

# Executive Summary of the UGC Sponsored Minor Research Project

Ref: Sanctioned Vide Letter vide UGC letter No. F.

**MRP-6362/16(SERO/UGC) proposal no 3672** dated: 5/3/2018

## Title of the Project

**“Studies on enhancement of secondary metabolites  
in *in vitro* through hairy roots and immobilized cell  
suspensions in medicinally important plant  
*Achyranthes aspera*”**

PRINCIPAL INVESTIGATOR

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## **Introduction**

Herbal drugs constitute a major part in all traditional systems of medicine. Through age long trial and error methods there is a treasure of information about plants used medicinally often in hidden ethnic groups. Old Indian medicinal systems like Ayurveda are using plants for many symptoms. In a newer study 23 medicinal claims were recorded for an important medicinal plant *Achyranthes aspera*, but not verified by experimental data. Though almost all parts of *A.aspera* are used in traditional systems of medicines, seeds, roots and shoots are the most important parts. The studies carried out till date with *Achyranthes aspera* tested the pharmaceutical efficacy of crude extracts using animal models. The ethanolic crude extracts of *A.aspera* proved to possess highly valuable medicinal properties. In many plants, the production of secondary metabolites can be enhanced by the treatment of the undifferentiated cells with elicitors such as methyl jasmonate, salicylic acid, chitosan and heavy metals, immobilization of plant cell suspensions etc. Even hairy roots reported to produce high levels of alkaloids (Srivastav et al2011, Namdev 2007, Sevo'n and Caldenty 2002). Hence, the present project proposal aims in optimizing the conditions for enhancement of secondary metabolites through immobilization of cell suspensions and *Agrobacterium rhizogenes* mediated hairy roots in *A.aspera*.

*Achyranthes aspera* (*A.aspera*) L. (Latjeera) is a herb belongs to the family Amaranthaceae, found all over India possessing valuable medicinal properties. In Chinese traditional medicine, the hot water extract of the plant has been used as an antiarthritic to alleviate arthritic pain.



**Fig 1.** *Achyranthes aspera* L plant with Inflorescence collected from a. Narapally forest department b. From Osmania University campus

### **Traditional uses:**

*A.aspera* plant extracts were useful in cough, bronchitis,rheumatism, malarial fever, dysentery, asthma, renal and cardiac dropsy,hypertension and diabetes mellitus. *A. aspera* reported to stimulate the immunity, enhance the antigen clearance, potentiate antibody production, elevate thyroid hormone levels, decrease hepatic lipid peroxidation and also possesses spermicidal,chemo preventive, anti-inflammatory, anti-arthritic and hypoglycaemicactivities. The leaf extracts known to also exhibit antimicrobial, antiviral,anticarcinogenic activity. Based on an ethnopharmacologicalsurvey of Gonda district forests (India) in 1994 and other tribes inindia, plants which are widely used infolk medicine, **A. aspera roots** werereported for their usage in snake bites, tooth brush, strained back, diarrheatec. *In vitro* and *in vivo* studies of the root extract have found the spermicidal activity. The dried leaf powder mixed with honey is useful in the early stages of asthma (Srivastav *et al* 2011).

### **Geographical source and Morphology**

It is found on road sides, field boundaries and waste places as a weed throughout India up to an altitude of 2100 m in South Andaman Islands. *A.aspera* is a perennial stiff erect herb, 0.2- 2.0 , is growing up to 1000 m height. Stems are square, leaves elliptic ovateor broadly rhombate, 5- 22 cm long, 2.5 cm broad, pubescent. The inflorescences are 8 - 30 cm long, with many single, white or red flowers, 3 - 7 mm wide.

### **Phytochemistry and pharmaceutical importance**

The literature on *Achyranthes aspera* reports that the plant has large number of medicinal properties as well as medicinally important chemicals like achyranthine, ecdysterone, betaine, pentatriacontane, 6-pentatriacontanone, hexatriacontane and tritriacontane. Chemical investigations of the seeds of *Achyranthes aspera* reported the isolation & identification of Saponins A and B. Along with these constituents certain other constituents were also isolated like oleanolic acid, amino acids and hentriacontane. (Hariharan & Rangaswami, 1970 and Ali, 1993). The alcoholic extract of the roots of *Achyranthes aspera*, showed anti-inflammatory activity in Wistar rats using carrageenan-induced paw edema method and cotton pellet granuloma test (Vijaykumar *et al* 2009).

Plants and/or plant cells *in vitro*, reported to show enhanced yields of natural secondary metabolites. *Agrobacterium rhizogenes* mediated hairy root cultures have been proven as being a natural source for secondary metabolites that are normally biosynthesized in roots of differentiated plants. Successful development of regeneration of shoots is a prerequisite for clonal propagation and for genetic transformation.

