Evaluation of antimicrobial activity of extracts of in vivo and in vitro grown Vinca rosea L. (Catharanthus roseus) against pathogens

Shagufta Naz, Rukhama Haq, Farah Aslam and Saiqa Ilyas
Department of Biotechnology and Microbiology, Lahore College for Women University, Lahore, Pakistan

Abstract: The antimicrobial activity of Vinca rosea was evaluated against pathogenic bacterial strains (Bacillus subtilis, B. licheniformis and Azotobacter sp.) and fungal strains (Aspergillus niger, Alternaria solani and Rhizopus oryzae) using agar well diffusion method. Methanolic extracts of in vivo leaf, in vitro leaf, in vitro calluses of leaf, nodal and fruit explants were used and exhibited antimicrobial activity as indicated by minimum inhibitory concentration (MIC). In vitro extracts showed better results as compared to the in vivo extracts for both the antibacterial as well as the antifungal activity. Among all the extracts, maximum zone of inhibition (30.3 mm±0.58) was formed by in vitro leaf callus extract concentration of 2.0mg/ml against B. licheniformis. Similarly in case of antifungal activity, maximum zone of inhibition (34.6mm ±0.57) was formed by in vitro leaf callus extract and MIC value is 6.0mg/ml against A. niger. Hence these results clearly depicts that V. rosea possess a great strength to fight against the microbial activity and can be used against various infections.

Keywords: Vinca rosea, antimicrobial activity, ILeaf callus, Aspergillus niger, Bacillus subtilis, Bacillus licheniformis.

INTRODUCTION

Medicinal plant products are useful in minimizing the adverse effects of various chemotherapeutic agents, in prolonging life span and attaining positive general health (Kaushik et al., 2000). It has been evaluated that approximately 75% of the total world population has been using plant-derived medicines (Gaines, 2004). The WHO estimates that up to 80% of people still believe on traditional remedies such as herbs for their medicines. The phytochemical extracts of the plants can be used in allopathic medicine as they are a potential source of antiviral, antitumor and antimicrobial agents (Nair et al., 2005). The most popular and valuable anti-cancer agents such as paclitaxel and vinblastine are derived solely from plants (Katzung, 1995; Pezzuto, 1996). Medicinal plants are the most important source of life saving drugs for the majority of the world's population (Shrivastava and Singh, 2011).

Among the valuable medicinal plants Vinca rosea L. possess an important and significant place in the list of medicinal plants. V. rosea L. belongs to family Apocynaceae is an herbaceous shrub also known as Madagascar periwinkle or Catharanthus roseus worldwide. It is cultivated mainly for its alkaloids, which possess anticancerous, antimicrobial and antidiabetic activities (Jaleel et al., 2006). It has been used in folk medicine as an antidiabetic, diuretic and antiulcerative, an anithemorrhagic and for wound healing (Pahwa, 2009).

Various in vitro techniques as micro propagation from existing and adventitious meristems or organ, tissue and cell cultures provide a large amount of V. rosea plant material for the isolation of alkaloids having medicinal properties (Pietrosiuk et al., 2007). The antibacterial potential in crude extracts of different parts (i.e., leaves, stem, root and flower) of C. roseus against clinically significant bacterial strains was reported (Muhammad et al., 2009). The tissue culturing of medicinal plants is widely used to produce active compounds for herbal and pharmaceutical industries (Sidhu, 2010).

C. roseus is an important antimicrobial plant for novel pharmaceuticals since most of the bacterial pathogens are developing resistance against many of the currently available antimicrobial drugs. The anticancer properties of C. roseus have been the major interest in all investigations. The antimicrobial activity has been checked against different microorganisms. The findings showed that the extracts from the leaves of this plant can be used as prophylactic agent in many of the diseases, which sometime are of the magnitude of an epidemic (Prajakta and Ghosh, 2010).

Keeping in view the medicinal importance of this plant, micropropagation technique was used for the growth of in vitro plants and evaluated the antimicrobial (antibacterial & antifungal) potential of the plant. The present research was focused to extraction of alkaloids with the help of organic solvents and their comparison of antimicrobial activity of both in vivo and in vitro plants.

