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The in vitro antitrypanosomal activity of Albizia gummifera leaf extracts

Abstract: For the control and treatment of trypanosomiasis, a limited number of chemotherapeutic drugs with mild side effects are available. As a result, a quest for a less toxic herbal treatment for trypanosomiasis is needed. Ethanolic extract of A. gummifera leaf (EEAL) and aqueous extract of Albizia gummifera leaf (AEAL) were tested for antitrypanosomal activity against Trypanosoma brucei brucei in vitro. We first compared the phytochemical concentrations of EEAL and AEAL and discovered that EEAL had higher phytochemical concentrations on average than AEAL: flavonoids (4.26 mg/g vs 2.50 mg/g); alkaloids (38.40 mg/g vs 19.80 mg/g); tannins (230.7 mg/g vs 45.74 mg/g) and saponins (128.66 vs 44.33 g/g). From the result of phytochemical concentrations of the two compounds, the higher values observed in flavonoids and alkaloid of EEAL led us to hypothesize that EEAL would have greater trypanocidal activity. Following that, EEAL and AEAL were tested for antitrypanosomal activity in vitro. Forty µl of blood holding in about 25±8 parasites/field was mixed with 20 µl of the EEAL and AEAL solutions of 100, 80, 60 mg/ml to produce an efficacious test concentration of 25, 20 and 15 mg/ml, sequentially. The extracts inhibited parasite motility and eliminated the organisms at the concentrations used in vitro, except for 15 mg/ml AEAL and 20 mg/ml AEAL. Following the screening, the Albizia gummifera ethanolic extract found to have positive in vitro trypanocidal activity. More research is needed to determine the concentrations of the extract for the in vivo test.

Keywords: Bioactive compounds, herbal extracts, phytochemicals, trypanosomiasis

1 Introduction

Human African Trypanosomiasis (HAT) and Animal African Trypanosomiasis (AAT) are diseases caused by trypanosomes. The trypanosome is flagellated haemoparasite, and it is an extracellular protozoan belonging to the genus Trypanosoma and is widely distributed in the tropics [1]. Tsetse flies of the genus Glossina, which are the only infected vectors, transmit the parasite. The risk of sleeping sickness transmission (i.e. the cow-fly-human transmission cycle) between species is high in areas where there is a high density of human, cattle, and fly cohabitation [2].

Since trypanosomiasis is one of the biggest barriers to livestock production in Africa, and since management and treatment of trypanosomiasis is costly, the HAT and AAT remain a zoonotic disease of public health and economic importance [2,3]. Resistance to AAT drugs has also grown to alarming levels, especially in areas with high trypanosomiasis transmission and high use of trypanocidal drugs [4]. Likewise, the lack of effectiveness and toxicity of commonly used HAT drugs, as well as the growing challenges of trypanosome resistance to currently available traditional drugs and a seriously impaired trypanocidal drug detection pathway, cause the discovery of new trypanocidal drugs with superior efficacy and safety attributes [5,6].

The treatment options for trypanosomiasis are limited to a few chemotherapeutic medications with mild to severe side effects. The drugs used to treat trypanosomiasis are old, expensive, and ineffective, and they are linked to severe side effects and drug resistance [7]. There is a need for a more reliable, readily available, less costly, and less toxic herbal therapeutic agent to combat trypanosomiasis [8].
since approximately 85 percent of the global populace relies solely on herbs for medication, the quest for plant-derived drugs has intensified in the last few years [9,10]. Some reports revealed the potentials of medicinal plants against trypanosomiasis [10]. For example, a 50 mg/kg aqueous extract of Peristrophe bicalyculata immobilized about 90% of T. brucei brucei in vitro after 60 minutes of incubation [11]. Other plants with antitrypanocidal activity against T. brucei brucei have been identified, including Carissa spinarum [12], Anchomanes difformis [13], and Saba florida [14].

A. gummifera is a common deciduous tree found in the tropics, and is thought to have medicinal properties for bacterial infections, malaria, skin disorders, and stomach pains, according to folklore [15,16]. A. gummifera’s antibacterial, antitrypanosomal, anti-plasmodial, and anticancer activities properties were related to spermine alkaloids, oleanane saponins and triterpenes [17–19]. Despite its widespread distribution, there are few records on A. gummifera leaf antitrypanosomal activity as compared to other tropical trees. Based on the earlier published works, we hypothesized that A. gummifera extracts would possess trypanocidal activities. We first determined the phytochemical concentration of different extracts and then tested their trypanocidal activity in vitro.

