In vitro antitrypanosomal activity of *Breonadia salicina* on *Trypanasoma brucei brucei*

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Abstract

Objectives: Trypanosomiasis is associated with human morbidity and mortality. The aim of the study was to evaluate the anitrypanocidal activity of 70 and 100% ethanolic extract of *B. salicina* on *Trypanasoma brucei brucei.*

Materials and Methods: The *B. salicina* extracts leaves were screened for phytochemicals and were subsequently subjected to in vitro antitrypanasomal activity bioassay.

Results: The results showed that the extracts of *B. salicina* contained alkaloids, saponins, tannins, carbohydrates and flavonoids, with the exception of alkaloids which was absent in 100% ethanolic extract. In addition, both the extract possess antitrypanasomal activity with 70% having a higher potentials

Conclusion: The antitrypanasomal activity of *B. salicina* was seen to be concentration and time dependent. Further research should be carried on the potentials of other plants should be screened for potential antitrypanasomal activity.

Keywords: Trypanasomiasis, *Breonadia salicina*, In vitro, Activity

1.0 Introduction

African trypanosomes are protozoans parasites responsible for Human African Trypanosomiasis and Nagana in cattle and are transmitted by the bite of an infected tse-tse fly. *Trypanosoma brucei brucei*, the causative agent of nagana is closely related to *Trypanosoma brucei rhodensiense* (East to South Africa) and *Trypanosoma brucei gambiense (*West and Central Africa) which causes Human African Trypanosomiasis or sleeping sickness. Sleeping sickness currently affects half of a million people in sub-Saharan African and an estimated 60 million people are at risk of contacting this disease which is fatal if left untreated [1]. The huge reproductive looses in livestock due to African Animal Trypanosomiasis (AAT) are attributed to low foetal weight, premature births and poor lactation [2].Trypanosomiasis is associated with human morbidity and mortality and it leads to decrease in productivity of livestocks, extreme weight loss, reduce growth rate and can lead to death.

The natural environment is endowed with plant materials and fruits which have been found to be of great medicinal and nutritional importance. There are many experimental pieces of evidence indicating the use of plants for medicinal purposes [3].Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medical practice for the treatment of various ailments. Plants have been usedas anti-paratistic, antimicrobial and anti-tumor agents for decades.

Breonadia salicina is monotypic genus of the flowering plant in the Rubiaceae family. The genus contains only one species *B. salicina*, which is found in tropical and southern African, the Abrabian Peninsula and Madagascar. The common names of *B. salicina* plants are: Matumi (English); Mingerhout (Afrikaans); Mohlome (N. Sotho), Umhlume (isiZulu); Multume[4]. In Nigeria the leaf decoction of *B. salicina* is used as a bath for treatment of yellow fever. The Hausa name is Kadanyar Kurmi and the plant was found to be used for the treatment of trypanosomiasis by the Fulani people.

Trypanocides are used for the control of the disease in 37 African countries where animal trypanosomiasis is endemic but the available drugs are old, expensive, less effective, and face the problem of drug resistance [5, 6, 7]. In addition, they have high level of toxicity, high cost, poor efficiency, undesirable route of administration and drug resistance [8]. Hence, there is a need for exploiting medicinal plants for efficient and cheaper trypanocides.

Similarly, there is little or scarcity of information on the medicinal value of *B.salicina*. As such, the use of *B. salicina* in treatment of disease caused by *T. b.brucei* remains unknown to our knowledge. Therefore the result

obtained from the research will provide a safer, affordable and effective substituent of trypanocidal drugs. The research was aimed at investigating anti trypanasomal activity of *B. salicina* ethanol leaf extract through phytochemical screening of the leaf and its in vitoantitrypanasomal activity.

2.0 Materials and Method

2.1 Study Area

The study was conducted at the Nigeria Institute for Trypanosomiasis Research, Department of Trypanosomiasis & Vector and Parasitology Department Kaduna State.

2.2 Plant Material and Sample Collection

A fresh leaf of *B. salicina* was collected from Galma town,GiwaLocal Government, Kaduna states Nigeria, and was brought to the Herbarium section of Nigeria Institute for Trypanosomiasis Research, Kaduna State where it was identified. The dried leaves were pounded to fine powder with a mortar and pestle. One hundred grams (100g) of powdered leaf of *B. salicina* was extracted with 500ml of absolute Ethanol (100%). The mixture was shaken vigorously for six hours. It was then allowed to stand for the next 18 hours, shaken again and filtered using size 1 whatman filter paper. The filtrate was placed in an electric drier and evaporated slowly at 45˚C to dryness as described by [9].

