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# *In vitro* trypanocidal activity of the Egyptian plant *Schinopsis lorentizii* against trypomastigote and amastigote forms of *Trypanosoma cruzi*

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**ABSTRACT**

Chagas' disease is a chronic illness caused by the protozoan *Trypanosoma cruzi.* According to estimates, approximately 16-18 million people are infected in Latin American. Plant extracts exhibit a wide variety of secondary metabolites and can play an important role in the discovery of new compounds with biological potential. The *in vitro* trypanocidal activity of the extracts obtained from six plant species collected in Egypt (*Parkia africana*, *Parkia roxburgi*, *Lagerstromeia speciosa*, *Schinopsis lorentzii*, *Lagerstromeia indica*, and *Sapindus saponaria)* was assayed against trypomastigote and amastigote forms of *T. cruzi*. The cytotoxic activity of the most active extract was also evaluated by conducting MTT assays. *S. lorentzii* and *S. saponaria* were the most active extracts against the trypomastigote form;$IC_{50}$ values were 9.9 and 27.34 µg/mL, respectively. The *S. lorentzii* extract was also evaluated against the amastigote form ($IC_{50}$ was 111.5 µg/mL).The *S. lorentzii* extract did not exhibit significant cytotoxic activity. The selectivity index value indicated that this extract was highly selective for the parasite. The *S. lorentzii* and *S. saponaria*extracts exhibit trypanocidal activity, probably as a result of the presence ofdifferent constituentsand their concentrations in the extracts.

## INTRODUCTION

Neglected tropical diseases are a group of lethal diseases or disabling infections affecting more than a billion people worldwide. Poor populations living in rural areas and urban slums are at higher risk of infection. Therefore, people suffering from these diseases constitute an unattractive market to the private research sector (Schmidt *et al.*, 2012). Seventeen neglected tropical diseases, including Chagas' disease, leishmaniasis, and African trypanosomiasis affect millions of people around the world. It is estimated an annual incidence of 28,000 cases of Chagas' disease in the region of the Americas, 6 to 8 million people infected and approximately 12,000 deaths per year (WHO). Chagas' disease, also known as American trypanosomiasis, is caused by the intracellular obligatory parasite *Trypanosoma cruzi*, which is transmitted to humans and other mammals by hematophagous insects (Rocha *et al.*, 2007). After infection, a short-term acute phase (4-8 weeks) with patent parasitemia that often goes undiagnosed is followed by a lifelong chronic phase that appears after a long latent period known as indeterminate form. The chronic phase of Chagas disease is characterized by scarce circulating parasites. These parasites cause symptomatic chronic cardiomyopathy and/or digestive symptoms in approximately 30% of Chagas disease patients (Marin-Neto *et al.*, 2007; Campi-Azevedo *et al.*, 2015). Although the discovery of Chagas' disease dates back to over one hundred years, the drugs that are currently available to treat infected individuals, Nifurtimox and benznidazole have serious drawbacks.

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These medications exhibit high toxicity and limited effect against different *T. cruzi* isolates and stages of the disease, which makes the discovery of novel pharmaceuticals a matter of utmost importance (Soeiro 2009; Andriani *et al.,* 2011).In this context, natural sources such as plants, which contain various secondary metabolites, can play an important role in the discovery of new substances with biological potential (Schimidt *et al.,* 2012).

In continuation of our previous works on the antiparasitic studies of Egyptian plants (Rashed *et al*., 2013a,b),we now report the evaluation of the *in vitro* trypanocidal activity of six Egyptian plant extracts against trypomastigote form of *T. cruzi*, which have not yet been described. Additionally, we have also assessed the trypanocidal activity against amastigote form of *T. cruzi* and cytotoxic activity against $LLCMK_2$ cells of the most active plant extract.

## MATERIALAND METHODS

### Plant material

*Parkia africana*, *Parkia roxburgi*, *Lagerstromeia speciosa*, *Schinopsis lorentzii*, *Lagerstromeia indica*, and *Sapindus saponaria*barks were collected from the Al-Zohiriya Garden in Giza, Egypt, in May 2011.

All the plants were identified by Dr. Mohammed El-Gebaly at the Department of Botany, National Research Centre (NRC), and by Mrs. Tereeza Labib, Consultant of Plant Taxonomy at the Ministry of Agriculture and director of the Orman Botanical Garden, in Giza, Egypt. A voucher specimen was deposited in the herbarium of the Al-Zohiriya Garden in Giza, Egypt.

