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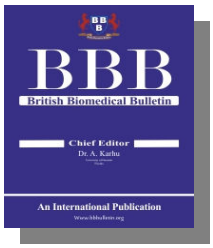

Original

In vitro Callus Regeneration and Biochemical Analysis in the Medicinal Plant *Phyllanthus niruri* L.

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ABSTRACT

Objectives
The main objective of the present study is to find out the callus initiation in the plant *Phillanthus niruri* and to determine its biochemistry.
Method
The callus formation was done with MS medium and its biochemical analysis was done with the standard methods [12 - 14].
Result
Callus growth was more in explants taken from apical bud and nodal segment nearer to the apical bud. But the callus growth was delayed in explants taken from mature leaves and nodal segments away from the apical bud. In the phytochemical analysis Amino acid, protein, total sugar, starch and phenol content was more in aerial part of the *P. niruri*.
Conclusion
The callus growth is more from apical bud than compared to matured leaves and nodal segments far from apical bud. The phytochemicals such as Amino acid, protein, total sugar, starch and phenol were more in apical parts than the matured portion.

Introduction

The genus *Phyllanthus* (Euphorbiaceae) has containing 550 to 750 species and most of them produce valuable secondary metabolites which have been extracted from whole plants[1]. In Brazil, infusion of leaves stems and roots of *Phyllanthus* sps are used in folk medicine for treating intestinal infections, diabetes, the hepatitis B virus and disturbances of the kindly and urinary bladder[2]. Several compounds such as alkaloids, tannins, falconoid, lignans, Phenols and terpenes

have been isolated and identified in various species of *Phyllanthus* and have shown anticipative action in mice and other therapeutic activities[3]. Antiviral effects against hepatitis B virus[4] and possibly against the reverse transcriptase of retroviruses hence also have been reported[5-6]. The few studies available on the tissue culture of *Phyllanthus, P. abnorms*[7], *P. Carolinians*, *P. tenellus*[8] and *P. niruri* and transformed root cultures of *P. nirur*[9]. Additional studies on callus and root extracts of these different species have shown the presence of phyllemblin, tannin which has antimicrobial activity, of possible hydrolysable tannins which inhibited DNA polymerase and reverse transcriptase, of grain and its derivatives which showed high activity in the inhibitions of HIV reverse transcriptase and angiogenesis - Converting enzyme involved in diabetic complications[10]. *Phyllanthus niruri* is also used in folk medicine by patients with urolithiasis. Previous reports showed that administering an infusion of Pn to patients with renal calculi was effective in promoting stone elimination and had an inhibitory effect on the formation of stones in as experimental model of calcium oxalate (Ca OX) Lithia sis in rats[9]. Chronic toxicity, supporting the therapeutic potential of Pn. Many factors are involved in the pathogenesis of urolithiasis.

Although the presence of a supersaturated milieu is necessary for precipitating CaOx (Present in most calculi) acting as a promoter of crystal formation, this is not enough to from a stone, as urine is normally a supersaturated solution and only some individuals are prone to this disease. One reason for this is the presence of inhibitors of litho genesis in urine, including macromolecules, proteins, citrate and magnesium [11] thus, an imbalance between the promoter and inhibitors may present a potential factor in litho genesis. The present study was undertaken to callus initiation and to determine bio chemistry of *Phillanthus niruri* callus and aerial part of the plant.

Materials and Methods

Plant material

The aerial parts of *Phillanthus niruri was* collected from Kollimalai hills which are situated on Eastern Ghats at an altitude of 1200 mts in the Namakkal District and are 45 km from Namakkal town. The Kollimalai Hills are known for medicinal herbs and plants that grow in abundance on the hill slopes. The collected plant parts were washed, stored in sterile ice box and taken to lab for the studies.

Phillanthus niruri grows up to 70 cm hight and has ascending herbaceous branches. Its bark is smooth and light green in colour. It bears numerous pale green flowers which are often which turn red while ripe. The fruits are small, smooth capsules and contain seeds.

Induction of callus from explants

The apical bud and nodal segment nearer to the apical bud of *Phillanthus niruri* were used as explants for initial culture Basal medium used for initial set of experiment for shoot proliferation consisted of MS salt with 3% (W/V) sucrose, and 0.8% (W/V) agar. The explants were cultured on MS medium supplemented with different concentration kinetin-BAP, IBA, IAA, NAA. In all cases, the pH of the media was adjusted to 5.7 – 5.9 before autoclaving at 121 ˚C for 15min. All cultures were incubated at 25˚C ± 2˚C under a 16-h photoperiod at 63 mol m−2 s−1 PAR at plant level produced by Philips TDL fluorescent light tubes.