2.3 Phytochemical Screening of Ethanol Leaves Extract of *B. Salicina*

The phytochemical was conducted using standard procedures as follows.

Test of Saponins

Frothing Test: Small quantity of *B. salicina* was dissolved in 10ml of distilled water in a test tube and it was shaken vigorously persistent frothy or honey comb formed which indicates that saponins is present in the sample extract [10].

Test for Tannins

Lead sub-acetate test: Three (3) drops of lead sub-acetate solution was added to a solution of *B. salicina*. A colour precipitate formed which indicates the presence of tannins in the extract sample [10].

Test for Alkaloids

Meyers Test: few drops of Meyer's reagent were added to a solution of *B. salicina* in a test tube and precipitate was formed which indicate the presence of alkaloids in the extract sample [10].

Test for Cardiac Glycosides

Kella-Killiani Test: 5ml of *B. Salicina* treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution, the test tube was held at an angle of 45^0C and 1ml of concentrated sulpharic acid was added down the test tube, purple ring colour at the interface indicates presence of cardiac glycosides [10].

Test for Anthraquinones

Bontrgers's Test: Small quantity of the *B. salicina* extract was shaken with 1ml of benzene and filtered. 5ml of 10% of ammonia solution was added to the filtrate and stirred. The production of the presence of pink-red colour indicates presence of anthraquinones in the sample [10].

Test for Flavanoids

Shinoda Test: To an alcoholic solution of the extract three pieces of magnesium chip were added followed by a drop of concentrated alcoholic acid. Appearance of a red to purple colour indicates the presence of flavonoids in the sample extract [10].

Test for Carbohydrates

Molisch's Test: Few drops of molisch reagent was added to the extract dissolved in 2ml of water, 1ml of concentrated sulpharic Acid was added and was allowed to run down the side of the tube to form a layer. Appearance of the violet ring when sulpharic acid was added indicates the presence of carbohydrates in sample extract [10].

2.4 Test Organism

T. b. brucei (Federe stain) was obtained from the Department of Trypanosomiasis, Vector & Parasitology Department, National Institute for Trypanosomiasis Research. Parasites were harvested from the blood of a donor rat at peak parasitemia and were diluted with the ethanol leaf extract of *B.salicina* then subsequently used for invitro antitrypanosomal assay.

2.5 Determination of Parasitaemia

Prasitaemia was monitored in blood obtained from the tail. The number of parasites was determined microscopically at (X400) magnification using the 'Rapid matching' method of [11].

2.6 In vitro Antitrypanosomal Activity of Crude Extracts

Assessment of in vitro antitrypanosomal activity of ethanol leaf extract of *B. salicina* was performed in micro titre plates. A stock solution of 200mg/ml of the ethanol leaf of *B. salicina* was prepared. Two hundred micro liter of blood containing parasites (25tryp/field) was incubated with equal volume of two hundred micro liter of the ethanol leaf extract of *B. salicina* solution of 20mg/ml, 10mg/ml, 5mg/ml, and 1mg/ml respectively to produce effective test concentrations of 10mg/ml, 5mg/ml, 2.5mg/ml, and 0.5mg/ml. A control was included which contained parasites suspended in 10mg/ml of samorin in phosphate buffer saline (PBS) only. The mixture were incubated at 37 degree Celsius for 5mins in wells of microscope (X400) for a drop in cessation of motility at 10, 20, 30, 40, 50, and 60 mins respectively [12].

3.0 Results

Table 1: Phytochemical constituents of hydro-ethanolic and ethanolic extracts of *B.salicina*

Classes of Phytochemicals	70% *B. Salicina*	100% *B. salicina*
Saponins	+	+
Alkaloids	+	-
Tannins	+	+
Anthraquinones	-	-
Flavonoids	+	+
Carbohydrates	+	+
Glycosides	-	-

Key: +: present -: absent

Table 2: Observed *T.b.brucei* Motility after Incubation in 70% Hydro-ethanolic Extract of *B.salicina*

Conc (mg/ml)	10 min	20 min	30 min	40 min	50 min	60 min
0.5	+++	++	*	*	*	*
2.5	+++	++	*	*	*	*
5	+++	*	*	*	*	*
10	+++	*	*	*	*	*
Control	*	*	*	*	*	*

Key:*= no motile parasites

+= weak parasites

++= slightly weak parasites

+++= actively motile parasites

++++= very active motile parasites

Table 3: Observed *T.b.brucei* Motility after Incubation in 100% Ethanolic Extract of *B.salicina*

Conc (mg/ml)	10 min	20 min	30 min	40 min	50 min	60 min
0.5	+++	+++	++	*	*	*
2.5	+++	+++	++	*	*	*
5	+++	+++	++	*	*	*
10	+++	++	++	*	*	*
Control	*	*	*	*	*	*