### Preparation of the extracts

Air-dried bark of the plants (280g) was extracted with methanol/distilled water 80:20 (v/v) several times (five times), at room temperature, by the maceration method. Each extract was concentrated under reduced pressure, to afford12 g, 14.5 g, 10.5 g, 16 g, 11.5 g, or 13 g ofdry (*P. Africana*, *P. roxburgi*, *L. speciosa*, *S. lorentizii*, *L. indica* and *S. saponaria*) extracts, respectively.

### Phytochemical analysis.

Phytochemical screening of each extract was conducted according to the methods described by Yadav and Agarwala (2011).

### Parasites and Life cycle

The Y strain of *T. cruzi* was used in the assays. This strain is characterized by thin forms and tropism for phagocytic mononuclear cells (Pereira da Silva and Nussenzweig, 1953). The Y strain of *T. cruzi* is routinely maintained by serial passages through BALB/c mice. The Ethics Committee for Animal Care of the University of Franca authorized all the experiments; all the experimental protocols were in accordance with the national and international accepted principles for laboratory animal use and care.

### Anti-trypomastigote assay

The *in vitro* trypanocidal assay was undertaken by using the trypomastigote form of *T. cruzi*, obtained by culturing in $LLMCK_2$ cell lineage (*Macaca mulatta* kidney cell). The cells were cultured in RPMI-1640 medium supplemented with L-glutamine (2 mM), $NaHCO_3$ (10 mM), penicillin (100 U/mL), streptomycin (100 µg/mL), and 5% inactivated fetal calf serum. The culture was kept in a 96-well microplate at 37°C, under atmosphere of 5% $CO_2$ and 95% humidity. Trypomastigotes obtained from the blood of infected animals (by cardiac puncture) at the parasitemic peak were added to the cell culture at a 5:1 ratio. After seven days, the culture supernatant was removed and centrifuged, and the free forms of the parasite were harvested for the tests. Each well was filled with $1x10^6$ cells, which were counted with the aid of a Neubauer chamber. The samples were dissolved in dimethyl sulfoxide (DMSO) and added to the wells to give final concentrations of 12.5, 25, 50, 100, and 200 µg/mL. The microplate was incubated at 37°C for 24 h, and the biological activity was evaluated by direct quantification of the parasites in a Neubauer chamber. RPMI 1640 medium plus DMSO were used as negative control; Benznidazole was used as positive control. All the experiments were performed in triplicate. Results are expressed as mean± SD.

### Anti-amastigote assay

The anti-amastigote assays were developed in $LLCMK_2$ cellcultures as described by Giorgio *et al.,* 1998. The cells were cultured in RPMI 1640 medium supplemented with glutamine (2 mM), $NaHCO_3$(10 mM), penicillin (100 U/mL),streptomycin (100 µg/mL), and 5% inactivated fetal bovine serum. The culture was kept in a 24-well microplate for 24 h, at 37 ºC, in 5% $CO_2$ with 95% humidity. Each well was filled with $1 \times 10^6$ cells, which were counted by using a Neubauer chamber. After 24 h, the trypomastigotes obtained from the cell culture were collected and centrifuged at 760 rpm for 8 min, at 12 ºC. The supernatant consisted of trypomastigotes, whereas the cells constituted the sediment. After a second centrifugation (at 3000 rpm for 30 min, at 12ºC), the trypomastigotes ($1 \times 10^6$) were added to the culture, and the microplate was incubated for 48 h. The extract was dissolved in dimethyl sulfoxide (DMSO) and added to the wells to give final concentrations of 12.5, 25, 50, 100, and 200µg/mL. After 96 h of incubation, the colorimetric assay was accomplished by the Giemsa-staining method. The infected cells were counted, and the percentage of parasitic reduction was determined by comparison with the negative control (DMSO 0.5%).

