Entada rheedii phaseoloidin, protocatechuic acid and entadamide A against protozoal diseases: trypanosomiasis and leishmaniasis

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ABSTRACT

Background: African plant extracts and their isolated constituents remains a hot area for discovering novel drugs. Entada rheedii Spreng. and its previously isolated major phytochemicals were evaluated for their antiprotozoal potency. Method: In vitro antiprotozoal activity against Trypanosoma brucei brucei, T. b. rhodesiense, T. cruzi and Leishmania infantum was determined, including cytotoxicity evaluation for the determination of selectivity. Results: The crude extract was inactive. Phaseoloidin exhibited pronounced activity against T. b. brucei, T. cruzi, T. b. rhodesiense, and L. infantum (IC50 of 9.70, 8.00, 7.83 and 6.96 μg/mL, respectively). Entadamide A showed pronounced activity against T. cruzi and L. infantum (IC50 of 8.98 and 10.77μg/mL, respectively). Protocatecheuic acid showed pronounced activity against T. b. brucei (IC50 of 8.12 μg/mL) and moderate activity against T. cruzi and T. b. rhodesiense (IC50 of 14.42, 12.23 μg/mL, respectively). All the active compounds exhibited low cytotoxicity score 2 (CC50>13 μg/mL). Conclusion: The major phytochemicals of the African E. rheedii seeds were potent against sleeping sickness, Chagas disease, and leishmaniasis. They acted in their pure form rather than acting collectively in the crude extract.

Keywords: Sulfuramide, phaseoloidin, antitrypanosomal, MRC-5stress.

INTRODUCTION

Protozoal infections are one of the major worldwide health problems especially, African sleeping sickness, Chagas disease and leishmaniasis are among the neglected tropical diseases that do not receive attention like many others. Neglected tropical diseases tend to thrive in developing countries where health care, water purity, and sanitation are poor. The WHO estimates that not less than one-sixth of people suffer from at least one neglected tropical disease and it is predicted that 7-8 million people have the Chagas disease [1]. Conventional medicines for neglected tropical diseases are unaffordable, especially for poor African people, and of course they can cause many side effects. This encouraged the authors to search for more effective and less harmful medicinal agents from medicinal plants that are thought to be an excellent source of new antiprotozoal drugs [2]. Africa is highly diverse ethnobotanically, the documentation of the African plant-based chemical components by in silico procedures to explore their mechanisms of action is nowadays hot research topic [3-7]. Many isolated compounds from African medicinal plants were evaluated in vitro and/or in vivo against parasitic protozoal infections [8-11]. In this...
study, the emphasis is laid on isolates from *Entada rheedii* Spreng. (Fabaceae) seed; an African medicinal plant for drug discovery and further development of new agents for parasitic diseases treatment especially sleeping sickness, leishmaniasis, and Chagas disease. No studies have been traced reporting *E. rheedii* phytochemicals antiprotozoal activity.

2. Materials and methods

2.1 Plant material, extraction, fractionation, and isolation of the major constituents

*E. rheedii* Spreng. seeds were purchased from Pharmacognosy Department, Faculty of Pharmacy, Cairo University experimental station. Then, the seeds were powdered and defatted with *n*-hexane. The marc was then extracted with ethanol (70%) and isolation of the major constituents was done as reported by Okba, et. al. [12].

2.2 Chemicals

All reference drugs were obtained from WHO-TDR and Sigma-Aldrich (Bornem, Belgium).

2.3 *In vitro* biological assays

Standard protocols used by the Lab of Microbiology, Parasitology and Hygiene (LMPH), Faculty of Pharmaceutical Sciences, Biomedical and Veterinary Sciences of the University of Antwerp, Belgium were applied [13,14]. Test plate production, antitrypanosomal and antileishmanial activity in addition to evaluation of cytotoxicity on MRC-5 cells (human embryonic lung fibroblasts) were all carried according to Abdel-Sattar et.al. [15].

2.4 HPLC characterization of the crude extract

HPLC apparatus Agilent Series 1100 equipped with Quaternary pump; and UV detector series 1100 was used for HPLC analyses. HPLC analysis was done on a Hypersil-ODS (4.6x250 mm, 5µm) column.

Isocratic elution was adopted with acetonitrile and 15% acetic acid (40:60 v/v) as mobile phase. The flow rate of the mobile phase was 1 ml/min, and the injection volume was 5 µl for both standards and sample extracts. Detection was carried out by a UV detector set at 270 nm for phenolic acids. Components of the samples were identified by comparing their retention times with that of the standards (prepared as 50-600 µg/ml solutions in methanol). Quantification was based on measuring the peak areas of both standards and samples related to external standards.

