

# Invitro Studies on *Achyranthes coynei* Santapau. for Micropropagation



**Dr. Pramod J Hurkadale** *M.Pharm., Ph.D., PDCR*

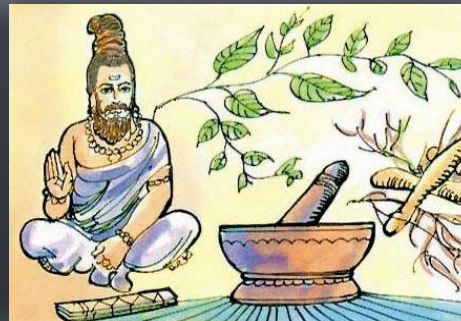
Professor & Head

Dept of Pharmaceutical Biotechnology  
K L E University's College of Pharmacy,  
Belagavi - 590 010, Karnataka, India.

[pramodh@kleuniversity.edu.in](mailto:pramodh@kleuniversity.edu.in)



Accredited 'A' Grade by NAAC  
Placed in Category 'A' by MHRD



Folk Herbal Medicine



Accredited 'A' Grade by NBA

# Study Area



**a: Semi-Evergreen Forests (*Plateau Near Kanakumbi*)**

**b: Moist & Dry Deciduous Forests (*Near Kinaye*)**

**c: Scrub & Thorn Forests (*Near Gokak During Rainy Season*)**





Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

journal homepage: [www.elsevier.com/locate/jep](http://www.elsevier.com/locate/jep)



## Ethnomedicinal plants used to treat bone fracture from North-Central Western Ghats of India

Vinayak Upadhyaya<sup>a</sup>, Harsha V. Hegde<sup>a,\*</sup>, Shripad Bhat<sup>a</sup>, Pramod J. Hurkadale<sup>b</sup>, S.D. Kholkute<sup>a</sup>, G.R. Hegde<sup>c</sup>

<sup>a</sup> Regional Medical Research Centre, ICMR, Nehru Nagar, Belgaum 590010, India

<sup>b</sup> College of Pharmacy, KLE University, Nehru Nagar, Belgaum 590010, India

<sup>c</sup> PG Department of Botany, Karnatak University, Dharwad 580003, India

### ARTICLE INFO

#### Article history:

Received 17 January 2012

Received in revised form

12 April 2012

Accepted 26 May 2012

Available online 2 June 2012

#### Keywords:

Bone fracture  
Ethno-medicine  
Medicinal plants  
Western Ghats

### ABSTRACT

**Ethnopharmacological relevance:** North Central Western Ghats in India comprises rich bio-cultural diversity and is also home to varied ethnomedicinal practices. The study was taken up for documentation and analysis of traditional knowledge regarding the practice and use of plants in the treatment of bone fracture. This is an effort to preserve the vanishing wealth of traditional knowledge. **Materials and methods:** Key informants identified in a preliminary survey and collection of information was through semi structured, open ended interviews. The details on age, place of practice, experience of key informants and learning of practice, disease they treat and mode of diagnosis, storage and usage of plants were collected. The identity of plants and their information was confirmed through repeated guided transect walks in different seasons with the informants and focus group discussions. Identified plants were deposited at the herbaria of Regional Medical Research Centre, Belgaum.

**91 medicinal plant species belonging to 51 families used to cure 64 types of diseases.**

reduction and speedy recovery and *Gmelina arborea* has the highest use value (0.27).

**Conclusions:** The results indicated the importance of traditional herbal practices in community for their health needs. Both conservational strategies and further validation studies are the need of the hour for better utilization and sustenance of the documented knowledge.

