Antifungal activity of Rhein isolated from Cassia fistula L. flower

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Abstract

Antifungal activity of rhein (1, 8-dihydroxyanthraquinone-3-carboxylic acid) isolated from the ethyl acetate extract of Cassia fistula flower was studied. Rhein inhibited the growth of many fungi such as Trichophyton mentagrophytes (MIC 31.25 µg/ml), Trichophyton simii (MIC 125 µg/ml), Trichophyton rubrum (MIC 62.5 µg/ml) and Epidermophyton floccosum (MIC 31.25 µg/ml).

Introduction

Plants used in traditional medicine usually constitute an important source of new biologically active compounds. Numerous useful drugs have been discovered from higher plants by following up ethnomedical uses [1]. During the past several years, there has been an increasing incidence of fungal infections due to a growth in immunocompromised population such as organ transplant recipients, cancer and HIV/AIDS patients. This fact coupled with the resistance to antibiotics and toxicity during prolonged treatment with several antifungal drugs, has been the reason for an extended search for newer drugs to treat opportunistic fungal infections [2,3]. Cassia fistula L., (Caesalpiniaceae), a semi-wild Indian Laburnum (also known as the Golden Shower), is distributed in various countries including Asia, South Africa, Mexico, China, West Indies, East Africa and Brazil. It is an ornamental tree with beautiful bunches of yellow flowers. C. fistula exhibited significant antifungal activity and showed properties that support folkloric use in the treatment of some diseases as broad-spectrum antifungal agent [4]. The whole plant is used to treat diarrhea; seeds, flowers and fruits are used to treat skin diseases, fever, abdominal pain and leprosy by traditional people [5]. This plant has a strong tendency to contain anthraquinone derivatives. From the genus Cassia many quinone derivatives such as Kaempferol have also been isolated; a proanthocyanadin has been isolated from the acetone extract of the flower [6]. A bianthraquinone glycoside, fistulin, together with kaempferol and rhein have been isolated from ethanol extracts of C. fistula flowers [7]. Besides phenolics and their derivatives, a certain amount of alkaloids have also been reported in the flowers; traces of triterpenes have been observed in both flowers and fruits [8,9].

Our preliminary evaluation of ethyl acetate extract from Cassia fistula flowers showed significant antifungal activity [10]. In the present work, we report the separation and identification of rhein from C. fistula flowers and its antifungal effect.

Materials and Methods

Plant material
Cassia fistula flowers were collected from Loyola College Campus, Chennai, India. It was authenticated by Dr. S. Amerjotthy, Department of Botany, Presidency College, Chennai, India. A voucher specimen (ERIC-D-73) is deposited at the herbarium of Entomology Research Institute, Loyola College, Chennai.

Preparation of crude extract
The extracts were taken using cold percolation method. Fresh flowers were collected (9kg) and shade dried at room temperature and ground in a manual mill. The powder (1kg) was extracted with 3 ltr (1:3 w/v) of hexane for 48 hours. The extract was filtered through a Buchner funnel with Whatman number 1 filter paper. The filtrate was evaporated to dryness under reduced pressure using rotary evaporator at 40°C. The remains of the plant material were extracted with chloroform (11g), ethyl acetate (17g), methanol (20) and water (13g) sequentially in a similar manner. The crude extracts were stored at 4°C until further use.

Isolation of active compound
The crude ethyl acetate extract (10 g) was subjected to column chromatography over silica gel (200 g-acme’s 100–200 mesh) and eluted with hexane followed by the combination of hexane: ethyl acetate ranging from 95:5 to 100. 117 fractions were collected in a 200ml conical flask. After checking TLC, the fractions were combined in to 24 fractions. Fraction 10 showed a crystal which was subjected to crystallographic analysis and identified reported.10 Fraction 18 showed single spot on TLC (RF = 0.36) and yielded 210 mg; this fraction was eluted using hexane: ethyl acetate (10:9) as mobile phase solvent system. The spot turned pink on exposure to ammonia vapor; it indicated the presence of

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anthraquinones. The compound was subjected to spectroscopic analysis.

