APPLICATION OF LEAF EXTRACTS OF PHLOGACANTHUS THYRSIFLORUS NEES. AGAINST SOME FUNGI PATHOGENIC TO ONION (ALLIUM CEPA L.)

Juri Devi et al.

KEYWORDS

Onion
Crude extract
MIC
NSave Nature to Survive

Phlogacanthus thyrsiflorus Nees, a plant with great medicinal value was evaluated for in vitro antifungal activity against three important pathogenic fungi including Aspergillus flavus, A. niger and Rhizopus stolonifer isolated from infected onion. Crude extracts of leaves were prepared by using methanol and water as solvent. The in vitro antifungal activity was evaluated at three different concentrations (20mg, 10mg and 5mg/mL) for each extract following agar well diffusion method and the inhibitory activity was dependent on concentration. The methanol extract was more effective in comparison to aqueous extract and minimum inhibitory concentration (MIC) ranged between 3.12 – 12.5 mg/mL. The results were compared with standard fungicide.

INTRODUCTION

Since ancient times medicinal plants have been used virtually in all cultures as a source of medicine. Use of herbal remedies as described in ancient text such as Vedas and Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties (Singh and Singh, 2010). In recent years there is an increasing demand in plant derived drugs. This increasing interest is mainly due to the fact that green medicine is safe and ecofriendly than the synthetic drugs many of which are known to possess adverse side effects (Nair and Chanda, 2007).

Plant extracts have been in use to control many diseases in plants and tuber crops (Okigbo and Nmeka, 2005). Current research mainly focuses on natural molecules and products from plants since these are easily accessible and can be selected based on their ethno medicinal use (Arora and Kaur, 2007). In India much work has been done on ethno medicinal plants with a view to develop new pesticides since plants are a source of potent natural pesticides.

Phlogacanthus thyrsiflorus Nees. commonly known as “Titaphul” belongs to the family Acanthaceae. The plant is distributed throughout the tropics and in the entire North East India. The whole plant possesses immense medicinal property. Inflorescence is used for vermicide and also as remedy for cough. Leaf juice is used in asthma, jaundice, dysentery, tuberculosis, malarial fever and in rheumatism (Kalita and Bora, 2008; Patwari 1992).

Onion is one of the most widely cultivated vegetables in the world and India occupies second position in onion production after China. But this important vegetable which is being exported to other countries is known to be infected by fungi after harvest degrading the quality as well as quantity of the produce. There is extensive literatures regarding post harvest fungal decay of onion around the world (Lee et al, 2001; Rafika et al. 2006; Shehu and Muhammad, 2011). Control of onion diseases by using biological agent is well established. But till now no work has been done to control the pathogens by using leaf extract of P. thyrsiflorus.

Keeping above aspects in view the present investigation was undertaken to assess the antifugal activity of this valuable medicinal plant against certain fungi pathogenic to onion.

MATERIALS AND METHODS

Collection of plant material
Fresh leaves of Phlogacanthus thyrsiflorus were collected from nearby forest areas of Guwahati (latitude 26º10’N and longitude 91º45’E), Assam, India, during growing season (December – April). The plant was identified by Dr. G.C Sarma, taxonomist, Department of Botany, Guwahati University.

Preparation of leaf extract
Fresh leaves were thoroughly washed under running tap water, powdered with a
mechanical grinder and stored in airtight bottles. Dried leaf powders were extracted using distilled water and methanol as the solvent.

**Aqueous extract**

10g of dried powder was added to 200mL of distilled water in a 250mL conical flask, plugged tightly and kept on a rotary shaker at 170 – 200 rpm for 24 h. Thereafter it was filtered through 8 layers of muslin cloth, supernatant was collected and solvent was evaporated using a rotary evaporator (Buchi R –124). The crude extract so obtained after complete evaporation of the solvent was stored at 4°C in a refrigerator in airtight bottles.

**Methanol extract**

10g of dried powder was mixed with 200mL of methanol in a 250ml conical flask, plugged tightly and kept on a rotary shaker at 170 – 200 rpm for 24 h. Supernatant was collected after filtration and solvent was evaporated using a rotary evaporator (Buchi R-124). The residue obtained was collected and stored at 4°C in a refrigerator in airtight bottles.

**Test fungi**

Four to five small fragments from infected onion bulbs were placed on Czapek Dox Agar (CDA) Media with streptomycin to isolate the fungal pathogens. After incubating the petriplates at 28±1ºC for 5 – 7 days the isolates were identified based on their morphological and cultural characteristics and were confirmed as *A.flavus*, *A.niger* and *Rhizopus stolonifer* (with the available literatures Agrios, 1988; Barnett, 1972; Bessey, 1950). The pathogenicity tests were carried out with afore said fungi (Koch, 1882). The test fungi were maintained on freshly prepared CDA medium, procured from Hi Media Laboratories Pvt. Ltd, Mumbai, India.

**Antifungal assay**

The antifungal activity of crude leaf extract was determined by Agar Well Diffusion Method (Soumya and Nair, 2012). Briefly, CDA plates were inoculated with 200μl of two days old culture of each test fungal suspension containing 1.0 × 10⁷ cells. The plates were evenly spread out with the help of a glass spreader. Agars well were made by scooping out the media with a sterile cork borer (6 mm in diameter). Three different concentrations (20mg, 10mg and 5mg/mL) of each extracts were prepared by dissolving in required amount of dimethly sulphoxide (DMSO) and each well was filled with 100 - 150μL of extracts. Bavistin (0.5mg/mL) was used as positive control while, agar well loaded with only DMSO (2%) was used as negative control. The petriplates were incubated at 28±1ºC for two days and the zone of inhibition was measured and compared with the control (i.e. both positive and negative). Three replicates were maintained in each case.

