ChemSearch Journal 12(2): 129 – 135, December, 2021
Publication of Chemical Society of Nigeria, Kano Chapter

Received:12/12/2021 Accepted: 22/12/2021
http://www.ajol.info/index.php/csj

In vitro Antiplasmodial, Antileishmanial, Antitrypanosomal and Antimicrobial Activities of Crude Extracts of *Alchornea cordifolia* Leaves

[1*]Vincent O. Imieje, [2]Christiana Roland Emem, [2]Mohammed Mahmoud Tanko, [1]Ese Ogaganefe, [1]Irene O. Oseghale and [1]Osayemwenre Erharuyi

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, 300001, Nigeria

[2]Department of Biochemical and Pharmaceutical Technology, Federal Polytechnic, Mubi, Nigeria

***Correspondence Email:** vincent.imieje@uniben.edu

ABSTRACT

Malaria, *leishmaniasis*, *trypanosomiasis* and pathogenic microorganisms infections are a public health burden worldwide. And the rapid development of resistance to the currently used drugs is a significant threat that necessitates the search for new anti-infective agents. *Alchornea cordifolia* (Schumach. and Thonn) Muell. Arg, family *Euphorbiaceae*, leaves have been used in folklore medicine to treat different disease conditions such as malaria, fevers, diarrhoea, infertility, wounds, inflammations, diabetes and cancers. This study aimed to assess the *in vitro* potentials of different crude extracts of the leaves against *Plasmodium falciparum*, *Leishmania donovani* (*promastigotes*, *axenic amastigotes*, and intracellular amastigotes), *Trypanosoma brucei* trypomastigotes and a panel of pathogenic bacteria and fungi, using established methods. Crude extracts of the powdered leaf sample of the plant extracted with various solvents were used for the experiment. The results of the antiplasmodial screening revealed that aqueous, methanol and ethanol extracts significantly inhibited two strains of *Plasmodium falciparum* parasites with IC_{50} values of 4.885 -18.094 µg/mL. The extracts also showed inhibitory activity against *T. brucei* trypomastigotes with IC_{50} of 8.68 -15.71 µg/mL, while chloroform was active against *L. donovani* blood-stage amastigotes. The extracts were equally effective against *Cryptococcus neoformans* (IC_{50} range of 32.258 – 161.853 µg/mL), and only aqueous extract was active against methicillin-resistant *S. aureus* (IC_{50} 199.054 µg/mL). The presence of bioactive compounds in the extracts may be responsible for the observed biological effects, and the plant could be further explored for lead compounds.

Keywords: *Alchornea cordifolia*, crude extract, malaria, neglected tropical diseases

INTRODUCTION

Infectious diseases, malaria, *leishmaniasis*, Human African *Trypanosomiasis* (HAT), as well as bacterial and fungal and microbial infections are major public health diseases affecting millions of people worldwide. Malaria currently affects over 40% of the world's population resulting in annual deaths of 1-2 million, mostly in sub-Saharan Africa (96%), Asia and South America, where children under five years (80%) and pregnant women are predominantly affected (WHO, 2020). The disease is caused by *Plasmodium falciparum* through a bite from infected female anopheles mosquitoes. Some recent figures indicating the gradual resistance of *Plasmodium falciparum* to the current ACTs based treatments is alarming and necessitate an urgent need for alternative treatment options (WHO, 2021). Leishmaniasis, a protozoa disease of public health concern caused by several parasites of the genus Leishmania, affect over 12 million people worldwide with high morbidity and mortality, mainly in Africa, Asia and Latin America, (Volpedo *et al*., 2021). Drugs such as pentavalent antimonials, pentamidine and amphotericin B, used in treating leishmaniasis have adverse side effects. Additionally, the usual parenteral route of administration makes compliance of the therapy a source of concern (Oliveira *et al*., 2011).

Human African Trypanosomiasis (HAT) is a vector-borne parasitic disease caused by trypanosomes transmitted through the bite of an infected tsetse fly (genus: Glossina). It is caused majorly by two pathogenic subspecies *Trypanosoma brucei* gambiense and *Trypanosoma brucei* rhodesiense that affect humans and livestock. Despite a gradual decline in cases, 992 new cases reported in 2019, it is still a significant public health concern (WHO, 2021). Some of the current drugs namely melarsoprol, pentamidine and D,L-α-difluoromethylornithine (DFMO) used in its management are highly toxic (Imieje *et al*., 2017; Atouguia and Costa, 1999). Generally, the current treatments used for the management of these three disease suffers from one disadvantage to the other, hence the need to explore alternative drugs which are potent, cost-effective agents with lesser side effects is paramount.

