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Research article

In vitro anti-trypanosomal activity of crude extract and fractions of *Trichoscypha acuminata* stem bark, *Spathodea campanulata* flowers, and *Ficus elastica* lianas on *Trypanosoma brucei brucei*

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Abstract: The low therapeutic index of available trypanocidal drugs and the increasing emergence of resistant *Trypanosoma* parasites indicate the urgent need to develop new strategies for trypanosomiasis control. One such strategy is the screening of medicinal plants as sources of new lead compounds. *Trypanosoma brucei brucei* is a sub-species only infecting animals and thus largely used to screen anti-trypanosomal potential of various substances. Therefore, the present study investigates the anti-trypanosomal activity of crude extract, hexane, dichloromethane, ethyl acetate, and aqueous fractions of *Spathodea campanulata* P. Beauv. flowers, *Trichoscypha acuminata* Engl. stem bark, and *Ficus elastica* Roxb. Ex Hornem lianas using the Alamar Blue assay. Overall results showed that the crude extract of *T. acuminata*, *S. campanulate*, and *F. elastica* did not significantly reduce the viability of *Trypanosoma brucei brucei* at the tested concentration of 25 µg/mL. However, the hexane and dichloromethane fractions of *T. acuminata* and the hexane fraction of *F. elastica* exhibited viability percentages of 23.2 ± 10.5, 18.2 ± 9.7, and 20.1 ± 13.1% with IC_{50} values of 5.5, 5.0, and 17.5 µg/mL, respectively. Further research to identify compounds responsible for the observed activity and their

mechanisms of action towards new leads in parasitical drug discovery is needed.

Keywords: *Spathodea campanulata*; *Ficus elastica*; *Trichoscypha acuminata*; anti-trypanosomal activity; *Trypanosoma brucei brucei*

1. Introduction

African Trypanosomiasis is a parasitic disease widely distributed in Africa, which is spread through the bite of the tse-tse fly, transmitting protozoan parasites of the genus *Trypanosoma* to humans or animals. Numerous Trypanosoma species and sub-species have been described and reported to pose a significant health challenge especially in Sub-Saharan Africa. These include *Trypanosoma brucei brucei*, which infects animals and causes immense economic losses to agricultural production. [1]. Several preventive and treatment strategies have been deployed to overcome the billions of dollars lost to animal African Trypanosomiasis (AAT) [2]. These strategies have resulted in slow progress in eradicating AAT compared with human African Trypanosomiasis (HAT). At the moment, there is no vaccine against AAT and sustained control of insect vectors is still difficult. Available treatments are antiquated and hampered by toxicity, limited diagnosis, drug resistance, high cost, and misuse [3,4]. Thus, the major strategy relies on the use of trypanocidal drug which is also challenged by the increasing emergence of resistance, indicating the need to search for new chemical entities that are effective against trypanosomes, safe, and affordable for disease-endemic countries [5,6]. In this regard, harnessing medicinal plants is required to feed the pipeline of drug development for trypanosomiasis control and elimination.

It has been confirmed by WHO that herbal medicines serve the health needs of about 80% of the world's population [7]. The use of plants for therapeutic purposes (herbal medicine) has a long history and is currently experiencing renewed public interest. It is possible to use whole plants or derived products from extraction. Previous studies reported the anti-trypanosomal activity of some Cameroonian pharmacopoeia plants including *Selaginella vogelii* Spring leaves [8], *Diospyros conocarpa* Gürke ex K. Schum. roots [9], *Terminalia mantaly* H. Perrier roots [10], and *Piptadeniastrum africanum* (Hook.f.) Brenan roots [11]. Pursuing the perspective of valorization of Cameroonian traditional medicine, the present work focused on *Spathodea campanulata* P. Beauv. (Bignoniaceae)*, Ficus elastica* Roxb. Ex Hornem (Moraceae), and *Trichoscypha acuminata* Engl. (Anacardiaceae). These plants species were chosen based on previous research which highlighted their antiparasitic (antiplasmodial) activity [12–14]. Also, *Spathodea campanulata* stem bark and *Ficus elastica* aerial roots wood have shown anti-trypanosomal activity [15,16]. Therefore, the present study was undertaken to evaluate in vitro the anti-trypanosomal activity of extract and fractions of *Trichoscypha acuminata* stem bark, *Spathodea campanulata* flowers, and *Ficus elastica* lianas.

