Antifungal and Antiaflatoxigenic activity of Aegle marmelos Linn.

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INTRODUCTION

The increased demand for safe and natural food, without chemical preservatives, provokes many researchers to investigate the antimicrobial and antiaflatoxigenic effects of natural compounds (1). Mycotoxins are secondary metabolites produced by specific filamentous fungi that contaminate agricultural products. They are toxic to humans and animals, cause significant reductions in crop yield and cause economic losses (2-3). Their occurrence in various countries has been well documented (4). When the fungi invade and grow in commodities such as peanuts, corn and the resulting contamination with aflatoxins often makes the commodities unfit for consumption. Consumption of mycotoxin contaminated foods has been associated with several cases of human poisoning, mycotoxicosis, sometimes resulting in death (5). Aflatoxins are known to be potent mutagenic, carcinogenic, teratogenic, hepatotoxic, immunosuppressive and also inhibit several metabolic activities (10). These toxins have been incriminated as the cause of high mortality in livestock and some cases of death in human being (11). Among all classes of aflatoxin, aflatoxin B1 (AFB1) is known to be the most significant in terms of animal and human health risk (12). Thus foods contaminated with these toxigenic fungi and presence of aflatoxin is a major concern, which has received worldwide attention due to their deleterious effects on human and animal health as well as their importance international food trade (13). Although there are many synthetic fungicides in use, their safety in foods are yet to be fully established and also the chances of obtaining newer fungicides that meet stringent environmental and food safety requirements through empirical synthesis do not appear to be high. The use of numerous plant extracts, spices, and their constituents may provide an alternative way to prevent fungal growth and aflatoxin formation (14). Therefore there has been a considerable interest by the food industry and a growing trend in consumer preferences for natural antifungals over synthetic compounds. The present report evaluates the antifungal and antiaflatoxigenic potential Aegle marmelos.

Aegle marmelos Linn. (Rutaceae), AM, commonly known as “Bael tree”, is a well known deciduous tree, 7-8...
m in height with trifoliate aromatic leaves and bisexual flowers, indigenous to India, Burma and Sri Lanka, often planted in the vicinity of Shiva temples. It grows wild all over the sub Himalayan forests, central India, its west coast and in dry hilly places. Fresh half ripe fruit is mild astringent, and used to cure dysentery, diarrhea, hepatitis, tuberculosis, dyspepsia and is good for heart and brain. The roots have antidiarrhoeic, antidote to snake venom, anti-inflammatory and wound healing properties. The leaves and seed oil have antifungal antiaflatoxigenic properties (15). In view of above information and folk lore use of roots and fruits of this plant as antidiarrhoeal and anti microbial agent, present study was undertaken to evaluate the antifungal and antiaflatoxigenic activity of ethanol extract of Aegle marmelos Linn.

**MATERIAL AND METHODS**

**Plant materials and extraction.**

The specimens of AM dried leaves were obtained from Toranmal forest, of Nandurbar district, Maharashtra, and authenticated by Dr. D.A. Patil, at the Pharmacognosy department of R.C. Patel College of Pharmacy and the herbarium specimen (RP-094) was prepared and deposited in the same department. The dried leaves of AM were coarsely powdered by using mixer. The powdered drug was passed through sieves of no 40 and powdered pass through the sieve was used for extraction. The extracts obtained were filtered twice and were concentrated under vacuum using rotary vacuum evaporator, (Butchi, Switzerland) and stored in desiccator until use. About 500 mg of AM leaves powder was extracted overnight by cold maceration in ethanol and water (7:3). The yield of ethanol water extract was 8.5 gm.

**Test Microorganisms**

Cultures were isolated from infected groundnut, maize by using spread plate technique and named as F1, F2, F3, and F4 respectively. The isolated strains were checked for aflatoxin production. The standard aflatoxigenic strains Aspergillus flavus, Aspergillus parasiticus were procured from NCIM, Pune and maintained on PDA agar slants at 4°C.

**Phytochemical screening**

The ethanol extract of AM leaves was subjected to preliminary phytochemical screening using standard methods (16). The plant extract was screened to detect the presence of major phytoconstituents like alkaloids, saponins, flavanoids, glycosides, coumarone glycosides, tannins etc.

**Determination of inhibition of fungal growth and aflatoxin production**

Spores from the strains preserved on Czapek dox agar plate were obtained and inoculated into 5ml of the sterile 2% Tween 80 solution to prepare inoculum. 5ml of inoculum was transferred into synthetic production media (gm lit⁻¹ of distilled water): Glucose, 20.00; yeast extract, 5.00; KH₂PO₄, 1.52; K₂HPO₄, 1.00; KCl, 0.52; MgSO₄.7H₂O, 0.52; CuNO₃.3H₂O, trace; ZnSO₄.7H₂O, trace; FeSO₄.7H₂O, trace; tryptone, 20.00; peptone, 20.00; cas-aminoacid, 20.00; temperature, 25°C; agitation, 160 rpm; pH 7.5 (after sterilization). The flask was then incubated on orbital rotary shaker (steelmate novatech, India) at 200 rpm at 25°C for 6 days and growth of fungi was monitored during incubation (4).

