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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 **Aim:** *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* (IC_{50} of 9.70, 8.00, 7.83 and 6.96 µg/mL, respectively). Entadamide A showed pronounced activity against *T. cruzi* and *L. infantum* (IC_{50} of 8.98 and 10.77µg/mL, respectively). Protocatecheuic acid showed pronounced activity against *T. b. brucei* (IC_{50} of 8.12 µg/mL) and moderate activity against *T. cruzi* and *T. b. rhodesiense* (IC_{50} of 14.42, 12.23 µg/mL, respectively). All the active compounds exhibited low cytotoxicity score 2 (CC_{50}>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

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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 IC_{50}-values calculation (µg/mL) adopted by LMPH (Table 1). IC_{50} of tested compounds exsepressed 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* (IC_{50} 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 IC_{50} of 8.98 and 10.77 µg/mL, respectively. Protocatecheuic acid also showed pronounced activity (score 3) against *T. b. brucei,* (IC_{50} of 8.12 µg/mL), while it's methyl ester was inactive. A moderate (score 2) antiprotozoal activity was given by protocatecheuic acid against *T. b. rhodesiense* and *T. cruzi* (IC_{50} 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 antiprotozoal 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 antiprotozoal drugs, but it is the first time to conduct an antiprotozoal 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 antiprotozoal activity. The current study enthuses further *in vivo* study on phasoloidin.

The pronounced antiprotozoal 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 antiprotozoal 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 IC_{50} of 6.96 µg/mL which is less than that of miltefosine IC_{50} (10.7 µg/mL).

5. Conclusion

Three different compounds were previously isolated from *E. rheedii* seeds crude extract. Those isolated compounds were more potent antiprotozoal 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.

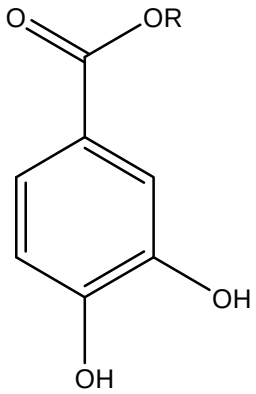
List of Abbreviations

CC_{50}: 50% cytotoxic concentration
IC_{50}: The half maximal inhibitory concentration
LMPH: Lab of Microbiology, Parasitology and Hygiene
SI: Selectivity Index (SI = CC_{50}/IC_{50})
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.

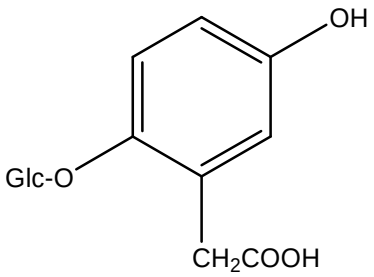
Tested sample	unit	MRC-5		*T. b. brucei*			*T. b. rhodesiense*			*T. cruzi*			*L. infantum*		
		CC_{50}	SC	IC_{50}	SI	SC	IC_{50}	SI	SC	IC_{50}	SI	SC	IC_{50}	SI	SC
Crude ethanol (70%) extract		> 64.00	1	> 64.00	>1.00	**=1**	> 64.00	>1.00	**<1**	> 35.51	> 1.80	=1	> 64.00	> 1.00	**<1**
Protocatechuic acid	µg/ml	32.22	**2**	8.12	3.97	=3	12.23	2.63	=2	14.42	2.23	=2	32.46	0.99	=1
	mM	0.209		0.053			0.079			0.094			0.211		
Protocatechuic acid methyl ester	µg/ml	> 64.00	**1**	41.21	> 1.55	=1	35.17	> 1.82	=1	34.90	> 1.83	=1	> 64.00	> 1.00	<1
	mM	0.380		0.245			0.209			0.207			0.381		
Phaseoloidin	µg/ml	31.41	**2**	7.83	4.01	=3	8.00	3.93	=3	9.70	3.24	=3	6.96	4.51	=3
	mM	0.951		0.024			0.024			0.029			0.021		
Entadamide A	µg/ml	34.27	**2**	37.65	0.91	=1	35.33	0.97	=1	8.98	3.82	=3	10.77	3.18	=3
	mM	0.212		0.234			0.219			0.055			0.067		
Standards:															
Tamoxifen		9.3		Nd			Nd			Nd			Nd		
Benznidazole		Nd		Nd			Nd			2.6			Nd		
Suramin		Nd		0.04			0.04			Nd			Nd		
Miltefosine		Nd		Nd			Nd			Nd			10.7		

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; CC_{50}: concentration causing 50% cytotoxicity; IC_{50}: concentration causing 50% inhibition; SI: selectivity index (SI = CC_{50}/IC_{50}); Sc: score; Activity score 1: inactive, 2: moderate, 3: pronounced activity.