3. Results

In a continuation of our interest in exploring plants with antiprotozoal potential [15-21], the ethanol (70%) extract of *E. rheedii* Spreng. seed and its major isolated phytochemicals [12] were tested against *T. cruzi*, *T. b. rhodesiense*, *T. b. brucei* and *L. infantum*, along with MRC-5 cell line for cytotoxicity together with an assessment of their selectivity. Analysis of the collected data is based on the scoring system [8] and IC50-values calculation (µg/mL) adopted by LMPH (Table 1). IC50 of tested compounds exspressed as µM/mL were recorded in Table 2. Structures of the previously [12] isolated compounds were illustrated in Figure 1.

Results in (Table 1) revealed that the total ethanol extract of *E. rheedii* Spreng. seeds was inactive (score 1) against all tested protozoa. On the other hand, phaseoloidin exhibited pronounced activity (score 3) against all tested protozoa; *T. b. brucei*, *T. cruzi*, *T. b. rhodesiense* and *L. infantum* (IC50 of 9.70, 8.00, 7.83 and 6.96 µg/mL, respectively). Entadamide A had pronounced activity (score 3) against *T. cruzi* and *L. infantum* only with an IC50 of 8.98 and 10.77 µg/mL, respectively. Protocatecheuic acid also showed pronounced activity (score 3) against *T. b. brucei* and *T. cruzi* (IC50 of 12.23 and 14.42 µg/mL respectively). All the active tested compounds have low toxicity against MRC-5 cell line (cytotoxicity score 2). The best selectivity index (SI) [2] was presented by phaseoloidin (4.51 *L. infantum*, 4.01 *T. b. brucei* and 3.93 *T. b. rhodesiense*).
Entadamide A showed the best SI (3.82) towards *T. cruzi*.

Results of the HPLC analysis enabled the quantification of the isolated compounds, being 3315.48 mg/100g protocatecheuic acid and 11487.7 mg/100g for phaseoloidin as markers for the total crude extract of *E. rheedii* seeds Figure (2).

4. Discussion

Parasitic diseases still represent a global threat, especially among poor countries. This is due to the absence of vaccines and the developed resistance against the available drugs. Nothing was reported in the literature concerning the antiprototozoal potency of *E. rheedii* seeds constituents. One study has reported the interesting activity of monomethyl ester-15- kolavic acid terpenoid isolated from *E. abyssinica* against *T. brucei* [22]. It has been reported that Fabaceae phenolics [23], terpenoids [22, 24], flavonoids [25-27] and crude extracts [24, 28] are good candidates for discovering novel antiprototozoal drugs, but it is the first time to conduct an antiprototozoal screening on Fabaceae plants sulphur compound; entadamide A. In addition to the antiviral activity of phasoloidin, the current study represented the first report on its antiprototozoal activity. The current study enthuses further *in vivo* study on phasoloidin.

The pronounced antiprototozoal activity of protocatecheuic acid against *T. b. brucei* and its moderate activity against *T. b. rhodesiense* and *T. cruzi* while the lack of activity of its methyl ester activity against all studied protozoa is in accordance with the reported data that protocatechuic acid is more potent than its methyl ester as an antibacterial agent against gram positive and gram negative bacteria, and *Mycobacterium* [12]. It is worthy to mention that this is the first study to test the activity of protocatecheuic acid and its methyl ester on *T. b. brucei*, *T. b. rhodesiense* and *L. infantum*. Protocatecheuic acid and its ethyl ester activity against *T. cruzi* was studied once before [29].

It is also worthy to note that phaseoloidin and entadamide A were, respectively, the major isolated phenolic compound and thioamide compound from *E. rheedii* seed and they both exhibited potent antiulcerogenic and antibacterial activities.

To conclude, the isolated compounds of *E. rheedii* were more active than the crude extract. This was in accordance with our finding during evaluation of the antiprototozoal activity of other Fabaceae plants [15].

Our study revealed that all the tested compounds were less active than the used reference standard drugs except for phaseoloidin which exhibited pronounced antileishmanial potency with an IC₅₀ of 6.96 µg/mL which is less than that of miltefosine IC₅₀ (10.7 µg/mL).

5. Conclusion

Three different compounds were previously isolated from *E. rheedii* seeds crude extract. Those isolated compounds were more potent antiprototozoal candidates than the crude extract. This indicate that *E. rheedii* may contain more active compounds yet to be discovered. However, all isolated compounds demonstrated non-specific activity. Translation of some *in vitro* results into *in vivo* follow-up studies is recommended in a future study.

6. Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

7. Ethical conduct of research

The use of laboratory rodents was performed in accordance to (European Union directive 2010/63/EU on the protection of animals used for scientific purposes and the Declaration of Helsinki) mandatory guidelines and was approved by the ethical committee (UA-ECD 2015-90) of the University of Antwerp.

