Microscopic Assessment of Antimicrobial Activity of *Duranta erecta* L. Plant Extracts and Preparation of their Antiibiogram

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**ABSTRACT**

The antifungal properties of methanolic extract of different parts of *Duranta erecta* was determined against *Aspergillus flavus*, *A. fumigates*, *A. niger*, *Alternaria sp.*, *Fusarium oxysporum*, *Penicillium sp.*, *Rhizopus sp.* and *Trichoderma sp.* using agar disc diffusion method. As a result of this study it was found that methanolic extract of leaf and stem of *Duranta erecta* was effective against *A. niger*, *A. flavus* and *A. fumigatus* at 1000 mg/ml concentration which recorded significant inhibition zone of 2.3 cm, 2.3 cm and 2.2 cm respectively. *A. fumigatus* stem also showed good activity at 500 mg/ml i.e. 2.0 cm and similar zone was observed by root extract at 1000 mg/ml against *A. fumigatus* and leaf extract at 1000 mg/ml against *Trichoderma sp.* *A. fumigatus* showed activity at all conc. by all parts while maximum test fungi did not show any activity by any part of the plant.

**Key words:** Antifungal properties, *Duranta erecta*, Agar disc diffusion method, *A. fumigates.*

**INTRODUCTION**

*Duranta erecta* Linn. (Syn. *Duranta plumieri* Jacq., and Eng: Golden dewdrop) is commonly known as pigeon berry and locally called ‘Kata mehedi’ belongs to the family Verbenaceae. It is shrubs, herbs or small tree usually 1 to 3 m. in height and also grown as a hedge plant in various parts of our country (David, 1981). The plant is not browsed by cattle and is believed to be poisonous (Nelson, 1996). Ethyl acetate and aqueous extracts of leaves showed significant antimalarial activity when administered to mice (Castro et al., 1996). The fruits are used in the treatment of malaria and intestinal worms (Whistler, 2000). The leaves are used in the treatment of abscess (Xiao, 1992). Previously, wide range of chemical compounds had been isolated from different parts of *Duranta repens*. Especially from stem durantosides I, II, III, duranterectoside A and lamiidoside were isolated (Takida et al., 1995) but there was no report on biological activities of these compounds. Thus, the aim of this investigation was to evaluate the in *vitro* antimicrobial activity crude methanolic extracts (Leaf, stem and roots) *Duranta erecta*.

**MATERIALS AND METHODS**

**Plant material**

The fresh and healthy leaves stem and roots of the plant were collected from the different localities of Jaipur (Rajasthan). A voucher specimen was prepared and submitted to the Department of Botany, University of Rajasthan, Jaipur, India.

The plant materials were washed thoroughly with running tap water, shade dried at room temperature and then powdered. The powdered plant materials were used as raw materials for the extraction of antimicrobial compounds from the plants.

**Preparation of plant extract**

The powdered material of leaves stem and roots were extracted in soxhlet apparatus using ethanol and methanol as solvent. The plant material was loaded in the inner tube of soxhlet apparatus and then fitted into a round bottomed flask containing methanol and ethanol separately. The solvents were boiled gently over a heating mantle using the adjustable rheostat. The extraction was continued for 24 hrs and the solvent was removed at the reduced pressure with the help of rotator vacuum evaporator to yield a viscous residue of each of ethanol and methanol.

**Culture used**

Total of 8 fungi were used for the antimicrobial study. They were as follow: *Aspergillus flavus*, *A. fumigates*, *A. niger*, *Alternaria sp.*, *Fusarium solani*, *Penicillium sp.*, *Rhizopus sp.*, *Trichoderma sp.* All the fungi were obtained from the plant pathology laboratory of Department of Botany, University of Rajasthan, India. They were sub cultured and identified for their sensitivity.

**Antimicrobial assay and antibiogram of plant extract**

Strains of fungi were grown on Potato Dextrose Agar growth media. Antimicrobial activity was determined by disc diffusion method. Plates with PDA were seeded with fungal inocula and then drained off. They were dissecated at room temperature for 15-20 min. The disc prepared from different concentrations (250, 500 and 1000 mg/ml) of herbal extract was placed in quadrangular manner in petri dishes. Then petri dishes were incubated at 27±2°C for 48 h. After 48 h, the results were noted for the zone of inhibition diameter (cm). As positive control for antifungal assay fluconazol was used.