Spectroscopic analysis

IR, 1H-NMR, 13C NMR and MASS were taken from Nicholas Primal Pvt. Ltd. Ennore, India and used to identify the isolated compound.

Fungi

Fungi, Trichophyton rubrum, T. rubrum 57/01, T. Mentagrophytes, T. simii, Epidermophyton floccosum, Scopulariopsis sp., Aspergillus niger, Botrytis cinerea, Curvularia lunata and Candida albicans MTCC 227 were used for the experiments. All cultures were obtained from the Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India.

Assay for antifungal activity

The antifungal activity of the isolated compound was determined using standard method [11]. The compound was tested by micro broth two-fold serial dilution technique. The crude extract and compound were dissolved in water + 2% dimethyl sulfoxide (DMSO). The initial concentration of extract was 1mg/ml; the initial concentration of the compound was 250µg/ml. The initial test concentrations were serially diluted two-fold. Each well was inoculated with 5 ml of suspension containing 104 spore/ml of fungi. Fluconazole and Ketoconazole were included in the assays as positive controls. The plates were incubated for 24, 48 or 72 h at 27 °C up to 9 days. MIC was defined as the lowest extract concentration showing no visible fungal growth after incubation time.

Results

The present study deals with the antifungal activity of crude ethyl acetate extract and an isolated compound from C. fistula flower. The crude ethyl acetate extract inhibited the growth of fungi T. mentagrophytes (MIC 250 µg/ml), T. simii (MIC 1000 µg/ml), T. rubrum (1000 µg/ml), T. rubrum 57 (MIC 500 µg/ml), E. floccosum (MIC 500 µg/ml), Scopulariopsis sp (MIC 500 µg/ml). The structural identification of compound was carried out using IR, MS, 1H NMR and 13C NMR spectra as follows: the EI-MS: m/z 284, 267, 256, 239, 228, 211, 183, 155, 142, 126. It showed the molecular ion at m/z 284, which was corresponding to the molecular formula C15H8O6 of rhein; its melting point was at 321ºC [12]. The 1H NMR spectrum revealed five aromatic protons of which two were broad singlets due to meta coupling. 1H NMR (75 MHz, DMSO): 11.9 (1H, brs, C1–OH), 11.5 (1H, brs, C8–OH), 8.13 (1H,brs, C2–H), 7.40 (1H, d, J = 8.5 Hz, C5–H), 7.73 (1H, m, C6–H), 7.75 (1H,brs, C4–H), 7.81(1H, d, J = 8.0 Hz, C7–H) corresponded to rhein [13]. 13C NMR [(300 MHz, dimethyl sulfoxide (DMSO)] δ ppm: 161.2 (C-1), 124.2 (C-2), 165.6 (C-3), 119.0 (C-4), 124.7 (C-5), 138.5 (C-6), 124.7 (C-7), 161.5 (C-8), 181.2 (C-9), 181.2 (C-10), 130.0 (C-4a), 118.9 (C-8a), 118.7 (C-9a), 133.3 (C-10a), 191.4 (3-COOH) which corresponded to those reported [12]. The active compound was identified as Rhein (1,8-dihydroxyanthraquinone-2-carboxylic acid) (Fig 1).

