Phytotoxic and leishmanicidal activity of *Salix nigra*

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**Abstract**

*Salix Nigra* is a member of family Salicaceae and has been planted frequently to help stabilize the banks of eroding streams, to hold the soil in road cuts and embankments, and to bind shifting sands. The plant has also been used for its antiperiodic, antipyretic, antiseptic, antirheumatic and analgesic effects. The current research was aimed to evaluate the *in vitro* phytotoxic and leishmanicidal effects of the methanolic extract of plant. Our results showed that the methanolic crude extract of *Salix Nigra* possess remarkable phytotoxic & leishmanicidal activity which might be due to the presence of bioactive constituents in the plant’s extract.

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**Introduction**

The old aphorism "we come on this earth as guests of plants" rightly summarizes the services rendered by plants to humans. As most generous hosts, they have contributed in each and every domain of human existence that includes provision of food, shelter, clothing, clean environment, etc. Yet another great obligation as a meek host is their use as remedies in the treatment of diseases. According to reports, 30% of the drugs are obtained from medicinal plants (Grabley and Thiericke, 1999) which play role in improving human health (Sahreen et al., 2010; Khan et al., 2009; Khan et al., 2010 a,b). Medicinal plants have been prescribed and used for centuries, with almost no change in the form or the way they have been used, and with a strong belief in their usefulness in diseases (Alkofahi et al., 1996).

Pakistan is gifted with a wide variety of medicinal plants which are being investigated for their allelopathic and or medicinal potential (Fujii et al., 2003; Khan et al., 2010c). It is desirable to make use of the therapeutic potential of higher plants to get new, less expensive, more effective and safer natural drugs. *Salix Nigra* has been used in traditional medicine system for its various therapeutic effects. However, no pharmacological studies have been carried out in detail so far on this plant. The aim of the current study was to assess the phytotoxic and antileishmanial activity of crude methanolic extract of *Salix Nigra*. The results showed that the methanolic crude extract of *Salix Nigra* possess significant phytotoxic & leishmanicidal activity.

**Material and method**

The plant of *Salix Nigra* was collected from the area of Ghoriwala, district Bannu, Khyber Pakhtunkhwa, Pakistan in the month of March. The plant was properly recognized by taxonomist, Botany department, UST Bannu. It was washed with water and shade dried at room temperature for 3 consecutive weeks. It was then grinded into powder mechanically by a local grinder machine.

100 gm powder of *Salix Nigra* was extracted in 1L commercial grade methanol (Merck Lab) and randomly shaked for 3hrs on shaker machine. It was then kept for 7 days at room temperature. After this time period, the extract was filtered by using whatman filter paper No1 and the filtrate was further concentrated under reduced pressure on rotavap at 38°C.

The concentrated methanolic crude extract was then applied to lyophilizer and converted into very fine powder form which was stored at 4°C in a Falcon tube.

**Preparation of Sample**

Sample was prepared by dissolving 5mg of crude extract powder in 5ml methanol. Form this stock solution; two sub-solutions were prepared i.e. 100µgm/ml and 1000µgm/ml for phytotoxic assay respectively.

**Phytotoxicity bioassay**

Protocol of McLaughlin and Rogers (1998) was used for checking the phytotoxic efficacy of methanolic fraction of plant extract. Two concentrations (100 and 1000 µgm/ml) in respective solvent were prepared and preceded. Maize seed were washed with dH₂O.

Filter paper was put in each autoclaved Petri plate. 2 ml of each fraction was poured in each plate and the respective solvent was evaporated. 4 seeds were placed in each plate and 5ml dH₂O sprayed over the plates and let them in a sterile medium for five days. After 3 and 5 days, root and shoot inhibition was noted and compared with the control.

**Antileishmanial assay**

Tissue samples were collected from cutaneous leishmaniasis patients in Bannu and D.I.khan districts, Khyber Pakhtunkhwa, Pakistan, by making a small notch with the help of sterilized lancet till the blood oozed out. The samples were potted in sterilized Eppendorf containing 0.9% normal saline solution. Tissue samples were inoculated on the liquid phase of Novy–McNeal– Nicolle medium and then sub cultured in Roswell Park Memorial Institute
(RPMI 1640) augmented with 10% of fetal calf serum. Furthermore, Penicillin G, streptomycin and kanamycin were mixed with media to shun bacterial infectivity. The cultures were incubated at 26°C and investigated for parasite growth using a microscope every 8th day until promastigotes (the flagellate stage of Leishmania parasites) were seen or up to 1 month before being disposed off as negative culture (Zhai et al., 1999). The plant samples of various concentrations (15, 25, 35 and 45 mg/ml) were added in test tubes, each having 5ml of RPMI 1640 culture media. After haemocytometer counting, promastigotes were suspended to yield 1x10^6 cells/ml in each culture tube. All the invitro experiments were run in triplicate and the results were expressed as the percentage inhibition in leishmania parasite number after different time intervals of 48 and 96 hrs.

