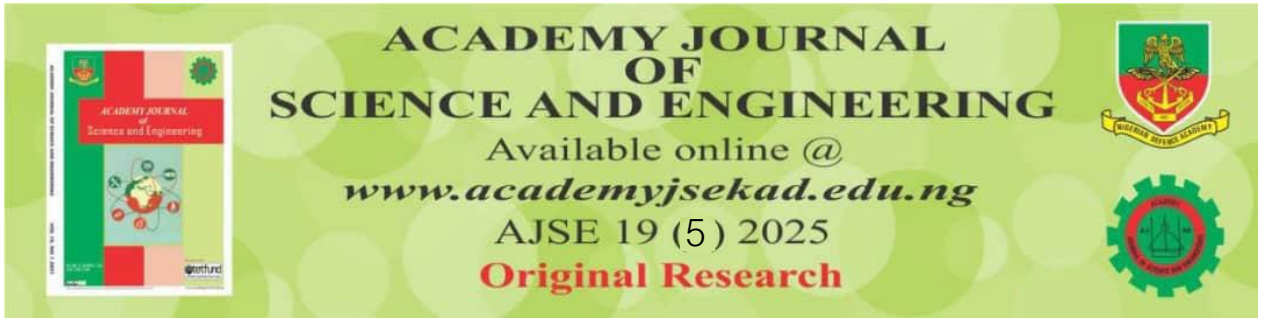

ANTI-TRYPANOSOMAL EFFECTS O7F SOME SELECTED NIGERIAN MEDICINAL PLANTS

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Abstract

Treatments of African trypanosomiasis have been dependent on the use of synthetic drugs which have several drawbacks. This is compounded by the emergence of drug-resistant parasites. These limitations have craved the urgent need for novel drugs from non-synthetic sources such as plants. The study, therefore, was aimed at evaluating in vitro antitrypanosomal activity of crude leaf extracts (CLE) of *Acalypha wilkesiana*, *Annona muricata*, *Calotropis procera, Hyptis sualovens*, *Momordica charantia*, *Artemisia annua*, and *Siphonochilus aethiopicus* using Hexane, ethyl acetate and methanol as solvents. Extraction was carried out by Soxhlet extraction method and different concentrations (100 µg/ml to 0.049 µg/ml) of the selected plants were evaluated for antitrypanosomal activity against trypomastigotes stage of *Trypanosoma brucei brucei* S427 in 96 well plates. Bioassay of CLE was assessed using Alamar blue ™ assay. *Hyptis sualovens* and *M. charantia*, displayed moderate activities against *T. b. brucei* S427 with an EC_{50} range of 11- 14 µg/ml. Poor activities (EC_{50} ranging between 19 - 53 µg/ml) were exhibited by all extracts of *A. annua, A. wilkesiana*, *A. muricata, C. procera* and *H. suaveolens* methanol extract when tested against the parasite. This finding confirms the efficacy of some of the plants that are used by local herdsmen in the treatment of animal trypanosomiasis.

Keywords: African Trypanosomiasis, Plants, Antitrypanosomal, *In vitro* assay, Treatment, Extractions

1.0 INTRODUCTION

Sleeping sickness, commonly known as African trypanosomiasis (AT), is a significant problem for both humans and livestock on the African continent, especially in Nigeria (WHO, 2012). African trypanosomiasis is caused by a single-celled, flagellated parasitic protozoan from the Trypanosomatidae family, also known as Old World trypanosomes. The protozoan parasite is spread by a tsetse fly of the genus *Glossina* which affects 37 African countries (Denbarga *et al*., 2012). Animal African trypanosomiasis (AAT) and human African trypanosomiasis (HAT) are two subtypes of African trypanosomiasis. HAT causative agents are *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* and AAT are caused by *Trypanosoma brucei brucei*, *Trypanosoma congolenses*, *Trypanosoma cruzi*, *Trypanosoma evansi*, and *Trypanosoma equiperdum*. *Trypanosoma evansi* is the cause of surra and the alleged "mal de cadeiras" that occurs in other areas aside Africa. *Trypanosoma vivax*, *Trypanosoma brucei,* and *Trypanosoma congolense* are the etiological agents of Nagana or its associated diseases in Africa and Asia.