2. Materials and methods

2.1. Sample collection

All plant materials were harvested in September 2019 and authenticated at the Cameroon National Herbarium by comparison with voucher specimen previously deposited. Thus, *S. campanulata* flowers

(n°50085/HNC), *F. elastica* lianas (n°65646/HNC), and *T. acuminata* stem bark (n°12964/HNC) were collected at Bafou (West region), Melen (Centre region), and Douala (Littoral region) respectively.

2.2. Extraction and fractionation

The harvested materials were reduced into small pieces, air-dried, and pulverized. The powder of *S. campanulata* flowers (800 g), *F. elastica* lianas (1200 g), and *T. acuminata* stem bark (2300 g) were separately macerated into 3 L with 95% methanol at room temperature during 48 h [17]. After evaporation under reduced pressure using a rotary evaporator at a speed of 60 rpm at 40 °C, the obtained crude extracts were weighing 56, 51, and 89.3 g for *S. campanulata* (TESCf), *F. elastica* (TEFEl), and *T. acuminata* (TETAsb) respectively.

Thereafter, part of each extract (TESCf, 15.6 g; TEFEl, 46.0g; and TETAsb, 74.3g) was mixed with, 200 mL distilled water in order to carry out successive liquid-liquid fractionation using a separating funnel with organic solvents of increasing polarity, namely hexane, dichloromethane, and ethyl acetate.

The total extractable contents (TECs) obtained from TESCf was 4.27, 4.64, 0.62, and 6.02 g, for the hexane (HFSCf), dichloromethane (DFSCf), ethyl acetate (EFSCf), and aqueous (AqFSCf) fractions respectively. For TEFEl, the TECs were found to be 14, 10, 9, and 12 g for the hexane (HFFEl), dichloromethane (DFFEl), ethyl acetate (EFFEl), and aqueous (AqFFEl) fractions respectively. The TETAsb exhibited a TECs of 6.05, 3.57, 17.05, and 47.63 g, for the hexane (HFTAsb), dichloromethane (DFTAsb), ethyl acetate (EFTAsb), and aqueous (AqFTAsb) fractions respectively.

2.3. Test organism and assessement of anti-trypanosomal activity

T.b. brucei was acquired from the Center for Chemico- and Biomedical Research, Department of Chemistry, Rhodes University, South Africa. *T.b. brucei* 427 trypomastigotes were cultured in Iscove's Modified Dulbecco's Medium (IMDM; Lonza) supplemented with 10% fetal calf serum, HMI-9 supplement, hypoxanthine, and penicillin/streptomycin at 37°C in a 5% CO_2 incubator [18].

The anti-trypanosomal activity of prepared crude extracts and fractions was tested using the resazurin assay, which is based on the reduction of blue, non-fluorescent resazurin by living cells to the red fluorescent metabolite resorufin. Briefly, 25 µg/mL of testing samples were introduced into 96-well plates, leaving the last rows as the drug-free negative control. Then, 10^5 *T. b. brucei* cells were added to each well, followed by incubation of the plates at 37°C, 5% CO_2 for 48 h, before adding a freshly prepared resazurin (0.15 mg/ml in sterile physiological water). The plates were further incubated for 24 h under the same conditions. Reduction of resazurin to resorufin by viable parasites was assessed by fluorescence readings (excitation 560 nm, emission 590 nm) in a Spectramax M3 plate reader. Fluorescence readings were converted to percent parasite viability relative to the average readings obtained from untreated control wells.

