and to explore "what can be done" with regard to the tissue culture of medicinal plants. Such a consideration necessarily includes an overview of tissue culture as a propagation tool. No in vitro studies reports are available from the literature of *C. gouriana* and it is very rare and endangered medicinal plant. In *N. zeylanica* only in vitro callus culture and detection of berberine was carried out by Praveendhar and Ashalatha 2003, and also biosystematically, this species holds much importance because only two stove-climbing species are reported in the genus *Naravelia* (Manjunath et al., 2004).

For the present investigation two medicinal plants namely, *Clematis gouriana* Roxb. and *Naravelia zeylanica* (L.) DC. (Ranunculaceae) have been selected for the derivation of in vitro protocol, phytochemical evaluation and pharmacological of screening of the crude extracts and the isolated constituents.

1. **In vitro studies**

Tissue culture is a means of preserving species that are rare and threatened and providing an alternative source of plants for commercial, horticultural and traditional medicinal trade. Tissue culture is the aseptic culture of plant protoplasts, cells, tissues or organs on a culture medium which is as defined as possible the cultures are maintained under controlled environmental condition.
Micropropagation, the growth of plant cells outside an intact plant, is a technique essential in many areas of the plant sciences. A culture of individual or groups of plant cells, and whole organs, contribute to understanding both fundamental and applied science and allows the production of large number of plants from small pieces of the stock plant in relatively short periods of time. Basically the technique consists of taking a piece of a plant (such as a stem tip, node, meristem, embryo, or even a seed) and placing it in a sterile, (usually gel-based) nutrient medium where it multiplies. The formulation of the growth medium is changed depending upon whether you are trying to get the plant to produce undifferentiated callus tissue, multiply the number of plantlets, grow roots, or multiply embryos for "artificial seed". The main two important criteria should be followed are, i) Selection of plant sources, where, some species, or even clones are easier to grow in culture than others. Some respond reluctantly to culture, some do not respond at all, and many plants have never been tried. ii) Choosing a growth medium (price, convenience, type of plant and purpose of the micropropagation all enter into this decision), how important are the kinds of hormones used? on limited scale, media ingredients are available. It is a fascinating and useful tool that allows the rapid production of many genetically identical plants using relatively small amounts of space, supplies and time.

Perhaps the most heavily researched area of tissue culture today is the concept of selecting disease, insect or stress resistant plants through tissue culture. Just as significant gains in the adaptability of many species have been obtained by selecting and propagating superior individuals, so the search for these superior individuals can be tremendously accelerated using in vitro systems. Such systems can attempt to exploit the natural variability known to occur in plants or variability can be induced by chemical or physical agents known to cause mutations.

Another purpose for which plant tissue culture is uniquely suited is in the obtaining, maintaining, and mass propagating of specific pathogen-free plants.
The concept behind indexing plants free of pests is closely allied to the concept of using tissue culture as a selection system. Plant tissues known to be free of the pathogen under consideration (viral, bacterial, or fungal) are physically selected as the explant for tissue culture. In most cases, the apical domes of rapidly elongating shoot tips are chosen. These are allowed to enlarge and proliferate under the sterile conditions of *in vitro* culture with the resulting plantlets tested for the presence of pathogen (a procedure called indexing).

Further plant tissue culture technique have advantages in metabolite production over intact plants due to the fact that (a) the rate of cell growth and biosynthesis in cultures initiated from a very small amount of plant material is quite high and the final product may be produced in a considerably short period of time. This is in contrast to large amounts of mature plant tissues processed to obtain a small quantity of a drug. (b) Plant cell cultures are maintained under controlled environmental and nutritional conditions, which ensure the continuous yields of metabolites. On the contrary continual decline in the natural habitats due to ecologically disturbed conditions may make the availability of source plants uncertain unless they are clonally propagated. (c) Suspension culture offers a more effective mechanism of incorporating precursors into cells than is found in whole plants. (d) New routes of synthesis can be recovered from deviant and mutant cell lines which can lead to the production of novel compounds not previously found in whole plants. (e) Some cell cultures have the capacity for biotransformation of specific substrates to more valuable products by means of single or multiple step enzyme activity. (f) Culture of cells may be more economical for those plants that take long periods to achieve maturity (Razdan, 2003).

a. **Leaf culture**

Culture of excised leaf primordia is valuable to study the effects of various nutrients, growth factors and changing environmental conditions on leaf
development under conditions divorced from the complexities of the intact plant. Leaf primordial or very young leaves are excised, surface sterilized and inoculated on an agar solidified medium, in culture leaf remains in healthy condition for a long period. Leaves can be taken from aseptically grown plants for culture. Since, leaves have a limited growth potential, so in culture the amount of leaf growth depends upon the stage of maturity at the time of excision. Leaf primordial or very young have more growth potential than nearly mature leaves (De, 2003). Many investigators were succeeded in deriving protocols for the regeneration of plantlets from the leaf culture of many medicinal plants.

The somatic tissue of higher plants is capable, under certain conditions, of regenerating adventitious plants. Adventitious buds are those which arise directly from a plant organ or a piece of thereof without an intervening callus phase. Induction of adventitious shoots directly on root, leaves, bulb scales and other organs of intact plants is a common method is particularly suitable to herbaceous species.

The requirement for exogenous auxin and cytokinin in the process varies with the tissue system, apparently depending on the endogenous levels of the hormones in the tissue. These observations led to the concept of totipotency, i.e. the capacity of all cells to regenerate a complete new plant even after differentiation within the somatic tissues of the plant. Every cell in the plant is derived from the original zygote through mitotic divisions and should contain the complete genome. Thus, the formation of adventitious organs will depend on the reactivation of genes concerned with the embryogenic phases of development.

In conventional propagation, the main stimulus for adventitious shoot formation arises from the physical separation of cutting from the parent plant, causing changes in the production and distribution of endogenous hormones. The same applies to explants used for in vitro procedures, and in some species shoot formation may occur spontaneously on a medium lacking any growth regulators.
But in most species the addition of growth regulators to the medium is required to initiate shoot formation. Two main types of growth regulating substances, auxins and cytokinins, are employed at different concentrations with respect to one another and depending upon the explant taken, age of the plant and growing conditions. Adventitious in vitro regeneration may give a much higher rate of shoot production than the possible proliferating axillary shoots. Adventitious shoot proliferation is the most frequently used multiplication technique in micropropagation system (Chawla, 2003).