# Plate: 1



a: *Abelmoschus esculantus* (L.) Moench.  
(Malvaceae)

b: *Actinopteris radiata* (Sw.) Link.  
(Actinopteridaceae)

c: *Aerva lanata* (L.) Juss.  
(Amaranthaceae)

d: *Aristolochia tagala* Cham.  
(Aristolochiaceae)

e: *Cassia fistula* L.  
(Caesalpiaceae)

f: *Catharanthus roseus* (L.) G. Don.  
(Apocynaceae)

g: *Cinnamomum tamala* (Ham.) Nees & Eberm  
(Lauraceae)

h: *Cissus quadrangularis* L.  
(Vitaceae)



# Plate: 2



a: *Pedalium murex* L.  
(Pedaliaceae)

b: *Rauvolfia serpentina* (L.) Benth. ex Kurz.  
(Apocynaceae)

c: *Rauvolfia tetraphylla* L.  
(Apocynaceae)

d: *Remusatia vivipara* (Roxb.) Schott  
(Araceae)

e: *Saraca asoca* (Roxb.) de Wilde  
(Caesalpiniaceae)

f: *Solanum nigrum* L.  
(Solanaceae)

g: *Tinospora cordifolia* (Willd.) Merrill.  
(Menispermaceae)

h: *Withania somnifera* (L.) Dunal  
(Solanaceae)



# Plate: 3



a: *Achyranthes aspera* L.  
(Amaranthaceae)

b: *Achyranthes coynei* Sant.  
(Amaranthaceae)

c: *Asparagus racemosus* Willd.  
(Asparagaceae)

d: *Asparagus gonoclados* Baker  
(Asparagaceae)

e: *Piper betle* L.  
(Piperaceae)

f: *Piper longum* L.  
(Piperaceae)

g: *Piper nigrum* L.  
(Piperaceae)

h: *Piper trichostachyon* (Miq.) C. DC.  
(Piperaceae)



# *Achyranthes coynei* Santapau.



a: *Achyranthes coynei* Habitat  
b: Petiole

c: Ventral View of Leaf  
d: Dried Leaves  
e: Leaf Powder

## Short Communication

# Phenolic Contents and Antioxidant Properties from Aerial Parts of *Achyranthes coynei* Sant

V. UPADHYA, S. R. PAI\*, G. ANKAD, P. J. HURKADALE<sup>1</sup> AND H. V. HEGDE

Regional Medical Research Centre, ICMR, Nehru Nagar, <sup>1</sup>KLES College of Pharmacy, KLE University, Nehru Nagar, Belgaum-590 010, India

Upadhyia, *et al.*: Antioxidant Properties of *A. coynei*

Aim of the study was to evaluate antioxidant activity and total phenolic content of *Achyranthes coynei*; an endemic plant used in treatment of several diseases in the same lines that of *Achyranthes aspera* by traditional practitioners of Belgaum region. Efficiency of extraction methods was studied for aerial parts (leaves, stem, and inflorescence) extracted in methanol using continuous shaking, microwave assisted and ultra sonic extraction technique, by exposing it for different time period. Total phenolic content was measured by Folin-Ciocalteu method and antioxidant activity using 2,2'-diphenyl-1-picryl hydrazyl radical scavenging assay and ferric reducing antioxidant power assay. Extracts of *A. coynei* revealed highest yield of total phenolic content in continuous shaking method compared to other methods. Significantly higher amount of phenolic content ( $467.07 \pm 23.35$  tannic acid equivalent and  $360.83 \pm 18.04$  caffeic acid equivalent mg/100 g FWD) was estimated at 360 min of continuous shaking.

**The present study reports TPC and antioxidant activity of *A. coynei* for the first time.**

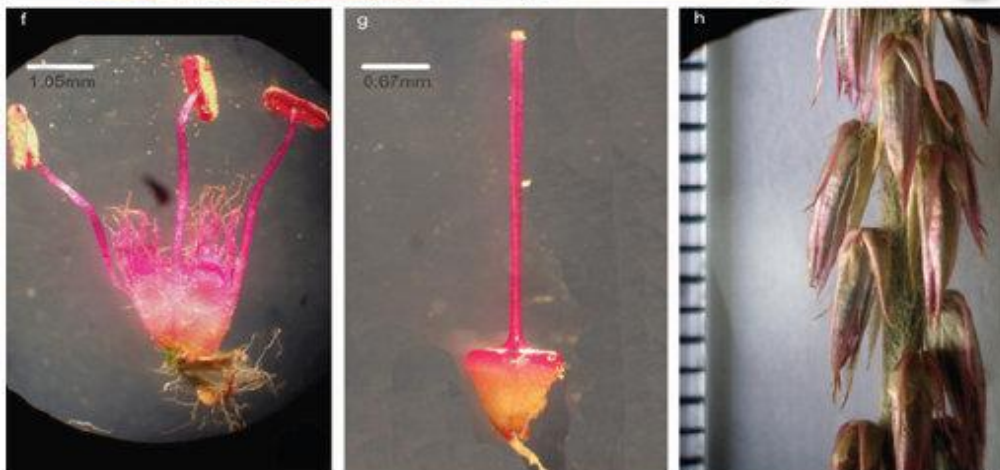
Key words: Antioxidant activity, *Achyranthes coynei*, endemic, total phenolic content