**Results**

**Phytotoxicity assessment**

**Effect on root growth inhibition**

Methanolic fraction of Salix Nigra showed significant inhibitory effect on root growth of maize. Phytotoxic (allelopathic) effect of Salix Nigra was evaluated against maize seed growth under control environmental condition at both 100 ppm and 1000 ppm, respectively as shown in Fig. 1 & 3.

**Table 1. Leishmanicidal activity of Salix Nigra.**

<table>
<thead>
<tr>
<th>Plant Sample</th>
<th>Concentration mg/ml</th>
<th>After 48 Hrs, Growth inhibition %</th>
<th>After 96 Hrs, Growth inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salix Nigra</em></td>
<td>15</td>
<td>3.0 ± 0.5</td>
<td>6.0 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>5.0 ± 1.0</td>
<td>25.0 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>12.5 ± 1.5</td>
<td>35.5 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>30.0 ± 3.5</td>
<td>42.0 ± 4.0</td>
</tr>
</tbody>
</table>

**Effect on Shoot growth inhibition**

*Salix Nigra* also affects the shoot growth of maize seeds. Results of the present study revealed that methanolic fraction of *Salix Nigra* has markedly inhibit the growth of maize at 5th day of treatment both at 100 and 1000 ppm respectively as shown in Fig. 2 & 4 respectively.

**Leishmanicidal activity**

The crude extract of *Salix Nigra* in a concentration-dependent manner (at 12, 25, 35, and 45mg/ml) caused 3.0 ± 0.5, 5.0 ± 1.0, 12.5 ± 1.5, 30.0 ± 3.5% inhibition of Leishmania tropica growth after 48 hrs, while after 96 hrs exhibited 6.0 ± 2.0, 25.0 ± 3.0, 35.5 ± 3.5 and 42.0 ± 4.0% inhibitory effect, respectively, as shown in Table 1.

**Fig. 1.** Effect of *Salix Nigra* on root growth of Maize seeds (3 days reading).

**Fig. 2.** Effect of *Salix Nigra* on shoot growth of Maize seeds (3 days reading).

**Discussion**

Including Pakistan, approximately in entire world, there is a marked reduction in crop production due to weeds. The extent of losses caused by weeds was
found to be more as compared to the insects and other diseases but their facts are usually ignored. Weeds control through synthetic drugs has caused various human health problems and soil water pollution (Barkatullah et al., 2001). So weeds control through harmless means is required to protect environment and to increase crop yield. Crude methanolic extract of Salix Nigra showed good phytotoxicity as compared to control which proved the presence of phytochemicals in the plant which can be further isolated and purified for future use.

Fig. 3. Effect of Salix Nigra on root growth of Maize seeds (5 days reading).

Fig. 4. Effect of Salix Nigra on shoot growth of Maize seeds (5 days reading).

Leishmaniasis is generally caused by trypanosomatid protozoan Leishmania (transmitted by the female phlebotomus sand fly), and is one of the major health troubles of tropical, subtropical and Mediterranean areas. Human infectivity is caused by about 21 of 30 species of the genus Leishmania that infects mammals. Approximately 12 million new cases of the most common forms, cutaneous (causes skin sores) and visceral (affects internal organs of the body such as spleen, liver and bone marrow) leishmaniasis, have been reported to crop up every year (Killick-Kendrick, 1999; Onocha et al., 2011). Cutaneous leishmaniasis is the commonest appearance, representing up to 75% of all new cases is caused by L. tropica and Leishmania major, wherein lesions appear on the face, arms and legs. Mucocutaneous leishmaniasis is caused by Leishmania braziliensis and is recognizable by nasal impediment, bleeding and production of painful mucosal lesions. Visceral leishmaniasis by Leishmania donovani and Leishmania infantum is manifested primarily by hepatosplenomegaly, anaemia, prolonged fever and can be deadly if left untreated (Desjeux, 2001; Herwaldt, 1999). The World Health Organization has identified leishmaniasis as a major public health dilemma. The control of leishmaniasis remains a serious problem. An effective vaccine against leishmaniasis does not exist, and chemotherapy is the only effective way to treat all forms of disease. However, current therapy is toxic, expensive and the resistance has emerged as a serious problem, which has forced the search for new anti-leishmanial agents (Monzote, 2009). In the present research, we observed that crude methanolic extract of Salix Nigra caused L. tropica growth inhibition in concentration-dependent manner. The Antileishmanial effect of the plant increased with increase in treatment duration (after 96 h), as expected.

**Conclusion**

Methanolic plant extract of Salix Nigra showed significant phytotoxic & leishmanicidal activity and this might be due to the presence of bioactive constituents in the plant extract which are further needed to be isolated and purified for future use.

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