Synergistic effect of *Pongamia pinnata* bark and *Tamarindus indica* fruit extract against aflatoxin producing fungi i.e. *Aspergillus flavus*

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Abstract

Aflatoxin is the most toxic of many naturally occurring toxins produced by fungi. *Aspergillus flavus* and *Aspergillus parasiticus* are the major causal organisms. These co-exist and grow on almost all crops. *Pongamia pinnata* (Karanj) and *Tamarindus indica* (Imli) and other tree species producing non edible oil were screened for their possible antifungal activity. Methanolic fraction were assayed to control the fungi and significant reduction in fungus growth was observed when applied synergistically than the individual plant extract. It was found that Combination of both extract were more effective than the individual extract when tested alone i.e 62% inhibition of fungal growth as compared to *Pongamia* bark alone (33.20%) and *Tamarindus indica* alone (34.12%).

Keyword: Aflatoxin, *Tamarindus indica*, *Pongamia pinnata*.

Introduction

Aflatoxin are the secondary metabolite i.e. the metabolite not required during the growth of microorganism and it was mainly produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* and in some cases by *Aspergillus nomius*. The most important group of toxigenic Aspergilli are the Aflatoxigenic molds, *A. flavus*, *A. parasiticus* and the recently described but much less common species *A. nomius* all of which are classified in *Aspergillus* section Flavi (Gams et al., 1985). Although these three species are closely related and shares many similarities a number of characteristics may be used in their differentiation. *A. flavus* is widely distributed in nature but *A. parasiticus* is less wide spread, the actual extent of its occurrence being complicated by the tendency for both species to be reported indiscriminately as *A. flavus*. In a wide ranging survey of the mycoflora of commodities in Thailand, *A. flavus* was the most common species in the peanut and second most common (after *Fusarium moniliforme*) in corn. *A. nomius* was reported from both commodities. Soyabean, mung bean, sorghum and other commodities also contained considerable population of *A. flavus* but *A. parasiticus*.

*A. flavus* and *A. parasiticus* have strong affinity with nuts and oil seeds, corn, peanuts and cotton seed are the most important crops invaded by these mold and in many instances, invasion takes place before harvest not during storage. Peanuts are invaded while still in the ground if the crops suffer drought stress or related factor (Cole et al., 1982; Pitt et al., 1991; Sanders et al., 1981). In corn insect damage to developing kernels allow entry of Aflatoxigenic molds but invasion can also occur through the silks of developing
ears (Liljehoj et al., 1980) cotton seeds invaded through nectaries. (Klich et al., 1984). Cereals and spices are common substrate for *A. flavus* (Pitt et al., 1991), but aflatoxin production in these commodities is almost always a result of poor drying, handling or storage and aflatoxin levels are rarely significant.

Significant amount of aflatoxin can occur in peanuts, corn and other nuts and oil seeds particularly in some tropical countries where crops may be grown under marginal condition and where drying and storage facilities are limited.

*A. flavus* can produce Aflatoxin B₁, B₂ and cyclopiazonic acid, but only a proportion of isolates are toxicogenic. *A. parasiticus* produces Aflatoxin B₁, B₂, G₁ and G₂ but not cyclopiazonic acid, and almost all the isolate are toxicogenic. *A. nomius* is morphologically similar to *A. flavus*, but like *A. parasiticus* produces B and G aflatoxin without cyclopiazonic acid. Because these species appear to uncommon, it has been little studied, so the potential toxigenicity of isolates is not known and practical importance of this species is hard to access.

There is an immense potential of active fractions from many biodiversity resources available in the country. The proposed study focuses on a systematic study with respect to antifungal potential of bioactive compounds of these resources in relation to fungal infestation and aflatoxin production in high risk groundnut, its oil and cake. *Pongamia pinnata* Pierre (Leguminosae) is commonly known as Karanja. It is distributed throughout Western Ghats and chiefly found in tidal forests of India (Krishnamurthi, 1969).

