Unilag Journal of Medicine, Science and Technology (UJMST) Vol. 7, No 2, 2019

PHYTOCHEMICAL ANALYSIS, ANTI-TRYPANOSOMAL, ANTIMALARIA AND CYTOTOXICITY POTENTIAL OF LEAVES OF *ACALYPHA GODSEFFIANA* MUELL ARG FROM EASTERN NIGERIA

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ABSTRACT

The incidence of death due to untreated trypanosomiasis and malaria in Nigeria and in Sub- Sahara African at large is on the increase yearly, hence, needs urgent attention. The emergence of drug-resistant trypanosomes and plasmodium has necessitated the search for novel therapy through medicinal plant. In this study, standard methods were adopted in evaluating the effects of solvents on phytochemical compositions, anti-trypanosomiasis (*in-vitro* on *T.b. brucei*), anti-malaria and cytotoxicity potential (overt method resazurium based raegent) of *Acalypha godseffiana* (leaves) was evaluated using standard methods. The qualitative and quantitative analysis revealed the presence of non-nutritive phytochemicals (%): alkaloid (3.07 ± 0.81), saponin (19.40 ± 0.61), phytic acid (0.92 ± 0.08) and oxalate (17.07 ± 0.45). The total phenolics results showed that methanol extract has highest concentration of flavonoid (379.66 mg quercetin g^{-1}), while acetone has the highest concentration of proanthocyanidin (264.67 mg catechin g^{-1}) and the highest concentration of phenolic compounds was found in ethanol extract (208.03 mg gallic g^{-1}). Water extracts has the least of all the total phenolics. The anti-trypanosomal activity revealed that acetone at 50 µg mL^{-1} significantly decrease the viability of the trypanosomes with IC_{50} of 67.02 µg mL^{-1}; while having no significant effect on the viability of the HeLa cells (indicating that the extract are not significantly cytotoxic). Meanwhile, none of the extracts significantly reduce the malaria viability at 50 µg mL^{-1}. The result revealed that *Acalypha godseffiana* leave possess anti-trypanosomal potential. The finding which is been reported for the first time for the plant can be incorporated into the pharmacopeia of traditional medicine.

Key words: *Acalypha godseffiana*, phytochemicals, anti-trypanosomal, cytotoxicity

INTRODUCTION

African trypanosomiasis (sleeping sickness) has been reported to be currently affecting about half a million populace of Sub-Saharan Africa; and about 60 million people are estimated to be at risk of contracting this disease which is fatal if untreated (WHO 1988; Peter *et al.,* 2009). A. trypanosomiasis is a disease caused by trypanosomes (protozoan parasites) and transmitted by tsetse flies (of the genus *Glossina*). It occurs in two form: the chronic form caused by

Trypanosoma brucei gambiense, (prevalent in West and Central Africa) and last for years; while the acute form caused by *T. b. rhodesiense*, (prevalent in Eastern and Southern Africa) last for only weeks before death occurs, if untreated (Gyapong, 2007).

The Nigerian land mass of about 80% has been infested by the tsetse fly parasite (Obaloto *et al.*, 2015). The disease is reported to be on increase in cattle, which can be attributed to the menace of tsetse flies, drug resistance and the presence of other haematophagous flies (Mann *et al.*, 2009).

Sleeping sickness epidemiology is a complex phenomenon. Its transmission cycles are subject to interactions of humans, tsetse flies and trypanosomes. Though re-emergent disease, much attention has not been given to it, may be due to its regional impact (Gyapong, 2007).