In the species of Torenia fournieri (Bajaj, 1972); Solanum nigrum, S. dulcamara and S. khasianum (Bhatt et al., 1979) shoot and root initials were successfully regenerated from leaf derived calli. The morphogenesis from the callus tissue is controlled by a balance between auxins and cytokinins (Skoog and Miller, 1957). Torrey (1961) advanced this hypothesis and stated that the organogenesis in callus tissue starts with the formation of cluster of meristemoids. The differentiation of plantlets from the leaf callus of Psophocarpus tetragonolobus was demonstrated by Gregory et al. (1980). Further they mentioned that the interaction of auxins in lower concentrations and cytokinins in higher concentrations would provoke shoot differentiation from the leaf callus.

b. Stem culture

Stem culture is an important tool for rapid multiplication of plants. During last decade, regeneration of plantlets has been achieved from the stem culture either from direct organogenesis or through differentiation from the callus. Mantell et al (1976) in Dioscorea alata and D. rotundata, estimated that, on a regular 14-20 day cycle it would be possible to obtain 65,000 plants from a single
node within six months. In 1998, Usha Yadav, investigated the *in vitro* protocol for the clonal propagation of *Syzygium cumini* L. Multiple shoots were obtained from nodal and shoot tip segments of 10 to 15 day old seedlings on revised MS medium supplemented with 6-benzyladenine singly or in combination with α-naphthalene acetic acid, indole 3-acetic acid or indole-3-butyric acid. Similarly many investigators has worked on clonal propagation of many plant species using axillary nodal explants viz., *Dalbergia latifolia* (Rai and Chandra, 1988); *Morus alba* (Sharma and Thorpe, 1990); *Prospis juliflora* (Nandwani and Ramawat, 1991); *Dalbergia latifolia* (Raghava Swamy et al., 1992); *Gardenia jasminoides* (George, et al., 1993); *Acacia auriculiformis* (Reddy et al., 1995); *Clerodendrum colebrookianum* (Mao et al., 1995); *Azadirachta indica* A. (Joshi and Thengane, 1996); *Pterocarpus santalinus* (Anuradha and Pullaiah, 1999); *Canavalia virosa* (Kathiravan and Ignacimuthu, 1999); *Litchi chinensis* (Das et al., 1999); *Valeriana jatamansi* (Kaur et al., 1999); *Ancistrocladus abbreviatus* (Bringmann et al., 1999); *Anoectochilus regalis* (Gangaprasad, 2000); *Phyllanthus carolinensis* (Catapan et al., 2000); *Gymnema sylvestre* (Komalavalli and Rao, 2000); *Eclipta alba* and *Eupatorium adenophorum* (Borthakur et al., 2000); *Hildegardia populifolia* (Anuradha and Pullaiah, 2001); *Paulownia fortunei* (Venkateswarlu et al., 2001); *Plumbago zeylanica* (Rout et al., 2001); *Lavandula viridis* L. (Dias et al., 2002); *Decalepis hamiltonii* (Anitha and Pullaiah, 2002); *Bacopa monnieri* (Upasana, et al., 2002); *Acacia catechu* (Thakur, et al., 2002); *Atropa acuminata* (Ahuja, et al., 2002); *Wedelia chinensis* (Martin, et al., 2003); *Enicostemma littorale* (Shanthi and Xavier, 2003); *Celastrus paniculatus* (Maruthi, et al., 2004); *Mentha piperita* (Kiran Ghanti, et al., 2004); *Centella asiatica* (Shashikala, et al., 2005); *Solanum nigrum* (Jabeen, et al., 2005); *Vitex negundo* (Sweta Das, et al., 2005); *Wedelia chinensis* (Mahantesh, et al., 2005); *Bacopa monnieri* (Escandon et al., 2006); *Glossocardia bosvallea* (Geetha and Gopal, 2007); *Citrullus colocynthis* (Maheshchandmeena and Vidya patni, 2007); *Ceropegia oculata* (Nikam and Savanth, 2007); *Cassia siamea* (Raja sreelatha et
al., 2007),* Allium sativum* (Ghanashyam bhanja, 2007); *Plumbago indica* (Rajasri Bhatacharyya et al., 2007); *Dioscorea hispida* (Shukla et al., 2007); Shahanaz Beegum et al., 2007) in *Ophiorrhiza prostrate*.


c. **Study of genetic variability**

Tissue-culture-induced phenotypic and genotypic variations are collectively termed 'somaclonal variation' (Larkin and Scowcroft, 1981). It has relevance in the clonal propagation of valuable or endangered plant germplasm, and in the production of transgenic plants. It may also be an effective means of generating useful mutants. Because of these reasons, somaclonal variation has been intensively studied by using various molecular markers in several plant species, including *Arabidopsis* (Polanco and Ruiz 2002) and rice (Kim et al., 2002). Nonetheless, few studies have addressed the molecular basis or nature of somaclonal variation (Al-Zahim et al., 1999; Yang et al., 1999).

Both RAPD and ISSR markers have been used to assess somaclonal variation in maize and were found to be highly efficient (Osipova et al., 2003).
Moreover, Kuznetskova et al. (2005) showed their reliability in analysing DNA polymorphisms generated by long-term culture and subsequent regeneration in pea. In this study, the RAPD molecular marker was shown to be efficient in determining the genetic changes induced by tissue culture.

RAPD stands for random amplification of polymorphic DNA. It is a type of PCR reaction, but the segments of DNA that are amplified are random. The scientist performing RAPD creates several arbitrary, short primers (8-12 nucleotides), then proceeds with the PCR using a large template of genomic DNA, hoping that fragments will amplify. By resolving the resulting patterns, a semi-unique profile can be gleaned from a RAPD reaction.