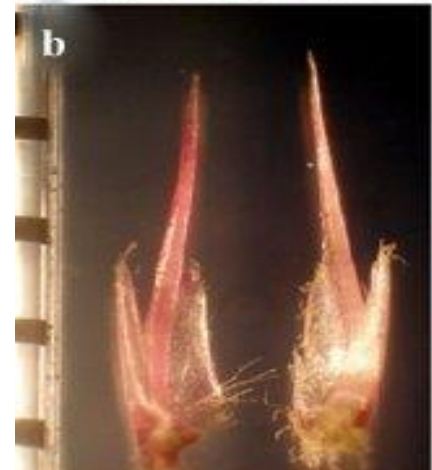
# Botanical Description of *Achyranthes coynei* Santapau.



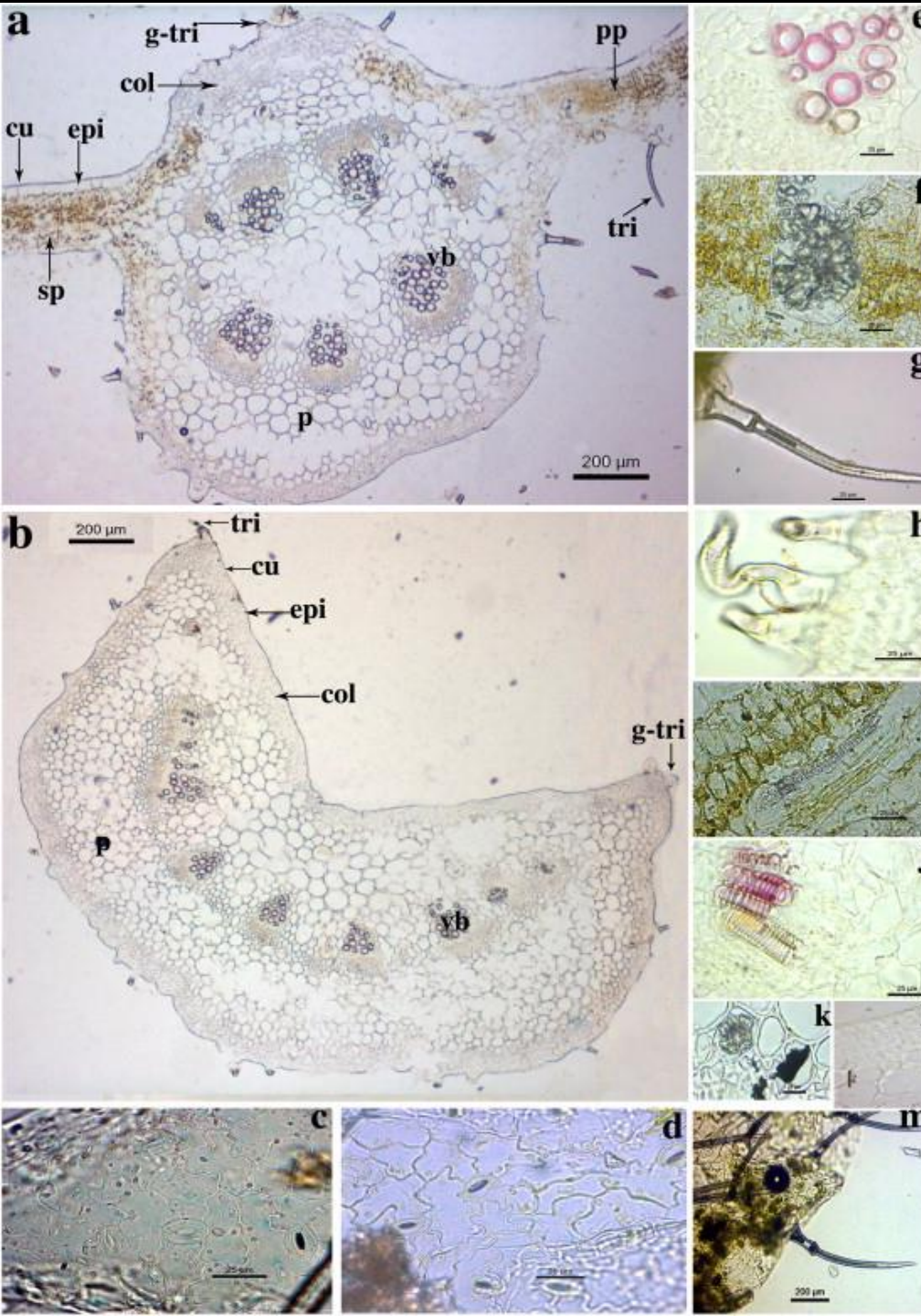
**(A);**  
a-habitat,  
b-inflorescence,  
c-flower,  
d-flower bud,  
e-bract,  
f-androecium,  
g-gynoecium,  
h-fruits.