Plants have been used from time immemorial by man to treat various diseases. In recent times, a large percentage of people in the developed and developing countries of the world are using complementary and alternative medicine for the management of different conditions, including cancers, (Mavar-Manga, 2006); diabetes, (Szkudelski, 2001); inflammations, (Osadebe and Okoye, 2003); microbial infections, (Okeke *et al*., 1999; Gasting *et al.,* 2008); and as antimalaria, (Musuyu Muganza *et al*. 2012).

Alchornea cordifolia (Schumach. and Thonn.) Muell. Arg, family Euphorbiaceae, is distributed widely in Africa's tropics (Nigeria, Senegal, Gabon, Sierra Leon, Ivory Coast, Burundi and Burkina Faso). In Nigeria, *Alchornea cordifolia*, (English: Dove wood or Christmas bush) is called Ewa Ipa (Yoruba), Mbom (Efik), Ukpaoromi (Ijaw) etc. *Alchornea cordifolia* parts are widely used to treat coughs, gonorrhoea, infertility, bacterial infections, diarrhoea, ulcers, pain, inflammation, fever and bronchial conditions, (Boniface *et al*., 2016). Various authors have reported that the decoction of the leaves of *Alchornea cordifolia* is taken as a sedative and anti-spasmodic for epilepsy, headaches, cough, sore throat and bronchial infections. The leaf is chewed as an appetizer and the stem bark is used as fish poison, (Igoli *et al*., 2005; Ishola *et al*., 2012) while the dried leaves are smoked to treat cough, (Kayode and Kayode, 2008). Phytochemical studies of the different parts of the plants have revealed the presence of bioactive secondary plant metabolites, alkaloids, flavonoids, triterpenoids, fatty acids, steroids, and phenolics, (Boniface *et al*., 2016) in different parts of this plant. This led to the isolation of guaijaverin, hyperin, and quercetin, (Ogungbamila and Samuelsson, 1990); acetyl aleuritolic acid, daucosterol, beta-sitosterol (Mavar-Manga *et al*., 2008); yohombine, N1,N2-diisopentenyl guanidine, N1,N2,N3-triisopentenyl guanidine, and indomethacin (Mavar-Manga *et al*., 2008). These compounds have demonstrated significant pharmacological activities in *in vitro* and *in vivo* studies.

This present study investigates the *in vitro* antiplasmodial, antileishmanial, antitrypanosomal, antimicrobial and cytotoxicity effects of different solvents extracts of the leaves of *Alchornea cordifolia*.

MATERIALS AND METHODS

Plant collection and preparation

Fresh mature *Alchornea cordifolia* leaves were collected in February 2019 from Ekosodin village, Ovia North East Local Government area, Edo State, Nigeria. The botanical identity was authenticated at the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Nigeria where a voucher specimen (PCG-FP261) was assigned. The leaves were washed, air-dried, pulverized into a coarse powder in a mechanical grinder. And the powdered sample was stored in an air-tight container until ready for use.

Extraction

The powdered (100 g) leaves of *Alchornea cordifolia* was subjected to successive maceration in different solvents (700 mL each) in increasing order of polarity n-Hexane (AC-HEX), chloroform (AC-CHL), ethyl acetate (AC-EAT), ethanol (AC-ETH), methanol (AC-MET) and water (AC-AQ) with intermittent agitations for 72 hours. Consequently, the different crude extracts were filtered, concentrated to dryness *in vacuo* at 40ºC with a rotary evaporator, and the percentage yields were calculated based on the initial weight of the air-dried powdered sample. The dried extract was stored in an air-tight container and kept in the refrigerator at 4ºC until further use.

Phytochemical Screening

The Phytochemical screening tests were carried out using standard methods (Sofowora 1993; Evans, 2002).

In vitro Activity Screening

The primary and secondary antiplasmodial effects of the crude extracts of *Alchornea cordifolia* leaves were investigated *in vitro* against *Plasmodium falciparum* (chloroquine-sensitive D6 and chloroquine-resistant W2 strains). The extracts' selectivity indices (a measure of samples' cytotoxicity on mammalian cells) were determined using Vero cell lines (monkey fibroblast). The extracts were also investigated against *Leishmania donovani* promastigotes, axenic amastigotes, blood-stage amastigotes (amastigotes in THP1 cells); *Trypanosoma brucei* promastigotes and against a panel of pathogenic microorganisms.

Antiplasmodial Assay

The *in vitro* antiplasmodial activity of the extracts was measured by a colourimetric assay that determine the parasite lactate dehydrogenase (pLDH) activity described by Makler *et al*. (1993) and Samoylenko *et al*. (2009). The effects of the test samples on plasmodial LDH activity were determined using Malstat reagent (Flow Inc, Portland, OR). DMSO (0.25 %) and chloroquine/artemisinin were included in each assay, serving as vehicle and positive control drugs.