No knowledge of the DNA sequence for the targeted gene is required, as the primers will bind somewhere in the sequence, but it is not certain exactly where. In recent years, RAPD is used to characterize, and trace, the phylogeny of diverse plant and animal species. Random amplified polymorphic DNA (RAPD) markers were used by many researchers to evaluate the somaclonal variation/genetic variability of micropropagated plants such as Hordeum spontaneum (Breiman, 1987); Allium sativum (Al-Zahim et al., 1991); Begonia (Bouman, 1992); Trifolium pratense L (Nelke, 1993); Trembling aspen and Bigtooth aspen (Liu et al., 1993); Begonia (Bouman, 1994); Populus deltoides (Rani, 1995); Picea abies (Heinze et al., 1995); sugar beet (Munthali et al., 1986); Picea glauca (Isabel, 1995); Quercus suber L. (Fallego et al., 1997); Fragaria xananassa (Popescu, 1997); Digitalis obscura L. (Nebauer et al., 1999); Dicrocoelium dendriticum (Sandoval et al., 1999); Scutellaria (Keizo et al., 2000); White spruce (Lamhamedi et al., 2000); Turmeric (Salvi et al., 2001); Begonia plants (Bouman et al., 2001); Mangifera indica L. (Hemanthkumar et al., 2001); Melia azedarach L. (Olmos et al., 2002); Asparagus spp. (Shasany et al., 2003); Lippia (Verbenaceae) (Vicini et al., 2004); Anthurium andraeanum Hort. (Prakash et al., 2005); Juchum et al., 2007).
In the present study the plantlets regenerated directly from the stem explant through adventitious organogenesis and indirectly through stem calli were maintained in the departmental garden. The occurrence of somaclonal variation in these plants has been comparatively evaluated with the in vivo mother plant using RAPD markers.

d. Callus Culture for secondary metabolite production

Natural products have been investigated and utilized to alleviate disease since early human history. In the early 1900s, before the "Synthetic Era", 80% of all medicines were obtained from roots, barks and leaves. The study of natural products involves isolation in a pure form of these compounds and investigation of their structure, formation, use, and purpose in the organism. Secondary metabolites appear to function primarily in defense against predators and pathogens and in providing reproductive advantage as intraspecific and interspecific attractants. Study of natural products has led to the discovery of a variety of useful drugs for the treatment of diverse ailments and contributed to the development of separation science and technology, spectroscopic methods of structure elucidation and synthetic methodologies that now make up the basics of analytical organic chemistry. More recently, the Vinca alkaloids, vinblastine (Loomis, 1972) and vincristine (Whittaker and Banthorpe, 1972) were isolated as antineoplastic agents from the Madagascar periwinkle, Catharanthus roseus, and subsequently derivatized to vinorelbine and vindesine, the drugs that are currently in use for cancer treatment (Newman, 2000). Similarly, a potent antimalarial drug, a sesquiterpenoid endoperoxide, named artemisinin (Croteau, 1987) was isolated from Artemisia annua. Embilin was isolated from in vitro derived calli of Embelia ribes (Kumaraswamy, 2006), a triterpene Lupeol was isolated from the leaf and leaf calli and a quinine derivative 2-methoxy dodecyl 2-methaoyx 6-decyl benzoquinone was isolated from the stem and stem calli of Celastrus paniculatus and also a saponin wedelolactone was isolated from the leaf and leaf calli Wedelia calendulacea (Harish, 2008).
It has been demonstrated that the biosynthetic activity of cultured cells can be enhanced by regulating environmental factors, as well as by artificial selection or the induction of variant clones. Some of the medicinal compounds localized in morphologically specialized tissues or organs of native plants have been produced in culture systems not only by inducing specific organized cultures, but also by undifferentiated cell cultures. The possible use of plant cell cultures for the specific biotransformation of natural compounds has been demonstrated (Cheetham, 1995; Scruggs, 1997; Krings and Berger, 1998; Ravishankar and Ramachandra Rao, 2000; Kavitha et al., 2007). Due to these advances, research in the area of tissue culture technology for production of plant chemicals has bloomed beyond expectations. In the present study calli induced from the leaf explants of *C. gouriana* and *N. zeylanica* were subjected for the evaluation of secondary metabolite.

2. Phytochemical screening

India has an ancient heritage of traditional medicine. The Materia Medica of India provides a great deal of information on the folklore practices and traditional aspects of therapeutically important natural products. Indian traditional medicine is based on various systems including Ayurveda, Siddha and Unani. These traditional systems of Indian medicine are each unique but there is a common thread in their fundamental principles and practices. With the emerging worldwide interest in adopting and studying traditional systems and exploiting their potential based on different health care systems, the evaluation of the rich heritage of traditional medicine is essential. The government and the private sectors in India are now exploring all of the possibilities for the perfect evaluation of these systems in order to effectively adopt the therapeutic approaches available in Indian systems of medicine as well as to help in generating data to put these products on the national health program.
Natural products have long been and will continue to be extremely important as sources of medicinal agents and model for the design of synthetic and semisynthetic novel substances for treating human diseases. The evaluation of these drugs is primarily based on phytochemical, pharmacological, and allied approaches including various instrumental techniques such as chromatography, microscopy, spectroscopy, X-ray crystallography etc.

In India, Department of Biotechnology has launched a Bio-prospecting and Molecular Taxonomy programme which opens avenues for sustainable utilisation and bio-prospecting of medicinal plant genetic resources and commercialization of the drugs originated from the medicinal plants. This has resulted in the characterization of intraspecific genetic diversity of many medicinal plants (Symplocos laurina, S. racemosa, Gaultheria fragrantissims, Eurya nitida, Vitex negundo, Podophyllum hexandrum and Rhododendron nilgiricum etc) (Anonymous, 2000). As a result of many bio-prospecting projects carried out worldwide, many therapeutic compounds have been discovered and introduced to the national as well as the international markets. But so far, only 90,000 natural compounds have been well studied which represent about 40% of total possible new drugs (Tyler, 2001). However, the increasing need for phytochemicals as a safe alternative or an adjunct to modern medicine is seriously felt particularly due to the widely perceived biohazardous side effects of the synthetic drugs (Jackson and Kanmaz, 2001). India can play a leading role in this context as it has got rich heritage of medicinal plants combined with vast storehouse of medicinal plant germplasm.

