Research Proposal on

IN VITRO PROPAPAGATION, ISOLATION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM SELECTED MEDICINAL PLANTS OF KARNATAKA

Details of the proposed project to be undertaken:

Origin of proposal

From the past decades all the developed countries turned their attitudes towards plant based pharmaceuticals because of their lack of side effects. Since the demand for raw materials for extraction of drugs has increased many folds, large number of traders have started exporting the wild plants indiscriminately with the result the population of certain medicinal plants are becoming endangered and rare and others are facing genetic erosion (Cunningham 1998).

Generally, it has been demonstrated that free radicals are detrimental to human health. They react with DNA, lipids or cell membranes, leading to various diseases (Namiki, 1990). Therefore, any event that removes free radicals in the human body is considered to be beneficial for human health. Hence there is an urgent need for validated natural products, which can potentially retard or prevent the oxidative process.

Thus, there is an urgent need to identify plants of medicinal importance, which have made their entry into the available literature as well as those, which are popular among the tribals and rustics and propagate through in vitro techniques and also evaluated their bio-efficacy.

Therefore, the present research proposal focused on in vitro propagation selected important rare and endangered medicinal plants of Western Ghats of Karnataka viz., Ceropogia spirallis, Ceropogia attenuata, Aristolochia tagala, Ophiorrhiza prostrata, Ophiorrhiza mungos.

I. Ceropogia L. (Asclepiadiaceae) a pantropical old world genus of about 200 species exhibits a tremendous diversity with reference to habit, flower architecture and ecological applications. Presently India represented 50 species of which about 38 occur in Western ghats. Several species of Ceropogia are narrow endemics and fall under one or another IUCN Red list
category (Nayar and Sastry 1987). Tuberous roots of many *Ceropegia* species are edible (Anonymous, 1992). The root tubers are the officinal parts known to contain an alkaloid called “Ceropegin” (Nadkarni, 1976); bitterness of the tubers was eliminated by boiling and then consumed. The *Ceropegia spiralis* root tubers known to contain starch, sugars, gum, albuminoids, fats, crude fiber and valuable constituents in many traditional Indian Ayurvedic drug preparations that are active against many diseases especially diarrhea, dysentery and the starchy tubers are useful as a nutritive tonic (Kirtikar and Basu, 1935; Chopra et al., 1956).

In recent years the genus has attracted an attention of several workers due to rarity of its species, medicinal importance and recognized importance of conservation of rare plants of the globe. Most of the endemic species of *Ceropegia*, by virtue of being restricted only to special habitat and narrow ecological niche, are highly vulnerable and merit of special consideration in their conservation. Conservation of *Ceropegia* species is a challenge to biologist, it need immediate attention and efforts of universities, forest departments and local communities to save through propagation programme.

1. *Ceropegia spirallis* Wight, Ic. Pl. Ind. Or. t.1267.1848. is an annual herb grown wildly in south India and is in endangered category (Nayar and Sastry, 1987; Madhav Gadgil, 2004). It is a tuberous, erect, sometimes twining, narrow leaved species with one flowered cyme. It has wide distribution in Peninsular India. It has flower with spirally twisted corolla lobes and architecture of the flower is of great ornamental importance. It needs ex situ and in situ conservation. Endemic to Peninsular India and also endangered. Distributed in Andhra Pradesh, Karnataka, Kerala and Tamil Nadu. So far our knowledge goes; no report has been published on *in vitro* propagation of *Ceropegia spirallis* (Murthy et al., 2010).

2. *Ceropegia attenuata* Hook. Ic. Pl. 9.t.867.1852. It is a tuberous, erect narrow leaved species with one flowered cyme found throughout shrubby open forest of North Canara (Karnataka). It grows in cervices of lateritic in hilly tract ranging in altitude between 300-700 m. Tubers are edible. Flowers are long and ornamental valve. Due to habitat destruction, the populations of the species are decreasing day by day. *Ex vitro* and *in situ* conservation are needed for this species (Yadv et al., 2008).

II. *Aristolochia* L. is a large genus of the Aristolochiaceae with about 120 species, distributed throughout the tropical and subtropical countries. Many of them of economic importance due to aristolochic acids and terpenoids. The phenolics such as terpenoids are the important components present in Aristolochiaceae plants namely *Aristolochia brasiliensis* Mart. and Zucc., *Aristolochia bracteolata* Retz., *Aristolochia indica* Linn., *Apama siliquosa* Lamk. and *Aristolochia tagala* Cham.
1. **Aristolochia tagala** Champ. is a twining herb of the family Aristolociaceae. In Bangladesh, it grows mainly in forest thickets and hill slopes of Chittagong Hill Tracts (CHT). The plant is valuable for its bioactive compound aristolochic acid, which is unique to this genus (Wu et al. 2004). Roots and leaves of this plant are widely used in the traditional tribal medicine in CHT to treat fever, dysentery, snakebite, rheumatism and toothache (Biswas 2006). Propagation of *A. tagala* relies only on seed but the viability of seed is very low due to the presence of scanty endosperm. For this reason, the natural propagation of this plant species is hampered (Murugan et al., 2006). Moreover, deforestation, jhum cultivation and over exploitation are causes of depletion of this medicinally important plant species (Ravikumar, and Ved, 2000). In these consequences, an alternative mass propagation system like in vitro propagation and reintroduction programme/technology can efficiently be applied to save this rare medicinally important plant to meet up the demand as well as for future conservation. only one report has been published on in vitro propagation of *A. tagala* (Biswas et al., 2007).