MATERIALS AND METHODS

Surface sterilization of explants and micropropagation

The used explants for micropropagation and callogenesis were obtained from Botanical Garden of Lahore College for Women University and Green House of Lawrence...
Garden, Lahore, Pakistan. Explants were washed with running tap water and detergent. Then excised explants were surface sterilized with the 40% bleach (sodium hypochlorite) for 20 min. Then several rinses were given with autoclaved distilled water till the removal of smell of bleach. The explants were then cultured on MS basal medium (Murashige and Skoog, 1962) supplemented with various concentrations of BAP and NAA for micropropagation and 2, 4-D and Kin for callogenesis. In vivo grown plants of *V. rosea* leaves, in vitro grown *V. rosea* leaves, leaf callus, nodal callus and fruit callus as explants were used for antimicrobial activity.

**Extraction procedures**

**Soxhlet extraction**

Soxhlet apparatus was used for the extraction of alkaloids from *in vivo* grown *V. rosea* leaves. The weighed fresh *in vivo* plant material was crushed in pestle and mortar first and then placed in the extraction thimble. The weighed amount was placed in an extraction chamber, which was suspended above the flask containing the solvent methanol and below a condenser. The flask was heated and the methanol evaporated and moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the plant material. The extraction chamber was designed so that when the solvent surrounding the sample exceeded a certain level it flowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the methanol extract was removed and methanol was evaporated by using rotary evaporator.

**Assay for antimicrobial activity**

Antimicrobial activity was tested by agar well diffusion method (Mukherjee *et al*., 1995). Different concentrations of the *Vinca* alkaloids were prepared in organic solvent i.e. methanol by using serial dilution method. 0.5ml of the 24h fresh cultures were poured into 35ml sterile molten nutrient agar in sterile petri plates and allowed to be solidified. After solidification, 7 mm wells were made using sterile borer. The wells were then filled with 0.1ml of the sample extract. The antibacterial assay plates were incubated at 37ºC for 24h and antifungal assay plates were incubated at 25ºC for 48h. After incubation, the zones of inhibition were measured. Each experiment was carried out in triplicate and mean diameter of the inhibition zone was recorded.

**Minimum inhibitory concentration (MIC)**

The extract which showed antimicrobial activity in agar well assay was subjected to MIC assay (Jones *et al*., 1985). In order to determine MIC, serial dilutions of the extract were prepared with concentration ranged from 2 mg/ml to 20 mg/ml and zone of inhibitions were checked. Minimum inhibitory concentration (MIC) as the lowest concentration of plant extracts inhibiting the growth of the organisms was determined.

**STATISTICAL ANALYSIS**

All experiments were arranged in a Completely Randomized Design and data were analysed using the Costat software. Data (mean ± SD) were collected from five experiments each with four replicates. The data thus generated were analysed through one way analysis of variance (ANOVA) and the treatments’ means were compared for significance by Duncan’s New Multiple Range (DMR) test at 0.05% P.

**RESULTS**

**Antibacterial activity**

Antibacterial activity of different parts of *Vinca rosea* was observed, MIC values and their zones of inhibitions were presented in fig. 1 and table 1 respectively. Extracts of *in vivo* leaf, *in vitro* leaf, *in vitro* leaf callus, *in vitro* nodal callus and *in vitro* fruit callus were tested against three bacterial strains i.e. *Bacillus subtilis*, *Bacillus licheniformis* and *Azotobacter sp*. The organic solvent used for extraction of all above-mentioned samples is methanol.

![Fig. 1: MIC values of antibacterial activity of different parts of *V. rosea*](image-url)
Aspergillus niger fruit callus were tested against three fungal strains i.e. Alternaria solani, Rhizopus oryzae and Bacillus subtilis in vitro extracts. Methanolic extracts of leaf, leaf callus and nodal callus of V. rosea were observed to have strong antibacterial activity as compared to in vivo extracts. In vitro leaf callus gave higher zone of inhibition against B. licheniformis (30.3mm ±0.58a) among all the tested microorganisms showed that all the extracts and followed by B. subtilis (28.7mm ±1.15b) in leaf callus. In vitro nodal callus also gave 28.6mm ±1.57b zone of inhibition against Azotobacter sp.