It is believed that herbal drugs are relatively safe and exhibit a remarkable efficacy in the treatment of chronic ailments. According to an estimate, of nearly quarter is being used for medicinal purpose (Anonymous, 2000). About 80% of people in developing countries depend on traditional systems of medicine for primary health care (Farnsworth et al., 1985). One fourth of the prescriptions filed
by pharmacies each year are for substances derived from plants, and when the
drugs obtained from microorganisms and animals are added in, the total rises to
40%. Some 120 chemicals extracted in pure form from about 90 species of higher
plants are used in medicine throughout the world; a wide range of plant species
being used locally for medicinal purposes (Dar and Farooq, 1997). The World
Health Organization has reported that about 4 billion people are relying on herbal
medicines, and listed over 21,000 plant names (including many synonyms) said to
be of medicinal use. However, only about 5,000 higher plant species have been
investigated as potential sources of new drugs. Examination of drugs used in the
traditional medicine in various countries is one of the priority programmes of
prescription data the natural products still play a major role in drug treatment, as
over 50% of the most prescribed drugs in the US had a natural product either as
the drug, or as a 'forebear' in the synthesis or design of the agent (Grifo et al.,
1997).

In India, Karnataka state is endowed with rich natural resources especially
along the Western Ghat ranges. The Western Ghats range arises abruptly in the
west from the Arabian Sea coast and descends gradually towards the dry Deccan
plains in the east. The total area of the Western Ghat is estimated to be about
20,000 sq. kms. The good climatic conditions and altitudinal gradients have
resulted in the development of a variety of forests from evergreen to semi
evergreen, moist deciduous to dry deciduous and scrub jungles. It is one of the
richest biodiversity centers and is considered as one among the eighteenth hot spot
of the world. This region comprises about 4000 species of angiosperms of which
2,280 species are endemic to this region (Pascal, 1982).

Phytochemically, the species of *Clematis* contains many secondary
metabolites. Many investigators successfully isolated some of the secondary
metabolites from the species of *Clematis*. Clemontanoside-C, a new hedragenin-
based saponin isolate from the stem of *Clematis montana* (Thapliyal and Bahuguna, 1993) and from the aerial part of *Clematis tibetana*, two new hederagenin, 28-O-bisdesmosides called lematibetosides A and C. A new gypsogenin 3, 28-O-bisdesmoside called clematibetoside B, were isolated together with ten known saponin (Kawata et al., 2001). Protoanamomin has been isolated from the Australian ‘Headache Vine’ *Clematis glycinoides* (Southwell and Tucker 1993). Ursolic acid a triterpene was isolated from *C. gouriana* for the first time structure was predicted with spectroscopic data.

In *N. zeylanica* only alkaloid berberine was detected from the methanol extract (Praveenndhar and Ashalatha, 2003). The Extract from flowering plants of *N. zeylanica* yielded three simple benzamides, 3, 4-methylenedioxybenzamide, 4-methoxybenzamide and 4-hydroxy-3-methoxybenzamide (Jaroszewski et al., 2005). No reports are available for extraction and isolation of phytoconstituents from leaves of this plant. The taraxerol and β-sitosterol from Petroleum ether extract and berberine from methanol extract were isolated for the first time and structure were predicted with spectroscopic data.

### 3. Pharmacological Evaluation

*Clematis gouriana* is used in the Indian system of medicine ‘Ayurveda’ to eliminate malarial fever and headache. Root and stem paste is applied externally for psoriasis, itches and skin allergies. The traditional medicine practitioners residing in the vicinity of Bhadra Wildlife Sanctuary, India, use the leaf and stem juices for treating infected wounds, psoriasis, dermatitis, blood diseases, leprosy and cardiac disorders.

*Naravelia zeylanica* is useful in the treatment of pitta, helminthiasis, dermatopathy, leprosy, rheumatalgia, odontalgia, colic inflammation, wounds and ulcers (Praveendhar and Ashalatha, 2003). The root and stems have a strong penetrating smell (Warrier et al., 1995) In the Indian system of medicine,
‘Ayurveda’, the plant is used to relieve malarial fever and headache while root and stem paste is applied externally for psoriasis, itches and skin allergy (Harsha et al., 2002). In Kerala, India *N. zeylanica* is used as a source of drug for intestinal worms, skin disease, leprosy and toothache (Sivarajan and Balachandran, 1958). The traditional medicine practitioners residing in the vicinity of Bhadra Wild Life Sanctuary, Karnataka, India are using the leaf and stem juices for treating psoriasis and dermatitis. Many pharmaceutical industries in India (Hindustan Liver Ltd., Mumbai; Himalayan Drug House, Bangalore) are engaged in the production of skin ointments from this plant.

No reports were available for the pharmacological evaluation of these diseases so we have conducted the pharmacological screening of both the plants on wound healing, hepatoprotective, *in vitro* and *in vivo* antioxidant activity, antimicrobial activity and anti cancer activity of the extracts and isolated constituents of both the plants.

a. **Wound healing activity**

The ‘wound’ refers to any opening in tissue due to either internal or external factor which results in cell death and cell injury. The word ‘healing’ means replacement of destroyed tissue by living tissue. Wound may be caused by trauma—either accidental or surgical, by physical, chemical and microbial agents or by ischaemia, which leads to infarction.

The process of healing involves two distinct processes:

i. **Regeneration**: In this healing takes place by proliferation of parenchymal cells and usually result in complete restoration of the original tissues.

ii. **Repair**: In this healing takes place by proliferation of connective tissue elements resulting in fibrosis and scarring.
Repair process involves following steps:

- **Formation of clot and crust**: The clot formed holds together the sides of the wound. If it reaches the surface, it dries to form a crust or scab. The crust prevents oozing of fluid from the wound and also serves as a mechanical barrier against bacteria from outside.

- **Formation of granulation tissue**: This is a composite step made up of 2 processes. i). Vascularization where in new blood vessels and lymphatics are formed in the area undergoing repair. ii). Proliferation of fibroblasts occurs.

- **Organization**: Newly formed capillaries and fibroblasts gradually replace the clot. The process is known as organization. The new tissue formed is known as granulation tissue.