2.4. Single concentration screening and dose response assays

The *T.b. brucei* culture incubated with plant samples (crude extracts and fractions) at concentrations ranging from 250 µg/mL to 0.11 µg/mL (3-fold-dilutions) for 48 h was used to determine the percentage of growth inhibition against untreated control. Experiments were performed

in triplicate and a standard deviation (SD) was derived. For comparative purposes, pentamidine (anti-trypanosomal drug) was used as a reference drug at concentrations ranging from 0.00001 μM to 100 μM. All measurements were performed using the Biotek Synergy MX microplate reader. The IC_{50} (50% inhibitory concentration) of tested samples was determined using dose-response graph which was plotted as percent cell viability vs. Log[compound] by non-linear regression using GraphPad software v. 5. 03 (GraphPad PRISM, Inc., San Diego, CA, USA). The samples were considered active when exhibiting an $IC_{50} < 10$ μg/mL.

3. Results

In vitro anti-trypanosomal activity of crude extracts and fractions of *S. campanulata* flowers, *T. acuminata* stem bark, and *F. elastica* liana is presented in Figure 1.

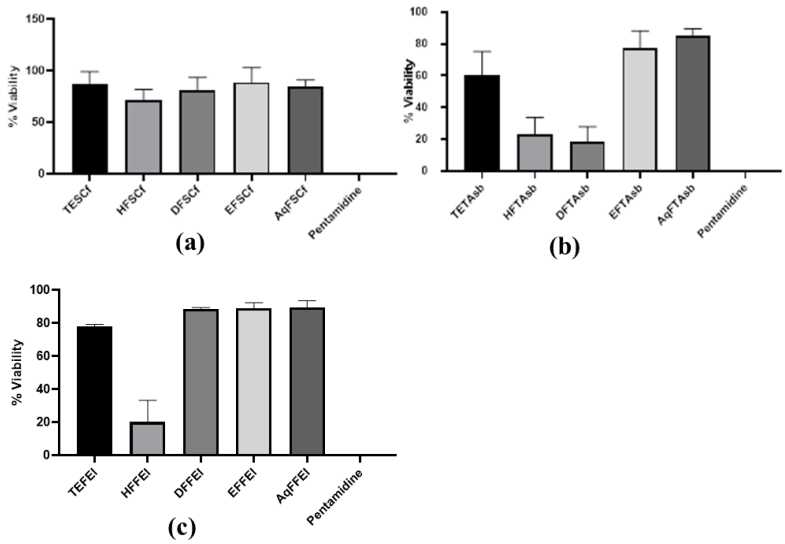

Figure 1. Effect of (a) *S. campanulata* flowers, (b) *T. acuminata* stem bark, and (c) *F. elastica* liana crude extracts and fractions on *T.b. brucei* viability. Each bar represents the means of 3 independent experiments with standard deviation bars. TESCf = total extract of *S campanulata* flowers; HFSCf = hexane fraction of *S. campanulata* flowers; DFSCf = dichloromethane fraction of *S. campanulata* flowers; EFSCf = ethyl acetate fraction of *S. campanulata* flowers; AqFSCf = aqueous fraction of *S. campanulata* flowers; TETAsb = total extract of *T. acuminata* stem bark; HFTAsb = hexane fraction of *T. acuminata* stem bark; DFTAsb = dichloromethane fraction of *T. acuminata* stem bark; EFTAsb = ethyl acetate fraction of *T. acuminata* stem bark; AqFTAsb = aqueous fraction of *T. acuminata* stem bark; TEFEl = total extract of *F. elastica* lianas; HFFEl = hexane fraction of *F. elastica* lianas; DFFEl = dichloromethane fraction of *F. elastica* lianas; EFFEl = ethyl acetate fraction of *F. elastica* lianas; AqFFEl = aqueous fraction of *F. elastica* lianas; Pentamidine = reference drug.