2 Materials and methods

2.1 Plant Collection, extraction and phytochemical analysis

Fresh leaves of the A. gummifera plant were plucked from farmland in Ogbese along Ise Ekiti Road, Ekiti State, Nigeria, between January and February 2018. The geographic coordinates of Ogbese are 7°27′36″N 5°25′12″E. A Botanist authenticated the leaf sample at the Department of Crop Soil and Pest, The Federal University of Technology, Akure (FUTA), Nigeria.

The collected A. gummifera leaves were washed under running tap water and air-dried at room temperature. The dried leaves were milled to form A. gummifera leaf powder (AGLP). The leaf extracts were obtained using techniques that had previously been reported [20, 21]. A 400 g AGLP was soaked in 2000 ml of 70% ethanol, and another 400 g of AGLP was soaked in 2000 ml of distilled water. The two preparations were shaken sequentially for six hours and left for 48 hours unshaken after that. Then, both preparations were filtered separately using Whatman No 1 filter paper. After that, the ethanolic and aqueous extracts were concentrated under a vacuum using a rotary evaporator (SCILOGEX SCI100-S 5L Rotary Evaporator, Vertical Coiled Condenser Manual Lift) at the temperature of 35-40°C. The dried ethanolic extract of A. gummifera leaf (EEAL) and aqueous extract of A. gummifera leaf (AEAL) were kept at -20°C until use. The phytochemical analysis was performed three times on EEAL and AEAL, with an average of three replicates recorded.

2.1.1 Quantification of Total flavonoids

The flavonoids were determined using the Surana et al. [22] procedure. Each dilution of standard rutin solution (10-100 g/ml) and 0.50 ml of each extract stock solution (1 mg/ml) were taken separately in test tubes to determine total flavonoid content. 1.50 ml methanol, 0.10 ml aluminium chloride solution, 0.10 ml potassium acetate solution, and 2.80 ml distilled water were applied to each test tube and shaken together. The sample blanks for all extracts and all dilutions of regular rutin were made in the same way but with distilled water instead of aluminium chloride solution. Until calculating the absorbance, all prepared solutions were filtered through Whatman filter paper No. 1. At 510 nm, absorbance was measured against a suitable blank.

The total flavonoid content was measured using a rutin calibration curve, and the result was expressed in mg rutin equivalent per gram dry weight extract.

2.1.2 Quantification of Total tannins

The FC technique defined by Biswas et al. [23], was used to estimate total tannins. In a 100 ml volumetric flask, the plant extract (1 ml) was diluted with 49 ml distilled water, 0.1 ml metaphosphoric acid, 1.7 ml 75 percent ethanol, 2.5 ml FC, and 10 ml (1.0 mol/ml) Na₂CO₃. The mixture was thoroughly mixed and kept at room temperature for 15 minutes. In a spectrophotometer, the absorbance of sample mixtures and regular solutions was measured against a blank at 680 nm. Tannic acid (TA) was used as a reference, and the total tannin content in the plant extract was stated as equal to TA (mg TA/g DW) using the standard curve (R² = 0.9972).

2.1.3 Total saponins quantification

Vanillin and concentrated sulfuric acid colourimetric approach were used to assess total saponin in the plant extract [24]. 0.1 ml of the plant extract was blended with 0.5 ml of 50% ethanol, 0.5 ml of freshly made 8% (w/v)
vanillin solution, and 4.0 ml of 77 per cent (w/w) sulfuric acid, in that order. The mixture was then warmed to 60°C in a water bath for 15 minutes; the absorbance was measured with a UV/Vis spectrophotometer at 545 nm after cooling to room temperature. The samples' total saponin content was measured using a tea saponin calibration curve and expressed as mg TSE/g DW.

2.1.4 Determination of alkaloids

The gravimetric method was done to analyse the alkaloid material, as described by Adeniyi et al. [25]. Using a weighing balance, 5 g of each sample was weighed and distributed in 50 ml of 10% acetic acid solution in ethanol. After a thorough shake, the mixture was left to sediment for about 4 hours before being filtered. On a hot plate, the filtrate was reduced to a quarter of its original volume. To precipitate the alkaloids, concentrated ammonium hydroxide was added drop by drop. The precipitate was filtered out using pre-weighed filter paper, which was then washed with a 1% ammonium hydroxide solution. The precipitate-containing filter paper was dried in an oven at 60°C for 30 minutes, and then moved to desiccators to cool before being reweighed until it reached a constant weight. The constant weight was kept track of. The alkaloid weight was defined as the fraction of the sample weight using the filter paperweight difference.

