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Original Article

Antifungal Activity of Areca catechu L

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Abstract

The purpose of this investigation was to study the antifungal activity of hot water extract of Areca Catechu nuts collected from coastal region of Kerala. The fungi (Mucor sp, Aspergillus niger, Cladosporium sp and Candida albicans) were inoculated on Sabouraud’s Dextrose Broth (100ml) and kept at room temperature for overnight incubation. 0.1 ml of this O/N culture was incubated along with different concentrations of the extract (10-1000µg/ml) for eight hours, in 3 ml of nutrient broth. The absorbance was read at 530 nm at different intervals. The experiment was done in triplicate. The Areca nut extract did not inhibit the growth of mycelial fungal forms such as Mucor sp, Aspergillus niger and Cladosporium sp but the growth of unicellular fungus Candida albicans was inhibited. The concentration needed for 100% inhibition was 16.67 µg/ml concentration. Disc diffusion method was used to evaluate the zone of inhibition against fungi by taking Nystatin disc as standard. The largest zone of inhibition was observed against Candida albicans. The most effective concentration needed for 85% inhibition of the production of aflatoxin by Aspergillus flavus was between 100-250 µg/ml.

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KEYWORDS:- Areca catechu; Sabouraud’s Dextrose Broth (SDB); Antifungal activity; Candida albicans; Aflatoxin

1. Introduction

Areca nut, which is also known as betelnut, is the seed of arecanut palm, which is an ornamental plant. Areca nut chewing is practiced in India as well as in many Asian Countries from time immemorial. In traditional practice it is used for cleansing the oral cavity [1]. A common practice among Asian elders is the chewing of betel quid commonly known as “nganga”, a combination of betel nut, betel leaves with lime or tobacco. Chewing betel quid is considered a past time and is a cultural tradition in different countries like India, Indonesia, and Arabian Peninsula, some of the Pacific islands such as Micronesia, Fiji, Solomon Islands and the Philippines [1]. Areca catechu L. is a straight solitary tree that has annular leaf scars. The leaves have leaflets that measure up to 4 cm. The ovoid fruit turns red or orange when ripe and has a fleshy pericarp and a fibrous mesocarp Seads are called as nuts; they are used for mastication [2]. The husks are used as an alternative to toothbrush and it is also used as a vermifuge in some parts of China [3]. However this property of betel nut chewing has not been scientifically proven. The antimicrobial effect of ethanol extract of Areca catechu L. seeds against mixed-oral flora and certain-gram negative clinical isolates were tested using agar-well diffusion method by [4]. Their work demonstrated positive antimicrobial properties of Areca catechu L. nut against Candida albicans but not against Gram-negative bacetria. Areca nut is reported to have antiinflammatory and antiproliferative properties. These activities are due to the presence of alkaloids in Areca nut. Four major alkaloids have been found in Areca catechu; they are arecoline, arecaidine, guvacoline, and guvacine. Arecoline is an oily-liquid that is soluble in water, alcohols and ether; this lipophilic nature can have an entry to the brain and intracellular spaces to give stimulatory effects. We carried out this work with a view to assess the antifungal activity of Areca catechu nuts. Since not much work has been found in the literature.

2. Materials and Methods:

2.1 Chemicals and media

Dimethyl sulphoxide (DMSO), Mueller Hinton Agar (MHA), Sabouraud dextrose agar (SDA), the antifungal agents Nystatin, Amphotericin B and Aflatoxin were obtained from Himedia, Mumbai, India. Methanol was obtained from Rankem Company, India.
Table 1 Effect of Areca nut extract on Candida albicans

<table>
<thead>
<tr>
<th>Concentration + strain</th>
<th>Percent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1h</td>
</tr>
<tr>
<td>10 µg/ml A.E + C albicans</td>
<td>0</td>
</tr>
<tr>
<td>20 µg/ml A.E + C albicans</td>
<td>0</td>
</tr>
<tr>
<td>50 µg/ml A.E + C albicans</td>
<td>100</td>
</tr>
<tr>
<td>100 µg/ml A.E + C albicans</td>
<td>100</td>
</tr>
</tbody>
</table>

Note: Concentration is expressed per 3 ml of medium; there was no inhibition against Mucor sp, Aspergillus niger, Cladosporium sp.

Table 2 Antifungal Activity of Areca nut extract

<table>
<thead>
<tr>
<th>Organism</th>
<th>Diameter of growth inhibition zone (mm)</th>
<th>Areca nut extract</th>
<th>Standard Amphotericin B</th>
<th>Nystatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucor sp.</td>
<td>12</td>
<td>16</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>14</td>
<td>20</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cladosporium sp.</td>
<td>13</td>
<td>16</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>18</td>
<td>16</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

All values are an average of three determinations. The concentrations of extracts employed were a 1000 µg/ml. Diameter of cups was 6 mm.