In the chemotherapy of African trypanosomiasis, six drugs have been employed which include: Pentamidine, Arsobal, Suramin, Melarsoprol Eflor- inithine and Mel B. These drugs have long been in use except new ones are recently developed (Steverding and Tyler, 2005). Meanwhile, the undesirable toxic side effects of these drugs have been reported, besides, the emergence of drug-resistant trypanosomes (Perez-Morga, 2007). Hence, the need for continual search and develop cheap and potent drugs to curb the menace of sleeping sickness disease. Natural products from plants have been observed to have offered novel possibilities for new drugs for treatments of diseases such as typanosomiasis (Hoet *et al.,* 2004). Researchers have reported African plants exhibiting anti-trypanosomal potential (Mann and Ogbadoyi, 2012). *Acalypha godseffiana* (Euphorbiaceae family) belongs to the genus *Acalypha* comprising about 570 species (Ogundaini, 2005). It is commonly called copperleaf, Joseph's coat, fire dragon, and match-me-if-you-can (Christman, 2004). It originated from Pacific Island, a fast growing bushy shrubs, an ever green plant with green leaf blade and creamy-white margins. The young developing leaf has pinkish tinge. In traditional medicine, the roots are used as cathartic and leaves reported to have laxative and vulnerary (Sahoo *et al.,* 2014). The expressed juice or boiled decoction of the leaves of *A. godseffiana* has been employed in traditional health care practice in Eastern Nigeria, for the management of gastrointestinal disorders, fungal skin infections, hypertension and diabetes mellitus. The leaf of species of *Acalypha* has also been reported to possess antimicrobial (Akinyemi *et al.,* 2005; Ogundaini, 2005; Oladunmoye, 2006) hypolipidaemic, hypoglycaemic (Quds *et al.,* 2012) diuretic and anti-hypertensive (Seebaluck *et al.,* 2015) properties. There is no

report on the antitrypanosomal potential of *Acalypha godseffiana*. In this study, the phytochemical analysis, antitrypanosomal, antimalarial and cytotoxic potentials of leaves of *Acalypha godseffiana* were investigated.

2.0. **MATERIALS AND METHODS**

2.1. **Plant Collection, Authentication and Preparation of Extract**

Leaves of *Acalypha godseffiana* were obtained from Imo State, Eastern Nigerian from October to December 2015. The plant was authenticated (LUH 2753) and deposited at the Herbarium, Department of Botany, Faculty of Science of University of Lagos Akoka. The plant part were air-dried for 5 days and pulverized to coarse powder. One hundred grams (100 g) each of the coarse sample were separately macerated with acetone, ethanol, and methanol for 72 h, while in lukewarm distilled water for 12 h. The solvent extracts were concentrated to dryness using rotary evaporator at 37 ^{o}C while water extract were freeze dried. All the extracts were kept at 4 ^{o}C until use.

2.3. **Phytochemical Analysis**

Crude alkaloid and saponin, phytic acid and oxalate were determined using already adopted methods.

2.3.1. **Alkaloid**

Five grams of the plant sample was placed in a 250 mL beaker and 200 mL of 10% CH_3CO_2H in C_2H_5OH was added. The mixture was covered and allowed to stand for 4 h. It was filtered and the filtrate was concentrated on a water bath until it reaches a quarter of its original volume. Concentrated NH_4OH was added until precipitation was complete. The mixture was allowed to settle and the precipitate collected on a weighed filter paper and washed with dilute NH_4OH. The precipitate, alkaloid, was dried and weighed. The percentage alkaloid was calculated by difference (Gracelin *et al.,* 2013).

2.3.2. **Saponins**

Twenty grams of plant sample was weighed into a 250 mL conical flask. 100 mL of 20% C_2H_5OH was added. The mixture was heated over a hot water bath for 4 h with continuous stirring at about 55 °C. It was afterwards filtered with a Whatman No.42 paper. The residue was re-extracted with another 200 mL of 20% C_2H_5OH. The combined extract was reduced to 40 mL over a water bath at about 90 °C. The concentrated extract was transferred into a 250 mL separator funnel and 20 mL of $(CH_3CH_2)_2O$ was added to the extract and shaken vigorously. The aqueous layer was recovered, while, the $(CH_3CH_2)_2O$ layer was discarded. This

purification process was repeated. 60 mL of n-butanol was added and the combined n-butanol extract was washed twice with 10 mL of 5% NaCl. The remaining solution was heated on a water-bath in a pre-weighed 250 mL beaker. After evaporation the residue was dried in an oven to a constant weight. The % saponin was calculated by difference (Gracelin *et al.*, 2013).

2.3.3. **Determination of Phytic Acid**

Phytic acid was determined using protocol as described by Olaleye *et al.* (2013). About 2 g of the sample was weighed into a 250 mL conical flask. Precisely, 100 mL of 2 % HCl was used to soak the sample for 3 h and filtered through Whatman No. 1 filter paper. About 25 mL aliquot of the filtrate was placed in a new 250 mL conical flask and 5 mL of 0.3 % ammonium thiocyanate solution added as an indicator; 53.5 mL of distilled water was added to give the desired acidity. The mixture was then titrated with standard iron III chloride solution which contains 0.00195 g of iron per mL until a brownish yellow colour persisted for 5 min. Phytic acid was calculated as: **Phytic acid (%)** = titre value × 0.00195 × 1.19 × 100.