The explants were surface sterilized with 60% alcohol for 5 min and 0.1% HgCl2 for 10 min, then rinsed three times in sterilized water. Explants were place on agar- solidified culture medium in the

culture tubes. The basal medium consisted of salts and vitamins of MS medium and solidified with 0.8% (w/v) agar. The medium was adjusted to pH 5.8 before adding agar, and then sterilized by autoclaving at 121ºC for 15 min. All the cultures were maintained at 25± 2ºC under 16/8 h light/ dark conditions. The leaf explants was cultured on MS medium supplemented with various concentration of auxins [NAA, IBA, and 2, 4-D (0.5 – 2.5 mg/l)] in combination with cytokinin [BAP and KIN (0.5 – 2.5 mg/l)] for callus induction. The effect of growth regulators on callus induction response was studied and an effort was made to determine the appropriate growth regulator combination for optimal callus growth. Callus induction could be observed after 40 - 50 days.

Bio chemical analysis

1. Estimation of starch[12]

Callus tissue 1gm is cut into piece and added with 20 ml of distilled water and ground well with the help of pestle. The centrifuged and filtered supernant was used as sample. With 3 ml callus tissue supernant sample, 2 ml of Iodine reagent were added, to measure in colorimeter at 660nm. All the reagents except the extract were mixed and used as blank.

2. Estimation of total sugars[13]

Callus tissue 1gm is cut into piece add distilled water 20 ml was ground in a pestle were centrifuge filtered with supernant sample. With 1 ml callus tissue supernant sample ii) 2 ml Anthrone reagent were added, To measured in colorimeter at 620nm. All the reagents except the extract were mixed and used as blank.

3. Estimation of the amino acid[14]

1gm of callus tissue was cut into piece and added 20 ml of distilled water. It was ground into pestle and centrifuged. 4 ml of the supernant of the sample was added with 1 ml of Nin hydrin reagent to produce pint colour. At last it was measured in colourimeter at 550 nm. All the reagents except the extract were mixed and used as blank.

4. Estimation of protein[15]

1gm of callus tissue was cut into piece and added 20 ml of distilled water. It was ground into pestle and centrifuged. 2 ml of callus tissue supernant sample was taken and 1ml TCA, O.1N NaOH and 2ml Alkaline Copper reagent were added to produce dark blue color, to measured in colorimeter at 650nm. All the reagents except the extract were mixed and used as blank.

5. Estimation of total phenols[16]

Cut pieces of callus tissue 1gm and distilled water 20 ml were ground in a pestle. It was centrifuged and filtered with supernant sample. 1 ml callus tissue supernant sample, 2 ml Sodium carbonate and 1ml Folin Ciocalteu's reagent were added to produce blue color. It was measured in colorimeter at 650nm. All the reagents except the extract were mixed and used as blank.

Results

In *phyllanthus niruri L.,* Callus initiation and Biochemical study was carried out for the present study. Various parts such as nodal segments from different nodes, apical buds, young and mature leaves were taken as explants.

Callus proliferation was varied from one expand, to another explants even when they were inoculated on M.S medium containing same concentration of different growth hormones. The callus growth was enhanced, an explants taken from apical bud and nodal segment nearer to the apical bud

when it was inoculated on M.S. medium containing 0.5 mg/1 NAA and 0.5mg/1IBA. However callus growth was similar on explants taken from young leaves (Plate – 1). when inoculated on M.S. Medium containing 1.0 mg/1 BAP + 1 .0mg/1 NAA and 0.5 mg/1 IBA. The callus proliferation was slow in explants taken from mature nodal segments and mature leaf bits, even when it was inoculated on M.S. medium containing 2.0mg/1 BAP+2.0mg/1 IBA+1.5mg/NAA. There was no such observation recorded by previous workers, in *Phyllanthus niruri L.* From this study, it is revealed that the callus proliferation was more in young tissue which is having more meristamatic cells, than the mature cells taken from nodal segments away from apical bud and matures leaves. However all the callus possesses green pigments; this may be due to activity proto chlorophyll during the culture period. All type of explants showed granular callus and are having active growth. Twenty five to thirty days old callus was taken for biochemical studies.

The biochemical study of callus was compared with aerial part of the *Phyllanthus niruri L.* The amino acid contents was 483.1 mg/g in aerial part of plant but it was 291.6mg/g in 25 days old callus protein content was 326.4mg/g in aerial part the plant and it was 208.08mg/g in 30 days old callus Total sugar was 287.8mg/g in aerial part of the plant and it was 196. 1mg/g in 27days old callus . The starch content was 181.3mg/g in aerial part of the plant and it was 126.9mg/g in 29days old callus The phenol content was 376.2mg/g in the aerial part of plant and it was 264.6mg/g in 30 days old callus (Figures – 1 to 4). The present study revealed that all the biochemical substances were more in the aerial part of the plant when compare to callus. Previous workers stated that the photochemical screening revealed the presence of steroids, terpenes and falconoid as the major photochemical groups, but not the same constituents belonging to these groups in all extracts. Alkaloids were detected in whole plant extract and were in traces in the intact epical stem extracts and callus extracts.