At the tested concentration (25 μg/mL), none of the fractions nor extract of *S. campanulata* induced significant *T. b. brucei* mortality (Figure 1(a)). The viability percentages were 87.7 ± 11.5%

for total extract (TESCf), 72.1 ± 10.0% for hexane fraction (HFSCf), 80.9 ± 12.6% for dichloromethane fraction (DFSCf), 88.8 ± 14.4% for ethyl acetate fraction (EFSCf), and 84.9 ± 6.4% for aqueous fraction (AqFSCf). The extract and fractions of *T. acuminata* stem bark exhibited various viability percentages. The total extract was found to have the highest activity (60.3 ± 14.8%) as compared to dichloromethane (77.4 ± 10.8%) or the aqueous fractions (85.4 ± 4.0%) respectively (Figure 1(b)). For *F. elastica* lianas, the viability percentages were found to be 78.1 ± 1.2, 88.4 ± 1.0, 89.1 ± 3.3, and 89.5 ± 4.0% for total extract (TEFEl), dichloromethane (DFFEl), ethylacetate (EFFEl), and aqueous fractions (AqFFEl) respectively (Figure 1c). Pentamidine used as a reference drug induced 100% mortality at 1 µM.

Further, tree samples namely HFTAsb, DFTAsb, and HFFEl which induced parasites viability percentages less than 50% (23.2 ± 10.5, 18.2 ± 9.7, and 20.1 ± 13.1% respectively) were tested for IC_{50} determination following graphical regression method on dose-response curves. As depicted in Figure 2, HFTAsb, DFTAsb, and HFFEl exhibited IC_{50} values of 5.54, 5.00, and 17.5 µg/mL respectively. Also, the IC_{50} of pentamidine was found to be 0,01 µM.

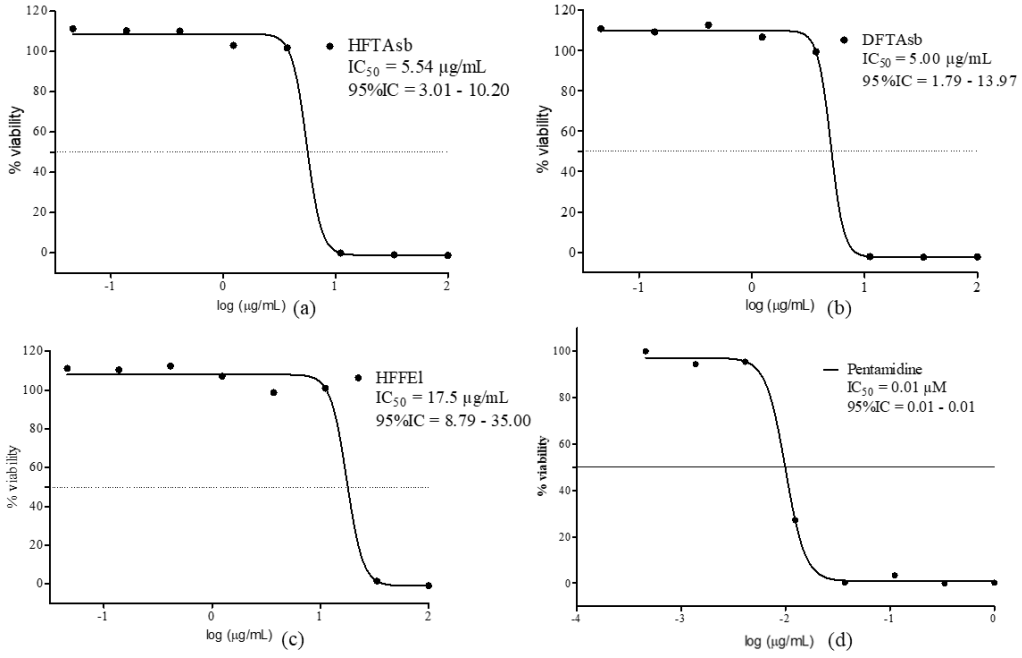

Figure 2. Dose-response curves of (a) hexane fraction of T. acuminata, (b) dichloromethane fraction of T. acuminata, (c) hexane fraction of F. elastica, and (d) Pentamine in anti-trypanosomal assay. HFTAsb = hexane fraction of T. acuminata stem bark; DFTAsb = dichloromethane fraction of T. acuminata stem bark; HFFEl = hexane fraction of F. elastica liana.