2.3.4. **Determination of Oxalate Content**

The modified titration method as described by Rafiq *et al.* (2016) was employed to determine the oxalate content of the plant. 1 g of the coarse sample was weighed into a conical flask. 75 mL of 3 M H_2SO_4 was added and stirred with a magnetic stirrer for an hour. This was filtered and 25 mL aliquot of the filtrate was collected and heated to 80 – 90 ^oC. This filtrate was kept above 70 ^oC at all times. The hot aliquot was titrated against 0.05 M of $KMnO_4$ until an extremely faint pale pink colour persisted for 15-30 seconds. The oxalate content was calculated by taking 1 mL of 0.05 M of $KMnO_4$ as equivalent to 2.2 mg oxalate.

2.3.2. **Analysis Total Phenloic Composition**

2.3.2.1. **Total Phenol Contents**

The phenol contents in the extracts were determined by Folin-Ciocalteu's phenol reagent (Adedapo *et al.*, 2009; Nabavi *et al.*, 2008). Briefly, 0.5 mL extract / standard (1 mg mL^{-1}) was mixed with 2.5 ml of 10 % Folin-Ciocalteu reagent (v/v) and 2 mL (75 g L^{-1}) Na_2CO_3 solution was added with mixing. The tubes were vortexed for 15 seconds and allowed to stand for 30 min at 40 °C for color development. Absorbance was then measured at 760 nm using UV-vis spectrophotometer. Gallic acid was used as standard. Extracts were evaluated at a final concentration of 1 mg mL^{-1}. Total phenolic content was expressed as mg g^{-}

[1]gallic acid equivalent using Y=13.417x+0.1919, R^2=0.9593 obtained from a calibration curve of gallic acid.

2.3.2.6. **Total Flavonoids**

Total flavonoids were determined using the method already adopted by Samatha *et al.* (2012). To 0.5 mL of the sample and standard (1 mg mL^{-1}), 2 mL distilled water was added and 0.15 mL of 5% of $NaNO_2$ and allowed to stand at 25ºC for 5 - 6 min. 0.15 ml of 10% $AlCl_3$ was added and allowed to stand for another 6 min. After which 1 mL 0f 4% NaOH to the mixture and make up to 5 mL with distilled water, vortexed for 15 min and colour change observed. The absorbance was measured at 420 nm using spectrophotometer. Total flavonoid contents were calculated as quercetin (mg g^{-1}) equivalent using Y=1.755x+.0237, R^2=0.977.

2.3.2.7. **Total Proanthocyanidins**

Determination of proanthocyanidin was based on the already adopted protocols (Sofidiya and Familoni, 2012). A volume of 0.5 mL of 1 mg mL^{-1} extract solution was mixed with 3 mL of 4% vanillin-methanol solution and 1.5 mL hydrochloric acid; the mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm. Total proanthocyanidin contents were expressed as catechin equivalents (mg g^{-1}) using the following equation based on the calibration curve: y = 1.0733x + 0.0226, R^2 = 0.9956, where x is the absorbance and y is the catechin equivalent (mg g^{-1}).

2.4. **Anti-trypanocidal Activity**

The protocol adopted by Bulus and Addau, (2013) was employed with modification to assess for anti-trypanocidal activity of extracts of *A. godseffiana*. Each extract was added to *in vitro* cultures of *T. b. brucei* in 96-well plates at a fixed concentration of 25 µg mL^{-1}. After an incubation period of 48 h, the numbers of parasites surviving drug exposure was determined by adding a resazurin based reagent. The reagent contains resazurin was reduced to resorufin by living cells. Resorufin is a fluorophore (Exc_{560}/Em_{590}) and were quantified in a multiwell fluorescence plate reader.

2.5. **Anti-malarial Activity**

For screening of extracts against malaria parasites, the methods of Zofou *et al.* (2011) were adopted with little modification. Fifty µg mL^{-1} of each extract was added to parasite cultures in 96-well plates and incubated for 48 h in a 37ºC CO_2 incubator. After 48 h the plates are removed from the incubator. Twenty µL of culture was removed from each well and mixed with 125 µL of a mixture of

Malstat solution and NBT/PES solution in a fresh 96-well plate. These solutions measured the activity of the parasite lactate dehydrogenase (pLDH) enzyme in the cultures. A purple product was formed when pLDH is present, and this product was quantified in a 96-well plate reader by absorbance at 620 nm (Abs_{620}). The Abs_{620} reading in each well was thus an indication of the pLDH activity in that well and also the number of parasites in that well.