### Cytotoxicity assay

The $LLCMK_2$ fibroblast cells were grown in RPMI 1640 medium supplemented with penicillin (100 U/mL), streptomycin (100 µg/mL), and 5% inactivated fetal calf serum, and maintained at 37°C in 5% $CO_2$. A cell suspension was seeded at a concentration of $1 \times 10^6$ cells/mL in a 96-well microplate containing RPMI 1640 medium. Thereafter, the cells were treated with *S. lorentzii* extract at different concentrations (12.5, 25, 50,

100, and 200µg/mL). The plates were incubated at 37°C for 24 and 96 h. The biological activity was evaluated by using the MTT colorimetric method [MTT; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] in a microplate reader at 540 nm. RPMI 1640 medium plus DMSO and RPMI 1640 medium were used as positive and negative controls, respectively. All the experiments were performed in triplicate. The percentage of cytotoxicity was determined by the formula: % cytotoxicity= {1−[(Y−N)/(N−P)]} ×100, where Y=absorbance of the well containing cells and the extract at different concentrations; N=negative control; P=positive control. The selectivity index (SI) was determined for trypomastigotes as the ratio of $CC_{50}$ to$IC_{50}$ values.

**Statistical analysis**

The statistical tests were performed with the Graphpad Prism (version 5.0) software. The data were statistically analyzed by one-way analysis of variance.

## RESULTS AND DISCUSSION

The biological potential of medicinal plants as a source of new drugs deserves further exploration. The use of plant extracts or plant-derived chemicals to treat several diseases has stood the test of time. In recent years, there has been growing interest in the use of medicinal plants in both underdeveloped and developing countries—natural drugs have been reported to be safe, especially when compared to synthetic drugs (Abirami *et al.*, 2012).

Table 1 lists the results obtained for the trypanocidal activity of the assayed plant extracts against *T. cruzi* trypomastigotes.

**Table 1:** *In vitro* trypanocidal activity of plant extracts against the trypomastigote form of *T. cruzi.*

| Samples | % lysis ± S.D. / concentration (µg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | 200 | 100 | 50 | 25 | 12,5 | $IC_{50}$ (µg/mL) |
| 1 | 69.5±1.0 | 63.0±4.3 | 50.7±4.9 | 27.8±4.1 | 32.6±2.1 | 54.5 |
| 2 | 44.4±2.5 | 25.0±1.6 | 21.6±1.6 | 10.0±3.3 | 6.1±0.9 | >200 |
| 3 | 46.0±3.1 | 33.3±1.5 | 24.8±0.9 | 21.1±2.4 | 5.8±1.8 | >200 |
| 4 | 97.8±1.0 | 95.2±0.6 | 73.9±4.3 | 75.0±4.7 | 55.4±1.0 | 9.9 |
| 5 | 61.3±3.1 | 16.7±2.0 | 9.7±1.3 | 13.2±2.0 | 0±0 | 170.8 |
| 6 | 92.3±0.6 | 79.4±1.7 | 61.3±3.1 | 50.1±1.0 | 30.5±1.7 | 27.34 |

1: *Parkia africana* exract, 2: *Parkia roxburgi* extract, 3: *Lagerstromeia speciosa* extract, 4: *Schinopsis lorentizii* extract, 5: *Lagerstromeia indica* extract, 6: *Sapindus saponaria* extract. Benznidazole (positive control): $IC_{50}$= 9.8 µg/mL.

The anti-*Trypanosoma cruzi* activity of the extracts was assessed at concentrations of 12.5, 25, 50, 100, and 200µg/mL for 24 hours. The trypanocidal activity was determined by comparing the count of trypomastigotes in each sample with the count of trypomastigotes in control groups. The *S. lorentzii* and the *S. saponaria* extracts were the most active against the Y strain of *T. cruzi*; $IC_{50}$was 9.9 and 27.34µg/mL, respectively. At 200 µg/mL, 100 µg/mL, and lower concentrations, the *S. lorentzii* extract provided 97%, 95%, and over 70% trypomastigote lysis, respectively.

Compared to the *S. lorentzii* and *S. saponaria* extracts, the *P. Africana* and *L. indica* extracts exhibited lower trypanocidal potential, with $IC_{50}$values of 54.5 and 170.8µg/mL, respectively, and 69% and 61% trypomastigotelysis, respectively. The*P. Roxburgi* and *L. speciosa* extracts did not display significant trypanocidal activity ($IC_{50}$>200µg/mL).