The Tropical Diseases Research Department of the World Health Organization considered Leishmaniasis and trypanosomiasis to be among the 10 most important diseases in tropical regions and among the three first-line diseases (African Trypanosomiasis, Chagas disease, and Leishmaniasis) (WHO, 2021). War, migration of carrier populations from active foci, environmental degradation, changes in the host preference of tsetse flies, genetic variability of the parasite, the presence of asymptomatic parasite-infected people, and lack of surveillance as well as maintenance of infection in animal reservoirs have all been linked to the disease re-emergence (Cordon-Obras *et al*., 2010). Worldwide, humans and animals including cattle, buffaloes, camels, sheep, goats, horses, donkeys, mules, pigs, cats, and dogs are all susceptible to trypanosomiasis, which is brought on by a protozoan parasite (Mirshekar *et al*., 2019). Trypanosomiasis is called 'neglected tropical diseases' because pharmaceutical companies have little interest in investing in them (Kande Betu Ku Mesu et al., 2021).

The infection can be spread through blood contact with infected people and animals, which happens when sharp objects like needles, razor blades, toothbrushes, and shaving tools are shared by drug users. *Glossina* species are susceptible to mutation, which can result in the formation of new strains that are resistant to drugs due to changes made to the parasites' drug-binding sites. Trypanosomiasis is treated with synthetic medications, which have numerous adverse effects and lose their effectiveness over time due to drug resistance. These and other considerations have led to a search for substitute medications made from plants or phytochemicals. Traditional medicines with local preparations are the predominant means of therapy for trypanosomiasis treatment, especially in many parts of the world where the disease is prevalent (Africa, India, China, the Middle East and South Asia) (Ahmad Khan and Ahmad, 2019).

According to Bashir *et al*. (2015) and Lawal *et al*. (2015), Africa is endowed with a wide range of natural compounds that can be employed as anti-infectives against various diseases. Igoli *et al*. (2011) demonstrated that potent anti-parasite components can be found in Nigerian medicinal herbs. Diverse plants have been shown to contain phytochemicals such as terpenoids (such as artemisinin) that have antiprotozoal and antiplasmodial properties (Kabiru *et al*., 2016; Taek *et al*., 2021). Therefore, this work is aimed at screening medicinal plants with antitrypanosomal activity used for the treatment of trypanosomiasis.

2.0 MATERIALS AND METHODS

Plants Collection

Plants selected for this study were based on information from herbalists and their potential for treating skin infections. Plants were identified at the Biological Sciences Department, Kaduna, NDA. Voucher numbers were assigned to each plant as follows: *Acalypha wilkesiana* (NDA/BIOH/2022/25), *Annona muricata* (NDA/BIOH/2022/24), *Calotropis procera* (NDA/BIOH/2022/22), *Hyptis sualovens* (NDA/BIOH/2022/23), *Momordica charantia* (NDA/BIOH/2022/26), *Artemisia annua* (NDA/BIOH/2022/28), and *Siphonochilus aethiopicus* (NDA/BIOH/2022/22).

Extraction Procedure

Leaves from each plant were air-dried and ground into powder using a grinder. Twenty grams (20g) of the powdered dried leaves were used for the extraction using the Soxhlet apparatus. Extraction was done using n-hexane, ethyl acetate, and methanol as solvents. Each of the extracts obtained was evaporated at 40ºC. The solvent and concentration of each of the extracts were recovered under a vacuum using a rotary evaporator connected to a condenser. Residual solvents were allowed to evaporate under the fume hood. Samples were labelled and stored at -20ºC before analysis (Ebiloma *et al*., 2017).