Different parts of the plant have been used in traditional medicines for bronchitis, whooping cough, rheumatic joints and to quench thirst in diabetes (Kirtikar et al., 1995). Previous phytochemical examination of this plant indicated the presence of furanoavones, furanoavonols, chromenoavones, avones, and furanodiketones (Talapatra et al., 1980, 1982; Murty et al., 1944; Rangaswami et al., 1942; Sharma et al., 1973; Pathak et al., 1983; Toshiyuki et al., 1992). In the present communication, we describe the isolation and characterization of three new furano-avonoid glucosides, pongamoides A-C (1–3), and a new avonol glucoside pongamoid D (4). Tamarind (*Tamarindus indica* L.) belongs to the family Leguminaceae and grows naturally in many tropical and sub-tropical regions. In Thailand, two types of Tamarind are found in abundance, the so-called sweet and sour varieties. Tamarind is an important food resource for the Thai population. The flower and leaf are eaten as vegetables, while the gum obtained from the seed is used for manufacturing Tamarind gum, which is well known as a component of jelly (Phakruchaphan, 1982). Tamarind seeds are also reported to contain phenolic antioxidants, such as 2-hydroxy-30, 40-dihydroxyacetophenone, methyl 3,4-dihydroxybenzoate, 3,4-dihydroxyphenyl acetate and epicatechin (Tsuda et al., 1994). Extracts exhibit antioxidant potential by reducing lipid peroxidation *in vitro* (Tsuda et al., 1993, 1994) and anti-microbial activity (De et al., 1999). Pumthong (1999) described the antioxidant activity of extracts of Tamarind pericarp, and reported the presence of mainly trimeric tannins and oligomeric procyanidins but these were not identified or quantitated. From this stand point it was of interest to compare the polyphenolic content in methanolic extracts of Tamarind pericarp and seeds, utilizing the methods of Owen et al. (2000a, 2003b).

**Material and Methods**

**Collection and extraction of plant materials**

*Pongamia* bark were collected from the campus of Banthara Research Station of National Botanical Research Institute (CSIR), Lucknow and Fruits of *Tamarindus* were purchased from the local market of
I. Lucknow, Pongamia bark were then air dried to a uniform moisture level. Fruits of T. indica obtained were extracted in methanol using Polytron homogenizer (PT 6100 KINEMETICA). Methanolic extracts collected by filtration (Whatman No. 1) were concentrated under vacuum at low temperature using Rotary Evaporator of Heidolph, Switzerland. The residue then dissolved in 15% ethyl alcohol to get the desired concentration for the activities.

Maintenance of fungal strains

The strain of Aspergillus flavus MTCC2799 were obtained from Microbial Type Culture Collection from IMTECH, Chandigarh. The culture was maintained at 4 ± 1°C on Slants of Potato Dextrose Agar (PDA)

Antifungal assay

Antifungal activity of P. pinnata and T. indica was tested against aflatoxin producing fungal strains of A. flavus obtained from IMTECH, Chandigarh, India.

Preparation of Inoculum

The spore suspension was prepared as described by Fan & Chen (1999). A. flavus was grown on PDA (HiMedia) slant for 5 - 7 days at 25 ± 3°C and the spore were harvested by adding 10 ml of sterile water and aseptically dislodging the spore with a sterile inoculating loop. This was diluted to obtain desired concentration of spore suspension.

Agar Well Diffusion Method

The Potato Dextrose Agar media (HiMedia) was cooled down up to 40-45°C after autoclaving and added desired amount or concentration of plant extract. Shaked it very well and poured the media in Petriplates. After solidifying the media, three wells of 8mm diameter were made in each Petriplates. Without addition of plant extract were used as controls, 40 μL of spore suspension contained 18 x 10^4 spores mL^-1 of A. flavus were added to each wells and incubated at 28°C for 5 days. Fungal growth of both the treated and untreated control plates was measured at every 24 hrs for 5 days (Perea et al., 1990).