- **Wound contraction**: Wound contraction is the shrinkage of the area of the wound. It depends on the reparative abilities of the tissue, type and extent of the damage and general state of health of the tissue. It is a normal process, which hastens healing. The mechanism of contraction may be (i) drying up of the fluid in the wound. (ii) contraction of granulation tissue and (iii) contraction of narrow band of tissue at the edge of the wound.

- **Epithelialization**: Epithelial cells from the margin of the wound, flatten, elongate and begin to migrate as a continuous sheet. It lasts till the gap is completely covered and the normal architecture of the cover is reestablished. The newly formed film of epithelium is only one or two layer thick. It becomes stratified and keratinized later.

- **Breaking strength of wound**: This is the strength of a healing wound and is measured experimentally by the amount of force required to disrupt it.
In the traditional systems of medicine, various plants have been used to promote wound healing. Wound healing effect of the various plant extracts has been reported by many investigators such as *Aloe vera* (Udupa et al., 1994); *Trigonella foenum graecum* (Taranaalli and Kuppast, 1996); *Centella asiatica* (Suguna et al., 1996); *Hypericum mysorenses* (Mukherjee and Suresh, 2000); *Nelumbo nucifera* (Mukherjee, et al., 2009); *Ginkgo biloba* (Bairy and Rao, 2001); *Aegle marmelos* (Jaswanth et al., 2001); *Polyscias scutellaria* (Divakar et al., 2001); *Opuntia ficus-indica* (Park and Chun, 2001); *Buddleja globosa* (Mensah et al., 2004); *Argemone mexicana* (Patil et al., 2001); *Glycorrhiza glabra* (Kishore Gnana Sam et al., 2001); *Thespesia populnea* (Nagappa and Cheriyan, 2001); *Polyscias scutellaria* (Divakar et al., 2001); *Indigofera enneaphylla* (Hemalatha et al., 2001); *Datura alba* (Priya et al., 2002); *Coronopus didymus* (Prabhaker et al., 2002); *Heliotropium indicum, Plumbago zeylanicum and Acalypha indica* (Suresh Reddy et al., 2002); *Gmelina arborea* Roxb. (Shirwaikar et al., 2002); *Bryophyllum pinnatum* (Mahamood and Patil, 2002); *Eucalyptus globulus* (Hukkeri et al., 2002); *Aristolochia bracteolata* (Shirwaikar et al., 2003); *Terminalia arjuna* (Madhura and Sushma, 2003); *Dodonaea viscosa* (Joshi et al., 2003); *Hyptis suaveolens* (Shirwaikar et al., 2003); *Lawsonia alba* (Mandawghade and Patil, 2003); *Cinnamomum zeylanicum* (Kamath et al., 2003); *Eucalyptus globulus* (Kusum et al., 2004); *Trigonella foenum-graecum* (Taranaalli et al., 2004); *Eclipta alba* (Patil et al., 2004); *Oxalis corniculata* (Taranalli et al., 2004); *Merremia tridentata* (Hatapakki et al., 2004); *Diospyros cordifolia* (Mankani et al., 2004) and *Saussurea lappa* (Ganachari et al., 2005); *Vanda roxburghii* (Nayak et al., 2005); *Plagiochasma appendiculatum* (Meenakshi et al., 2006); *Terminalia arjuna* (Minakshi and Sushma, 2006); *Centella asiatica* (Shetty et al., 2006); *Cyperus rotundus* (Puratchikody et al., 2006); *Embelia ribes* (Kumar Swamy et al., 2007); *Lycopodium serratum* (Manjunatha et al., 2007); and the wound healing activity was screened for synthesized compounds by (Prakashnaik et al., 2008).
b. Screening of Caseinolytic activity by proteases

Several investigators has worked on Caseinolytic activity of medicinal plants derived compounds for the screening for proteases viz., Wilson et al., (1993, 1997); Varani et al., (1978); Nath and Ledford, (1972) Divergent patterns of matrix metalloproteinase activity during wound healing in ileum and colon of rats by Seifert et al., (1996); Pirilia et al., (2004); Ryan et al., (2006); Nagaraju et al. (2007). Plant extracts of Vitis vinifera L. (Vitaceae) seed methanol extract has been studied for its ability to inhibited the caseinolytic, hyaluronolytic and fibrinogenolytic activities of the venom (Mahadeswaraswamy et al., 2008).

c. Antioxidant Activity

Oxidation process is one of the most important routs for producing free radicals in food, drugs and even living systems. Those free radicals include superoxide radical anion (\(\cdot O_2^-\)), hydroxyl radicals (\(\cdot OH\)), singlet oxygen (\(1O_2\)), and hydrogen peroxide (\(H_2O_2\)). In cellular oxidation reactions, superoxide radical normally is formed first, and its effects can be magnified because it produces other kinds of cell-damaging free radicals and oxidizing agents. The damaging action of the hydroxyl radical is the strongest among free radicals (Liu and Ng, 2000). Reactive oxygen species produced by ultraviolet light, ionizing radiation, chemical reactions, and metabolic processes have numerous pathological effects, such as causing lipid peroxidation, protein peroxidation, DNA damage and cellular degeneration related to cardiovascular disease, ageing, cancer, inflammatory diseases, and a variety of other disorders (Bauerova and Bezek, 1999; Finkel and Hollbrook, 2000; Visioli et al., 2000).

Oxidation involves the transfer of electrons from one atom to another. The oxidized molecule loses an electron while the receiving molecule is reduced. Oxidation reactions are an essential part of aerobic metabolism, since oxygen is an electron acceptor in the electron flow system that produces energy (Lee et al., 2003). Oxidation becomes a problem when reactions become uncoupled and free radicals are formed.
Free radicals are molecules that are highly reactive and unstable because they contain an unpaired electron. Electrons are most stable in pairs, hence the free radicals tend to attach to or receive hydrogen ions from molecules with lower bond dissociation energy like unsaturated fatty acids or phenolic antioxidants. The free radicals due to environmental pollutants, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins (Halliwell, 1994). Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Kumpulainen and Salonen, 1999; Cook and Samman, 1996).

Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity (Barlow, 1990).