2.6. **Cytotoxicity Assay of the Extracts**

The overt cytotoxicity of the extracts was determined using resazurin based reagent as described by Merghoub *et al.* (2009). About 50 µg mL^{-1} of each extract was incubated in 96-well plates containing HeLa (human cervix adenocarcinoma) cells for 48 h. The number of cells that survived after the exposure was determined by resazurin based reagent and the readings obtained using resorufin fluorescence in a multiwell plate reader.

3.0. RESULTS

3.1. Phytochemical Analysis

The results of phytochemical screening show the presence of all the tested phytochemicals in methanol extract, while alkaloid, terpenoid, flavonoid, tannin, phenols and gardiac glucoside were present in acetone extract, with the absence of saponin and steriods (Table 1). The quantitative phytochemical study revealed that the leaves of *Acalypha godseffiana* plant have high saponin and oxalte content (Table 2a). The total phenolic analysis revealed that the extracts of leaves of *A. godseffiana* were abundant in proanthocyanidin (condensed tannin; Table 2b). The total phenolics results showed that methanol extract has highest concentration of flavonoid (379.66 mg quercetin g^{-1}), while acetone has the highest concentration of proanthocyanidin (264.67 mg catechin g^{-1}) and the highest concentration of phenolic compounds was found in ethanol extract (208.03 mg gallic g^{-1}). Water extracts has the least of all the total phenolics.

3.2. **Anti-trypanosomal Assay**

The result of activity of the extracts against trypanosomes (Figure. 1 and 2, table 3) revealed that acetone and water extracts at concentration of 50 mg/ml significantly affected the viability of trypanosomes with viability (%) as 15.17 and 15.63 respectively. The IC_{50} of acetone having the least viability was 67.02 µg mL^{-1}

3.3. **Anti-malaria Assay**

None of the extracts at 50 mg mL^{-1} significantly affect the growth of Malaria parasites (*Plasmodium falciparum* strain 3D7) (Figure 4)

3.4. **Cytotoxicity Potential**

The results of the cytotoxicity of the acetone, ethanol, methanol and aqueous extracts of *A. godseffiana* (leaves) are as shown (Figure 3, Table 5). The extracts exhibited high cell viability from 72.14 % to 96.02 %, depicting non- toxic potential of extracts on cells lines.

FIGURES

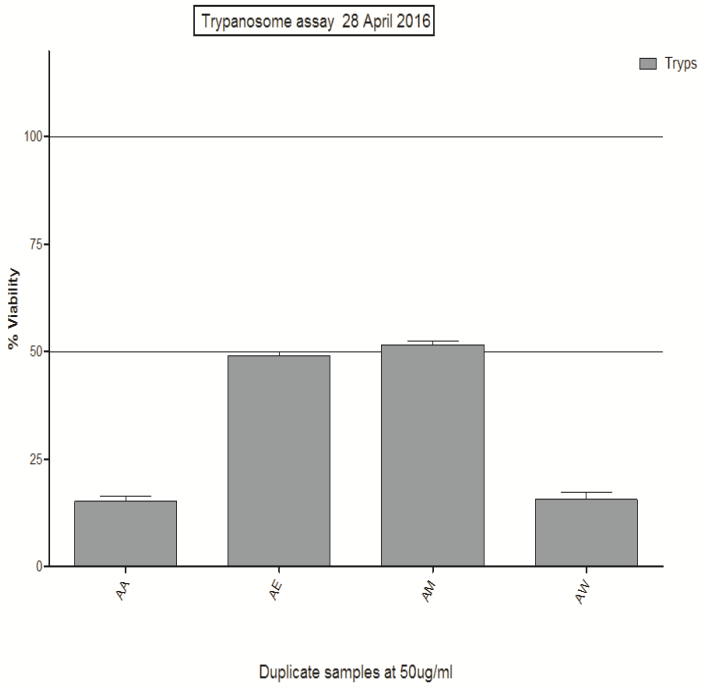

Figure 1: The Anti-trypanosomal Activity of Extracts of *A. godsffiana*

***Key**: **AA** = *Acalypha* Acetone; **AE** = *Acalypha* Ethanol; **AM**= *Acalypha* Methanol; **AW**= *Acalypha* Water