Antileishmanial Assay

The crude extracts were evaluated against *L. donovani* promastigote, *L. donovani* axenic amastigote, and *L. donovani* amastigote in THP1 according to the protocol described by Jain *et al*. (2012), which uses the Alamar Blue colourimetric assay method described by Mikus and Steverding (2000). Pentamidine and amphotericin B standard antileishmanial drugs were used as positive controls. The IC_{50} and IC_{90} values were computed from response curves using XLFit®.

Antitrypanosomal Assay

The antitrypanosomal assay was carried out according to a method previously reported by Jain *et al*. (2016).

Cytotoxicity assay

The cytotoxicity of the test samples was also tested against transformed human monocytic (THP1) cells. The assay method adopted for this study was previously described by Jain *et al.* (2016). This assay was done to assess the cytotoxicity of the plant extracts against Vero cell line (monkey kidney fibroblast) and their inherent antiplasmodial activity against *Plasmodium falciparum* in order to establish their selectivity indices (SI), which is the ratio of IC_{50} values of test samples against *Plasmodium falciparum* to that of Vero cell line.

In vitro Antimicrobial Assay

The crude extracts of *Alchornea cordifolia* were subjected to *in vitro* susceptibility testing against a panel of pathogenic organisms: the fungi include *Candida albicans* (ATCC 90028), *Aspergillus fumigatus* (ATCC 204305), *Cryptococcus neoformans* (ATCC 90113), while the bacteria were methicillin-resistant bacterium *Staphylococcus aureus* (MRSA; ATCC 33591), *E. coli* (ATCC 35218), *Klebsiella pneumonia* (ATCC 43816), Vancomycin resistance *Enterococcus faecium* (*ATCC* 49532) and *Pseudomonas aeruginosa* (*ATCC 27853)* using a modified version of the NCCLS methods (CLSI, 2008). The fungi and bacteria used in this experiment were obtained from the American Type Culture Collection (ATCC), Manassas, VA. All the test samples were dissolved in DMSO (0.25 %). Fluconazole, Amphotericin B and Ciprofloxacin were used as positive control drugs.

Statistical analysis

The results were presented as mean ± standard deviations (SD). IC_{50} values relative to controls were obtained using XL fit 4.2 software (IDBS, Alameda, CA).

RESULTS AND DISCUSSION

Phytochemical Constituents

The results of the phytochemical screening of the crude extract of *A. cordifolia leaf* revealed the presence of alkaloids, carbohydrates, reducing sugars, deoxysugars, saponins, tannins, phenolic compounds, flavonoids, terpenoids and proteins. Apparently, the findings were in agreement with Osadebe *et al*. (2012) and Amos-Tautua *et al*. (2011). Studies have established the biological importance of secondary metabolites shown to possess different pharmacological activities exhibited by extracts and fractions of medicinal plants (Oseghale *et al*., 2020).

Table 1: Inhibition of *Plasmodium falciparum* in an *in vitro* assay by crude extracts of *A. cordifolia* leaves at a test concentration of 15.8667 µg/mL.

Extracts	Percentage inhibition
AC-CHL	30
AC-AQ	87
AC-EAT	27
AC-ETH	57
AC-HEX	6
AC-MET	86

Note: Fractions exhibiting ≥ 50 and above percentage inhibition of parasites were further subjected to secondary screening to determine their IC_{50} values and selectivity indices (SI). AC-HEX = n-Hexane, AC-CHL= chloroform, AC-EAT = ethyl acetate, AC-ETH = ethanol, AC-MET = methanol, and AC-AQ = water.

Antiplasmodial Activity

The extracts were first subjected to primary antiplasmodial screening against the two strains of *plasmodium falciparum*, chloroquine sensitive (D6) and chloroquine resistance (W2). This was to determine the extract that inhibited ≥ 50% parasites growth at a single concentration of the extracts (15.8667 µg/mL). The extracts were further evaluated in the secondary screening to determine their IC50 values. The result of the primary screening is shown in Table 1. In this study, the aqueous (AC-AQ), methanol (AC-MET), and ethanolic (AC-ETH) extracts of *A. cordifolia* significantly inhibited *Plasmodium falciparum* growth with percentage inhibition of 87 %, 86 %, and 57 %, respectively, in the primary screening assay. The other extracts were less effective against the parasite. These extracts were further subjected to a secondary antiplasmodial screening assay.

Table 2: Antiplasmodial activity of crude extracts of *A. cordifolia* leaves and their SI values at 47.6-0 – 0.19588 µg/mL.