Many investigators reported that plant-derived products contain a wide range of phytochemicals and phenolic compounds that possess substantial antioxidant activities (Shaftidi, 2004). These phytochemicals and phenolics provide protection against harmful effects of free radicals and are known to reduce the risk of certain types of cancer, CHD, CVD, stroke, atherosclerosis, osteoporosis, inflammation and other neurodegenerative diseases associated with oxidative stress (Hertog, 1993; Ness and powles, 1997; Mazza et al., 1999; Joseph et al., 1999; Chu et al., 2000; Surh, 2003). Evaluation of antioxidant efficacy in plant products cannot be carried out accurately by any single universal method or extraction solvent due to the complex nature of the phytochemicals present.
Numerous methods (Siriwardhana and Shahidi, 2002; Amarowicz et al., 2004; Prior et al., 2005; Decker et al., 2005) have been used to evaluate and estimate the antioxidant efficacy of foods and dietary supplements, and these relate to measurement of free radical scavenging activity, reducing power, and chelation of pro-oxidative metal ions. Kumar and Gupta (2002) showed that the aqueous, methanolic, chloroform and petroleum ether extracts of seeds of Celastrus paniculatus were investigated for their effect on antioxidant in rats. Bang et al. (2001) reported that the isolated flavones from ethyl acetate-soluble extract of the dried aerial parts of Celastrus orbiculata was found to exhibit significant in vitro antioxidant effects. Praful et al., (2003) investigated that the free radical scavenging capacity of three aqueous extracts obtained from seeds aqueous extracts of Celastrus paniculatus exhibit a dose-dependent free radical scavenging capacity for DPPH and also for superoxide-generated assays. The antioxidant activity of methanol extract of leaves and stem of C. paniculatus and the ethanolic extract of Wedelia calendulacea was evaluated by Harish (2008). Schuler (1999) reported that besides well known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices. Pourmorad et al., (2006) carried out a systematic record of the relative free radical scavenging activity in plant extracts (Mellilotus officinalis, Equisetum maximum, Plantago major, Adiantum capillus-veneris and Urtica dioica) having phenol and flavonoid contents. Kumpulainen and Salonen, (1999) found that due to depletion of immune system natural antioxidants in different maladies, consuming antioxidants plant extract as free radical scavengers may be necessary.

Several investigators has worked on medicinal plants which possess potent antioxidant activity viz., Rubus idaeus, Rubus occidentalis, and Fragaria ananassa (Shiow and Hsin-Shan, 2000); Cordyceps sinensis (Li et al., 2001); Emblica officinalis (Kaur and Kapoor, 2002); Morinda officinalis (Soon and Tan, 2002); Satureja hortensis (Güllüce et al., 2003); Allium cepa, Illicium religiosum,
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*Fagopyrum esculentum*, *Origanum officinalis*, *Rosmarinus officinalis*, *Pyrus pyrifolia*, *Acanthopanax senticosus*, *Eugenia caryophyllata* and *Erigeron annuus* (Young and Kyong, 2003); *Ardisia compressa* (Sonia and de Mejia, 2004); *Theobroma cacao* (Osman et al., 2004); *Aframomum danielli*, *Allium cepa*, *Allium sativa*, *Capsicum frutescens*, *Citrus sinensis*, *Curcuma longa*, *Justicia flava*, *Ocimum gratissimum*, *Piper guineense* (Odukoya et al., 2005); *Fagopyrum esculentum* (Ting and Chi-Tang, 2005); *Rhodiola sacra*, *Polygonum multiflorum* and *P. multiflorum* (Chi-Chun et al., 2006); *Garcinia mangostana* (Weecarangsan et al., 2006); *Gentiana lutea* (Kussar and Zupaneie et al., 2006); *Lycium barbarum* (Li et al., 2007); *Ocimum basilicum* (Ilhami et al., 2007) and *Zanthoxylum piperitum* (Yamazaki et al., 2007) *Celastrus paniculatus* (Harish et al., 2008); *Grewia tiliaefolia* (Khadeer ahamed et al., 2008); *Embelia ribes* (Kumar Swamy et al., 2008) the antioxidant activity was screened for synthesized compounds by Prakashnaik et al., 2008.

d. **In vivo antioxidant activity**

Hepatitis is an inflammation and/or necrosis of liver cells. There are two types of hepatitis, Acute (sudden in onset) infection of the liver e.g. infective hepatitis, serum hepatitis and toxic hepatitis. The common causes of acute hepatitis are hepatotropic viruses A, B, C, D and E, hepatotoxins and drugs.

Acute viral hepatitis is a systemic infection affecting the liver predominantly. Almost all cases of acute viral hepatitis are caused by one of the five viral agents: hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), hepatitis E virus (HEV). A sixth agent hepatitis G virus (HGV) has been discovered, but its role in acute viral hepatitis remains to be established. All these human hepatitis viruses are RNA viruses except for hepatitis B which is a DNA virus. Jaundice due to drugs and chemicals arise as a result of accidental, suicidal or therapeutic exposure to the agent. The
exposure may be of an acute or a slow, prolonged and chronic type. Certain drugs and chemicals, which are poisonous to human system, produce jaundice by hepatocellular damage. Hepatitis continues to be a major cause of illness and death among all communicable diseases (Pradeep, 1999). In the developing countries like India majority of the population rely upon the plant based traditional medicines to cure jaundice. The allopathic medical care is too costly and the interferon treatment is highly sophisticated and beyond the reach of people living below the poverty line.

The liver regulates many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions (Wolf, 1999). But there are not much drugs available for the treatment of liver disorders (Karan et al., 1999; Chatterjee, 2000). Among the various mechanisms involved in the hepatotoxic effect of carbon tetrachloride (CCl4), one is oxidative damage through free radical generation (DeLeve and Kaplowitz, 1995). The antioxidant property is claimed to be one of the mechanisms of hepatoprotective effect (Bhatt and Bhatt, 1996).