**RESULT AND DISCUSSION**

Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant (Nascimento et al., 2000). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the present study, methanol was selected for the
plant extraction. From the study it was found that methanolic extract of leaf and stem of Duranta erecta was effective against A. niger, A. flavus and A. fumigatus at 1000 mg/ml concentration which recorded significant inhibition zone of 2.3 cm, 2.3 cm and 2.2 cm respectively. A. fumigatus stem also showed good activity at 500 mg/ml i.e. 2.0 cm and similar zone was observed by root extract at 1000 mg/ml against A. fumigatus and leaf extract at 1000 mg/ml against Trichoderma sp. A. fumigatus showed activity at all conc. by all parts while maximum test fungi did not show any activity by any part of the plant.

A. fumigatus is exceptional amongst the aspergilli in being both a primary and opportunistic pathogen as well as a major allergen associated with severe asthma and sinusitis [Gugnani, 2003, Denning, 1998, Latge, 1999]. It was first reported to cause opportunistic invasive infection about 50 years ago [Rankin, 1953]. In immunocompromised patients, mycelia growth can proliferate throughout pulmonary or other tissues causing invasive aspergillosis. A. fumigatus has been demonstrated to be a primary pathogen of the airways, sinuses, lungs, damaged skin and subcutaneous tissues (Pasqualotto et al., 2006). However the present study of in vitro antifungal evaluation of plant forms a platform for further phytochemical and pharmacological studies to discover new antibiotic drugs against this fungus.

Microscopic evaluation of activities of methanolic extracts of Duranta erecta

A. niger, A. flavus and A. fumigatus were selected for microscopy due to comparatively rapid growth and higher sensitivity towards methanolic extract of selected plant. The slides of the fungi were prepared from different plates having antimicrobial activity at different concentrations and observed under microscope. After treatment with leaf extract, A. flavus displayed an extensive, interlaced network of largely vegetative hyphae with a profound reduction in conidiophore formation. This reduction was dependent on extract concentration. Control samples of A. flavus displayed heavy sporulation with long chains of conidiospores masking the underlying conidiophores and mycelia. Typically, conidiophores from the control culture displayed normal vesicles covered with chains of conidia. In contrast, the treated culture exhibited, when present, very few conidiophores with altered morphologies and reduced numbers of conidiospores (Figure 1; a, b & c).

The growth of A. fumigatus treated with crude extract of Duranta erecta stem showed that growth of hyphae was inhibited. There was direct relation between concentration and damaging of the cell wall/membrane of microorganism as shown in Figure (B: a, b & c). It showed that high concentration of extract ruptured the cell wall of hyphae, collapse of cytoplasm and damaged the conidia, and resulted in broken hyphae.

In contrast to control, microscopic observation of leaf extract on A. niger fungal hyphae and conidia showed drastic morphological alterations with shrunken and collapsed form (Figure: C; a, b & c). This could be due to the leak in the cell wall or perhaps some alteration in the membrane permeability.

Table 1: Impact of CH₂OH extract of D. erecta against fungi measured by the diameter of the inhibition zone (DIZ) cm.

<table>
<thead>
<tr>
<th>Plant parts used</th>
<th>Conc. (mg/ml)</th>
<th>A. flavus</th>
<th>A. fumigatus</th>
<th>A. niger</th>
<th>F. oxysporum</th>
<th>Penicillium sp.</th>
<th>Alternaria sp.</th>
<th>Rhizopus sp.</th>
<th>Trichoderma sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>250 mg/ml</td>
<td>ND</td>
<td>1.3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Stem</td>
<td>ND</td>
<td>1.8</td>
<td>0.9</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Root</td>
<td>ND</td>
<td>0.8</td>
<td>1.5</td>
<td>1.7</td>
<td>0.7</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Leaf</td>
<td>500 mg/ml</td>
<td>1.5</td>
<td>1.5</td>
<td>1.7</td>
<td>0.7</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Stem</td>
<td>2</td>
<td>1.5</td>
<td>1.5</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Root</td>
<td>2</td>
<td>1.5</td>
<td>1.5</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Leaf</td>
<td>1000 mg/ml</td>
<td>2.3</td>
<td>1.6</td>
<td>2.3</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2</td>
</tr>
<tr>
<td>Stem</td>
<td>2</td>
<td>2.2</td>
<td>1.8</td>
<td>2.4</td>
<td>1.5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.7</td>
</tr>
<tr>
<td>Root</td>
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<td>2</td>
<td>2.2</td>
<td>2</td>
<td>2.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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</tr>
<tr>
<td>Fluconazole (Control)</td>
<td>3.5</td>
<td>2</td>
<td>2</td>
<td>2.3</td>
<td>2.8</td>
<td>1.8</td>
<td>ND</td>
<td>ND</td>
<td>2.1</td>
</tr>
</tbody>
</table>

ND= Not detected.
Studies are in progress to determine the specific active compounds responsible for these alterations, and such active compounds potentially could be used as natural fungicides.

REFERENCES

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