According to Osório *et al*. (2007), plant extracts with $IC_{50}$ < 10 µg/mL, $IC_{50}$ > 10 µg/mL and < 50 µg/mL, $IC_{50}$ > 50 µg/mL and < 100 µg/mL, and $IC_{50}$ > 100 µg/mL can be classified as highly active, active, moderately active, and non-active with regard to their antiprotozoal activity, respectively. Based on this classification, the *S. lorentzii* ($IC_{50}$ = 9.9 µg/mL) and the *S. saponaria* ($IC_{50}$ = 27.3 µg/mL) extracts are highly active and active, respectively. Flavonoids and triterpenes, classes of constituents of these extracts, have been shown to exhibit trypanocidal action (Uchiyama, 2009; Izumi *et al.*, 2011) and may account for the observed activity.

Table 2 shows data concerning the activity of the *S. lorentzii* extract (the most active against *T. cruzi* trypomastigotes) against *T. cruzi* amastigotes. Compared with its activity against *T. cruzi* trypomastigotes, the *S. lorentzii* extract was less active against *T. cruzi* amastigotes: $IC_{50}$ was 111.5µg/mL, and parasite lysis was around 60% and 44% at 200 and 100 µg/mL, respectively. The lower susceptibility of amastigotes to the extract can be rationalized as follows: to gain access to the intracellular parasite (amastigote), the extract has to cross the host cell membrane and the parasitophorous vacuole membrane, whereas the extracellular trypomastigote is directly exposed to the extract.

**Table 2:** *In vitro* trypanocidal activity of plant extracts against the amastigote form of *T. cruzi*

| Sample | % lysis ± S.D./concentration (µg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | 200 | 100 | 50 | 25 | 12,5 | $IC_{50}$ (µg/mL) |
| *S. lorentizii* | 60.5±4.0 | 44.4±0.6 | 40.9±2.8 | 27.5±3.4 | 19.1±1.7 | 111.5 |

Positive Control: Benznidazole ($IC_{50}$ = 19.1 µg/mL); Negative Control: DMSO 0.5%

MTT assays on $LLCMK_2$ fibroblast cells allowed us to ascertain the cytotoxicity of the *S. lorentzii* extract; the assayed concentration range was chosen on the basis of *in vitro* studies conducted with *T. cruzi*. The cell cultures were treated with the extract at concentrations of 12.5, 25, 50, 100, and 200µg/mL for 24 and 96hours. Cell viability was determined by the ratio between the absorbance values obtained in the treated and untreated (control) groups (Fig. 1).

The *S. lorentzii* extract was not significantly cytotoxic to $LLCMK_2$ cells within the first 24 hours of the experiment:$CC_{50}$ was greater than 400µg/mL, and cell viability remained around 79% at the highest tested concentration. Similarly, after 96 hours of treatment, $CC_{50}$ was greater than 400 µg/mL, and the percentage

of viable cells was around 78% at the higher assayed concentrations (400 and 200 μg/mL).

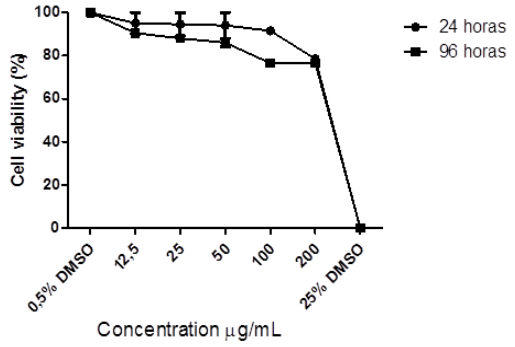


**Fig. 1:** Effects of *S. lorentizii* extract on the viability of $LLCMK_2$ fibroblast cells. Cytotoxicity was determined by the MTT assay after 24 and 96 h of treatment with the indicated concentrations. Values are expressed as mean ± S.D.

The selectivity index represents the selectivity of a compound for a parasite cell and for a normal cell line. This index indicates the potential use of a given compound in clinical trials. To compare the trypanocidal activity and the toxicity of the *S. lorentzii* extract, we used mammalian cells to estimate the selectivity index (SI) of this extract. According to Lenta *et al*. (2007), SI values greater than 10 suggest that a compound is safe for use in mammals. The *S. lorentzii* extract gave SI of 40, which indicated that it was highly selective for the parasite.

