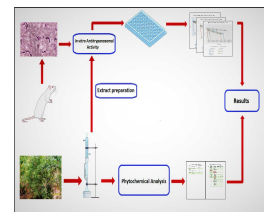


Nov 13, 2023

Assessment of the in vitro trypanocidal activity

DOI

<https://dx.doi.org/10.17504/protocols.io.81wgbxqd1lpk/v1>



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DOI: <https://dx.doi.org/10.17504/protocols.io.81wgbxqd1lpk/v1>

External link: <https://www.mdpi.com/2036-7481/8/1/6963>

Protocol Citation: Muhammad Muhsin Fathuddin 2023. Assessment of the in vitro trypanocidal activity. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.81wgbxqd1lpk/v1>

Manuscript citation:

Fathuddin, M. M., & Inabo, H. I. (2017). In vitro antitrypanosomal potential of chloroform leaf extract of Punica granatum L. on Trypanosoma brucei brucei and Trypanosoma evansi. Microbiology Research, 8(1). MDPI AG. Retrieved from <http://dx.doi.org/10.4081/mr.2017.6963>

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Protocol status: Working

We use this protocol and it's working

Created: November 06, 2023

Last Modified: November 13, 2023

Protocol Integer ID: 90433

Keywords: Antitrypanosomal activity, Trypanosoma brucei brucei, Rapid Matching Method, trypanosoma species, trypanosomiasis, trypanosoma brucei brucei, trypanocidal activity, including trypanosoma brucei brucei, trypanosoma evansi, trypanocidal activity the aim, plant extract

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Abstract

The aim of this experiment is to assess the potential of a plant extract to fight off Trypanosoma species, including *Trypanosoma brucei brucei*, *Trypanosoma evansi*, etc., by testing it at concentrations ranging from 006.25 to 400.00 mg/mL. The results obtained indicate that the plant extract may be a useful tool in the management of trypanosomiasis, but further studies are required to confirm its effectiveness.

Guidelines

The experimental animals need to be handled in accordance with Good Laboratory Practices and Guidelines for Laboratory Animal Facilities and the recommendations of the Committee on Animal Use and Care



Safety warnings

- ⚠ Handling the experimental animals may require training to reduce the risk of injury to the animal and the researcher

Ethics statement

This experimental work needs the Committee on Animal Use and Care approval before commencement

Before start

The experimental animals should be screened for any ailment at the commencement of the experiment using the laboratory's recommended procedure and cleared of any ailment.



Extract Preparation

- 1 To produce a stock  Sample solution of  100 mg/mL for the extract, weigh  1 g ( 1000 mg) of the extract, which was solubilized in 1 mL of dimethylsulfoxide (DMSO) solution and made up to  9 mL in dextrose saline/normal saline.
- 2 Serial dilutions were made for the remaining concentration of  50 mg/mL ,  25 mg/mL ,  12.5 mg/mL and  6.25 mg/mL of the extracts were made in Dextrose saline (freshly prepared).

Antitrypanosomal Activity

5m

- 3 The infected rat to undergo euthanasia must have attained a blood parasitemia of log 8.4 or higher

Citation

Herbert W.J., Lumsden W.H.R. (1976)
. *Trypanosoma brucei*: a rapid "matching" method for estimating the host's parasitemia. *Experimental Parasitology*.

[https://doi.org/10.1016/0014-4894\(76\)90110-7](https://doi.org/10.1016/0014-4894(76)90110-7)

LINK

- 4 Euthanatized animal's blood was dissolved in heparin ( 1 mL of heparin per  10 mL of blood) and mixed with glucose ( 0.1 g of glucose per  10 mL of blood).
- 5 Then, aseptically, a clean micropipette transfers the blood ( 50 μ L) to a clean, sterile microtiter plate into multiple wells.
- 6 To the two wells containing blood, the same volume (i.e.,  50 μ L) of the highest drug concentration was added, respectively.



- 7 Step 4 was repeated for the other different concentrations available.
- 8 The negative control blood was mixed with the dilutant used to prepare the various concentrations (i.e., dextrose-saline or water).
- 9 The positive tests were also performed with the standard recommended concentrations of a commercially available trypanocide drug.
- 10 At  00:05:00 5-minute interval, a drop of blood is taken from each well and smeared on a clean glass slide. then observe under a microscope at 400x for signs of motility unique to *Trypanosoma* spp.

Citation

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LINK

Citation

Chappuis F, Loutan L, Simarro P, Lejon V, Büscher P (2005)
. Options for field diagnosis of human african trypanosomiasis.
Clinical Microbiology Reviews.

<https://doi.org/10.1128/cmr.18.1.133-146.2005>

LINK

- 11 The above step is repeated with each well. Till motility stopped in all wells



Protocol references

Herbert, W. J., & Lumsden, W. H. (1976). Trypanosoma brucei: a rapid "matching" method for estimating the host's parasitemia. *Experimental parasitology*, 40(3), 427–431. [https://doi.org/10.1016/0014-4894\(76\)90110-7](https://doi.org/10.1016/0014-4894(76)90110-7)

Chappuis, F., Loutan, L., Simarro, P., Lejon, V., & Büscher, P. (2005). Options for field diagnosis of human African trypanosomiasis. *Clinical microbiology reviews*, 18(1), 133–146. <https://doi.org/10.1128/CMR.18.1.133-146.2005>

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Citations

Step 10

Herbert W.J., Lumsden W.H.R.. Trypanosoma brucei: A rapid "matching" method for estimating the host's parasitemia
[https://doi.org/10.1016/0014-4894\(76\)90110-7](https://doi.org/10.1016/0014-4894(76)90110-7)

Step 10

Chappuis F, Loutan L, Simarro P, Lejon V, Büscher P. Options for field diagnosis of human african trypanosomiasis.
<https://doi.org/10.1128/cmr.18.1.133-146.2005>

Step 3

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[https://doi.org/10.1016/0014-4894\(76\)90110-7](https://doi.org/10.1016/0014-4894(76)90110-7)