In order to check aflatoxin production in presence of AM extract, the test organism was grown at increasing concentration of AM powdered extract (0.5-2.0 mg/ml). The effect of extract was checked by monitering growth of mycelia and aflatoxin production. After incubation mycelia was separated by filtration and centrifugation of filtered broth was carried out to remove impurities.

**Detection of aflatoxin by using TLC**

Thin layer chromatography of broth was carried out with a objective to characterize the amount of aflatoxin produced, on precoated plates of Silica gel G 60 of dimension 10×10 cm. The filtered broth was tested for aflatoxin by using TLC and observing fluorescence at 360 nm by using the mobile phase Toluene: ethylacetate: chloroform: 90% formic acid (7:5:5:2). The sample (Fermented broth) was applied with the help of capillary about 2 cm from the bottom of the TLC plate. Chamber saturation was done by pouring the mobile phase (Toulene: Ethyl acetate: Chloroform: 90% Fomic acid (7:5:5:2) into the glass chamber, covered it with glass lid and saturation was done for 30 minutes. The spotted plates, after air drying were kept in saturated chamber and solvent was allowed to run after 2/3 run. After that plates were removed, air dried and examined visually under U.V. light at 365 nm the solute front and solvent front were recorded to calculate Rₕ values and Rₛ values were compared with reference compound.

**RESULT AND DISCUSSION**

In the present investigation 4 fungal strains were isolated from the infected groundnut sample. Out of 4 strains a
strain designated as F4, showed blue color fluorescence on TLC plate at 360 nm. To ensure toxigenic potential, production of aflatoxin was carried out by using shake flask method. The screened strain showed production of aflatoxin at shake flask level (Table 1). The plant extract showed the presence of phytoconstituents like alkaloids, saponins, flavanoids, glycosides, gums, and tannins with organic and inorganic constituents. The effect of different

### Table 1. Screening of aflatoxin producing strains from infected ground nut and maize

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Growth</th>
<th>Aflatoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>F2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>F3</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>F4</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+, growth; - no growth)

### Table 2. Effect of Aegle marmelos extract on growth and aflatoxin production on strain F4

<table>
<thead>
<tr>
<th>Broth containing plant extract (mg/ml)</th>
<th>Empty petridish weight (gm)</th>
<th>Petridish with dried mycelia (gm)</th>
<th>Weight of mycelia (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Control)</td>
<td>48.140</td>
<td>56.64</td>
<td>8.5</td>
</tr>
<tr>
<td>0.5</td>
<td>48.140</td>
<td>55.86</td>
<td>7.72</td>
</tr>
<tr>
<td>1.0</td>
<td>48.140</td>
<td>55.02</td>
<td>6.884</td>
</tr>
<tr>
<td>1.5</td>
<td>48.140</td>
<td>53.92</td>
<td>5.784</td>
</tr>
<tr>
<td>2.0</td>
<td>48.140</td>
<td>50.68</td>
<td>2.54</td>
</tr>
<tr>
<td>Standard Griseofulvin (100 μg/ml)</td>
<td>48.140</td>
<td>54.14</td>
<td>6.00</td>
</tr>
</tbody>
</table>

### Table 3. TLC profile of strains F4 control and test flask at 2mg/ml of AM extract

<table>
<thead>
<tr>
<th>Fungal strain</th>
<th>Fluorescence under UV at 365 nm Control</th>
<th>Fluorescence under UV at 365 nm (Test)</th>
<th>RF values (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Light Blue</td>
<td>Absent</td>
<td>0.525</td>
</tr>
<tr>
<td></td>
<td>Light Blue</td>
<td>Absent</td>
<td>0.524</td>
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<td>Absent</td>
<td>0.524</td>
</tr>
</tbody>
</table>

concentration of extract of AM leaves on growth of strain F4 is showed in (Table 2). The results show that inhibition of the fungal growth is related to the concentration of the AM extract. The growth of mycelia was maximum (8.5 gm) in a flask without AM extract whereas it was lowest (2.54 gm) in a flask containing 2mg/ml of AM extract and 6.00gm in flask containing standard antifungal griseofulvin at concentration of 100 μg /ml . TLC profile of the control and test broth of the strain F4 is summarized in (Table 3). No fluorescence in the test flask containing 2 mg/ml AM extract while it shows light blue color fluorescence was observed in the control flask (Table 3). The results show that antifungal activity of the extract is comparable to standard antifungal griseofulvin. The result shows that the ethanol extract of leaves of AM has potential to inhibit the fungal growth as well as it has profound antiaflatoxigenic activity. Our findings match with earlier studies of Kumar et. al, (17) and Mishra et.al (18). However systemic and concentration dependent study is required for the commercial exploitation of this plant as a natural remedy for controlling food born aflatoxicosis.

**ACKNOWLEDGEMENTS**

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REFERENCES


