INTRODUCTION
I. INTRODUCTION

Medicinal plants are the sources of important therapeutic aid for alleviating human ailments. With increasing realization of the health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics the interest in the use of plants and plant-based drugs has revived throughout the world. A large number of medicinal plants are yet to be investigated for their possible therapeutic properties. Most of the pharmaceutical industries are highly dependent on the wild populations for the supply of raw materials for the extraction of medicinally important compounds. Due to lack of proper cultivation practices, destruction of plant habitats, and the illegal and indiscriminate collection of plants from their habitats, many of the species are severely threatened. Advanced biotechnological methods of culturing plant cells and tissues should provide a new means of conserving and rapidly propagating valuable, rare, and endangered medicinal plants. Further, application of biotechnology on the evaluation of genetic diversity of the clones has contributed a lot in the cultivation, conservation and improvement of medicinal plant species.

Nature has always been a first-rate drug store house, with its enormous range of plants that are known to have effective therapeutic qualities. The medicinal plants have played a significant role in the most convenient and effective health care because these are not only natural, easily available, cost effective, safe and regenerative, but also the inherited knowledge of tribes and traditional medicine men as a result of their long term association with the forests for time immemorial.

In spite of, tremendous advances made in the modern medicine, there are still a large number of ailments for which suitable drugs are yet to be found. There is an urgent need to develop safer drugs for the treatment of hepatitis, arthritis, diabetes, psoriasis, herpes, leucoderma, leukemia, aids etc. The Vedic literatures
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also mentioned the identity and utility of herbs/herbal products in treating human ailments. Only a few plants have been subjected to rigorous phytochemical and pharmacological studies and many of the plants are yet to be investigated. The ability and maintenance of medicinal plants is low cost, already available with village populations and provides an effective way of treating illness without having to consume dangerous costly drugs and pills manufactured in remote factories. These can also be grown and maintained in herbal gardens at their own houses.

The knowledge of the biological activities and the chemical constituents of plant are desirable, not only for the discovery of new therapeutic agents, but also disclosing the value of new sources of economically useful materials. Thus a careful and extensive study on medicinal plants is necessary to identify newer plant products to evaluate the efficacy of the compound to combat the diseases. Scrutiny of medical indications by the source of compounds has demonstrated that natural products and related drugs are used to treat 87% of all categorized human diseases, including antibacterial, antipyretic, hepatoprotective, anticancer, anticoagulant, anti-inflammatory, antiparasitic, wound healing and immunosuppressant agents, among others. There was no introduction of any natural products or related drugs for 7 drug categories (anesthetic, antianginal, anti histamine, anxiolytic, chelator and antidote, diuretic, and hypnotic) during 1981 to 2002. In the case of antibacterial agents, natural products have made significant contributions as either direct treatments or templates for synthetic modification. Of the 90 drugs of that type that became commercially available in the United States or were approved worldwide from 1982 to 2002, approximately, 79% can be traced to a natural product origin (Newman et al., 2003).

India is endowed with vast resources of medicinal and aromatic plants. These plants have been used in traditional Indian Health systems over two thousand years for human welfare in the promotion of health, as drugs and
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fragrance materials. The World Health Organization (WHO) estimated that about 80% of the population in developing countries depends on traditional medicines, mostly plant drugs for their primary health care needs. The All India Ethnobiological Survey carried out by the Ministry of Environment and Forests, revealed that 7,500 plant species belonging to 386 families and 2200 genera are used by 4,635 ethnic communities for human and veterinary health care across the country. India is one of the major suppliers of medicinal plants with an annual turnover of herbal products worth Rs.23 billion. Of this, the domestic market of medicinal plants is about Rs.3 billion, which includes condiments and food additives (14%) herbal extracts (22%), essential oils, gums and resins (19%) and crude drugs (45%).