The percentage of inhibition was calculated using the following formula (Rasooli et al., 2004)

\[ I = \frac{C - T}{C} \times 100 \]

Where \( I \) = percentage inhibition

\( C = \) radial growth in control

\( T = \) radial growth in treatment

Result and Discussion

Production of toxin are linked to fungal growth and the environment in which the grains/ cereals are stored (especially Relative Humidity and Temperature). Fungal growth and subsequent mycotoxin product in stored grain can be inhibited by physical method (aeration, modified atmosphere, etc.) or by fungistatic of which is propionic acid, acetic acid and sorbic acid are the most common used (Paster et al., 1988). Report by several authors (Monzumi, 1978; Azzouz and Bullerman, 1982; Bahk and Marth, 1983; Bullerman et al., 1980; Yin & Cheng, 1998; Hitokoto et al., 1980) supports the fact that the extract of certain spices and herbs

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of medicinal importance exhibit antifungal property. These natural antifungal agents can be potential exploited in controlling the growth of fungi consequently inhibiting aflatoxin formation (Yin and Cheng, 1998; Grayer & Harborne, 1994).

Methanolic extract of Pongamia bark were tested for their antifungal activity against aflatoxin producing fungi. Fungal growth was significantly reduced in all the treatments as compared to that of control. Antifungal activity varied significantly among different treatments (Table 1). Methanolic extract of bark inhibited fungal growth from 26.44% to 33.20%.

Different concentrations i.e 500ppm, 1000ppm, 1500ppm and 2000ppm of methanolic extract of tamarind fruits were tested for their efficacy against aflatoxin producing fungi. All the concentrations tested were found to decrease fungal growth as compared to that of control. Percentage inhibition of fungal growth ranged from 11.60 to 34.12% at 500ppm and 2000ppm concentration, respectively (Table 2). Another experiment was also set to study the synergistic effect of both extract. Percentage inhibition of fungal growth ranged from 39.75 to 62.72% at 500ppm and 2000ppm concentration, respectively it was found that at 2000 ppm i.e percentage inhibition increased approximately 50 times higher than individual extract.

Table 1. Efficacy of bark of P. pinnata against aflatoxin producing fungal strain A. flavus

<table>
<thead>
<tr>
<th>Plant parts used</th>
<th>Concentration ( in ppm)</th>
<th>Radial dia in mm after 72 hrs</th>
<th>Percentage inhibition in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bark</td>
<td>Control</td>
<td>30.21 ± 1.436</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>22.22 ± 0.780</td>
<td>26.44</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>21.78 ± 1.080</td>
<td>27.9</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>20.35 ± 0.800</td>
<td>32.6</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>20.18 ± 0.980</td>
<td>33.20</td>
</tr>
</tbody>
</table>

Table 2. Efficacy of polar fraction of Tamarindus indica against A. flavus.

<table>
<thead>
<tr>
<th>Plant parts used</th>
<th>Concentration ( ppm)</th>
<th>Radial dia in mm after 72 hrs</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit</td>
<td>Control</td>
<td>30.21± 1.436</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>26.70 ± 0.025</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>25.03 ± 0.095</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>23.13 ± 0.145</td>
<td>23.4</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>19.9 ± 0.295</td>
<td>34.12</td>
</tr>
</tbody>
</table>
Synergistic effect of *Pongamia pinnata* bark

Table.3 Combined effect of methanolic extract *Tamarindus* Fruit and *Pongamia* bark against aflatoxin producing *A. flavus*.

<table>
<thead>
<tr>
<th>Plant parts used</th>
<th>Concentration (ppm)</th>
<th>Radial dia in mm after 72 hrs</th>
<th>Percentage inhibition in %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>30.21 ± 1.436</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>18.20 ± 0.010</td>
<td>39.75</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>16.13 ± 0.085</td>
<td>46.6</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>12.45 ± 1.055</td>
<td>58.78</td>
</tr>
<tr>
<td>Fruit + Bark</td>
<td>2000</td>
<td>9.75 ± 0.470</td>
<td>62.72</td>
</tr>
</tbody>
</table>

In conclusion, the present study reports the antifungal properties of *P. pinnata* and *T.indica* extracts, which can be commercially exploited and applied to food systems. Further studies are needed on the isolation and characterization of individual compounds to elucidate their different antifungal components.

References


(105)

Environment Conservation Journal


