In Vitro Anti Trypanosomal Activity of Crude and Fractionated Ethanolic Extracts of *Chrysophyllum albidum* (African Star Apple) Leaves

Zubairu Maimuna[1], Munira Shehu Abdullahi[2]

[1, 2]Department of Biochemistry, Faculty of Science, Kaduna State University, Kaduna – Nigeria

Abstract: ***This research work is aimed at evaluating the crude and fractionated ethanolic extracts of Chrysophyllum albidum (African Star Apple) leaves for its anti-trypanosomal potentials. This was done by crude ethanolic extraction and fractionation of the extract using column chromatography where four fractions were obtained. Antitrypanosomal potential was assessed by monitoring the effect on Trypanosoma brucei brucei parasite motility of the infected blood with an electron microscope for one hour with fraction four showing the highest antitrypanosomal activity. The crude extract of C. albidum and the fractions showed varying degrees of antitrypanosomal activity. The 4th fraction showed the highest antitrypanosomal activity as parasites were found dead within 20mins post-incubation. The crude extract, 1st, 2nd and 3rd fractions also showed varying degrees of antitrypanosomal activity. Hence, Chrysophyllum albidum (African Star Apple) leaves has the potential of leading to the development of cheap, safe and effective drug for the treatment or management of African trypanosomiasis.***

Keywords: Antitrypanosomal, motility, chromatography, microscope, *Chrysophyllum albidum*

1. Introduction

Trypanosomes of the genus *Trypanosoma* are protozoa affecting man and animals resulting in the disease trypanosomiasis, also called sleeping sickness, borne by the purely African genus *Glossina* (tsetse flies). They have been found in the blood plasma of a great variety of vertebrates. Many of them appear to produce no symptoms but a few are of great pathogenic importance and continue to pose grave economic and social problems in Africa (Nigeria inclusive) and other affected areas of the world. In the blood of rats and mice, the trypanosomes multiply rapidly, producing high parasitaemia which may kill the hosts in a few days. The manifestation of the disease varies according to the strain and host of a parasite, being generally characterize *Trypanosoma brucei brucei* and *Congolense* are unicellular parasites transmitted by the bite of tsetse fly and is the causative agent of sleeping sickness in humans and other related diseases in animals (Warren, 1998 and Kuzoe, 1993). d by fever, anemia and cachexia. Thus, it has a pathogenic effect on the host. The disease caused by the *Trypanosoma brucei-brucei* subgroup is associated with anemia, hepatocellular degeneration and glomerulo nephritis, (Umar, *et al*., 2008) which is largely attributed to the large amount of free radicals and peroxides generated by the trypanosomes that attack membrane polyunsaturated fatty acids and proteins, resulting in cellular injuries and consequently affecting vital tissues and organs of the infected animals.

In recent times, drastic measures were taken to cut down the epidemic of trypanosomiasis in the affected areas of Africa with more tsetse fly nets put up in several forests and habitats. Fumigation of swampy areas and modern drugs has also been used to treat cases of human and animal trypanosomiasis. These drugs however have their toxic effects and consequently harm the body and some do not prevent relapses. Some are effective only in the latter stages of infection when the nervous system is involved while others are effective only in the initial stages of infection. Moreover, these drugs are very expensive and are only now within reach due to aids from the World Health Organization (WHO). But with phototherapy research work springing up all over Africa, highlighting our richly endowed forests, the need to screen local medicinal plants for anti-trypanosomal potentials cannot be over-emphasized. Some of these plants are already being used to cure fevers, infertility diseases and so on. If a herbal cure for sleeping sickness is found, suffering victims can have drugs faster and cheaper without side effect-threats as is the case with synthetic drugs like Suramin and Eflornithine treatment. The phytochemical screening and quantitative estimation of the crude yield of chemical constituents of the *C. albidum* leaves studied showed that they are rich in alkaloids, flavonoids, tannins, cardiac glycosides and saponins. They were known to show medicinal activity as well as exhibiting physiological activity (Sofowara, 1993). Tannins, flavonoids, terpenoids, proteins, carbohydrates and renins are the phytochemicals that have been reported to be present in *C. albidum* (Akaneme, 2008).

2. Materials and Methods

Materials

Chloroform, 98% Ethanol, 98% Ethyl acetate, 99% n-Hexane, Commercially available silica gel for column chromatography, Acetone, Phosphate Buffer Saline (PBS), Phosphate Buffer Saline with glucose(PBSG), Infected blood from animals (Rats), D. glucose, EDTA, Methylated spirit, Standard drug [Diaminazene aceturate (berenil)], *C. albidum* leaves extract. Ninety six (96) wells micro titer plate (Flow laboratories Inc., Mclean, VA, USA), Micro pipette, Other syringes (2ml, 5ml, 2 pieces), Desiccator, Refrigerator, Scissors and surgical blades, Pin, Hand gloves, Glass slides and cover slip, Timer, Pasteur pipette, Face mask, Lancet, Microscope (Olympus Model CHA), Water bath (Equitron, Media Instrument 400013 Mumbai India), pH meter (Jenway 3505), Measuring cylinder (500 ml), Analytical weighing balance, Beaker (100ml, 250ml, 500ml and 1000ml), Spatula

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, Pipettes, Filter paper, Cotton wool, Glass column, Retort stand, Eluent or fraction collection bottles.