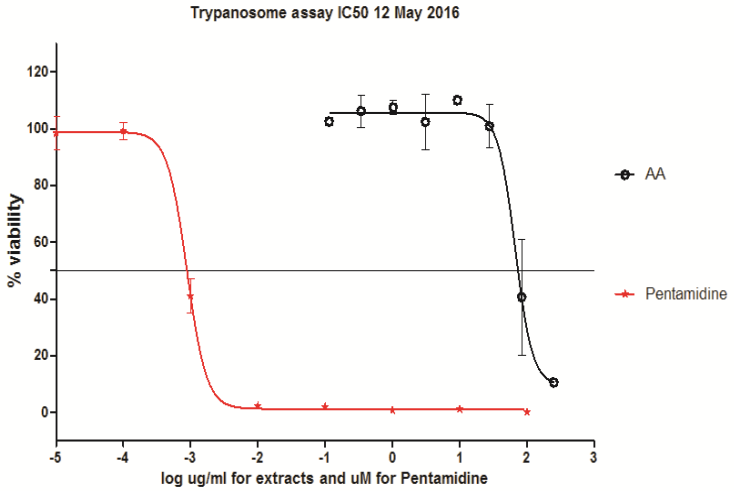

Figure 2: The IC_{50} of Anti-trypanosomal Activity of Acetone Extract of *A. godsffiana*

***Key**: **AA** = *Acalypha* Acetone

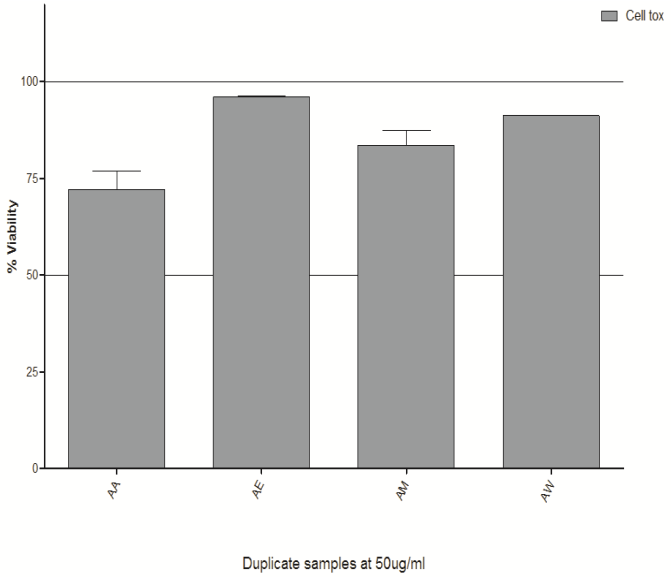

Figure 3: The Cytoxicity Activity of Extracts of *A. godsffiana*

***Key**: **AA** = *Acalypha* Acetone; **AE** = *Acalypha* Ethanol; **AM**= *Acalypha* Methanol; **AW**= *Acalypha* Water

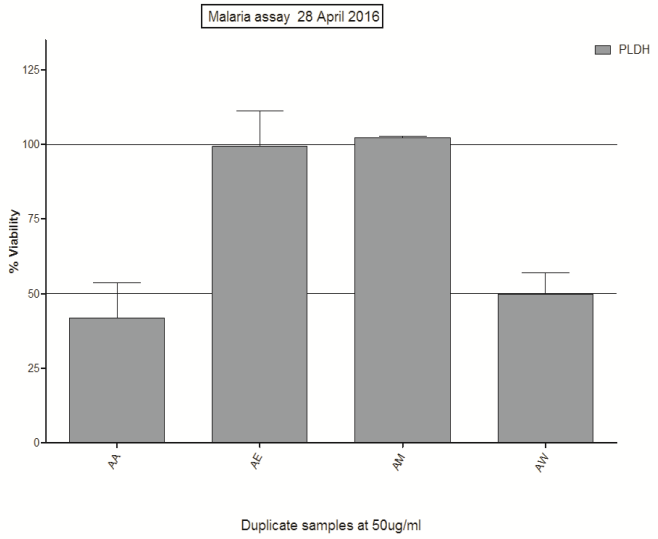

Figure 4: The Anti-malarial Activity of Extracts of *A. godsffiana*

***Key**: **AA** = *Acalypha* Acetone; **AE** = *Acalypha* Ethanol; **AM**= *Acalypha* Methanol; **AW**= *Acalypha* Water