2.2 Test organism in vitro trypanocidal activity

Trypanosoma brucei brucei (T. brucei brucei) was acquired from Nigerian Institute for Trypanosomiasis Research Vom, Nigeria. The parasite was kept alive in the Laboratory by constant passage into albino rats kept in standard laboratory conditions, fed standard rat chow, and provided free access to clean water until required. The parasite was sustained in the Laboratory by constant passage into albino rats until needed. The passage was performed four days after infection, and ten fields were examined with microscopy to determine the average parasitemia level (25±8) per field [26].

The evaluation of the in vitro trypanocidal activity of EEAL and AEAL was carried out in triplicates in 96 well microplates. Forty micro-litre (40 µl) of blood holding in about 25±8 parasites per field was mixed with 20 µl of the extracts (EEAL and AEAL) solutions of 100, 80, 60 mg/ml, to produce efficacious test concentration of 25, 20 and 15 mg/ml, sequentially. A 20 µl of commercial trypanocidal drug Centre-Diminal Plus® (Diminazine diaceturate (1.05 g); Antipyrine (1.31 g); Vitamin B12 (1 mg); manufactured by Aether Centre Biology Co. Ltd. Beijing. P.R., China) was used as the positive control; while the 20 µl glucose phosphate-buffered saline was used as the negative control.

At 5 minute incubation in a water bath at 37°C, 5 µl of the test mixtures were deposited on disparate microscope slides and covered with a coverslip. Every 15 minutes of a total of 60 minute analysis, the parasite count was observed and tracked under a microscope at X400 magnification. Parasites that did not move were presumed to be lifeless. The parasite’s mortality in extract-treated blood compared to that of the parasite-loaded control blood suspended in glucose phosphate-buffered saline without extract was taken as a measure of percentage of parasite mortality or trypanocidal activity [27]. The IC50 value of the standard drug and each extract was determined by recording the concentration at which 50% of the trypanosomes were cleared by each treatment [28].

2.2.1 Ethical approval

The research related to animals’ use has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

2.2.2 Statistical analysis

The data obtained from phytochemical analysis were calculated as means and expressed in descriptive statistics using the Microsoft Excel spreadsheets. All data in the trypanocidal analysis were subjected to a one-way ANOVA with SPSS version 20. Duncan multiple range test of the same package was used to assess the variations in means (P <0.05).

3 Results

Table 1 reveals the qualitative phytochemical composition of EEAL and AEAL. The EEAL has a high concentration of saponins, flavonoids, cardiac glycosides, and a low concentration of tannins and alkaloids. The AEAL has a moderate concentration of saponins and cardiac glycosides and low concentrations of tannins, flavonoids and alkaloids.

The quantitative phytoconstituents of EEAL and AEAL are shown in Figure 1. The concentration of the phytochemicals detected was higher in EEAL when compared to AEAL: flavonoids (4.26 mg/g vs. 2.50 mg/g);
Deborah Adebukola Oloruntola

Table 1 Qualitative phytochemistry of ethanolic and aqueous extracts of *Albizia gummifera* leaf

| Chemical constituents | Ethanolic extract of *Albizia gummifera* | Aqueous extract of *Albizia gummifera* |
|-----------------------|-----------------------------------------|----------------------------------------|
| Saponins              | +++                                     | ++                                     |
| Tannins               | +                                       | +                                      |
| Flavonoid             | +++                                     | ++                                     |
| Alkaloids             | +                                       | +                                      |
| Cardiac glycosides    | +++                                     | ++                                     |

Note: +: Low concentration; ++: Moderate concentration; +++: High concentration; -: Absent

No total seizure of motility was observed in the control (glucose phosphate-buffered solution) and 15 mg/ml AEAL treatment groups. An IC50 value of 15.73-20 mg/ml was found for the standard drug (Centre-Diminal Plus). The IC50 value of the EEAL was 15.5-25 mg/ml, while the IC50 value of the AEAL was 23.4-25 mg/ml.