Test Organism and Plant Materials

T. b. brucei was obtained from stabilates maintained at Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna. The parasites were maintained in the laboratory by continuous passage in rats until required. Passage was considered necessary when parasitaemia was in the range of 16-32 parasites per field (usually 3-5 day post infection). The leaves of matured *C. albidum* were collected from Zaria Local Government Area of Kaduna State. The leaves after collection were identified and authenticated by the herbarium unit of the department of Biological sciences, Ahmadu Bello University Zaria, and a Voucher Number "2680 " was deposited.

Methods

Sample Preparation and Extraction

The leaves of *C. albidum* was plucked and air dried in the laboratory after which it was thoroughly washed to remove debris and dirt, air dried to a constant weight, pounded to fine powder using the laboratory mortar and pestle and the powder stored in the right container until needed. 100g of the pounded dried plant material was weighed and extracted with 400ml n-Hexane by reflux followed by extraction with Ethanol. The extract was dried in vacuo and stored in the refrigerator at 4 degree Celsius until required (Nok *et al.*, 1993). The percentage yield of the extract obtained was calculated using the formular:

% yield = Amount of Extract/ Amount of Initial Sample×100.

Fractionation of the Crude Extract of *C. albidum* Leaves using Column Chromatography

Initially the column was prepared by mounting it tightly on a retort stand after which 50g of commercially available silica gel for Column Chromatography was weighed and poured into a beaker, Ethanol and water at a ratio of 50:50 was added and mixed to make slurry, which was transferred gently to the mounted column and 5g of powdered crude plant extract from the leaves of the plant was weighed and dissolved in 30ml ethanol then added to the prepared column which was sequentially eluted with Ethyl acetate/Ethanol, n-Hexane/Ethanol and Chloroform/ethanol and water/ethanol until the extract washed off. The fractions were collected in separate beakers, concentrated using a water bath and stored in the refridgerator for further use.

Determination of Parasitaemia

Parasitaemia was monitored in blood obtained from the tail of infected rats, pre-sterilized with methylated spirit. The number of parasites was determined microscopically at x400 magnification using the "Rapid Matching'' method of Herbert and Lumsden (1976). The method involved the microscopic counting of parasites per field in pure blood obtained from tails of infected rats.

***In vitro* Test for Anti-trypanosomal Activity**

Assessment of *in vitro* trypanocidal activity was performed in triplicates in 96 well micro titre plates. 40µl of blood containing about 20-25 parasites per field obtained as described under "determination of parasitaemia" was mixed with 20 µl of crude extract solution and also 20µl each of the different fractions of extract obtained was mixed with infected blood inside the wells of the micro titre plates. To ensure that the effect monitored is that of the crude extract's and fractions alone, a set of control was included which contains the parasite suspended in phosphate buffer saline with glucose serving as positive control. For reference, tests was also performed with the same concentration of Diminal^{R} (445mg diminazene diaceurate + 55mg phenazone/g) a commercial trypanocidal drug. Parasite count was then monitored on a glass slide covered with a cover slip and observed under the microscope at *400 magnification. The percentage of motile parasites was counted at 10minutes interval for 1hour. Cessation or drop in motility of the parasites in extract treated blood compared to that of parasite loaded control without extract was taken as a measure of antitrypanosomal activity.

3. Analysis of Results

The electron microscope at *400 magnification was used to view the parasites motility which was counted per field at different time intervals.

4. Results and Discussion

The Effects of Crude and Fractionated Extract of *C. albidum* Leaves on *Trypanosoma brucei brucei* Parasite Motility

The table 1 below shows the percentage of motile parasites and incubation time of the crude and phytochemical fractions of *C. albidum* which showed varying degrees of antitrypanosomal activity. The 4th fraction showed the highest antitrypanosomal activity as parasites were found dead within 20 mins post-incubation. The crude extract, 1st, 2nd and 3rd fractions also showed varying degrees of antitrypanosomal activity where parasites were found dead at 40 minutes post-incubation in crude extract while parasites were cleared in 3rd fractions after 60minutes post-incubation. The 1st and 2nd fractions were not very effective as parasite motility was still observed after 60mins incubation. For the negative, there was no significant drop in the number of motile parasites, unlike the standard which showed significant effect on the number of motile parasites by totally inhibiting motility of parasites at 10mins post incubation. Cessation or drop in number of trypanosomes served as a measure of antitrypanosomal activity of the extract when compared to the control. The result indicates a decrease in the percentage motile parasite with increase in the time of incubation.

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Table 1: The Effects of Crude and Fractionated Extract of *C. albidum* Leaves on *Trypanosoma brucei brucei* Parasite Motility

Time (Min)	Percentage of Motile Parasites(%)						
	Crude Extract	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Diminazene Aceturate	Positive control
10	84	92	96	80	20	0	100
20	63	80	87	71	0	0	94
30	28	73	80	53	0	0	92
40	0	66	76	24	0	0	88
50	0	51	65	0	0	0	85
60	0	40	54	0	0	0	80

5. Conclusion / Recommendation

The result obtained showed that the plant *C. albidum extracts* have varying degrees of antitrypanosomal activity with the 4th fraction having the highest antitrypanosomal activity. It is however, recommended that an *in vivo* study of this work should be carried out to ascertain whether the crude and fractionated extracts of *C. albidum* leaves will exhibit *in vivo* activity since a plant with high *in vitro* antitrypanosomal activity may have no *in vivo* activity and vice-versa because of the peculiarities in the plants constituents (Umar *et al*., 2000).

The mechanism by which the plant extract and its fractions exerts its trypanocidal activity should also be determined.

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