The earlier reports investigated the in vivo antioxidant activity of important medicinal plants Young et al., 1986 (Wedelia chinensis); Gulati et al., 1991 and Chandra et al., 1991 (Boerhaavia diffusa); Chattopadhyay et al., 1992 (Ocimum sanctum); Raj and Shalini 1999 (Cytisus scoparius); Mehta et al., 1999 (Trialthema portulacastrum); Babalola, 2001 (Vernonia amygdalina); Oh et al., 2002 (Cnidium monnieri); Krishna et al., 2004 (Boerhaavia erecta, Boerhaavia rependa and Diospyrus cordifolia); Srinivas, 2004 (Vitex negundo); Nagaraja and Krishna, 2004 (Andrographis alata); Krishna et al., 2005 (D. cardifolia).

Silymarin is a flavonoid isolated from Silybum marianum that kindled widespread world research on hepatoprotective agents (Wagner et al., 1968; Abraham et al., 1970; Pelter and Hansel, 1975). Other important antihepatotoxic drug discoveries from plant sources include cynarin from Cynara scolymus.
(Panizzi and Scarpti, 1954) and schizandrin from *Schisandra sphenanthera* (Liu *et al.*, 1978). The discovery of diverse chemical compounds from the natural products and synthetic compounds used in protective liver therapy such as phospholipids, sugar alcohols, pyrimidine, purine derivatives, vitamins, cysteine, glutathione, corticoids, androgens, penicillamine, ricinin etc., does not confine the activity to any particular class of compounds (Shirwaikar *et al.*, 1994), but emphasizes once again the complexity of liver disorders in addition to the different action, mechanisms of different pharmaceutical preparations. Silymarin is a complex mixture of four flavonolignan isomers, namely silybin, isosilybin, silydianin and silychristin with an empirical formula $C_{25}H_{22}O_{10}$. The structural similarity of silymarin to steroid hormones is believed to be responsible for its protein synthesis facilitatory actions. Among the isomers silybin is the major and most active component and represents about 60-70 per cent, followed by silychristin (20%), silydianin (10%), and isosilybin (5%). Silipide is the silybin-phosphatidylcholine complex which ensures a large increase in the bioavailability of silybin (Vailati *et al.*, 1993).

So far only feasible reports on the evaluation of hepatoprotective activity of the isolated constituents of the medicinal plants are available. In the present investigation also an attempt was made to screen the crude extracts, isolated compounds of leaves of *C. gouriana* and *N. zeylanica* against CCl$_4$ induced toxic hepatitis.

e. Evaluation of Antimicrobial activity

In light of the recent emergence of bacteria which are resistant to multiple antimicrobial drugs posing a challenge for the treatment of infections (Service, 1995), the need to discover new antimicrobial substances for use in combating such microorganisms become pertinent. Thus there is a constant and urgent need to develop new antimicrobial drugs for the treatment of infectious diseases from
medicinal plants (Cordell, 2000). Plant based natural constituents can be derived from any part of the plant like bark, leaves, roots, fruits, seeds, fruit rind, etc (Gordon and David, 2001); i.e. any part of the plant may contain active components.

Infections due to multidrug-resistant gram-negative microorganisms pose an important clinical problem, resulting in significant morbidity and mortality worldwide (Carmeli et al., 1999; Hsueh et al., 2002; Livermore, 2002).

Important pathogens in this study

_Pseudomonas aeruginosa, Staphylococcus aureus_ and _Klebsiella pneumonia_ are the important Gram-positive and Gram-negative human pathogenic bacteria that cause nosocomial infections. In particular, _P. aeruginosa_ is a predominant respiratory pathogen among cystic fibrosis patients producing chronic pulmonary infection and progressive deterioration in lung infection (Van Delden, 2004; Van Delden and Iglewski, 2005). The _Pseudomonas aeruginosa_ has been implicated in infections of respiratory and urinary tract, suppurative infections in sinuses and middle ear, septicemia, etc., whereas _Klebsiella pneumonia_ has been associated with a range of clinical conditions which include, infections of wounds, infections of urinary tract and eyes, septicemia etc. and Gram-positive _Staphylococcus aureus_ has been implicated in Abscess in immunodeficiency, Septicemia, Food poisoning patients and five clinically isolated pathogenic fungi such as _Trichophyton rubrum, Microsporum gypseum, Microsporum audouini, Trichophyton tonserans, _and Candida albicans_ were implicated in infections of Cutaneous mycoses, Scaring of the scalp, ringworm infections and Opportunistic mycoses candidosis.

Antibiotic resistance

The development of resistant microorganisms on prolonged exposure to existing antimicrobial agents has been known for a long time (Weisser et al.,
Extra-chromosomal genes were found responsible for these antimicrobial resistant phenotypes that may impart resistance to an entire antimicrobial class. These resistant genes have been associated with plasmids which are large, transferable, extra-chromosomal DNA elements. Other DNA mobile elements, such as transposons and integrons, are present on plasmids. These DNA mobile elements transmit genetic determinants for antimicrobial resistance mechanisms and may cause rapid dissemination of resistance genes among different bacteria (McDermott et al., 2002). The emergence of multiresistant bacteria to antimicrobial drugs has increased the need for new antibiotics or modifications of older antibiotics (Tollefson and Miller, 2000).