8. Conflict of interest

We wish to confirm that there are no known conflicts
of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

List of Abbreviations

CC₅₀: 50% cytotoxic concentration
IC₅₀: The half maximal inhibitory concentration
LMPH: Lab of Microbiology, Parasitology and Hygiene
SI: Selectivity Index (SI = CC₅₀/IC₅₀)
WHO: World Health Organization
WHO-TDR: Special Program for Research and Training in Tropical Diseases

Table 1. Antiprotozoal activity of E. rheedii Spreng. seed crude extract and isolates and their cytotoxicity against MRC-5 cells.

<table>
<thead>
<tr>
<th>Tested sample</th>
<th>unit</th>
<th>MRC-5</th>
<th>T. b. brucei</th>
<th>T. b. rhodesiense</th>
<th>T. cruzi</th>
<th>L. infantum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CC₅₀</td>
<td>IC₅₀</td>
<td>SI</td>
<td>CC₅₀</td>
<td>IC₅₀</td>
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<tr>
<td>Crude ethanol (70%) extract</td>
<td></td>
<td>&gt; 64.00</td>
<td>&gt; 64.00</td>
<td>&gt; 64.00</td>
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<td>&gt; 64.00</td>
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<tr>
<td>Protocatechuic acid µg/ml</td>
<td></td>
<td>32.22</td>
<td>2</td>
<td>8.12</td>
<td>3.97</td>
<td>=3</td>
</tr>
<tr>
<td>Protocatechuic acid methyl ester µg/ml</td>
<td></td>
<td>&gt; 64.00</td>
<td>1</td>
<td>41.21</td>
<td>&gt; 1.55</td>
<td>=1</td>
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<tr>
<td>Phaseoloidin µg/ml</td>
<td></td>
<td>31.41</td>
<td>2</td>
<td>7.83</td>
<td>4.01</td>
<td>=3</td>
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<tr>
<td>Entadamide A µg/ml</td>
<td></td>
<td>34.27</td>
<td>2</td>
<td>37.65</td>
<td>0.91</td>
<td>=1</td>
</tr>
<tr>
<td>Standards:</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>Tamoxifen</td>
<td></td>
<td>9.3</td>
<td>Nd</td>
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<td>Benznidazole</td>
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</tr>
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<tr>
<td>Miltefosine</td>
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<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>10.7</td>
</tr>
</tbody>
</table>

Scores adopted by LMPH for assessment of antiprotozoal and cytotoxic activities T. cruzi, score 1: >30, 2: >11, 3: >4; T. brucei brucei, score 1: >24, 2: >9, 3: >3; T. brucei rhod, score 1: >24, 2: >9, 3: >3; L. infantum, score 1: >30, 2: >11, 3: >4; Cytotoxicity scores: non-cytotoxic score 1: >37, low cytotoxicity score 2: >13, moderate cytotoxicity score 3: >5, high cytotoxicity: score 4: >1.8; MRC-5: diploid human embryonic lung fibroblasts; CC₅₀: concentration causing 50% cytotoxicity; IC₅₀: concentration causing 50% inhibition; SI: selectivity index (SI = CC₅₀/IC₅₀); Sc: score; Activity score 1: inactive, 2: moderate, 3: pronounced activity.
protocatechuic acid
protocatechuic acid methyl ester
R=H
R=CH₃

phaseoloidin

entadamide A
R=H

Figure (1): Structures of the *Entada rheedii* major phytochemicals

Figure (2): HPLC chromatogram of phenolics in *E. rheedii* Spreng. crude extract.
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Entadamide A و protocatechuic acid و phaseoloidin تنشط مركبات الإنتادة ريدياي


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ملخص

الخلفية العلمية: تبقى المستخلصات النباتية الأفريقية والمركبات المفسولة منها مجالاً مهماً للكشف أدوية جديدة.

الهدف: تقييم نشاط نبات (إنتادا ريدي) والمواد الكيميائية المفسولة منه ضد الكائنات الأولية. 

الطرقية: النشاط تم لـ Leishmania infantum و Trypanosoma, T. b. rhodesiense, T. cruzi brucei brucei مضاد الكائنات الأولية ضد تحديده في المختبر بالإضافة إلى تقييم النمذجة الخلوية لتحديد الانتفاضات. النتائج: الخلاصة الحرفية للمضادات كانت غير T. b. rhodesiense, T. cruzi, T. b. brucei نشاط واضح ضد Phaseoloidin نشطة. أظهر المركب الفينول الفاصوليدين


الخلاصة: المواد الكيميائية النباتية الرئيسية المفسولة من بذور الإنتادة ريدياي الأفريقية فعاله ضد مرض النوم، ومرض ناغاس، وداء الليشمانيات. تأثير المركبات الوفى ضد الكائنات الأولية يظهر في شكل مركبات النقلة المفسولة وليس له تأثير بشكل جماعي في المستخلص الحرف.

الكلمات الدالة: السلفوراميد، فاصوليدين، مضاد المثقفين