**(B);**  
a- inflorescence:  
flower first erect,  
later patent,  
finally deflexed.  
b-bractioles.



# Microphotography



- a: TS of leaf
- b: TS of petiole
- c: Lower epidermis
- d: Upper epidermis
- e: Vascular bundles
- f: Calcium oxalate crystal
- g: Long, multi-cellular warty trichome
- h: Glandular trichome with blunt apex
- i: Palisade & spongy parenchyma cells
- j: Xylem vessels
- k: Calcium oxalate crystals
- l: Collenchyma cells
- m: Epidermis with trichomes
- col: Collenchyma cells
- cu: Cuticle
- e: Epidermis
- g-tri: Glandular trichome with blunt apex
- p: Parenchyma
- pp: Palisade parenchyma
- tri: Long, multi-cellular warty trichome
- vb: Vascular bundle
- sp: Spongy parenchyma.



HOSTED BY

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

ScienceDirect

journal homepage: <http://ees.elsevier.com/ejbas/default.asp>

Full length article

# Pharmacognostic evaluation of *Achyranthes coynei*: Leaf



Gireesh M. Ankad<sup>a</sup>, Sandeep R. Pai<sup>a</sup>, Vinayak Upadhya<sup>a,\*</sup>,  
Pramod J. Hurkadale<sup>b</sup>, Harsha V. Hegde<sup>a</sup>

<sup>a</sup> Regional Medical Research Centre, Indian Council of Medical Research, Nehru Nagar, Belgaum 590 010, Karnataka, India

<sup>b</sup> College of Pharmacy, KLE University, Nehru Nagar, Belgaum 590 010, Karnataka, India

## ARTICLE INFO

### Article history:

Received 22 October 2014

Received in revised form

2 December 2014

Accepted 6 December 2014

Available online 19 December 2014

### Keywords:

Amaranthaceae  
*Achyranthes coynei*  
Endemic  
Pharmacognosy  
Physicochemistry  
HPTLC

## ABSTRACT

*Achyranthes coynei* Sant., (Amaranthaceae) is a rare, medicinal shrub, endemic to India. The plant especially, leaves are used in treatment of various disorders by folk healers. It has been scientifically evaluated for its antimicrobial and antioxidant properties. Pharmacognostic studies have not been carried out so far in this plant. So, the present study was undertaken to evaluate pharmacognostic characters of leaf of *A. coynei*. The studies were carried out in terms of morphological, microscopic characters and physicochemical parameters using standard methods. Leaf size (15–23 × 7–10 cm) and petiole nature (pink above and green beneath) were the distinguishing morphological character observed in the present study. Transverse section of leaf and petiole showed presence of large multi-

# First Time Reported

micro elements and radioactive factors were examined. Leaf powder showed fluorescence under the influence of different solvents. Preliminary phytochemical screening showed the presence of alkaloids, saponins and triterpenoids. High Performance Thin Layer Chromatographic (HPTLC) analysis yielded eight bands in leaf extract. The study forms the first report on pharmacognostic characters for *A. coynei*, which could be useful for identification and authentication of the plant.