Antibiotic resistance of *P. aeruginosa* is caused by environmental factors such as exposure to subinhibitory concentrations of antibiotics or limiting concentrations of divalent cations (Gunn et al., 1998; Livermore, 2002). Several investigators have worked on antimicrobial activity using medicinal plant extracts viz., *Eupatorium perfoliatum* (Habtemariam and Maepherson, 2000); *Mezoneuron benthamianum* (Binutu and Cordell, 2006); *Pterocarpus osun* (Ebi and Ofoefule, 2000); *Dichrostachys cinerea* (Eisa, 2000); *Enantia polycarpa* (Ajali, 2000); *Ricinus communis* (Parameswari and Tulasi Latha, 2001); *Drynaria quercifolia* (Ramesh et al., 2001); *Eupatorium cannabinum* (Senatore et al., 2001); *Bixa orellina* (Castello et al., 2002); *Datura alba* (Priya et al., 2002); *Feronia limonia* (Mukhlesur and Alexander, 2002); *Aerva lanata* (Chowdhury et al., 2002); *Embelia ribes* (Chitra et al., 2003); *Crotalaria pallida* (Muthusamy et al., 2003); *Cocos nucifera* (Srinivas et al., 2003); *Artemisia douglasiana* (Setzer et al., 2004); *Melissa officinalis* (Mimica-Dukic, 2004); *Solanum stramoenifolium* Jacq., *S. seaforthianum* Andr. and *S. violaceum* Ortg (Manjunatha et al., 2004); *Eupatorium glandulosum* (Sasikumar et al., 2005); *Quercus infectoria* (Basri and Fan, 2005); *Vigna angularis* (Hori et al., 2006); *Bauhinia variegata* and *Plumbago zeylanica* (Jigna et al., 2006); *Mentha longifolia*, *Melissa officinalis* and *Rosa*
damascene (Bassam et al., 2006); The antimicrobial activity of the extracts of Bidens pilosa L., Bixa orellana L., Cecropia peltata L., Cinchona officinalis L., Gliricidia sepium H.B. & K, Jacaranda mimosifolia D.Don, Justicia secunda Vahl., Piper pulchrum C.DC, P. paniculata L. and Spilanthes Americana Hieron (Jhon J Rojas et al., 2006); Cleome rutidosperma (Bose et al., 2007); Combretum molle, Peltophorum africanum, Piper capense, Terminalia sericea and Zanthoxylum davyi (Steenkamp et al., 2007); Exploring of Antimicrobial Activity of Triphala (Biadar et al., 2008); Capparis zeylanica (Chopade et al., 2008); Baccharis Species (Glaucoro Morales et al., 2006) and Hyptis suaveolens L. (Mandal et al., 2008).

The plants possess innumerable number of secondary metabolites which are usually produced under stress conditions and often in response to infections. These secondary metabolites possess profound antimicrobial potency. Many workers have isolated different types of active constituents and studied for their antimicrobial potency. Alkaloids (Burdock, 1971); phenolic compounds (Mason and Wasserman, 1987); tannins (Scalbert, 1991); flavanones and flavonoids (Panilio, et al., 1992); sesquiterpenes (Topcu, et al., 1993); anthroquinone (Kazmi, 1994); flavonoid glycosides (Hasan and Ahmad, 1996); triterpene acid glycosides (Kirmizigul et al., 1996); monoterpenes (Meng et al., 2000); diterpenes (Ulubelen et al., 2000); triterpenes (Akbar and Malik, 2002). These active constituents isolated from medicinal plants showed significant antibacterial effect.

f. Evaluation of Anti cancer activity

Cancer is a very widespread disease, which is responsible for millions of deaths each year worldwide. Chemotherapy is an essential strategy for the treatment of disseminated cancers. This observation stimulates the search for new anticancer agents, and in this regard, the investigation of naturally originating compounds could be very valuable. However, as a contribution towards the
chemotherapy of cancer, plant secondary metabolites in particular play a very important role (Cragg, 1997; Shu, 1998; Cragg, 2004; Newman, 2000). After several plant-derived natural products demonstrated prominent anticancer activity in patients with advanced malignancies in the 1950s and 1960s, the microtubule was recognized as a subcellular target of major strategic importance with regard to anticancer therapeutics. The first widely used class of antimicrotubule agents, the Vinca alkaloids (Cragg, 2004), has been the mainstay of both palliative and curative regimens for treating both hematopoietic and lymphoid malignancies for several decades. More recently, a large number of plant derived compounds such as the epothilones, camptothecin and discodermolide have been identified, with yet even more distinctive antimicrotubule and anticancer activities (Newman, 2000). Camptothecin isolated from the wood and bark of a Chinese tree, *Camptotheca acuminata* by Wani and Wall in 1966 (Tang, 1992). It is a pyrrolo [3, 4-b]-quinoline alkaloid that was extracted using ethanol from the stemwood of the plant (Ghisalberti, 1993). They stabilize topoisomerase-DNA cleavable complexes by hindering the DNA relegating step of the catalytic reaction (Wall, 1966; Giovanella, 1999), thus resulting in DNA cleavage stimulation, which lead to apoptosis (Binaschi, 1992). Topotecan and irinotecan are the two synthetic analogs of Camptothecin (Grabley, 1999) which shows activity towards ovarian cancer (topotecan) and colorectal cancer (irinotecan) (Creemers, 1996; Bertino, 1997). Flavopiridol is a flavone inhibitor of the cyclin-dependent kinase (CDK) family that was semi-synthesized from rohitukine, a plant derived natural product (da Rocha, 2001). It appears to be non-selective towards any particular CDK. The drug is in the early stages of clinical trials, but it is creating excitement because of its interesting mechanism of action (Newman, 2000). The progression of the cell cycle is blocked during stages of growth after the compound interferes with the kinase phosphorylation step (Kelland, 2000) which ultimately causes cell death. Homoharringtonine obtained from the seeds of a Chinese evergreen (*Cephalotaxus harringtonia*) widely used in China for traditional medicine and
known for efficiency as a cytotoxic anti-leukemia drug (Powell, 1970; Warber, 1999). This drug was a product of discovery through an extensive research program carried out by the National Cancer Institute in the 1960s, and in 1993, it was classified as one of the NCI’s investigational new drugs (Warber, 1999; Cordell, 1993). Homoharringtonine is thought to function during the cell cycle when proteins are being elongated by peptidyl transferase (Warber, 1999). This interruption of protein synthesis leads to apoptosis and differentiation of cancer cells (Warber, 1999; Zhou, 1995-96). A non-alkaloid bioactive compound from different plant species like *Podophyllum* and *Juniperus* that deserves some attention is podophyllotoxin. It is isolated from the roots and identified as an antitumor dimeric lignan in 1880 (Kuo, 2001). The epimer of podophyllotoxin is epipodophyllotoxin, giving rise to two semi-synthetic compounds with high activities and clinical applications, etoposide and teniposide (Williams, 1987). Like many anticancer drugs, etoposide functions by inhibiting topoisomerase II (Liu, 1989).

The present investigation undertaken because so far, no systematic study has been reported regarding the cytotoxic activity of *C. gouriana* and *N. zeylanica*, an effort has been made to screen *in vitro* cytotoxic activity of extracts and isolated constituents of *C. gouriana* and *N. zeylanica* using SRB assay.