Conclusion

In this studies it is concluded that callus proliferation was varied from one expand to another even though they were inoculated on M.S medium containing same concentration of different growth hormones. The callus proliferation was slow in explants taken from mature nodal segments and mature leaf bits. From this study, it is also revealed that the callus proliferation was more in young tissue.

The amino acid content was high in aerial part than the matured portions. Protein content was noted to reduce towards the age of the callus. Total sugar, starch and phenol contents were higher than the old callus. The present study revealed that all the biochemical substances were more in the aerial part of the plant when compare to callus.

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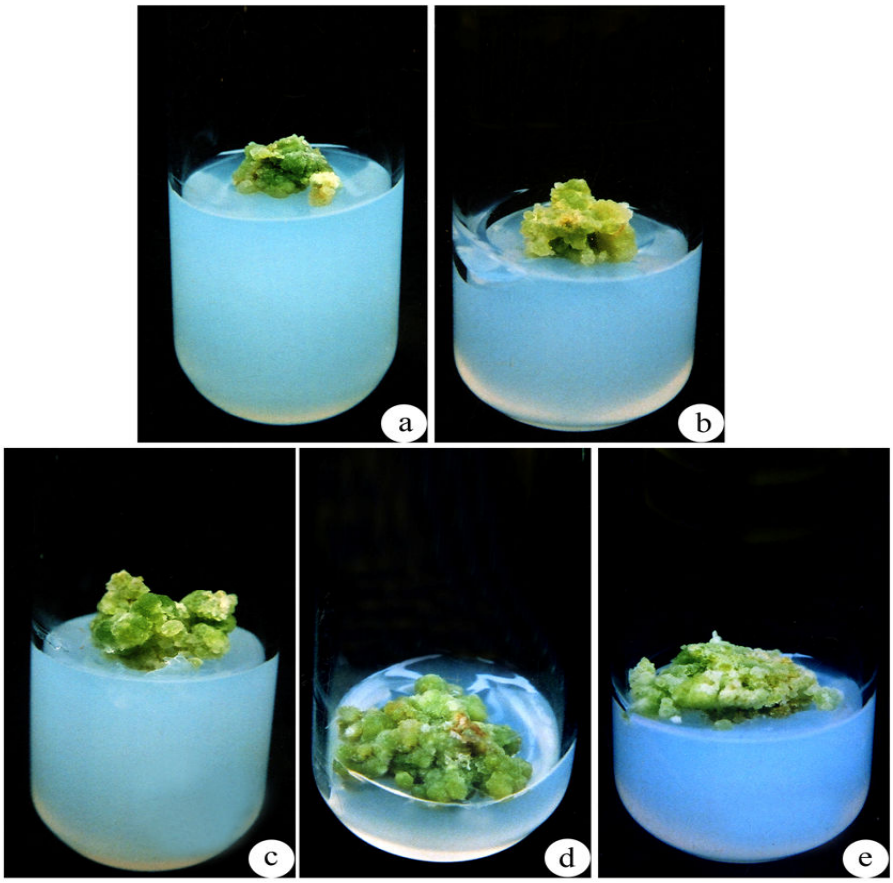
Plate - 1



Callus induction in *Phyllanthus niruri* L.

a) Callus derived from matured leaf in MS medium fortified with 2.0mg/l BAP + 2.0 mg/l IBA + 1.5mg/l NAA.
b) Callus derived from nodal explant in MS medium fortfied with 2.0mg/l BAP 2.0mg/l IBA + 1.5mg/l NAA.
c) Callus derived from young leaf in MS medium fortified with 1.0mg/l BAP+ 1.0mg/l NAA + 0.5 mg/l IBA.
d) Callus derived from apical bud explants in MS medium fortified with 0.5mg/l BAP + 0.5mg/l NAA + 0.5mg/l IBA.
e) Callus derived from nodal explants in MS medium fortified with 0.5mg/l BAP+ 0.5mg/l NAA + 0.5mg/l IBA.