Copyright 2014, Mansoura University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/>

# Need for the Study

- Amaranthaceae family consists of 850 sps. belonging to 71 genera ,out of which 60 sps under 20 genera are found in India.
- *A.coynei* ,an endemic plant (survey report by Pai SR.et al. (2011), it was found indifferent localities of Belagavi district).
- Possesses antimicrobial activity against pathogen like *S.epidermis*, *B.subtilis*,*S.aureus*,*P.aeruginosa* and *A.niger*.
- It is an endangered species and requires immediate action.
- Collection of plant material from the natural habitat is restricted to a few months in a year.
- The plants falls in 'RARE' category of IUCN.
- Hence ,it is worth to work on *in vitro* plant tissue culture.



# Objectives

- Collection of plant material
- Standardization of surface sterilization
- Initiation and establishment of cultures.
- Initiation of multiple shoots.
- Initiation of rooting.
- Hardening and acclimatization.
- HPLC analysis for determination of betulinic acid, oleanolic acid and ursolic acid contents in *in vitro* raised plants.

# Methodology

1

- Sterilization of equipments and glasswares
- 121°C /15 lbs pressure / 20 min.

2

- Preparation of media
- Preparation of stock solution and working media

3

- Explant preparation
- Surface sterilization

4

- Standardization of protocol
- Shoot and root induction).

5

- Maintenance 25±2°C /60-70% humidity under 16 h light / 8 h dark photoperiod (cool white fluorescent light; 40lmol m<sup>-2</sup>s<sup>-1</sup>).
- Sub culturing
- Hardening and acclimatization



# Shoot Initiation



- MS Media fortified with combination of cytokinin 3mg/L, 0.5mg/L TDZ & 0.5mg/L KN
- Cytokinins BAP, KN, TDZ with different conc. (0.5, 1.0, 1.5, 2.0, 3.0 mg/L) were used for shoot initiation and multiple shooting.
- Subculturing done routinely in fresh medium with same concentration
- Showed synergistic effects with higher efficiency of multiple shoot induction.
- Shoots were raised with different combinations of cytokinins.

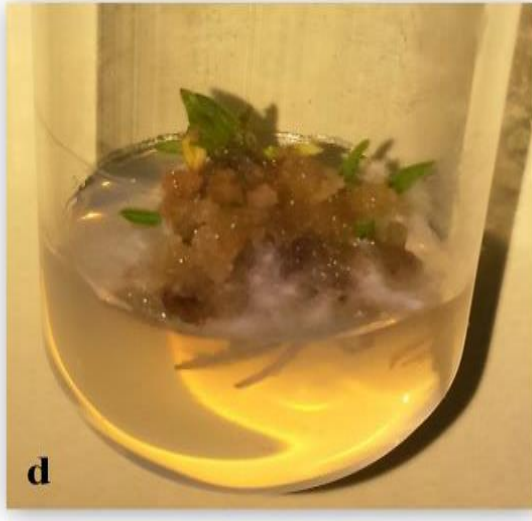
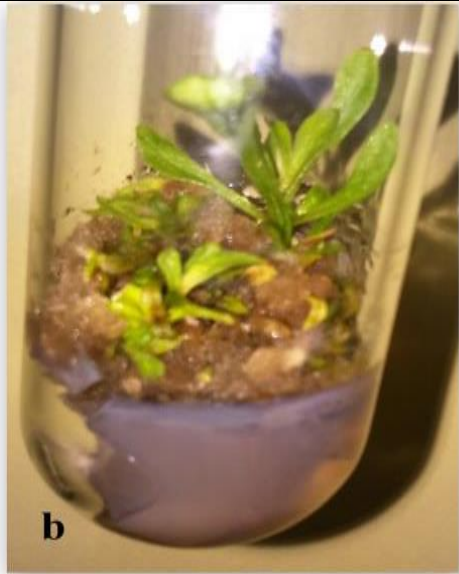
# Elongation of Shoots and Roots in MS media containing Combination of 3.0 mgL<sup>-1</sup> BAP, 0.5 mgL<sup>-1</sup> TDZ and 0.5 mgL<sup>-1</sup> KN





Root induction and callus formation on MS medium with 0.5 mgL<sup>-1</sup> NAA.

