Screening and Evaluation of in vivo and in vitro Generated Insulin Plant (Vernonia divergens) for Antimicrobial and Anticancer Activities

Santosh Kumar, Anand Prakash, Kanak Sinha, and Anita K Verma

Abstract—Vernonia divergens Benth., commonly known as “Insulin Plant” (Fam: Asteraceae) is a potent sugar killer. Locally the leaves of the plant, boiled in water are successfully administered to a large number of diabetic patients. The present study evaluates the putative anti-diabetic ingredients, isolated from the in vivo and in vitro grown plantlets of V. divergens for their antimicrobial and anticancer activities. Sterilized explants of nodal segments were cultured on MS (Murashige and Skoog, 1962) medium in presence of different combinations of hormones. Multiple shoots along with bunch of roots were regenerated at 1mg l⁻¹ BAP and 0.5 mg l⁻¹ NAA. Micro-plantlets were separated and sub-cultured on the double strength (2X) of the above combination of hormones leading to increased length of roots and shoots. These plantlets were successfully transferred to soil and survived well in nature. The ethanol extract of plantlets from both in vivo & in vitro sources were prepared in soxhlet extractor and then concentrated to dryness under reduced pressure in rotary evaporator. Thus obtained concentrated extracts showed significant inhibitory activity against gram negative bacteria like Escherichia coli and Pseudomonas aeruginosa but no inhibition was found against gram positive bacteria. Further, these ethanol extracts were screened for in vitro percentage cytotoxicity at different time periods (24 h, 48 h and 72 h) of different dilutions. The in vivo plant extract inhibited the growth of EAC mouse cells in the range of 65, 66, 78, and 88% at 100, 50, 25 & 12.5μg mL⁻¹ but at 72 h of treatment. In case of the extract of in vitro origin, the inhibition was found against EAC cell lines even at 48h. During spectrophotometric scanning, the extracts exhibited different maxima (λ) - four peaks in in vitro extracts as against single in in vivo preparation suggesting the possible change in the nature of ingredients during micropropagation through tissue culture techniques.

Keywords—Anti-cancer, Anti-microbial, EAC mouse cell, Tissue culture, Vernonia divergens.

I. INTRODUCTION

Nature has been a good source of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from them. Various medicinal plants have been used for years in daily life to treat diseases all over the world. The use of traditional plant extracts as well as other alternative forms of medicinal treatments have been getting momentum since the 1990s [1]. Vernonia divergens Benth (Fam.: Asteraceae), commonly known as insulin plant, is a potent sugar killer and is used as an excellent medicine for diabetes mellitus. India is known as a diabetogenic country. Diabetes is one of the most common diseases in human population. In many developing countries of the world, interest in native plant remedies has continued to increase as a result of the growing awareness of the importance of medicinal plants in health care delivery [2]. This interest now extends to many urban and developed communities including parts of Europe and America[3, 4]. In Nigeria and many other poor African countries, the belief in traditional herbal medicine is strong [5, 6]. Natural products are generally known to be efficacious with minimal side effects, but some natural compounds have been reported to act as mutagens and/or carcinogens. These genotoxic insults often lead to cancer, Alzheimer’s disease and other chronic degenerative diseases, such as atherosclerosis and heart diseases, which are the leading cause of death in human and animal populations. Plant derived compounds are a major area of interest for safer and more effective antimicrobial and anticancer agents.

The present work is targeted to screen and evaluate the in vivo and in vitro generated plantlets, Vernonia divergens for antimicrobial and anticancer activity.

II. MATERIALS & METHODS

The plant, Vernonia divergens, grown in the SAP garden of DRS Department of Botany, B.R.A. Bihar University, Muzaffarpur, established under UGC- SAP scheme was used as experimental material. Nodal segments (2-3 cm) as explants were excised from young plants. The explants were washed with 5% (v/v) teepol solution for 10 min, surface sterilized with 0.2% HgCl₂ for 2-3 min and rinsed 3-4 times with sterile double distilled water. Explants cultured with solid MS medium [7] containing 0.8% agar, 3% sucrose an supplemented with different concentrations of auxin and cytokinin. The pH of each medium was adjusted to 5.8 before
C. In Vitro Cytotoxicity Assay

Cytotoxicity assay was carried out in accordance with previously published protocol [8]. EAC (Ehrlich’s Ascitic Carcinoma) cells (5 x 10^3 cells/well) were cultured on a flat-bottomed 96 well plate. After 48 hours incubation, 2µL of stock solution was added to each well of the assay plate, which was then incubated for 2 hours at 37°C. After incubation, the formazan crystals formed by the reduction of tetrazolium salt by the mitochondria of living cells, were dissolved in DMSO. The plates were read in ELISA plate reader at wavelength of 540 nm.

D. Antimicrobial Activity (Agar well diffusion method)

The extracts form leaf, were studied for antimicrobial activity. A loop full of standard strains were inoculated in 30mL nutrient broth in a conical flask and incubated for 24 hr to activate the strains. In agar well diffusion method [9] the media and test bacteria cultures were poured into Petri dishes. The test strain, 0.25mL was inoculated into the media. Care was taken to ensure the proper homogenization. The experiment was performed under strict aseptic condition. After the medium was solidified, a well was made in the plates with sterile bore (6mm). The extracted compound (0.05mL & 0.1mL) was introduced into the well and the plates were inoculated at 37°C for 24 hr. All samples were tested in triplicates. Microbial growth was determined by measuring the diameter of the zone of inhibition against control i.e. without test compounds. The results were observed as zone of inhibition in mm.