### Table 1: Comparison of antibacterial activity of different extract concentrations from different parts of V. rosea.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extract concentrations (mg/ml)</th>
<th>Tested Microorganism</th>
<th>In vivo leaf zone of inhibition (mm)</th>
<th>In vitro leaf zone of inhibition (mm)</th>
<th>Leaf callus zone of inhibition (mm)</th>
<th>Nodal callus zone of inhibition (mm)</th>
<th>Fruit callus zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Bacillus subtilis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. licheniformis</td>
<td>-</td>
<td>28.7±1.15b</td>
<td>14.0±1.00a</td>
<td>21.3±0.58d</td>
<td>25.0±0.00a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Azotobacter sp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>B. subtilis</td>
<td>-</td>
<td>30.3±0.58a</td>
<td>23.3±1.53a</td>
<td>19.0±1.00a</td>
<td>20.7±1.15b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. licheniformis</td>
<td>4.3±0.58c</td>
<td>8.6±0.58b</td>
<td>8.3±1.52b</td>
<td>28.6±0.57b</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Azotobacter sp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>B. subtilis</td>
<td>-</td>
<td>17.7±1.15f</td>
<td>20.7±2.08e</td>
<td>14.0±1.00b</td>
<td>21.3±0.58e</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. licheniformis</td>
<td>26.3±1.52c</td>
<td>11.0±1.73e</td>
<td>19.3±0.58e</td>
<td>20.0±0.00e</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Azotobacter sp.</td>
<td>7.3±2.08a</td>
<td>12.0±1.00b</td>
<td>11.3±0.58e</td>
<td>25.0±0.00e</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>B. subtilis</td>
<td>5.7±0.58b</td>
<td>14.7±1.53f</td>
<td>5.7±0.58e</td>
<td>11.0±0.00b</td>
<td>23.7±1.53e</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. licheniformis</td>
<td>3.0±0.00d</td>
<td>25.0±0.00a</td>
<td>3.0±0.00d</td>
<td>17.0±1.73b</td>
<td>23.7±1.52e</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Azotobacter sp.</td>
<td>-</td>
<td>20.7±0.58c</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

It is evident from the Table 1 that in vitro extracts have strong antibacterial activity as compared to in vivo extracts. In vitro leaf callus gave higher zone of inhibition against B. licheniformis (30.3mm ±0.58a) among all the extracts and followed by B. subtilis (28.7mm ±1.15b) in leaf callus. In vitro nodal callus also gave 28.6mm ±1.57b zone of inhibition against Azotobacter sp.

### Table 2: Comparison of antifungal activity of different extract concentrations from different explants of V. rosea.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extract concentrations (mg/ml)</th>
<th>Tested Microorganism</th>
<th>In vivo leaf zone of inhibition (mm)</th>
<th>In vitro leaf zone of inhibition (mm)</th>
<th>Leaf callus zone of inhibition (mm)</th>
<th>Nodal callus zone of inhibition (mm)</th>
<th>Fruit callus zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Aspergillus niger</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alternaria solani</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhizopus oryzae</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>A. niger</td>
<td>-</td>
<td>30.0±0.00b</td>
<td>18.7±1.15a</td>
<td>8.0±0.00b</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A. solani</td>
<td>-</td>
<td>-</td>
<td>2.0±0.00f</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. oryzae</td>
<td>1.0±0.00c</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>A. niger</td>
<td>-</td>
<td>34.6±0.57c</td>
<td>5.0±0.00c</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A. solani</td>
<td>-</td>
<td>-</td>
<td>4.0±0.15c</td>
<td>5.0±0.00c</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. oryzae</td>
<td>4.3±0.58c</td>
<td>-</td>
<td>2.1±0.32f</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>A. niger</td>
<td>5.0±1.00c</td>
<td>30.3±1.15b</td>
<td>2.0±1.00c</td>
<td>4.6±0.57a</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A. solani</td>
<td>-</td>
<td>8.0±0.00b</td>
<td>10.7±0.58a</td>
<td>4.0±0.10b</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. oryzae</td>
<td>8.0±1.00c</td>
<td>10.3±0.58a</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

It is evident from the Table 1 that in vitro extracts have strong antibacterial activity as compared to in vivo extracts. In vitro leaf callus gave higher zone of inhibition against B. licheniformis (30.3mm ±0.58a) among all the extracts and followed by B. subtilis (28.7mm ±1.15b) in leaf callus. In vitro nodal callus also gave 28.6mm ±1.57b zone of inhibition against Azotobacter sp.