Extensive root system on MS medium with 0.5 mgL<sup>-1</sup> IBA.

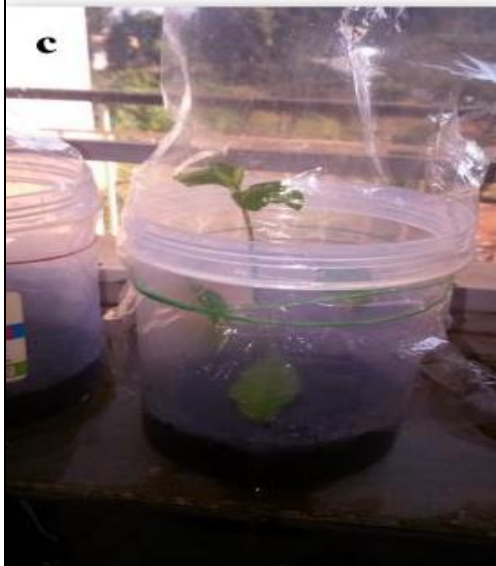


# Measurement of Shoot Length & Root Length





# Hardening and Acclimatization

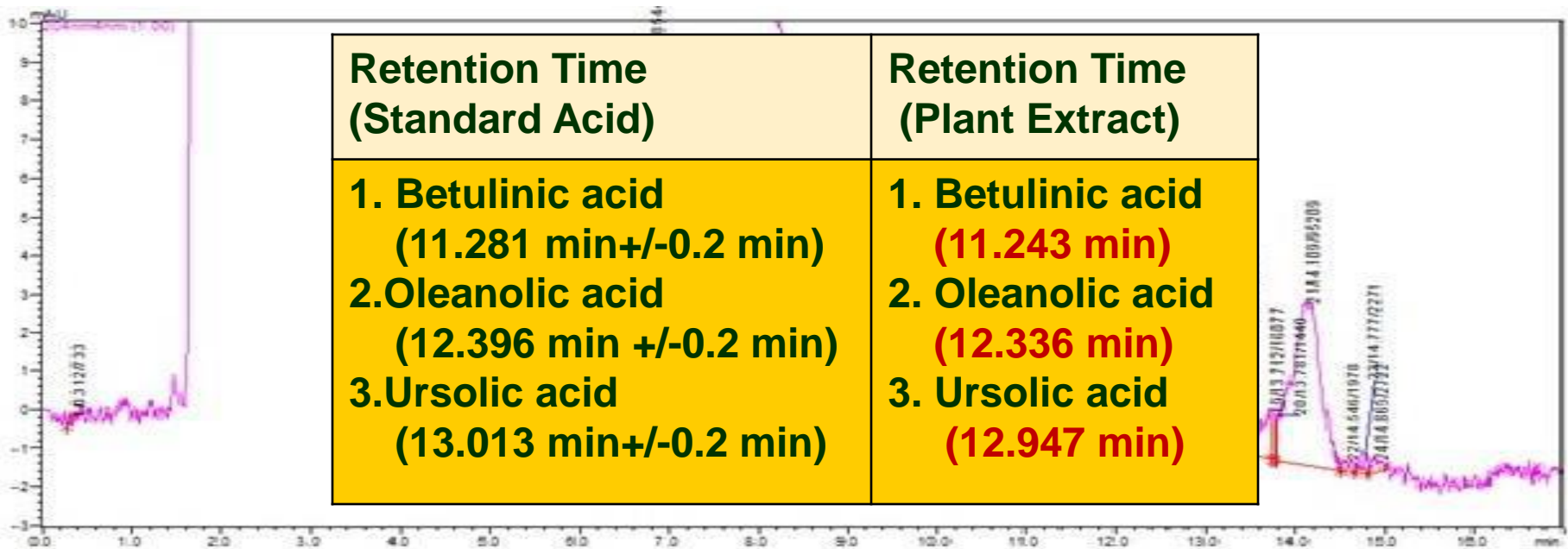


Mixture of Soil, Sand & Peat (4:3:3)

Survival Rate - 60%

# Analysis

- Determination of Betulinic acid, Oleanolic acid, Ursolic acid from the methanolic extract of in vitro tissue culture plants of *Achyranthes coynei* was performed.