Anti-Trypanosomal activity

Plant extracts were investigated for their anti-kinetoplastid effects on promastigotes of *Trypanosoma brucei brucei* S427. Alamar blue™ 96 well microplate test was used to assess the activity of the plant extracts. Test compounds (2x the maximum concentration) were serially diluted 1:1 (i.e., from 100 µg/ml as the top concentration to 1.56 µg/ml) in HMI-9 medium (14 g/L β mercaptoethanol, and 3.0 g/L $NaHCO_3$ adjusted to pH 7.4). Using a multichannel pipette, the samples (100 L) were then added to the cultivated cells and left for 48 hours in the incubator (37ºC, 5% CO2). After 48 hours of incubation, 5 mM resazurin sodium salt (Sigma Aldrich, UK) was added (20 µL per well) and the plate was incubated for a further 24 hours. The fluorescence intensity (λex/em: 544 and 590 nm) of the plant extracts was determined using a FLUOstar OPTIMA (Ebiloma *et al*., 2018).

3.0 RESULTS AND DISCUSSION

Yields of Hexane, Ethyl Acetate, and Methanol Extractions from Leaves of Selected Plants

Crude plant extract yields obtained using hexane, ethyl acetate, and methanol as solvents are presented in Table 1. *Alcalypha wilkesiania, Hyptis suaveolens, Momordica charantia,* and *Calotropis procera* yielded the highest crude extracts of 7.060g, 2.679g, 5.246g, and 2.1869 respectively when extraction was carried out using methanol as a solvent than using either hexane or ethyl acetate solvent. The yield of *Annona muricata* and *Siphonochilus aethiopicus* were more when extraction was carried out by Hexane than when extraction methods were done by either ethyl acetate or methanol. However, ethyl acetate yielded less crude plant extracts for all the sampled plants except for *Artemisia annua* which yielded more crude plant extracts (2.481g) than when methanol and hexane were used as solvents.

Table 1: Crude extract yields of sampled plants

S/N	Plant	Hexane	Ethyl acetate (g)	Methanol

		(g)		(g)
1.	*Annona muricata*	3.304	0.4814	3.164
2.	*Acalypha wilkesiana*	3.574	0.3887	7.060
3.	*Hyptis suaveolon*	2.5768	1.229	2.679
4.	*Momordica charantia*	0.9937	1.583	5.246
5.	*Artemisia annua*	2.304	2.481	1.154
6.	*Calotropis procera*	1.704	1.105	2.186

Anti-Trypanosomal Activity of Crude Ethyl Acetate, Methanolic, and Hexane Plants Extracts on Wild Type *Trypanosome brucei brucei* S427

The anti-kinetoplastid activity of crude ethyl acetate, hexane, and methanol extracts of *Artemisia annua*, *Annona muricata*, *Alcaiypha wilkesiana*, *Calotropis procera*, *Hyptis suaveolon,* and *Momordica charantia* on wild type ***T****rypanosome brucei* S427 is presented in Table 2. Generally, in the treatment of *T b. brucei* S427 with the ethyl acetate, hexane and methanol crude extract, the control drug yielded the most significant result (0.01±0.00 µg/ml). Within the plant's crude extracts tested, *Hyptis suaveolen* (11.63±1.22 µg/ml) and *Momordica charantia* (11.40±1.40 µg/ml) yielded the most among plants with moderate significant difference exhibited, *Artemisia annua* (13.82±0.52 µg/ml) and *Alcaiypha wilkesiana* (22.07±1.95 µg/ml) had intermediate values between the moderate and least significant difference while both *Calotropis procera*, 40.46±7.66 and *Annona muricata*, 43.76±4.81 yielded the least significant values.

Similarly, hexane crude extract treatment on T. b brucei showed *Hyptis suaveolen* (14.36±0.95 µg/ml) and *Momordica charantia* (14.54±1.16 µg/ml) yielded the most among plants with moderate significant difference exhibited, *Artemisia annua* (26.56±2.52 µg/ml) and *Alcaiypha wilkesiana* (22.17±1.92 µg/ml) had intermediate values between the moderate and least significant difference while both *Calotropis procera*, 45.51±1.15 and *Annona muricata*, 53.50±3.47, yielded the least significant values.