The growing interest in commercialization of plant-based medicines has lead to over exploitation of the plants. Hence, India has banned the export of several wild species in the form of raw materials, although the export of finished products containing the material is allowed. Despite this, about 95% medicinal plants collected in India are gathered from the wild, which includes trees (33%), herbs (32%), shrubs (20%), climbers (12%) and others constitute 3%. These collections are mostly destructive (72%) which include the entire plant (16.3%), reproductive parts like flowers, fruits and seeds (22.1%) or tuber, root and stem (39.5%). Such destructive and non sustainable collection methods coupled with low regeneration and habitat destruction have posed serious threat to the survival and availability of various medicinal plants in the wild. United Nations Industrial Development Organization (UNIDO) recommended the systematic cultivation of many medicinal plants with species-specific cultural practices, agronomic requirements, research and development to formulate good agricultural practices including proper selection and identification, propagation methods, cultivation techniques, harvesting and quality control of raw materials.
Valuation of biodiversity is desirable, as this not only highlights the contribution of biodiversity to society but also helps in developing an efficient conservation strategy for this precious natural resource. Economic valuation of Biodiversity and its different components in terms of use and option values induces efficiency in decision making criteria. Medicinal plants and herbs are one of the crucial components as far as the contribution of biodiversity to society is concerned. With a progressive loss of biodiversity all over the world, especially in the tropics, society is not only losing present benefits from current use but is being deprived of the option of future availability known as option value. Medicinal plants provide meaningful inputs for drugs. Their loss through extinction could lead to considerable loss to the society. Hence, monetization of the components of biodiversity, such as for medicinal plants in a cost-benefit framework helps to understand how the conservation of biodiversity affects the level of human welfare, medicinal uses of plant and animal species have been practiced for centuries in many parts of the world but valuation of such benefits by economists has commanded attention in the last two decades possible due to the alarming rate of species extinction.

Biotechnology plays a major role in the mass multiplication, germplasm conservation, secondary metabolite production and sustainable use of medicinal plants. The application of tissue culture for large-scale plant production meant for commercial purposes is well demonstrated in the case of several crops and horticulture species. Many varieties have grown remarkably well in vitro, allowing various kinds of experimental manipulations.

As early in the period 1493-1541, the great philosopher Poracelsun said that the purpose of chemistry is not to make gold, but to cure diseases. This can be considered as one of the major turning point in the history of drugs. At the end of eighteenth century and the beginning of nineteenth century, many scientists worked on natural products and described the procedure for the isolation of active
constituents from the crude drugs. Simultaneously these led to the development of the biologically active constituents that are also chemically synthesized in the large quantities by plant precursors.

In the last 50 years a phenomenal growth occurred in Phytochemistry. These developments have not only changed the face of biochemical research, integrated pharmacology and clinical pharmacology, but have made the discovery of drugs. Many Indian researchers like, Nadakarni (1954), Chopra (1956), Kirthikar and Basu (1987), Nayar and Shastri (1982) and Rastogi (1993) have done laborious and commendable work in compiling the details of Indian Medicinal Plants. However, many of the medicinal plants are yet to be subjected to rigorous phytochemical studies.

Most of the herbal medicinal plants can be cultivated at various parts of the country. Extracts obtained from the plants like Catharanthus roseus, Taxus baccata, Solanum khasiaum, Artemisia annua, Digitalis lanata, Commifora mukal, Cassia angustifolia, Andrographis paniculata, Celastrus paniculatus and Embelia ribes etc., have found a tremendous export potential. Many companies like CIPLA, Hindustan Lever Ltd., Dabur India Ltd., Himalaya drug House, Vimta Lab., Reddy’s Lab, Tamilnadu Dada, Tamilnadu Herbals Ltd., etc were actively engaged in the production of herbal drugs to cater the health needs of the country.

Presently there has been more demand all over the world regarding the use of the herbal drugs in the place of modern drugs. Even after 60 years of independence, a large population of India had faith on herbal medicaments, but scientifically cannot merely accept the use of the plants for specific disorders, unless, corroborated by clinical studies. The way in which the drug acts and the manner in which important organ of the body are stimulated has to be proved through clinical experimental evidences.
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*In vitro* regeneration usually results in high genetic and phenotypic variability in the individuals derived from cultures, which are called somaclonal variants. Somatic variation can be beneficial in medicinal plant improvement especially on traits for which somaclonal mutants can be enriched during *in vitro* culture, including tolerance to environmental or chemical stress, as well as for increased production of secondary metabolites. The Random Amplified Polymorphic DNA (RAPD) technique (Williams *et al.*, 1990; Welsh and McClelland 1990) is becoming a frequently used tool for study of genetic variability. RAPD technology is rapid, precise and sensitive technique for mapping of the genotypes (Martin *et al.*, 1991; Welsh *et al.*, 1991). A large numbers of regenerant individuals have been analyzed by RAPD markers to obtain a reliable estimate of allele frequencies in a population.