Discussion

Fungal diseases have increased dramatically in recent years. The treatment of mycoses has lagged behind and fewer antifungal than antibacterial substances are available. Therefore, a search for new antifungal drugs is extremely necessary [16]. Many plants are now used to treat various infectious diseases. In this study we examined the antifungal activity of C. fistula flower ethyl acetate extract and isolated compound rhein. Traditional uses of this plant favor its use as antifungal drug. Ethyl acetate extract of C. fistula showed promising antifungal activity. This indicated that there may be some active compound involved. So we selected ethyl acetate extract for isolation of active compound. Isolated active compound was confirmed by comparing the IR, 1H NMR, 13C NMR, MASS and m.p., data with that of the known compound rhein. Previously the same compound was reported against Botrytis cinerea [17]; Candida albicans, Trichophyton mentagrophytes [18]. However, rhein was not yet tested against T. rubrum, T. rubrum 57/01, T. simii, E. floccosum, Scopulariopsis sp., A. niger, M. grisea and C. Albicans were not inhibited.
In our findings rhein inhibited the growth of T. rubrum at 62.5 µg/ml. Generally anthraquinone derivatives, which include emodin, chrysophanol, rhein, aloe-emodin, physcion, and their glucosides, and important active components with various pharmacological actions such as purgation, antibacterial, antifungal and antitumor activity [21]. Antifungal activities of anthraquinones and naphthoquinones isolated from natural sources have been reported [22-25]. Rhein was isolated from the leaves of Cassia reticulata and tested against Neisseria gonorrhoeae. It exhibited significant inhibitory activities [26]. Novel anthraquinone (3,4-dihydroxy-1-methoxyanthraquinone-2-carboxaldehyde) was isolated from ethanolic extract of Saprosmia fragrans and tested against T. mentagrophytes (12.5 µg/ml) [27]. Our results showed that the isolated compound significantly inhibited the growth of T. mentagrophytes at 31.25 µg/ml and E. floccosum 31.25 µg/ml. Kanokmedhakul et al. [28] have isolated seven anthraquinone and triterpenoids from Prismatomeris fragrans and also reported antifungal activity for isolated compounds. Previously rhein was also reported to inhibit oral bacteria [29].

Conclusion

Rhein can be used as antifungal agent. The present findings show that rhein exhibited good activity against fungi.

References

Illustrations

Illustration 1

Figure 1: Rhein (1, 8-dihydroxyanthraquinone-3-carboxylic acid) isolated from ethyl acetate extract
**Illustration 2**

Table 1: Antifungal activity of Rhein (MIC µg/ml)

<table>
<thead>
<tr>
<th>Tested Fungi</th>
<th>EA</th>
<th>C</th>
<th>Fl</th>
<th>Ket</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichophyton mentagrophytes</em> 66/01</td>
<td>250</td>
<td>31.2</td>
<td>25</td>
<td>&lt;12.5</td>
</tr>
<tr>
<td><em>T. simii</em> 110/02</td>
<td>1000</td>
<td>125</td>
<td>&lt;12.5</td>
<td>&lt;12.5</td>
</tr>
<tr>
<td><em>T. rubrum</em> MTCC 296</td>
<td>1000</td>
<td>62.5</td>
<td>&lt;12.5</td>
<td>&lt;12.5</td>
</tr>
<tr>
<td><em>T. rubrum</em> 57/01</td>
<td>500</td>
<td>62.5</td>
<td>25</td>
<td>&lt;12.5</td>
</tr>
<tr>
<td><em>Epidermophyton floccosum</em> 73/01</td>
<td>500</td>
<td>31.2</td>
<td>12.5</td>
<td>&lt;12.5</td>
</tr>
<tr>
<td><em>Scopulariopsis</em> sp. 101/01</td>
<td>500</td>
<td>250</td>
<td>&lt;12.5</td>
<td>&lt;12.5</td>
</tr>
<tr>
<td><em>Aspergillus niger</em> MTCC 1344</td>
<td>&gt;1000</td>
<td>&gt;250</td>
<td>100</td>
<td>&lt;12.5</td>
</tr>
<tr>
<td><em>Curvularia lunata</em> 46/01</td>
<td>&gt;1000</td>
<td>&gt;250</td>
<td>&lt;12.5</td>
<td>&lt;12.5</td>
</tr>
<tr>
<td><em>Magnaporthe grisea</em></td>
<td>&gt;1000</td>
<td>&gt;250</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td><em>Botrytis cinerea</em></td>
<td>&gt;1000</td>
<td>&gt;250</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td><em>Candida albicans</em> MTCC 227</td>
<td>&gt;1000</td>
<td>&gt;250</td>
<td>&gt;100</td>
<td>25</td>
</tr>
</tbody>
</table>

EA- Ethyl acetate (Crude extract) C- Rhein; Fl – Fluconazole (antifungal agent); Ket- Ketoconazole (antifungal agent); nt- not test
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