For methanol crude extract treatment on *T. b*

brucei, only *Momordica charantia* (11.57±0.54 µg/ml) displayed a moderate significant difference, *Artemisia annua* (26.56±2.52 µg/ml) and *Alcaiypha wilkesiana* (22.17±1.92 µg/ml) and *Hyptis suaveolens* (19.69±0.64 µg/ml) had intermediate values between the moderate and least significant difference while both *Calotropis procera*, 32.45±2.76 µg/ml and *Annona muricata*, 34.29±0.85 µg/ml yielded the least significant values.

Table 2: Antitrypanosomal effect of Ethyl Acetate, Hexane and Methanol Crude Extracts on *Trypanosoma brucei brucei* S427

S/NO	PLANT	ETHYL ACETATE Mean±SD	HEXANE Mean±SD	METHANOL Mean±SD
1.	*Hyptis suaveolens*	11.63±1.23 ^{b}	14.36±0.95 ^{b}	19.69±0.64 ^{cd}
2.	*Artemisia annua*	13.82±0.52 ^{bc}	26.56±2.52 ^{c}	15.79±4.58 ^{bc}
3.	*Alcalypha wilkesiania*	22.07±1.95 ^{c}	22.17±1.92 ^{c}	23.33±4.23 ^{d}
4.	*Momordica charantia*	11.40±1.40 ^{b}	14.54±1.16 ^{b}	11.57±0.54 ^{b}
5.	*Calotropis procera*	40.46±7.66 ^{d}	45.51±1.15 ^{d}	32.45±2.76 ^{e}
6.	*Annona muricata*	43.76±4.81 ^{d}	53.50±3.47 ^{e}	34.29±0.85 ^{e}
7.	Diminazene acetate (Control drug)	0.01±0.00 ^{a}	0.01±0.00 ^{a}	0.01±0.00 ^{a}

Note: SD = Standard Deviation. Superscripts with different letters are significantly different at $p \leq 0.05$

Discussion

The present study showed the anti-trypanosomal effect of Ethyl Acetate, Methanol, and Hexane crude extract of *Artemisia annua*, *Annona muricata*, *Acalypha wilkesiana*, *Calotropis procera*, *Hyptis suaveolons* and *Momordica charantia* on Wild Type *Trypanosoma brucei brucei* S427.

The high extraction yield observed in methanolic extract when compared to the hexane and ethyl acetate, indicates that the extraction efficiency favors the highly polar solvents. This could be because the *Alcalypha wilkesiania, Hyptis suaveolens, Momordica charantia,* and *Calotropis procera* plant materials contain high levels of polar compounds that are soluble in solvents with high polarity. According to Truong *et al*. (2019), different extraction solvents result in various extraction yields due to differences in the

polarity of these extraction solvents that cause a wide variation in the level of extract. This result finding is consistent with the extraction yield of *Limophila aromatica* (Do *et al*., 2014), *Severinia buxifolia* (Truong *et al*., 2019), and some other medicinal plants (Kuppusamy *et al*., 2016). Studies have shown that the efficiency of the extraction technique is also strongly affected by the extraction method, temperature, extraction time, the composition of phytochemicals, and the solvent used (Do *et al*., 2014; Ngo *et al*., 2017).

Momordica charantia results for the three tested extraction solvents revealed that a moderate significant difference was exhibited against *T. b. brucei*. The plant leaves exhibited comparable effects on *T. b. brucei* with EC_{50} values of 11.40 µg/ml and 11.57 µg/ml for ethyl acetate and methanol, respectively. This supports the finding of Phillip *et al*. (2013), who reported *M. charantia* as a promising source of trypanocidal agents. *Momordica charantia* L. leaf methanolic extract was also reported to be effective in suppressing malaria at the highest dose of 200 mg/kg (Akanji *et al*., 2016). The plant was also reported of its traditionally used for treatment against diabetes, HIV, coughs, skin diseases, sterility in women, parasiticide, antipyretics, and a purgative e.t.c (Akanji *et al*., 2016). Flavonoids, one of the phytochemicals found in plants have been identified to exhibit antiparasitic potentials against trypanosomes and *Leishmania* parasites (Tasdemir *et al*., 2006)