Key: *= no motile parasites

+= weak parasites

++= slightly weak parasites

+++= actively motile parasites

++++= very active motile parasites

4.0 Discussion

The two plant extracts, 70% hydro-ethannolic and 100% ethanolic extracts of *B. salicina* were screened for seven classes of phytochemical. Alkaloids, saponins, tannins, carbohydrates and flavonoids were present in both the plant extracts with the exception of alkaloids which was absent in 100% ethanolic extract (Table 1). Previous reports attributed the trypanocidal activity of a number of tropical plants to the flavonoids (azaanthraquinone), highly aromatic planar quaternary alkaloids, barbarine and harmaine [13, 14].

Parasite motility constitutes a relatively reliable indicator of viability of most zooflagellate parasites [15]. Drop or cessation in motility of trypanosomes can serve as a measure of anti–trypanosomal activity of the crude extract when compared with the control.

Table 2 showed *T. b. brucei* motility after incubation with 70% extract of *B. salicina* across time. At 10 mins of treatment with 0.5, 2.5, 5 and 10mg/ml, the parasites were observed to be actively motile. However, in the control set up, no motile parasites were seen. In treatments with 1 and 5mg/ml of the 70% extract, the parasites were observed to be slightly weak at 20mins. However, treatments of 5 and 10mg/ml reveal no motile parasites at 20 mins after incubation. Similarly, treatments of 0.5, 2.5, 5 and 10 mg/ml showed no motile parasites at 30, 40, 50 and 60 mins after incubation.

Table 3 showed observed T. b. brucei motility after incubation in 100% extract of *B. salicina* for 60 mins. Parasites were observed to be actively motile in 0.5, 2.5, 5 and 10 mg/ml at 10 and 20 mins with the exception of 10mg/ml at 20 mins in which the parasites were seen to be slightly weak. Similarly, the parasites were seen to be slightly weak in 0.5, 2.5, 5 and 10 mg/ml at 30 mins. No motile parasites were observed in 0.5, 2.5, 5 and 10 mg/ml treatments at 40, 50 and 60 mins after incubation. Control set up shows the same response indicating no motile parasites after treatment with somarin.

Treatment with 70% has been found to be more effective because it has removed the parasites after 20 mins at concentration of 5 and 10 mg/ml. However, with 100%, absence of parasites was first reported after 40 mins. The low antritrypanosomal activity of 100% extract might be attributed to the absence of alkaloids.

From the results obtained in this work, the ability of the plant extracts to immobilize or reduce trypanosome motility was seen to be both concentration and time dependent (Tables 2 to 3). [16] reported that both the aqueous and ethanolic extracts of the various parts of *K. senegalensis* showed in vitro antitrypanosomal activity in a dose-dependent fashion with the ethanolic extracts seemingly exhibiting a higher activity against the *T. evansi* parasites.

Furthermore, [17] suggested that many natural products exhibit their trypanocidal activity through interference with redox balance of the parasites acting either on the respiratory chain or on the cellular defenses against oxidative stress. This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alterations in redox balance. It is also known that some agents act by binding with the kinetoplast DNA of the parasite [12]. Some plant extracts have been demonstrated to contain potential trypanocidal constituents (18, 19, 20]. From the above, it is evident that *B. salicina* possess antitrypanasomal potentials. In addition, [21] revealed that acetonic extract of *B. salicina* along with *B. buceras*, *V. infausta* and *X. Kraussiana* had good antifungal activity against *A. fumigatus*.

Conclusion

From the results obtained, the two plant extracts, 70% hydro-ethannolic and 100% ethanolic extracts of *B. salicina* contained alkaloids, saponins, tannins, carbohydrates and flavonoids with the exception of alkaloids which was absent in 100% ethanolic extract. It shows that 70% and 100% ethanolic extract of *B. salicina* exhibit antitrypanasomal activity against *T. b. brucei* parasites. However, 70% ethanolic extract has higher potential to reduce parasites motility and the activity was seen to be concentration and time dependent. This might be attributed to the presence of alkaloids. Further research should be carried out to isolate, identify, characterize and elucidate the structure of the bioactive compounds present so as to have the complete picture in terms of its antitrypanasomal activity. Other plants should be screened for potential antitrypanasomal activity.

Authors contribution

Concept and design of research- Ali Sani and Deepa Singh Laboratory and collection of data- Fatima Hassan and Asmau Mahe, Write up and critical analysis- Umar Abdullahi Zakariyya, Final approval- Ali Sani

Conflict of Interest

Nil

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