**Table 3**: Results of the phytochemical screening of the plant extracts.

| Botanical name | Plant Family | Plant part | Phytoconstituints |
|---|---|---|---|
| *Parkia africana* | Leguminoseae | Bark | Triterpenes/Sterols<br>Carbohydrates/<br>glycosides<br>Flavonoids<br>Tannins |
| *Parkia roxburgi* | Leguminoseae | Bark | Triterpenes/Sterols<br>Carbohydrates/<br>glycosides<br>Flavonoids<br>Tannins |
| *Lagerstromeia speciosa* | Lythraceae | Bark | Triterpenes/Sterols<br>Carbohydrates/<br>glycosides<br>Flavonoids<br>Tannins |
| *Schinopsis lorentizii* | Anacardiaceae | Bark | Triterpenes/Sterols<br>Carbohydrates/<br>glycosides<br>Flavonoids<br>Tannins |
| *Lagerstromeia indica* | Lythraceae | Bark | Triterpenes/Sterols<br>Carbohydrates/<br>glycosides<br>Flavonoids<br>Tannins |
| *Sapindus saponaria* | Sapindaceae | Bark | Triterpenes/Sterols<br>Carbohydrates/<br>glycosides<br>Flavonoids<br>Tannins<br>Saponins |

The phytochemical analyses of the investigated plant extracts showed the presence of triterpenes, flavonoids, tannins, and carbohydrates; the *S. saponaria* extract also contained saponins (Table 3). Several studies have reported on the trypanocidal activity of natural flavonoids (Takeara *et al.*, 2003; Sulsen *et al.*, 2007; Mai *et al.*, 2015). The lyophilized aqueous extract from *Lychnophora pinaster* Mart (Asteraceae) exhibits trypanocidal action (113.62 μg/mL); chemical characterization of this extract by HPLC revealed the presence of caffeic acid, isochlorogenic acid, vitexin, isovitexin, and quercetin (Silveira *et al.*, 2005).

Flavonoids like hispidulin and santin have significant trypanocidal and leishmanicidal activities. These flavonoids could serve as potential lead compounds for the development of more efficient drugs to treat leishmaniasis and Chagas disease (Sulsen *et al.*, 2007).

Terpenoids are a class of natural substances with various biological applications (Roberts, 2007). Their *in vitro* and *in vivo* trypanocidal potential has been extensively studied (Duarte *et al.*, 2002; Rosas *et al.*, 2007; Ferreira *et al.*, 2013).

Recently, Santos *et al*. (2013) have described the leishmanicidal and trypanocidal activities of two triterpenes, maytenin and pristimerin, isolated from *Maytenus ilicifolia.* The compounds are effective against *Leishmania amazonensis* and *Leishmania chagasi*as well as *Trypanosoma cruzi*. Both compounds have $IC_{50}$ lower than 0.3 nM against *T cruzi* epimastigotes.

Oleanolic and ursolic acids are ubiquitous triterpenoids in the plant kingdom, particularly in medicinal herbs, and are an integral part of the human diet. Previous studies reported by our group have shown that these triterpenes display significant *in vitro* trypanocidal activity (Cunha *et al.*, 2003, Cunha *et al.*, 2006; Ferreira *et al.*, 2010).

The industrial applications and properties of vegetable tannins stem from their ability to form complexes with proteins via hydrogen bonds. Tannins can inhibit microorganism growth by irreversibly deactivating enzymes (Venter *et al.*, 2012). These compounds exhibit leismanicidal and trypanocidal activities (Kolodziej and Kiderlen, 2005; Ogbadoyi *et al.*, 2007).

Shuaibu *et al.* (2008) have reported on the *in vitro* anti-trypanosomal activity of *Anogeissus leiocarpus* and *Terminalia avicennoides* methanolic extracts against four *Trypanosoma* species strains. Hydrolyzable tannins present in these extracts display trypanocidal action (MIC = 7.5-27.5 μg/mL or 14-91 μM). Moreover, these compounds are not significantly toxic to fibroblasts.

Cejas *et al.* (2011) authored one of the few literature studies on the antiparasitic activity of *S. lorentzii*. More specifically, these authors evaluated whether a commercially available polyphenolic vegetable extract from *S. lorentzii* (Bioquina®) reduced coccidiosis in broiler chicks. These authors suggested that the extract may have an impact against avian coccidiosis, but they stated that further studies on the potential

value of this product as a therapeutic or prophylactic anticoccidial agent are necessary.