### Antifungal activity

Antifungal activity of different samples including both in vivo & in vitro grown plant parts of V. rosea was observed and their zones of inhibitions were calculated (table 2). MIC values of all different extracts can be seen in the fig. 2. Methanolic extracts of in vivo leaf, in vitro leaf, in vitro leaf callus, in vitro nodal callus and in vitro fruit callus were tested against three fungal strains i.e. Aspergillus niger, Alternaria solani and Rhizopus oryzae.

The comparison of different extract concentrations against all tested microorganisms showed that all the in vitro extracts showed better clear zones of inhibitions. In vitro leaf callus gave higher zone of inhibition against A. niger (34.6mm±0.57a) and ranged from 1.0mm-34.6mm in diameter. In vivo leaf extract did not show any sensitivity against A. niger and A. solani except R. oryzae which gave very low zone of inhibitions ranging from 1.0mm-8.0mm.

### DISCUSSION

#### Antibacterial activity

It has been reported that Gram-positive bacteria are more sensitive to plant oil and extract (Cosentino et al., 1999; Karaman et al., 2003). In vivo leaf extracts were less sensitive to tested microorganism. The zone of inhibition for different bacterial strains and different extracts ranged from 3.0mm -30.3 mm in diameter. Many of the researchers has been studied the effect of plant extracts on the bacterial growth in different parts of the world (Reddy et al., 2001; Erdourul, 2002; Ates and Erdourul, 2003). Muhammad et al., (2009) reported the antibacterial potential in crude extracts of different parts of V. rosea against clinically significant bacterial strains.
Evaluation of antimicrobial activity of extracts

It was previously reported that the pattern of inhibition largely depends upon extraction procedure, plant part, physiological and morphological state of plant, extraction solvent and microorganism tested (Goyal et al., 2008; Thongson et al., 2004).

**Antifungal activity**

The best response was shown by *A. niger* followed by *A. solani* and *R. oryzae*. Antifungal activity of forty nine botanical extracts were assayed and the data on effect of plant extracts on the growth of *A. niger* was also presented by Bobbarala et al., 2009. Hence it has been supported earlier that the compounds isolated from the medicinal plants possess remarkable toxic activity against bacteria and fungi and possess pharmacological significance (Banso and Adeyemo, 2007). *V. rosea* is an important medicinal plant which might be useful as antimicrobial agents (Jayakumar et al., 2010).

![Fig. 2: MIC values of antifungal activity of different parts of *V. rosea*.](image)

The present work also clearly showed that the *in vitro* extract concentrations of all the parts of *V. rosea* exhibited far better results and resistance against both the bacteria and fungi, hence *in vitro* possess greater antimicrobial activity as compared to *in vivo* parts. According to the Roepke et al., (2010) and Cowan, (1999), the reason behind this may be that *in vitro* plant parts (leaves & calli) were either too young or immature whereas *in vivo* plant parts (leaves) were mature enough for accumulation of alkaloids. The appearance of low levels of catharanthine within older leaves is also reported by Roepke et al. (2010). The alkaloid production may be dependent on undifferentiated and immature cells which are mostly present in young leaf and in calluses as compared to the mature and differentiated cells which are present in mature leaves. By this it can be concluded that *in vitro* part (calluses) can be the ideal plant part for the production of antimicrobial compounds.

**CONCLUSION**

Hence it can be concluded that *in vitro Vinca rosea* propagation can be very useful for the production of antimicrobial compounds, which can prove useful in the medical industry. Further research is needed to isolate many new compounds or alkaloids from *V. rosea*, which are significant medicinally.

**REFERENCES**