4 Discussion

The study of the phytoconstituent of *A. gummifera* leaf defined some bioactive compounds of pharmacological importance such as flavonoids, alkaloids, tannins, saponins and cardiac glycosides in both the ethanolic and aqueous extracts, corroborating previous findings, as reviewed by Kokila et al., [15]. These classes of phytochemicals had been reported to exert therapeutic and antioxidant properties [29,30] and trypanocidal activity [26,31]. In addition, the trypanocidal activity of flavonoids, saponins tannins and cardiac glycoside was reported by Atawodi et al. [32] and Nwodo et al. [10]. The concentration of these phytochemicals being higher in the ethanolic extract than the aqueous extract suggests the existence of variation in the extraction yield of the various extraction solvents [33]. However, the higher extraction yield recorded by the ethanol, compared to aqueous in this study, disagreed with Truong et al. [33].

Immobilility of parasite is a reliable index to describe the antitrypanosomal effect of the plant extracts in vitro. In this study, the highest trypanocidal activity was recorded in ethanolic extract of *A. gummifera* leaf at 25 mg/ml concentration, where the complete cessation of parasite motility was observed at 30 minutes. These conform to the work of Bashir et al. [34], who reported that twenty-three West African plants inhibit Trypanosoma parasite motility within 31-60 minutes. Therefore, this study’s plant extract activity may be attributed to the phytoconstituents such as flavonoid, alkaloids, tannins present in the plant extracts, which had been implicated in trypanosomal activities [1]. The trypanocidal mechanism exhibited by the plant’s bioactive compounds is still unclear due to diversifying these bioactive compounds [1,35]. However, earlier study propounds that the phytochemicals or bioactive compounds exhibit trypanocidal activity by interfering with the parasites’ redox balance by acting on their cellular defences against oxidative stressor respiratory chain [36]. Furthermore, natural products contain structures that generate radicals, which can cause oxidative damage to trypanothione reductase, which is extremely sensitive to redox balance changes [28,31].
The in vitro antitrypanosomal activity of Albizia gummifera leaf extracts

**Table 2**  *In vitro* trypanocidal activity (% mortality) of ethanolic and aqueous extracts of Albizia gummifera leaf

| Time (minutes) | Control | Standard drug | 15 mg/ml EEAL | 20 mg/ml EEAL | 25 mg/ml EEAL | 15 mg/ml AEAL | 20 mg/ml AEAL | 25 mg/ml AEAL | P value |
|---------------|---------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------|
| 0             | 0.00±00 | 0.00±00       | 0.00±00       | 0.00±00       | 0.00±00       | 0.00±00       | 0.00±00       | 0.00±00       |         |
| 15            | 0.00±00 | 63.66±2.33a   | 18.00±0.57i   | 22.66±0.88k  | 41.33±0.88j  | 0.00±00       | 0.00±00       | 0.00±00       | 0.00±00 |
| 30            | 0.00±00 | 100.00±0.00a  | 43.00±1.73b   | 100.00±0.00a | 0.00±00       | 0.00±00       | 0.00±00       | 0.00±00       | 0.00±00 |
| 45            | 0.00±00 | 100.00±0.00a  | 46.00±3.46i   | 100.00±0.00a | 0.00±00       | 0.00±00       | 0.00±00       | 0.00±00       | 0.00±00 |
| 60            | 9.00±00 | 100.00±0.00a  | 100.00±0.00  | 100.00±0.00  | 8.00±0.58p   | 8.33±1.20+    | 100.00±0.00a | 0.00±00       | 0.00±00 |

Standard drug: Centre-Diminal Plus; EEAL: Ethanolic extract of Albizia gummifera leaf; AEAL: Aqueous extract of Albizia gummifera leaf; SEM: Standard error of the means; a-e Means within a row with different letters are significantly different (P<0.05).
The trypanocidal activity of A. gummifera leaf extracts demonstrated in this study was limited to a controlled setting for in vitro testing and may or may not be the same in a living being. In order to determine the anttrypanosomal role of A. gummifera leaf extracts in a living organism, further in vivo research is needed.

5 Conclusions

It could be deduced that the screened ethanolic extract of A. gummifera leaf at 25 mg/ml concentration exhibits positive in vitro trypanocidal activity. This observation is attributed to the bioactive compound in the extract. More research is required to determine the concentrations of the extracts for the in vivo test and to evaluate the extracts’ cytotoxic effects.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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