According to Barberis *et al*. (2012), the species of the genus *Schinopsis* have numerous applications, especially in animal husbandry and cattle ranching as well as logging activities. Several authors have reported that species of this genus contain phenols that can control gastrointestinal parasites in ruminants and rodents (Paolini *et al.,* 2003; Max *et al.,* 2005; Athanasiadou *et al.,* 2007). Moreover, tannins present in *S. lorentzii* can also reduce helminths in birds (Marzoni *et al.,* 2005). Thus, the tannins identified in the most active extracts of the present study could also account for the antiparasitic activity displayed by these extracts.

Given the important limitations of the currently available treatment for Chagas disease, especially the low efficacy of the medication in the chronic phase, new steps have been taken toward the search for chemotherapeutic approaches that can improve the control of this disease (Urbina, 2010).

Investigations into natural products can potentially provide positive results, particularly in the case of diseases that are difficult to treat and for which there is no cure. Determining the efficacy or optimum concentration of a tested drug is extremely important and acquiring information about its effect on parasite biology and parasite interaction with the host cell is of great value (Maya *et al*., 2007).

## CONCLUSION

This work has demonstrated the trypanocidal activity of *S. lorentzii* on the two evolutive forms of *T. cruzi* for the first time. The trypanocidal activity observed for the extracts of *S. lorentzii* and *S. saponaria*can be result of the presence of different constituents and their concentrations in the extracts. The low toxicity of the *S. lorentzii* extract to mammalian cells associated with its significant trypanocidal action makes this extract a promising candidate for the discovery of new trypanocidal drugs. Further studies on the isolation, identification, and bioassays of active compounds against the trypomastigote and amastigote forms of *T. cruzi* and *in vivo* assays are essential to elucidate structure-activity relationships, unveil the mechanisms of parasite death induced by the most promising substances, and identify their putative intracellular targets. Hopefully, such studies will culminate in the development of novel therapeutic agents to treat Chagas' disease.

**Conflict of Interest Statement:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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## REFERENCES

Abirami P, Gomathinayagam M, Panneerselvam R. Preliminary study on the antimicrobial activity of *Enicostemmalittorale* using different solvents. Asian Pac J Trop Med,2012; 5:552-555.

Andriani G, Chessler A-DC, Courtemanche G, Burleigh BA, Rodriguez A.Activity *in vivo* of anti-*Trypanosomacruzi* compounds selected from a high throughput screening. PLoS Negl Trop Dis, 2011; 5:1-6.

Athanasiadou S, Githiori J, Kyriazakis I. Medicinal plants for helminth parasite control: facts and fiction. Animal, 2007; 1:1392-1400.

Barberis IM, Mogni V, Oakley L, Alzugaray C, Vesprini JL, Prado DE. Biología de especies australes: *Schinopsis balansae* Engl. (Anacardiaceae). Kurtziana,2012; 37:59-86.

Campi-Azevedo AC, Gomesa JAS, Teixeira-Carvalho A, Silveira-Lemos D, Vitelli-Avelar DM, R Sathler-Avelar, Peruhype-Magalhães V, Béla SR, Silvestre KF, Batista MA, Schachnik NCC, Correa-Oliveira R, Eloi-Santos SM, Martins-Filho OA. Etiological treatment of Chagas disease patients with benznidazolelead to a sustained pro-inflammatory profile counterbalanced bymodulatory events. Immunobiology, 2015; 220:564-574.

Cejas E, Pinto S, Prosdócimo F, Batallé M, Barrios H, Tellez G, Franceschi M. Evaluation of quebracho red wood (*Schinopsis lorentzii*) polyphenolic vegetable extract for the reduction of coccidiosis in broiler chicks. Int J Poult Sci, 2011; 10:344-349.

Cunha WR, Crevelin EJ, Arantes GM, Crotti AE, Silva MLA, Furtado NA, Ferreira DS. A study of trypanocidal activity of triterpene acids isolated from *Miconia* species. Phytother Res, 2006; 20:474-478.

Cunha, WR, Martins C, Ferreira DS, Crotti, AEM, Lopes NP, Albuquerque S. *In vitro* trypanocidal activity of triterpenes from *Miconia* species. Planta Med, 2003;69:468-470.