The activity index (Arya et al., 2010) of crude extract was calculated by the following formula:

\[ AI = \frac{\text{Mean of zone of inhibition of the extract}}{\text{Zone of inhibition of the standard antibiotic drug}} \]

Percent of inhibition (Tejesvi et al., 2008) was calculated by the formula:

\[ \% \text{ of inhibition} = \frac{\text{inhibition zone in mm}}{\text{*control}} \times 100 \]

(* Growth zone is equal to plate diameter i.e. 80mm as growth occurs all over the agar plate)

**Determination of minimum inhibitory concentration (MIC)**

MIC of the extract was carried out using Broth Dilution Method (Ibekwe et al., 2001) with slight modification. CDA broth was prepared (100mL) and 10mL of each broth was dispensed into separate test tubes, sterilized at 121±1ºC and allowed to cool. Stock concentration of 100mg/mL of extract was prepared and serial dilution was done in the broth to obtain concentrations 50, 25, 12.5, 6.25, 3.12, 1.56, 0.7, 0.3mg/mL. 0.1mL of inoculum suspension of each test fungi was then inoculated into different concentrations of the extracts in the broth. The test tubes were then plugged properly and incubated at 28±1ºC for 1 – 4 days and observed carefully.

**Statistical analysis**

Antifungal activity was determined by measuring zone of inhibition i.e the mean of triplicates ± standard deviation of three replicates with the help of Computer programme Excel.

**RESULTS AND DISCUSSION**

In our study methanol extract exhibited efficient antagonistic response in all the test fungi as compared to aqueous extract. Also application of high dose (20mg/mL) showed better antifungal activity in contrast to lower dose. Overall maximum antagonistic activity of crude extract of *P.thrysiflorus* was found against *A.flavus* (zone of inhibition 30mm) followed by *A.niger* (zone of inhibition 18.6mm) and *Rhizopus stolonifer* (zone of inhibition 8.6mm) respectively (Table 1). The reason behind higher antifungal activity of methanol extract could be due to their intrinsic bioactivity or their ability to dissolve or diffuse in the media used in the assay (Parekh et al., 2005). Lack of solubility of the active constituents in aqueous solution might be another reason for higher antifungal activity of methanol (Parekh and Chanda, 2007).

The minimum inhibitory concentration of the extract was recorded (Table 2) as 3.12mg/mL in Aspergillus flavus, 6.25mg/mL in case of A.niger and 12.5mg/mL in case of Rhizopus stolonifer. Activity index (AI) and inhibition percentage (%) were also determined and presented in Table 3. Maximum activity index (1.6) and inhibition percentage (37) was found in case of A.flavus whereas minimum was obtained in case of

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Bavistin(0.5mg/mL)</th>
<th>5mg/mL</th>
<th>10mg/mL</th>
<th>20mg/mL</th>
<th>MIC(mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.flavus</td>
<td>18</td>
<td>14 ± 3.4</td>
<td>24 ± 3.6</td>
<td>30 ± 1.4</td>
<td>3.12</td>
</tr>
<tr>
<td>A.niger</td>
<td>13</td>
<td>7 ± 2</td>
<td>12.3 ± 4</td>
<td>18.6 ± 1.1</td>
<td>6.25</td>
</tr>
<tr>
<td>R.stolonifer</td>
<td>10</td>
<td>5 ± 1</td>
<td>6.3 ± 2</td>
<td>8.6 ± 1.5</td>
<td>12.5</td>
</tr>
</tbody>
</table>

*The datas are the standard deviations of three replicates; expressed as mean ± SD.*
Table 2: Antifungal activity of leaf extract of *P. thyrsiflorus* compared with standard antifungal drug (Bavistin) using agar well diffusion method

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Bavistin (0.5mg/mL)</th>
<th>5mg/mL</th>
<th>10mg/mL</th>
<th>20mg/mL</th>
<th>MIC (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. flavus</em></td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>8.6 ± 1.5</td>
<td>-</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>13</td>
<td>-</td>
<td>-</td>
<td>4.3 ± 1.5</td>
<td>-</td>
</tr>
<tr>
<td><em>R. stolonifer</em></td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*The datas are the standard deviations of three replicates; expressed as mean ± SD, '-' indicates no activity.

Table 3: Activity index (A.I.) and inhibition percentage (%) of *P. thyrsiflorus* leaf extract against test fungi

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Activity index</th>
<th>Inhibition percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. flavus</em></td>
<td>0.7 1.3 1.6</td>
<td>17 30 37</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>0.5 0.9 1.4</td>
<td>8 15 23</td>
</tr>
<tr>
<td><em>R. stolonifer</em></td>
<td>0.5 0.6 0.8</td>
<td>6.7 10</td>
</tr>
</tbody>
</table>

Rhizopus stolonifer (activity index = 0.8; inhibition percentage = 10). The antifungal activity of the crude extract was compared to the standard fungicide Bavistin.

In recent years, the search for plant based drugs has been on the rise due to their potential use in the treatment of various chronic and infectious diseases (Govindappa et al., 2011). Due to risk of adverse effects encountered with the use of synthetic drugs, medicinal plants may provide an alternative way for antimicrobial agent with significant activity against pathogenic microorganisms. In addition, a number of antibiotics have lost their effectiveness due to the development of resistant strains (Berahou et al., 2007).

Present investigation showed the antifungal potential of leaf extract of *P. thyrsiflorus* against some important pathogenic fungi associated with disease. This study confirms the use of leaf extract of this plant as protectant pesticide as a substitute of chemical pesticide. This paves the way for further research to elucidate the exact mechanism of action by which extracts exert their antifungal effect since this approach to plant disease management is economically viable and possess little environmental risk.

REFERENCES