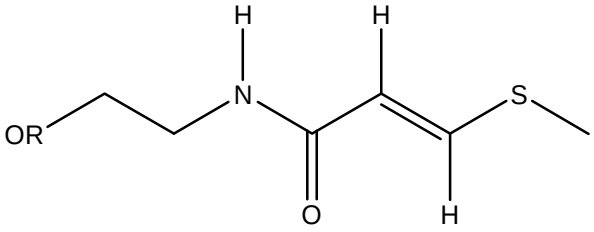


protocatechuic acid
protocatechuic acid methyl ester

R=H
R=CH_3



phaseoloidin



entadamide A
R=H

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

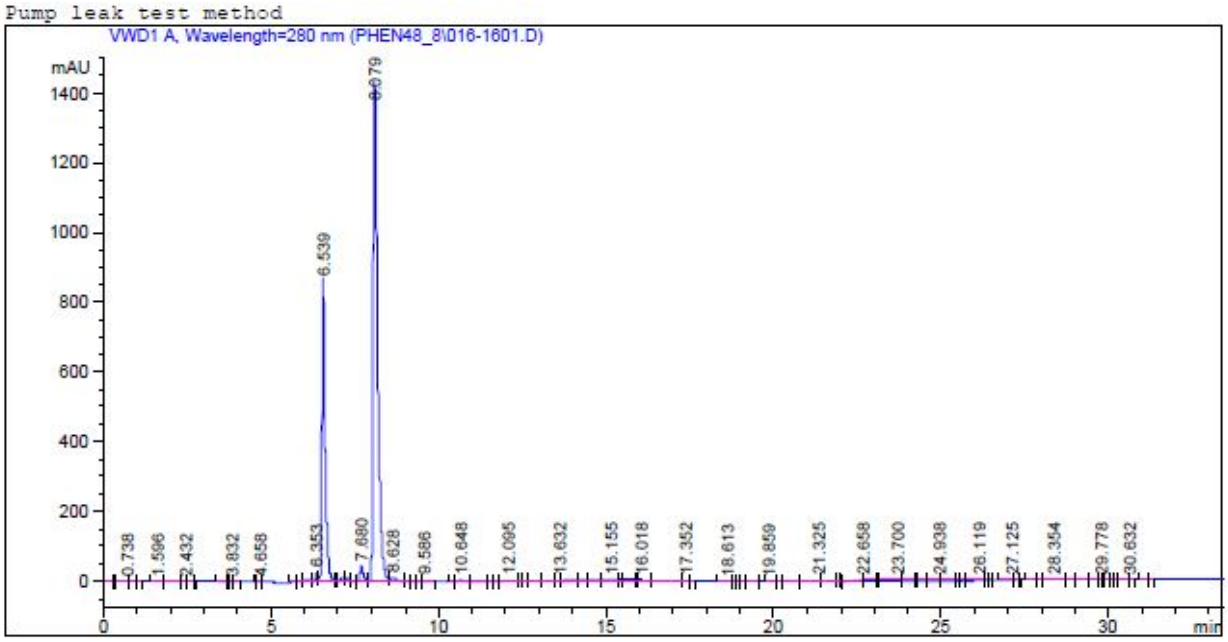


Figure (2): HPLC chromatogram of phenolics in *E. rheedii* Spreng. crude extract.

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نشاط مركبات الإنتادا ريدياي phaseoloidin وprotocatechuic acid وEntadamide A المضاد للكائنات الأولية: داء المثقبيات وداء الليشمانيات

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ملخص

الخلفية العلمية: تبقى المستخلصات النباتية الأفريقية والمركبات المفصولة منها مجالا مهما لاكتشاف أدوية جديدة. **الهدف:** تقييم نشاط نبات (*إنتادا ريدياي*) والمواد الكيميائية المفصوله منه ضد الكائنات الأولية . **الطريقة:** النشاط المضاد للكائنات الأولية ضد *Trypanosoma, T. b. rhodesiense, T. cruzi brucei brucei* و *Leishmania infantum* تم تحديده في المختبر بلأضافة الي تقييم السمية الخلوية لتحديد الانتقائية . **النتائج:** الخلاصة الخام للنبات كانت غير نشطة. أظهر المركب الفينولي الفاصوليدين Phaseoloidin نشاط واضح ضدT. b. brucei ، T. cruzi، T. b. rhodesiense و L. infantum (9.70 ، 8.00، 7.83و 6.96 IC_{50} ميكروجرام / مل على التوالي). كما أظهر مركب الثايو أميد Entadamide A نشاط واضح ضد *T. cruzi* و L. infantum (التركيز المانع للنص 8.98 و 10.77 IC_{50} ميكروجرام / مل على التوالي). أظهر حمض البروتوكاتيشوك Protocatecheuic نشاط واضح ضد *T. b. brucei* (8.12 IC_{50} ميكروجرام / مل) ونشاط متوسط ضد كلا من*T. cruzi* و*T. b. rhodesiense* (14.42 و12.23 IC_{50} ميكروجرام / مل على التوالي). أظهرت جميع المركبات النشطة درجة سمية خلوية منخفضة (CC_{50}>13 µg/mL) . **الخلاصة:** المواد الكيميائية النباتية الرئيسية المفصولة من بذور *الأنتادا ريدياي* الأفريقية فعالة ضد مرض النوم ، ومرض شاغاس ، وداء الليشمانيات. تأثير المركبات القوي ضد الكائنات الأولية يظهر في شكل مركباته النقية المفصولة وليس لهم تأثير بشكل جماعي في المستخلص الخام.

الكلمات الدالة: السلفوراميد phaseoloidin، فاصوليدين، مضاد المثقبيات MRC-5.

تاريخ استلام البحث 2019/2/24 وتاريخ قبوله للنشر 2019/12/11.