Duarte LP, Vieira Filho SA, Silva GDF, Sousa JR, Pinto AS. Anti-trypanosomalactivityofpentacyclictrierpenesisolated from*Austroplenckia populnea* (Celastraceae). Rev Inst Med Trop São Paulo, 2002; 44:109-112.

Ferreira DS, Esperandim VR, Marçal MG, Neres NBR, Cunha NL, Silva MLA, Cunha WR.Natural products and Chagas' disease: the action of triterpenes acids isolated from *Miconia* species. Univ Sci, 2013; 18:243-256.

Ferreira DS, Esperandim VR, Toldo MPA, Saraiva J, Cunha WR, Albuquerque S. Trypanocidal activity and acute toxicity assessment of triterpene acids. Parasitol Res, 2010; 106:985-989.

Giorgio S, Barão SC, Augusto O, Kwee JK. *Leishmania amazonensis* infection is reduced in macrophages treated with guanine ribonucleosides. Acta Trop, 1998; 70:119-122.

Izumi E, Ueda-Nakamura T, Filho BPD, Júnior VFV, Nakamura CV. Natural products and Chagas disease: a review of plant compounds studied for activity against *Trypanosoma cruzi*. Nat Prod Rep,2011;28:809-823.

Kolodziej H, Kiderlen AF. Antileishmanial activity and immune modulatory effects of tannins and related compounds on Leishmania parasitised RAW 264.7 cells. Phytochemistry, 2005; 66:2056-71.

Lenta BN, Vonthron-Senecheau CR, Sohd F,Tantangmo F, Ngouela S, Kaiser M, Tsamo E, Anton R, Weniger B. *In vitro* antiprotozoal activities and cytotoxicity of some selected Cameroonian medicinal plants. J Ethnopharmacol, 2007; 111:8-12.

Mai LH, Chabot GG, Grellier P, Quentin L, Dumontet V, Poulain C, Espindola LS, Michel S, Vo HTB, Deguin B, Grougnet R. Antivascular and anti-parasite activities of natural and hemisynthetic flavonoids from New Caledonian Gardenia species (Rubiaceae). Eur J Med Chem, 2015; 93:93-100.

Marin-Neto JA, Rassi Jr A, Morillo CA, Avezum A, Connolly SJ, Sosa-Estani S, Rosas F, Yusuf, S. Rationale and design of a randomized placebo-controlled trial assessing the effects of etiologic treatment in Chagas' cardiomyopathy: the benznidazole evaluation for interrupting trypanosomiasis. Am Heart J, 2007;156:38-43.

Marzoni M, Castillo A, Romboli I. Dietary inclusion of Quebracho (*Schinopsis lorentzii*) tannins on productive performances of growing pheasant females. Ital J Anim Sci, 2005, 4:507-509.

Max RA, Wakelin D, Craigon J, Kassuku AA, Kimambo AE, Mtenga LA, Buttery, PJ. Effect of two commercial preparations of condensed tannins on the survival of gastrointestinal nematodes of mice and goats *in vitro*. S Afr J Anim Sci, 2005; 35:213-220.

Maya JD, Cassels BK, Iturriaga-Vásquez P, Ferreira J, Faundez M, Galanti N, Ferreira A, MORELLO A. Mode of action of natural and synthetic drugs against *Trypanosomacruzi* and their interaction with the mammalian host. Comp Biochem Phys A, 2007; 146:601-20.

Ogbadoyi EO, Abdulganiy AO, Adama TZ, Joseph IO. *In vivo* trypanocidal activity of *Annona senegalensis* Pers. leaf extract against *Trypanosoma brucei brucei.* J Ethnopharmacol, 2007; 112:85-89.

Osorio E, Arango GJ, Jiménez N,Ruiz G, Gutiérrez D, Paco MA, Giménez A, Robledo S. Antiprotozoal and cytotoxic activities *in vitro* of *Colombian Annonaceae*. J Ethnopharmacol, 2007; 111:630-635.

Paolini V, Bergeaud JP, Grisez C, Prevot F, Dorchies P, Hoste H. Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus*. Vet Parasitol, 2003; 113:253-261.

Pereira da Silva LH, Nussenzweig V. Sobre uma cepa de *Trypanosoma cruzi* altamente virulenta para o camundongo branco. Folia ClinBiol, 1953;20:191-208.