Hyptis suaveolens methanol extract showed low trypanocidal activity than its ethyl acetate and hexane extracts. Similarly, the methanol crude extract of *Hyptis suaveolens* was reported to exhibit low antimalarial activity when compared to the result of hexane, acetone, and aqueous crude extracts (Aremu *et al*., 2022). The moderate activity exhibited by hexane and ethyl acetate crude extracts are in tandem with Olukwode *et al*. (2021) where *H. suaveolens* displayed no acute toxicity with an LD_{50} of 5000mg/Kg and moderate trypanocidal activity. The plant's phytochemicals have been reported to be rich in alkaloids, carbohydrates, glycosides, terpenoids, protein, steroids, flavonoids, phenols, and tannins.

Artemisia annua and *Acalypha wilkesiana* values obtained revealed low antitrypanosomal activities for all extracted solvents texted against *T. b. brucei*. Although *Artemisia annua* possesses one of the best antimalarial compounds artemisin (Tariq *et al*., 2009) and was also effective against *S. mansoni*, *F. hepatica*, and *Echinostoma caproni* parasites (Ferreira *et al*., 2011), it showed poor antitrypanosomal activity. Under our findings, the plant was potent against *Plasmodium falciparum* but displays moderate antitrypanosomal activity with $1C_{50}$ values of 99.4 µg/ml and 41.05 for methanol and dichloromethane extracts, respectively (Nibret & Wink, 2010). *Acalypha wilkesiana* (Red Hot Cat's Tail) leaf extract was only documented to be antitrypanocidal against *Trypanosoma brucei* when the time for the *invitro* exposure to the parasite was increased from 5 minutes (which other tested plants were effective) to 26 mins (Saleh *et al*., 2015). This signifies that plants with low antitrypasomal activity can be considered for treatment if the tested concentration of extracts used is increased.

However, *Calotropis procera* and *Annona muricata* exhibited the least activity against wild-type *Trypanosome brucei brucei* S427 ranging between 30 µg/ml and 51 µg/ml for all the solvents. This differs from the findings of Onyeli and Aliyoo, 2015 where *Anona muricata* hampered trypanosomal motility in 10 minutes, this difference could be a result of different extraction solvents used since chloroform solvent was used for their extraction. *Calotropis procera* result is in tandem with the findings from Hassan *et al*. (2008) where the plant

exhibited an *invivo* antitrypanosomal activity in the neutrophils rats with EC_{50} of 33.4 µg/ml.

Plant extracts of *Alcalypha wilkesiana, Calotropis procera,* and *Annona muricata* plants used in this study with low antitrypanosomal activity might possess other medicinal activities such as antimicrobial, antihypertensive, antidiabetic, antiviral, antidepressant, anti-inflammatory, antioxidant or they are antiparasitic against other species of parasites not analyzed in this work. Although *in vitro* bioactive screening remains a useful technique for the pre-selection of plants and bioassay-guided fractionation for the isolation and identification of active principles against biological infections, it should not be the only criterion as *in vivo* studies should be carried out to obtain additional evidence for the presence of bioactive principles.

4.0 CONCLUSION

A variety of medicinal plants and plant extracts have been reported for their significant role in anti-trypanosomal activity. *Hyptis Suaveolens* and *Momordica charantia* showed moderate activity against the parasite, while *Alcalypha wilkesiana, Artemisia annua, Calotropis procera,* and *Annona muricata* exhibited low antitrypanosomal activity. The study provides evidence for the use of the active plant crude extracts that exhibited moderate activity. Further studies are recommended to determine the active plant's bioactive compounds, efficacy in animal models, and possible safety to animal tissues.

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