These two medicinal climbers are very rare and endemic having few species in the family. In the genus *Naravelia* holds much importance because only two stove-climbing species are reported (Manjunatha *et al.*, 2004) because of that we were applied a biotechnological application to conserve these plants by micropropagation technique. These plants having high medicinal properties and using by traditional practitioners, these has been screened systematically on wound healing, hepatoprotective, antimicrobial activity, antioxidant activity and anticancer activity.

In the present investigation two endemic medicinal climbers have been selected for micropropagation, Phytochemical evaluation and screening of pharmacological activities.

*Clematis gouriana* Roxb. (Ranunculaceae)

*Clematis gouriana* is woody climber rarely found in the moist deciduous forest of Western Ghats, leaves opposite, trifoliately compound, petiole often twining, Inflorescence a panicle, flowers bisexual, bracts linear, perianth 4-8, valvate, petaloid, stamens many, filament linear, anther short, pistils apocarpous, fruit is an aggregate of achenes (Fig 1a).
Fig 1

a. A Twig of *Clematis gouriana* plant showing leaves and flowers.

b. A Twig of *Naravelia zeylanica* plant showing leaves and flowers.
The Ethanomedical survey in the Western Ghats region of Karnataka revealed that the *C. gouriana* are being used by the traditional practitioners to cure cut wounds, inflammations and as antipyretic medicine. In the Indian system of medicine ‘Ayurveda’ the roots of this plant are used to alleviate malarial fever and headache (Harsha *et al.*, 2002). Root and stem paste of *C. gouriana* is applied externally for psoriasis, itches and skin allergy. The traditional medicine practitioners residing in the vicinity of Bhadra Wild Life Sanctuary, India are using the leaf and stem juices for treating infectious old wounds, psoriasis, dermatitis, blood diseases, leprosy, wound healing, and cardiac disorders (Manjunatha *et al.*, 2004).

*Naravelia zeylanica* (L.) DC.

*N. zeylanica* is a woody climber, very rarely found in Western Ghats, Leaves opposite, pinnate; first pair of leaflets normal while the terminal one modified into 3-fid tendrils, Leaflets entire, Inflorescence is axillary panicle, flowers regular, bracts and bracteoles present, Sepaloid segments 4-5, caducous. Petaloid staminodes 10-14, club shaped, stamens many, connective produced beyond the anther, Fruit is an aggregate of achenes, fruit style feathery, Twisted 2-3 cm long (Fig 1b).

*N. 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 possess a strong penetrating smell (Warrier *et al.*, 1995). 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 Ethanomedical survey in Western Ghats region of Karnataka revealed that this plant are traditionally used for many diseases and infections but the availability of these plants in the Western Ghats is very rare and In the genus
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Naravelia holds much importance because only two stove-climbing species are reported (Manjunatha et al., 2004). In view of its medicinal importance, threatening status, lack of tissue culture reports, the present work focuses on the micropropagation, screening of genetic diversity between \textit{in vivo} plants and \textit{in vitro} regenerants by using RAPD markers, Screening of Phytochemical constituents and pharmacological activities.

The main objectives of the present investigation are as follows

1. To study the effect of growth regulators on the morphogenic potentialities of the leaf and stem explants of \textit{Clematis gouriana} Roxb. and \textit{Naravelia zeylanica} (L.) DC. under \textit{in vitro} conditions.

2. To standardize the protocol for rooting of the microshoots and hardening of \textit{in vitro} derived regenerants.

3. To evaluate the genetic variability among the stem explant regenerants of \textit{C. gouriana} and \textit{N. zeylanica} derived both from direct organogenesis and indirect organogenesis using RAPD markers.

4. To evaluate phytochemical constituents comparatively from the \textit{in vivo} leaves and their \textit{in vitro} derived calli of \textit{C. gouriana} and \textit{N. zeylanica}.

5. To screen the pharmacological property of the crude extracts of the leaves of \textit{C. gouriana} and \textit{N. zeylanica} and their isolated constituents for wound healing, \textit{in vitro} and \textit{in vivo} antioxidant and anti-cancerous properties.

6. To evaluate effect of crude extracts and the constituents on the formation of matrix metalloproteases which play an important role in tissue remodeling and wound healing process.

7. To evaluate antibacterial and antifungal activity of crude extracts and constituents of \textit{C. gouriana} and \textit{N. zeylanica} against the ATCC and clinical strains.