TABLES

Table 1: Qualitative Phytochemical Analysis of *A. godseffiana*

Phytochemicals	**Acetone**	**Ethanol**	**Methanol**	**Water**
Alkaloid	+	++	++	++
Flavonoid	++	++	++	+
Saponin	-	-	++	+
Tannin	++	+	+	+
Terpenoids	+	+	+	-
Phenols	++	++	++	+
Steriods	-	+	++	-
Cardiac glycoside	+	+	++	++

***Key:** + = present; ++ abundant; - absent

Table 2a: Non- Nutritive Phytochemicals of *A. godseffiana* (leaves)

Non- Nutritive	**Compositions**
Alkaloid	3.07 ± 0.81
Saponin	19.40 ± 0.61
Oxalate	17.07 ± 0.45
Phytic acid	0.92 ± 0.08

Table 2b: Total Phenolic Components of *A. godseffiana* (leaves)

Phytochemicals	Acetone	Ethanol	Methanol	Aqueous
Total Phenols (mg gallic acid g-1)	133.20 ± 0.001	208.03 ± 0.003	189.17 ± 0.002	53.52 ± 0.002
Total Flavonoid (mg quercetin g^{-1})	350.60 ± 0.002	304.25 ± 0.001	379.66 ± 0.001	156.11 ± 0.002
Total Proanthocyanidin (mg catechin g^{-1})	264.67 ± 0015	45.09 ± 0.002	75.84 ± 0.001	13.73 ± 0.001

*Values = triplicate determinations expressed as mean ± standard deviation of per g of plant extracts

Table 3: Anti-Trypanosomal Potentials of *A. godseffiana* (Leaves)

Extracts	% Viability
Acetone	15.17 ± 1.17
Ethanol	49.13 ± 0.89
Methanol	51.70 ± 0.77
Water	15.63 ± 1.65

Table 4: Cell toxicity of Crude Extracts of *A. godseffiana* (leaves)

Extracts	% Viability
Acetone	72.14 ± 4.90
Ethanol	96.02 ± 0.42
M ethanol	83.59 ± 3.88
Water	91.20 ± 0.07

DISCUSSION

The acetone extract of leaves of *Acalypha godseffiana* at 50 µg mL^{-1} significantly decresased the viability of the trypanosomes with IC_{50} of 67.02 µg mL^{-1}. This results is in line with the reports of Atawodi *et al*. (2003) of the trypanocidal potential of crude extracts from plants.

Natural products have been reported as source of potent trypanocidal agent (Rosenkranz, and Wink, 2008; Chan-Bacab, and Peña-Rodríguez, 2001). Flavonoids have been reported to exhibit trypanocidal activity (Hoet *et al.,* 2004). Similarly, the antitrypanosomal activity of alkaloids such as Azanthraquinone was reported by Nok, 2002; to potentially inhibit the respiration trypanosome. The quantification of secondary metabolites revealed that acetone extract was rich in flavonoids, proanthocyanidin and phenols, with highest concentration of proanthocyanidin (264.67 mg catechin equivalent g^{-1}) as compared to methanol (75.84 mg catechin equivalent g^{-1}), ethanol (45.09 mg catechin equivalent g^{-1}) and water extract (13.73 mg catechin equivalent g^{-1}). The decrease in viability of trypanosomes by acetone extracts can therefore be attributed to its high content of proanthocyanidin in synergy with alkaloids, terpenes and other polyphenols (Hoet *et al.,* 2007). The antimalarial study confirmed that extracts of leaves of *A. godseffiana* did not significantly reduce the malaria viability at 50 µg mL^{-1}; hence, possess no antimalaria activity. The cytoxicity of the extracts confirm that all the extracts employed in this study had no significant effects on the viability of the HeLa cells, making safe on cell lines.

CONCLUSION

The results obtained from the present investigation revealed the potent trypanocidal activity of *A. godseffiana* which was reported for the first time, to the best of our knowledge. The observed trypanocidal potential of *A. godseffiana* with its safety on cell lines; made it a potential anti-trypanosomal plant which can be incorporated into the pharmacopeia of the traditional medicine.

ACKNOWLEDGEMENT: This research project was supported by Central Research Grant, University of Lagos, Nigeria. And the South African Medical Research Council (MRC) with funds from National Treasury under its Economic Competitiveness and Support Package, and Rhodes University SandisaImbewu."

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