## **Objectives of the Study**

- To standardize reproducible *in vitro* protocols for efficient callus induction, initiation of cell suspensions using media manipulations from various explants of *Achyranthes aspera*.
- To standardize and establish immobilized cell suspensions.
- To standardize a reproducible protocol for induction of hairy roots from different explants using *Agrobacterium rhizogenes*(wild strain) of rapid propagation of transgenic hairy roots at lab scale

## Methodology

1. Collection of *Achyranthes aspera* germplasm from at least three to four locations in Hyderabad, for selecting the material with high contents of secondary metabolites like alkaloids, saponins, flavonoids, glycosides, achyranthine using biochemical tests etc.
2. *In vitro* establishment of protocols for callus induction and cell suspensions : Optimization of media by manipulations using media additives and hormonal combinations, culture conditions etc.
3. Induction of Hairy roots from various explants of *in vitro* developed plantlets of *A.aspera*
4. Analysis of extracted samples : Biochemical and phytochemical analysis of the natural and *in vivo* developed plant extracts for the presence of alkaloids, glycosides, saponins etc.using standard procedures available in the literature (Edeoga *et al*2005, Harborne 1998).



*Fig A.aspera* dehusked seeds growing on sterilized filter paper.

## **Major Findings**

1. The plant materials were collected from various locations in Hyderabad and outside Hyderabad district.
2. The collected material was identified taxonomically as *Achyranthes aspera* by taxonomists at department of Botany, Osmania University, Hyderabad.
3. The shade dried materials were subjected to extraction using various solvents using Soxhlet apparatus.
4. In *in vitro* studies, seeds and field grown leaves were used and the surface sterilization protocol has been standardized
5. A protocol was optimized for efficient germination of *Achyranthes* seeds after testing various techniques of culture methods
6. In vitro induced callus from leaves was found to undergo browning after 20 days
7. Hence tested with activated charcoal and ADS for optimizing regeneration frequency efficiently
8. Two strains of *Agrobacterium rhizogenes* have been procured from MTCC, (MTCC 2364, MTCC532). Chandigarh, maintained and simultaneously being tested for their efficiency in hairy root induction using leaf and nodal explants
9. The regeneration frequency of callus was enhanced upto 90% compared to control 50% using activated charcoal in regeneration medium
10. The biochemical studies of leaf, root and stem extracts showed positive results inferring to contain alkaloids and tannins in *A.aspera*
11. The cell suspensions were established from leaf derived callus as an alternate for immobilization of callus for obtaining secondary metabolites.
12. Hairy roots were induced from leaf explants of *A aspera* using MTCC2364 strain.



# ICTPA 2019



**40<sup>TH</sup> MEETING OF PLANT TISSUE CULTURE ASSOCIATION - INDIA  
&  
INTERNATIONAL CONFERENCE ON TRENDS IN PLANT SCIENCES AND  
AGROBIOTECHNOLOGY 2019**

## Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms ..... Dr. Govidugari Vijaya Laxmi .....  
from ..... Chaitanya Bharathi Institute of Technology, Telangana, India ....., attended and participated in  
the "International Conference on Trends in Plant Sciences and Agrobiotechnology 2019" held at Indian  
Institute of Technology Guwahati, during February 14 - 16, 2019.

Organized by  
Department of Biosciences & Bioengineering and Centre for Rural Technology, IIT Guwahati, India  
In association with  
Plant Tissue Culture Association - India (PTCA-I)

Prof. Gautam Biswas  
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PLANT TISSUE CULTURE  
ASSOCIATION - INDIA  
(PTCA-I)

INTERNATIONAL CONFERENCE  
ON TRENDS IN PLANT SCIENCES  
AND AGROBIOTECHNOLOGY 2019  
(ICTPA-2019)



FEBRUARY 14 - 16, 2019

Organized by: Department of Biosciences & Bioengineering and Centre for Rural Technology, IIT Guwahati, Assam, India  
In association with: Plant Tissue Culture Association - India (PTCA-I)

November 22, 2018

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**Subject:** International Conference on Trends in Plant Sciences and Agrobiotechnology-2019 (ICTPA2019) and 40<sup>th</sup> Meeting of Plant Tissue Culture Association-India (PTCA-I), February 14-16, 2019, at Indian Institute of Technology Guwahati, Assam, India

**Abstract No.** ICTPA10086, *In vitro* studies in *Achyranthes aspera*, a medicinally important weed.

Dear Dr Govidugari Vijaya Laxmi:

Thank you for your interest in ICTPA 2019 Conference. The Program Committee for the above mentioned conference has reviewed your abstract and accepted it for presentation. The presentation type of your abstract will be provided to you at a later date.

On behalf of Organizing Team for the ICTPA 2019 Conference, we invite you to attend this most important conference concerning all phases of Plant Biotechnology. You will have the opportunity to participate in discussions during the presentations of approximately 350 scientific papers, including the excellent Keynote and Plenary Lectures.

Please visit our website at <http://www.ictpa2019.in/> for more information about registration and accommodation. All registrants are responsible for their own travel, accommodation, and registration costs.

We would be most pleased to have you join us at IIT Guwahati for International Conference on Trends in Plant Sciences and Agrobiotechnology.  
With best wishes

Rakhi Chaturvedi Conference Convener