4. Discussion

The in vitro anti-trypanosomal activity of *Spathodea campanulata* flowers*, Trichoscypha acuminata* stem bark, and *Ficus elastica* lianas against *Trypanosoma brucei brucei* is reported in the present work. The three plants species are used in Cameroonian traditional medicine and thus were

selected. The stem bark of *T. acuminata* is used for the management of children's stomachs, infertility, dysmenorrheal, rheumatism, bleeding during pregnancy, bronchial ailments, headaches, feverish stiffness, side pain, and as a vermifuge and aphrodisiac [19]. *S. campanulata* is used in the fight against *Aedes* mosquitoes and to cure hemorrhoids, colorectal, skin, cervical cancers, and malaria [20–25]. Similarly, *Ficus elastica* is used as a diuretic and to treat skin diseases, allergies, and microbial infections [14]. Furthermore, previous studies highlighted their parasiticidal property against *plasmodium falciparum* chloroquine-sensitive 3D7 strain [12–14]. However, scientific evidence of their anti-trypanosomal activity remains scarce.

In this study, the total extract and fractions of *S. campanulata* flowers exhibited *T. b. brucei* viability percentages far higher than 50%, indicating their lower trypanocidal potential. In contrast, the stem bark extract is reported to have moderate activity during in vivo experiment, inducing a significant reduction of parasitemia after 8 days in infected mice [15]. Such observations could be explained by the phytochemical contents of part used as the components of a plant varied between parts [26] or by the experimental approach and condition (in vitro versus in vivo). Regarding *T. acuminata*, only the hexane and dichlorohexane fractions exhibited *T. b. brucei* viability percentages less than 50%, showing moderate anti-trypanosomal effect. This activity might be attribute to secondary metabolites such as tannins, polyphenols, flavonoids, triterpenes, and heterosides which were previously detected in theses fractions [12] and reported to possess numerous biological activities including anti-parasitic activity. Similar observations have been made by Fouekeng et al. while studying the anti-trypanosomal activity of the hexane fraction of *Antrocaryon klaineanum* stem bark [9]. Also, *F. elastica* extract and fractions were screened for their anti-trypanosomal potential. Again, only the hexane fraction showed potent activity, exhibiting an IC_{50} value of 17.5 µg/mL. Previous studies showed that hexane fraction of *F. elastica* lianas contains alkaloids which were yet to be isolated [14] but are well-known to possess significant anti-parasitic activities. This group of compounds said to affect trypanosomes by DNA intercalation in combination with the inhibition of protein synthesis [27]. Mbosso et al. showed that the total extract of *Ficus elastica* aerial roots wood which contained alkaloids exhibited trypanocidal activity [8]. Therefore, the observed activity could be attributed to this class of compounds. The standard drug pentamidine, which is one of the most employed trypanocidal drugs for its ability to cross the blood-brain barrier despite its partial retention by the capillary endothelium thus failing to reach the healthy or trypanosome-infected brain [28], was tested as a positive control. At the concentrations ranged from 0.00001 µM to 100 µM, the drug showed significant effect on the parasite viability, exhibiting an IC_{50} value of 0.01 µM. These findings indicate that the drug is not yet at an alarming level of resistance and should be under surveillance.

5. Conclusions

Despite the fact that crude extract of *S. campanulata* flowers, *T. acuminata* stem bark, and *F. elastica* lianas did not significantly reduce *T.b. brucei* viability at the concentration of 25 µg/mL, the hexane fractions of *T. acuminata* and *F. elastica* lianas exhibited moderate trypanocidal activity. Also, the dichloromethane fraction of *T. acuminata* exhibited potent in vitro anti-trypanosomal activity. The obtained results highlight the first anti-trypanosomal activity of *Trichoscypha acuminata* stem bark, *Spathodea campanulata* flowers, and *Ficus elastica* lianas and indicate that these Cameroonian medicinal plants should further be investigated towards identification of new leads compounds in trypanocidal drug discovery.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare no conflict of interest in this manuscript.

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