Table 3 Inhibition of Aflatoxin production by Areca nut

<table>
<thead>
<tr>
<th>Concentration of Areca nut/ml</th>
<th>Concentration of Aflatoxin (ppb)</th>
<th>Percent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>35 ppb</td>
<td>-</td>
</tr>
<tr>
<td>10-50 µg/ml</td>
<td>20 ppb</td>
<td>42.85%</td>
</tr>
<tr>
<td>100-250 µg/ml</td>
<td>5 ppb</td>
<td>85.71%</td>
</tr>
</tbody>
</table>

2.2 Extraction of Areca catechu L

Areca nuts were procured from the coastal areas of Kerala. Hot water extract of the arecanut were prepared by boiling 100g nuts in 500 ml of distilled water for one hour. The extract was then concentrated by evaporation. The yield of the extract was found to be 6.4%. The extract was then resuspended in distilled water and diluted to the desired concentration. The extract was stored at 4°C until further use.

2.3 Determination of antifungal activity of the extract:

2.3.1 Preparation of Inoculum

The filamentous fungi were grown in Sabouraud’s dextrose agar (SDA) slants at 28°C for 10 days and the spores were collected using sterile doubled distilled water and homogenized. Yeast was grown on Sabouraud’s dextrose broth (SDB) at 28°C for 48 h.

2.3.2 Effect of Areca nut extract on Candida albicans by Tube method

A loopful of fungi were inoculated in Sabouraud’s Dextrose Broth (100 ml) and kept at room temperature for overnight incubation. 0.1 ml of this O/N culture was incubated in the various concentrations of the extract (10-1000µg/ml) for eight hours, in 3 ml of nutrient broth. The absorbance was read at 530 nm at various time intervals. The experiment was done in triplicate.

2.3.3 Antifungal activity of Areca nut extract by disc diffusion method

The antifungal activity was screened by disc diffusion method [5]. The Sabouraud’s dextrose agar plates were inoculated with the fungal culture (72 hr culture). The following fungal pathogens were used for the experiments: Mucor sp, Aspergillus niger, Cladosporium sp and Candida albicans. Sabouraud Dextrose Broth cultures were prepared in test tubes. Using sterile cotton swab, the surface of Sabouraud’s Dextrose Agar plates were swabbed to prepare lawn cultures. 5 min after the agar surface had dried, wells were dug using cork borer aseptically. Wells were saturated with arecanut extract, concentrations ranging from 1000 µg/ml to 1000 µg/ml. The plates were incubated at 28°C for 24-48 hrs. The diameters of zones of inhibition were measured using a scale to the nearest millimeter [6].

2.3.4 Determination of the effect of Areca nut extract on Aflatoxin production by Aspergillus flavus

A loopful of A. flavus culture, which was originally grown on Sabouraud’s Dextrose Agar, was uniformly suspended in 5 ml of glucose ammonium nitrate medium containing the mineral supplements. It was incubated at 37 °C for six days. 100 µl of this suspension was inoculated in test tubes containing 2 ml of glucose ammonium nitrate medium with different concentrations of arecanut extract, ranging from 10-250 µg/ml. All the tubes were kept in triplicates and incubated at 30°C for six days. Aflatoxin was extracted using Modified Pons’ method [7] The toxin concentrations were estimated in correlation with the intensity of fluorescence of standard aflatoxin on TLC plates [8].

3. Results and Discussion

The antifungal activity of Areca nut hot water extract was studied. [9] Used the tube method to study the growth
characteristics of fungi by inoculating the culture in different Medias at different temperature, pH and NACL concentration. In another study, [10] reported the inhibition of C. albicans by germ tube assays in different medicinal plants namely such as Piper bredemeyeri Taq and Lippia origanoides oils. In the present study the tube method was used to see the effect of Areca nut extract against fungi by inoculating the cultures in nutrient broth and incubating at various concentrations for eight hours. There was 100% inhibition of growth at 50 and 100μg/ml from the first itself for Candida albicans. The growth of other fungi were not inhibited (Table 1). In the present study the plate method was employed for Mucor sp, Aspergillus niger, Cladosporium sp and Candida albicans to assess the zones of inhibition. Areca nut extract showed minimum zone of inhibition against Mucor sp (12mm), Aspergillus niger (14mm) and Cladosporium sp (13mm) but it showed good zone of inhibition against Candida albicans (18mm) at 16.67 µg/ml concentration (Table 2). In another study, [11] evaluated the antimicrobial activity of Areca nut seed extract against S. mutans and found 100% inhibition at 100μg/ml. [4] demonstrated the inhibitory effects of Areca catechu L against Candida albicans with zones of inhibition measuring 6 mm in diameter in MHA agar using 50 μg/ml concentration. [12] Reported that Areca catechu root ethanol extract showed antifungal activity against Aspergillus niger with zone of inhibition of 28 mm at 500 μg/ml. [13] reported that the acetone extract of Aloe vera leaves in concentration of 2000μg/ml significantly inhibited (100%) the growth of A. flavus and at the lowest concentration (2μL), the growth inhibition was 51.72%. In the present study the inhibition of aflatoxin by arecanut extract was 85.71% at the concentration of 100-250μg/ml (Table 3). [14] Areca nuts had collected from different regions of South Asia. Among them Indonesian and Sri Lankan samples inhibited Aflatoxin production in the range between 3.3–39.2 and 6.5–103.4 μg kg–1 respectively. This study gives scientific evidence for the antifungal property of Areca nut extract.

4. Conclusion
Areca Catechu nuts possess antifungal activity. The present investigation indicated that the hot water extract of Areca catechu exhibited antifungal activity against C. albicans, Mucor sp. Aspergillus niger, Cladosporium sp. Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antifungal activity.

References

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