Antifungal activity of Rhein isolated from Cassia fistula L. flower

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**Competing Interests:**
We have no competing interest  
**Additional Files:**
- Full manuscript  
- Cover letter
Antifungal activity of Rhein isolated from Cassia fistula L. flower

Author(s): Duraipandiyan V, Ignacimuthu S

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 \mu g/ml), Trichophyton simii (MIC 125 \mu g/ml), Trichophyton rubrum (MIC 62.5 \mu g/ml) and Epidermophyton floccosum (MIC 31.25 \mu g/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. (Caesalpiniaeae), 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 bianthaquinone glycoside, fistulin, together with kaempferol and rhein have been isolated from ethanol extracts of C. fistula flowers [7].

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\degree 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\degree 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 (RI = 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...
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 1 mg/ml; the initial concentration of the compound was 250 µg/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).

**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, C. lunata and M. grisea. Rhein inhibited the growth of T. rubrum, T. rubrum 57, T. simii, E. floccosum and Scopulariopsis sp. The anthraquinone derivative rhein is significantly antiseptic and is especially toxic to the pathogen [19].

Trichophyton rubrum is the most prevalent pathogenic fungus; worldwide it represents 80% of clinical isolates...
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 Saposma 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 Å,Åµg/ml)

<table>
<thead>
<tr>
<th>Tested Fungi</th>
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<tr>
<td><em>Trichophyton mentagrophytes</em> 66/01</td>
<td>250</td>
<td>31.2</td>
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<td><em>T. simii</em> 110/02</td>
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<tr>
<td><em>T. rubrum</em> 57/01</td>
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<td>62.5</td>
<td>25</td>
<td>&lt;12.5</td>
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<td><em>Epidermophyton floccosum</em> 73/01</td>
<td>500</td>
<td>31.2</td>
<td>12.5</td>
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<td><em>Scopulariopsis</em> sp. 101/01</td>
<td>500</td>
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<td><em>Aspergillus niger</em> MTCC 1344</td>
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<tr>
<td><em>Curvularia lunata</em> 46/01</td>
<td>&gt;1000</td>
<td>&gt;250</td>
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<td><em>Magnaporthe grisea</em></td>
<td>&gt;1000</td>
<td>&gt;250</td>
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<td><em>Botrytis cinerea</em></td>
<td>&gt;1000</td>
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<tr>
<td><em>Candida albicans</em> MTCC 227</td>
<td>&gt;1000</td>
<td>&gt;250</td>
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EA- Ethyl acetate (Crude extract) C- Rhein; Fl – Fluconazole (antifungal agent); Ket-
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Reviews

Review 1

Review Title: appropriate

Posted by Lead Faculty Dr. Gurpreet K Randhawa on 17 Dec 2011 06:11:43 AM GMT

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Rating: 6

Comment:
comparative comments on antifungal activity of rhein, ketoconzole and fluconazole can be given under result heading.
Future prospects concerning further animal/ clinical studies while concluding the study can be added

a graphical representation of above comparison will be welcomed

Competing interests: no

Invited by the author to make a review on this article?: No

Experience and credentials in the specific area of science: 10 years

Publications in the same or a related area of science: No

How to cite: RU A.appropriate[Review of the article 'Antifungal activity of Rhein isolated from Cassia fistula L. flower ' by ]WebmedCentral 1970;2(12):WMCRW001278
Review 2

Review Title: Comments on Antifungal activity of Rhein isolated from Cassia fistula L. flower published in Webmed Central

Posted by Dr. M Ayyanar on 14 Feb 2011 04:13:42 PM GMT

1. Is the subject of the article within the scope of the subject category? Yes
2. Are the interpretations / conclusions sound and justified by the data? Yes
3. Is this a new and original contribution? Yes
4. Does this paper exemplify an awareness of other research on the topic? Yes
5. Are structure and length satisfactory? Yes
6. Can you suggest brief additions or amendments or an introductory statement that will increase the value of this paper for an international audience? Yes
7. Can you suggest any reductions in the paper, or deletions of parts? Yes
8. Is the quality of the diction satisfactory? Yes
9. Are the illustrations and tables necessary and acceptable? Yes
10. Are the references adequate and are they all necessary? Yes
11. Are the keywords and abstract or summary informative? Yes

Rating: 7

Comment:
7. No, there is a no need for any reduction or deletion in any parts of the paper.