Rashed K, Da Cruz MG, Souza JM, Cunha WR, Magalhães LG.Evaluation of schistomicidal and leishmanicidal activities from *Alnus rugosa* (L.) and phytochemical composition. IJIPSR, 2013a; 1:227-234.

Rashed K, Da Cruz MG, Souza JM, Magalhães LG, Cunha WR.Evaluation of schistomicidal and leishmanicidal activities from *Dodonaeae viscosa* (L.) and phytochemical profile. Int J Inv Pharm Sci, 2013b; 1:462-466.

Roberts, SC. Production and engineering of terpenoids in plant cell culture. Nat Chem Biol, 2007; 3:387-395.

Rocha MO, Teixeira MM, Ribeiro AL.An update on the management of Chagas cardiomyopathy.Expert RevAntiInfectTher, 2007; 5:727-743.

Rosas LV, Cordeiro MSC, Campos FR, *et al*. *In vitro* evaluation of the cytotoxic and trypanocidal activities of *Ampelozizyphus amazonicus* (Rhamnaceae). Braz J Med Biol Res, 2007; 40:663-670.

Santos VAFFM, Leite KM, Siqueira MC, Regasini LO, Martinez I, Nogueira CT, Galuppo MK, Stolf BS, Pereira AMS, Cicarelli RMB, Furlan M, Graminha MAS. Antiprotozoal activity of quinonemethide triterpenes from *Maytenus ilicifolia* (Celastraceae). Molecules, 2013; 18:1053-1062.

Schmidt TJ, Khalid SA, Romanha A,Alves TMA, Biavatti MW, Brun R, Da Costa FB, de Castro SL, Ferreira VF, de Lacerda MVG, Lago JHG, Leon LL, Lopes NP, das Neves Amorim RC, Niehues M, Ogungbe IV, Pohlit AM, Scotti MT, Setzer WM, Soeiro MNC, Steindel M, Tempone AG. The potential of secondary metabolites from plants as drugs or leads against protozoan neglected diseases – Part I. Curr Med Chem, 2012;19:2128-2175.

Shuaibu MN, Wuyep PT, Yanagi T, Hirayama K, Ichinose A, Tanaka T, Kouno I.Trypanocidal activity of extracts and compounds from the stem bark of *Anogeissus leiocarpus*and *Terminaliaavicennoides*. Parasitol Res, 2008;102:697-703.

Silveira D, Wagner H, Chiari E, Lombardi JA, Assunção AC, de Oliveira AB, Raslan DS. Biological activity of the aqueous extract of *Lychnophora pinaster* Mart. Rev BrasFarmacogn, 2005; 15:294-297.

Soeiro MNC, de Castro SL. *Trypanosoma cruzi* targets for new chemotherapeutic approaches. Expert Opin Ther Targets, 2009; 13:105-121.

Sülsen VP, Cazorla SI, Frank FM, Redko FC, Anesini CA, Coussio JD, Malchiodi EL, Martino VS, Muschietti LV. Trypanocidal and leishmanicidal activities of flavonoids from Argentine medicinal plants. Am J Trop Med Hyg, 2007; 77:654-659.

Takeara R, Albuquerque S, Lopes NP. Trypanocidal activity of *Lychnophora staavioides* Mart. (Vernonieae, Asteraceae). Phytomedicine, 2003; 10:490-493.

Uchiyama N. Antichagasic activities of natural products against *Trypanosoma cruzi*.J Health Sci, 2009; 55:31-39.

Urbina JA. Specific chemotherapy of Chagas' disease: Relevance, current limitations and new approaches. Acta Trop, 2010; 115:55–68.

Venter PB, Senekal ND, Amra-Jordaan M, Bonnet SL, Van der Westhuizen JH. Analysis of commercial proanthocyanidins. Part 2: An electrospray mass spectrometry investigation into the chemical composition of sulfited quebracho (*Schinopsis lorentzii* and *Schinopsis balansae*) heartwood extract. Phytochemistry, 2012; 78:156-169.

World Health Organization: Available at: http://www.paho.org/hq/index.php?option=com_topics&view=article&id=10&Itemid=40743 [Accessed 23 November 2015].

Yadav RNS, Agarwala M.Phytochemical analysis of some medicinal plants. J Phytol, 2011; 3:10-14.

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