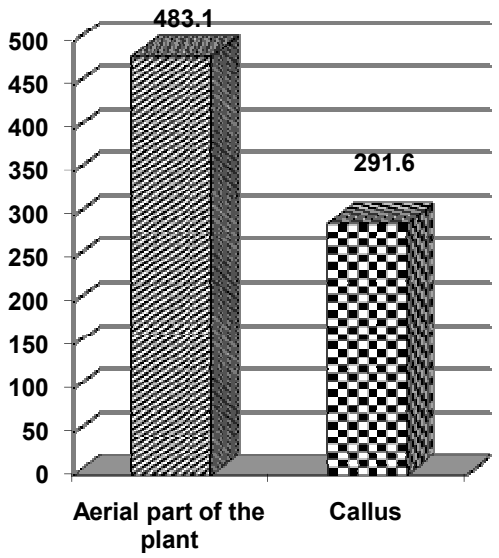


Figure 1. Estimation of amino acids in *Phyllanthus niruri .L*

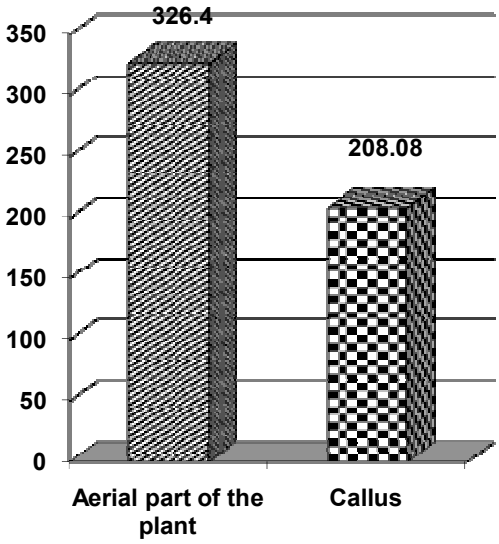


Figure 2. Estimation of protein in *Phyllanthus niruri. L.*

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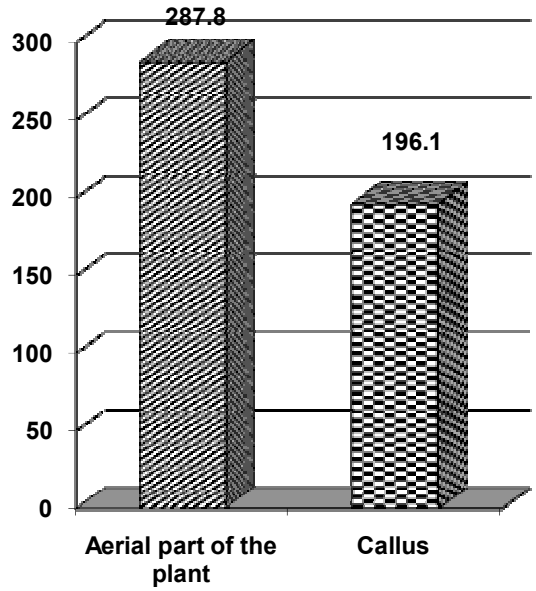

Figure 3. Estimation of sugar in *Phyllanthus niruri* L.

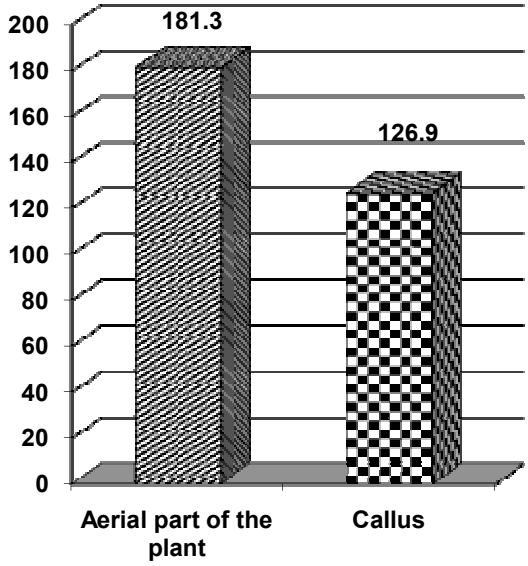

Figure 4. Estimation of starch in *phyllanthus niruri* L.

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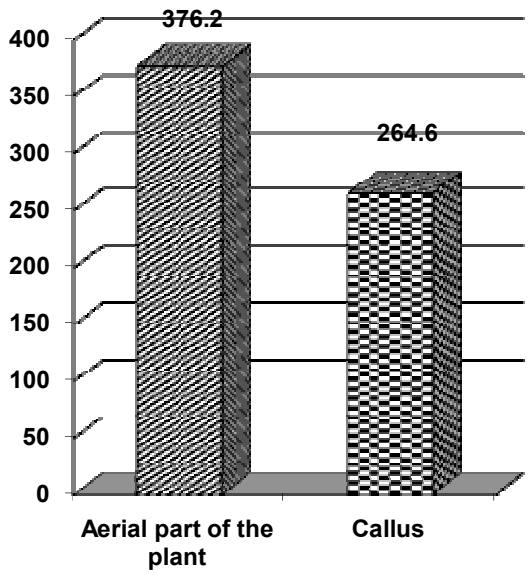

Figure 5. Estimation of total phenols in *Phyllanthus niruri* L.