Invited by the author to make a review on this article? : No

Experience and credentials in the specific area of science:
I am working in the field of complementary and Alternative medicine in the last seven years. In addition, I have adequate knowledge in the areas of Plant Taxonomy and Plant Tissue Culture.

Publications in the same or a related area of science: Yes


Review 3

Review Title: Antifungal activity of Rhein isolated from Cassia fistula L. flower

Posted by Dr. C Sreenathkumar on 02 Feb 2011 09:22:14 AM GMT

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Rating: 8

Comment:
Respect Editor

I thoroughly review the paper entitled "Antifungal activity of Rhein isolated from Cassia fistula L. flower". The introductory part of the paper is well presented. Rhein isolation and antifungal activity was presented in well defined manner.

I strongly recommend to the above paper published in your journal.

Competing interests: -

Invited by the author to make a review on this article? : Yes

Experience and credentials in the specific area of science:
10 years

Publications in the same or a related area of science: No

How to cite: Sreenathkumar C. Antifungal activity of Rhein isolated from Cassia fistula L. flower[Review of the article 'Antifungal activity of Rhein isolated from Cassia fistula L. flower ' by ].WebmedCentral 1970;2(2):WMCRW00432
Review 4

Review Title: Antifungal Activity Of Rhein Isolated From Cassia Fistula L. Flower

Posted by Dr. K Balakrishna on 03 Jan 2011 04:03:15 AM GMT

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<td>Are the keywords and abstract or summary informative? Yes</td>
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Rating: 5

Comment:
The Genus Cassia is well known for anthraquinones. Anthraquinone, in general quinones show marked antimicrobial activity. The authors have reported antifungal activity of one such anthraquinone viz. Rhein. Thus they have made an important contribution towards the antimicrobial activity of anthraquinones. The paper is acceptable for publication.

Thank you for selecting me as a referee.

your sincerely

Dr.K.Balakrishna

Competing interests: I have no competing interest

Invited by the author to make a review on this article? : No

Experience and credentials in the specific area of science:
40 years

Publications in the same or a related area of science: Yes


How to cite: Balakrishna K. Antifungal Activity Of Rhein Isolated From Cassia Fistula L. Flower[Review of the article 'Antifungal activity of Rhein isolated from Cassia fistula L. flower ' by ].WebmedCentral 1970;2(1):WMCRW00316
Review 5

Review Title: Antifungal activity of Rhein isolated from Cassia

Posted by Prof. Ary Fernandes Junior on 04 Nov 2010 10:49:27 AM GMT

1. Is the subject of the article within the scope of the subject category? Yes
2. Are the interpretations / conclusions sound and justified by the data? Yes
3. Is this a new and original contribution? Yes
4. Does this paper exemplify an awareness of other research on the topic? Yes
5. Are structure and length satisfactory? Yes
6. Can you suggest brief additions or amendments or an introductory statement that will increase the value of this paper for an international audience? Yes
7. Can you suggest any reductions in the paper, or deletions of parts? No
8. Is the quality of the diction satisfactory? Yes
9. Are the illustrations and tables necessary and acceptable? Yes
10. Are the references adequate and are they all necessary? Yes
11. Are the keywords and abstract or summary informative? Yes

Rating: 0

Comment: The study is important in the area of bioprospecting products with antimicrobial activity, particularly antifungal activity. The paper was prepared according to scientific standards and has the merit for the tests using a biological major compound in the plant under study. However, we believe that the article could be presented as short communication if it is considered a regular publisher on natural products.

Invited by the author to make a review on this article? Yes

Experience and credentials in the specific area of science: Microbiology and antimicrobial activity of natural products

Publications in the same or a related area of science: Microbiology and antimicrobial activity of natural products

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