III. RESULTS AND DISCUSSION

Plant micropropagation via direct shoots regeneration allows large scale multiplication of plantlets in vitro by preventing clonal variation as opposed to regenerated from calli alone, which often leads to somaclonal variation [10]. In this study, nodal segments and were excised from 1 year old plant of Vernonia divergens (Fig.1) and were tested for their response to plant growth regulators at different concentrations (Fig. 2).
Fig. 1 *Vernonia divergens* Benth. In SAP Garden of DRS Department of Botany, B R A Bihar Univ., Muzaffarpur.

Fig. 2 Development of shoot from nodal explants on MS basal medium; mark green & healthy shoot: 25 days old culture.

Fig. 3 Nodal explant showing devt of direct multiple shoots on MS+ 1mg L\(^{-1}\) BAP +0.5 mg L\(^{-1}\) NAA.

Fig. 4 Culture showing rooting in bunch on MS + 1mg L\(^{-1}\) BAP + 0.5 mg L\(^{-1}\)NAA  Showing separated microplantlets.

Fig. 5 Showing separated microplantlets.

Fig. 6 30 days old plantlet of *Vernonia divergens* on transfer to two separated tray bags.
Multiple shoots along with bunch of roots were regenerated directly from nodal segments at 1mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA and the best morphogenic response (4 to 5 per explants) was observed (Fig. 3 & 4, Table I). Generally the usage of BAP is considered as most suitable hormone for accentuating the large scale multiplication and micropropagation of various plant species [11]. Micro-plantlets were separated (Fig. 5) and sub-cultured on the double strength (2x) of the above combination of hormones leading to increased length of roots and shoots. Rooting was achieved in bunch of multiple shoot at the same combination of hormone. These plantlets were successfully transferred to soil and survived well in nature (Fig. 6).

The concentrated ethanol extracts were screened for in vitro percentage cytotoxicity at different time periods (24 h, 48 h, and 72h) of different dilutions [12]. The in vivo plant extract inhibited the growth of EAC mouse cell lines in the range of 65, 66, 78, and 88% at 100, 50, 25 & 12.5µg mL\(^{-1}\) but at 72 h of treatment. On the other hand, the extract of in vitro origin, the inhibition was found against EAC cell lines even at 48h (Fig.7). However, the inhibitory action was less by 20% in all treatments in case of the ethanol extract of in vitro derived plantlets [13]. Therefore, further experiments have been designed to develop in vitro plantlets under stress of salt.

**ECA Mouse Cell Lines: Vernonia Cytotoxicity Assay**

![Image](image1.png)

**Fig. 7 Growth inhibitory effect of the in vivo & in vitro ethanol plant extracts against EACMouse cell line**

The antimicrobial activity of in vivo and in vitro extracts of *V. divergens* were tested against *Escherichia coli* and *Pseudomonas aeruginosa* but no inhibition was found against gram positive bacteria like *Staphylococcus aureus* and *Streptomyces* spp. 0.1ml concentration of in vitro extracts were found to be effective against both the above gram negative bacteria. Thus it could be of paramount importance since gram negative bacteria were found to be more prone to cause health problems in human population [14]. On spectrophotometric scanning within the visible range (400-700 nm), the result suggested the different nature of the ingredients of ethanol extracts; there were 4 peaks in in vitro extract as compared to only one in in vivo extract of *V. divergens* (Fig. 8 & 9).

![Image](image2.png)

**Fig. 8 Spectrophotometric scan of the ethanol extract of in vivo grown plants with single peak(λ) at 416.8**

![Image](image3.png)

**Fig. 9 Spectrophotometric scan of the ethanol extract of in vitro grown plants with with four peaks (λ) at 433.6, 478.0, 535.6, and 580.0 of Vernonia divergens.**

**TABLE I**

<table>
<thead>
<tr>
<th>Growth regulators Shoot formation from Nodal segments of Vernonia divergens after 25 Days of MS medium containing 0.8% agar and 5% sucrose</th>
<th>No. of shoots</th>
<th>Ht. of shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP NAA</td>
<td>(mg l(^{-1}))</td>
<td>(%)</td>
</tr>
<tr>
<td>- -</td>
<td>45 ±1.00</td>
<td>1.32±0.10</td>
</tr>
<tr>
<td>1 -</td>
<td>48 ±1.00</td>
<td>1.65±0.12</td>
</tr>
<tr>
<td>2 -</td>
<td>55 ±1.00</td>
<td>2.25±0.13</td>
</tr>
<tr>
<td>3 -</td>
<td>50 ±1.50</td>
<td>1.95±0.12</td>
</tr>
<tr>
<td>1 0.5</td>
<td>100 ±0.00</td>
<td>4.49±0.09</td>
</tr>
<tr>
<td>1 0.7</td>
<td>100 ±0.00</td>
<td>4.20±0.10</td>
</tr>
<tr>
<td>1 0.9</td>
<td>100 ±0.00</td>
<td>4.10±0.08</td>
</tr>
<tr>
<td>2 0.5</td>
<td>95 ±0.00</td>
<td>3.58±0.05</td>
</tr>
<tr>
<td>2 1.0</td>
<td>97 ±1.00</td>
<td>3.65±0.07</td>
</tr>
<tr>
<td>2 1.5</td>
<td>93 ±1.00</td>
<td>3.42±0.03</td>
</tr>
</tbody>
</table>
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REFERENCES