HPLC chromatogram of methanolic extract of in vitro tissue culture plants of *A. coynei*

A:Betulinic acid, B:Oleanolic acid, C: ursolic acid



# Conclusion



- Protocol described is useful for *in vitro* micro propagation of *Achyranthes coynei*.
- Shoot tips as explants were good source for shoot multiplication, rooting and plant regeneration.
- A combination of ( $3\text{mgL}^{-1}$  BAP,  $0.5\text{mgL}^{-1}$  TDZ &  $0.5\text{mgL}^{-1}$  Kinetin) in MS medium showed maximum no. of shoots.
- Shoots thus formed, when placed in ( $0.5\text{mgL}^{-1}$  IBA) showed maximum rooting.
- Different concentrations of auxins produced callus and  $0.1\text{mgL}^{-1}$  NAA showed a good callus growth.
- Successful hardening response of 60% was reported.
- First report regarding the micro propagation of *A.coynei*.

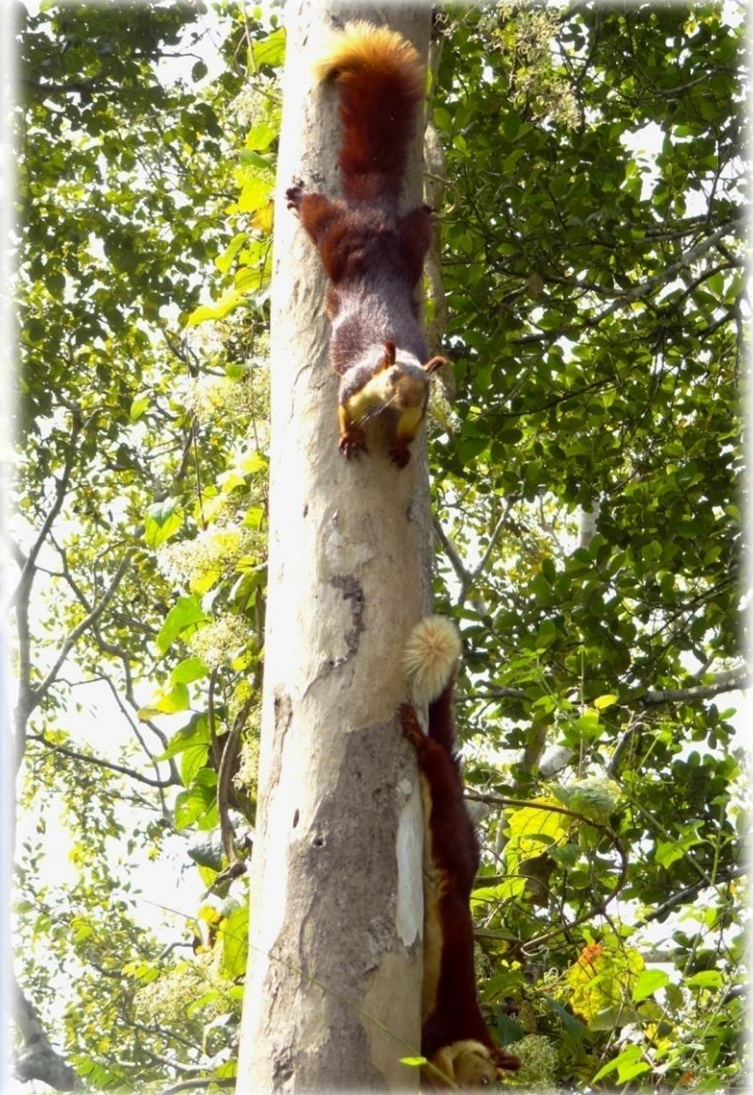
# “Globalizing Local Knowledge & Localizing Global Technologies.”



“National Level Field Workshop on Medicinal Plants in Western Ghats”  
Venue: Nature Camp, Kulgi, Dandeli, Karnataka. 6th to 8th January 2015



# Endangered Species from Western Ghats





# Sykes Point @ Dandeli, Uttara Kannada



**Explore, Experiment, Protect and Conserve**



# Thank You



Visit us @ [www.kleuniversity.edu](http://www.kleuniversity.edu)