III. **Ophiorrhiza** The genus *Ophiorrhiza* belongs to the family Rubiaceae, which comprises 150 species. The roots of *Ophiorrhiza* species, have been reported as the sources of anticancer drug: CPT and 10-methoxycamptothecin (Tafur et al. 1976; Saito et al. 2001; Sudo et al. 2002; Watase et al. 2004). The *Ophiorrhiza* spp. is also used to provide remedies for ulcers, helminthiasis, snake poison, poisonous wounds, gastropathy, leprosy, and hydrophobia (Kirtikar and Basu, 1975).

1. **Ophiorrhiza prostrata** D. Don is an herbaceous perennial medicinal plant, exploited for the production of camptothecin, which is accumulated mainly in the roots. The rate of plant propagation is critical to meet the pharmaceutical demand for camptothecin. A slow propagation rate in *O. prostrata*, because of low seed viability and germination rate as well as a small number of propagules (stem cuttings), has restricted the natural dissemination of the plant. In addition, the destruction caused by harvesting the roots as a source of the drug has threatened the survival of the plant. Thus, the large-scale demand necessitates rapid multiplication of the plant within a short timeframe without a negative impact on the natural resources. Inadequate reports have been published on in vitro propagation of this species (Beegum et al., 2007; Martin et al., 2008).

2. **Ophiorrhiza mungos** L. Commonly known as mongoose plant is very important medicinal plant found in Western Ghats. The roots are used for the treatment of cancer and snake bite. The roots containing cytotoxic quinoline alkaloid, campothecin (CPT), a high value anticancer compounds. It is half-woody, erect, smooth, plant up to 30 centimeters in height. The leaves are very thin, elliptic or elliptic-lanceo-latte, and pointed at both ends. The cymes are flat-topped, 2 to 7.5 centimeters in diameter, and smooth or hairy; their branches are subumbellate and very spreading.
The calyx-teeth are very short. The corolla is white and smooth, with very short, obtuse lobes which are keeled at the back. The pedicelled capsules are 2 to 5 millimeters in diameter. The seeds are many, minute, and angled. Only one report has been published on in vitro propagation of this plant (Binoy and Satheeshkumar 2004). This plant distributed in Western Ghats of Karnataka, Tamil Nadu and Kerala.

To best of our knowledge there are only few or inadequate reports on in vitro propagation and reintroduction of Ceropegia spirallis (Murthy et al., 2010), Ceropegia attenuata, Aristolochia tagala (Biswa et al., 2007), Ophiorrhiza prostrata (Beegum et al., 2007; Martin, 2008), Ophiorrhiza mungos (Jose, B and K Satheeshkumar 2004).

Thus the present proposal may be useful for mass multiplication, conservation and bioprospecting potentiality of these important medicinal plants. The protocol also could reduce the reliance on the natural population for extraction of herbal drugs, which in turn will stop these plants from becoming extinct.

**Objectives of the proposed project**

Main objectives of the research proposal, to standardize the reproducible in vitro propagation protocol for mass multiplication, acclimatization of in vitro propagated plants and reintroduction of selected medicinal plants into their natural habitat.

1. To know the distribution pattern and natural habitat, selected medicinal plants survey and documentation will be carried out in Western Ghats of Karnataka.
2. To evaluate the different explants for micro propagation on different types of nutrient media.
3. To standardized the protocol for morphogenic callus induction, regeneration of shoots and somatic embryogenesis.
4. To standardized procedure for acclimatization and transfer lab –to-land.
5. To evaluate the morphological characters of tissue cultured plants.
6. To compare the antioxidant properties and anticancer activity of in vitro cultures with its natural sources.
7. To isolate and characterization of probable antioxidant and anticancer compounds

**a. Work plan (including detailed methodology)**

Work plan (methodology/experimental design to accomplish the stated aim)

**To maintain germplasm for provide source for in vitro studies**

1. Select quality seeds from different agroclimatic location of Karnataka
2. Collected seeds and plants from different locations will be grown and maintained in the department garden.

**To evaluate the explants for in vitro propagation**

3. Explants viz., shoot tip and nodal, internodal leaf and cotyledons collected from seedlings and mature plants will be tested for multiple shoot induction, callus induction, shoot regeneration and somatic embryogenesis.

**To test the response of explants on different media with different concentrations and combinations of growth regulators**

4. Different types of nutrient media fortified with different concentrations and combinations of growth regulators (auxins and cytokinins) will be tested for multiple shoot induction, callus induction and somatic embryogenesis.

**To induce the rooting on medium different concentrations of auxins**

5. *In vitro* derived shootlets will be rooted on different concentrations of auxins

**Standardized techniques for acclimation to transfer to fields and evaluate the morphological characters**

6. Well established plant-lets will be acclimatized and transferred to ambient condition.

7. Morphological observation with respect to growth of the plant will be evaluated

**To compare the antioxidant and anticancer properties of *in vitro* cultures and natural plant extracts.**

8. Different assays will be used to screen for antioxidant activity (like DPPH assay, Reducing power assay, nitric oxide (NO) radical scavenging activity, lipid peroxidation assay, liposome model assay, linolic acid model, β-carotene assay, Hydroxyl radical assay, microsomal lipid peroxidation assay Thiobarbituric acid assay, Superoxide radical scavenging assay MTT assays etc )

9. Probable antioxidant and anticancer compound will be isolated and characterized using TLC, HPLC, NMR studies.

**Statistical analysis and compilation of Data**

10. The data obtained from the research proposal will be compiled for preparation of final technical report.

**References:**


Ravikumar, K. and Ved, D. K., 2000. *100 Red Listed Medicinal Plants of Conservation Concern in Southern India*, Foundation for Revitalization of Local Health Traditions, Bangalore,