Extract/Fractions	*P. falciparum* D6		*P. falciparum* W2		**VERO**
	IC_{50} (µg/mL)	**SI**	IC_{50} (µg/mL)	**SI**	IC_{50} (µg/mL)
AC-AQ	6.491	>7.3	4.885	>9.7	>47.60
AC-ETH	18.094	>2.6	11.088	>4.3	>47.60
AC-MET	8.180	>5.8	5.252	>9.1	>47.60
Artemisinin	<0.106	>9.0	<0.106	>9.0	>16.859
Chloroquine	<0.093	>9.0	0.4698	>1.4	>14.881

In the parasite Lactate dehydrogenase assay, extracts AC-AQ, AC-ETH and AC-MET were tested against the two strains of *Plasmodium falciparum* (D6 and W2) used in this study and the half-maximal inhibitory activity is shown in Table 2. All the extracts were active against both plasmodium strains, i.e. chloroquine-sensitive (D6) and resistant (W2) strains. Careful observation of the results showed that the antiplasmodial activity of the extracts is polarity dependent; with the aqueous (water) extract exhibiting the highest parasites inhibitory activity with IC_{50} values of 6.491 and 4.885 µg/mL against D6 and W2 compared to the methanol extract (AC-MET) IC_{50} of 8.180 and 5.252 µg/mL, and IC_{50} 18.094 and 11.088 µg/mL for the ethanol (AC-ETH) extract. The antiplasmodial activity of *A. cordifolia* has been reported by many researchers against different strains of Plasmodium species. According to Mesia et al. (2008) the methanol extract was active against the Ghanaian strain of *Plasmodium falciparum* with IC_{50} value of 2.8 µg/mL, while Tona et al. (2007) investigated the same extract against the chloroquine-sensitive strain and recorded an IC_{50} value range 1-3 µg/mL. In another study, the ethanol leaves extract exhibited significant *in vitro* antiplasmodial activity against *Plasmodium falciparum* as reported by Banzouzi *et al*. (2002). In a more recent study, the crude methanol extract of *Alchornea cordifolia* exhibited significant ($p < 0.05 – 0.001$) and dose-dependent *in vivo* activity against *Plasmodium berghei* in mice Nnamdi *et al*. (2017). All the extracts were not cytotoxic to mammalian cells, as shown by their selectivity (SI) indexes, Table 2.

Antileishmanial and Antitrypanosomal activity

Extracts of *Alchornea cordifolia* were subjected to *in vitro* screening against *Leishmania donovani* (promastigotes, axenic amastigotes, and intracellular amastigotes in THP1 cells) and blood-stage promastigotes of *Trypanosoma brucei*. The result of this screening (Table 3), showed that only sample AC-CHL exhibited significant inhibition of *L. donovani* blood stage amastigotes (infective stage of the parasite) with IC_{50} value of 12.92 µg/mL at final test concentrations of 20-0.8 µg/mL. Similarly, extracts AC-AQ, AC-EAT, AC-ETH, and AC-MET were effective against *T. brucei* trypomastigotes with IC_{50} values of 8.68, 15.71, 9.19, 9.32 µg/mL, respectively. The aqueous extract demonstrated the lowest IC_{90} value (17.11 µg/mL). All the extracts except AC-CHL at test concentrations of 20 – 8 µg/mL did not show activity against the promastigotes, axenic amastigotes and intracellular amastigotes.

Table 3: Leishmanicidal and Trypanosomicidal effects of crude extracts of *A. cordifolia* leaves at concentration range 20-0.8 µg/mL.

Extracts	*L. donovani* Promastigotes		*L. donovani* axenic amastigotes		*L. donovani* amastigotes/THP		*T. brucei*	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
AC-CHL	>20	>20	>20	>20	12.92	>20	>20	>20
AC-AQ	>20	>20	>20	>20	>20	>20	8.68	17.11
AC-EAT	>20	>20	>20	>20	>20	>20	15.71	>20
AC-ETH	>20	>20	>20	>20	>20	>20	9.19	18.33
AC-HEX	>20	>20	>20	>20	>20	>20	>20	>20
AC-MET	>20	>20	>20	>20	>20	>20	9.32	17.34
Amphotericin B#	0.2315	0.2705	1.233	-	0.1937	0.3365	-	-
Pentamidine#	4.4004	7.9519	29.366	-	9.303	15.263	0.0058	0.0088
DFMO#	-	-	-	-	-	-	15.658	40.214

NOTE: # = µM concentration

The positive control drugs, Pentamidine and DMFO, possess better activity against these protozoa. However, it was observed that some of the extracts showed better activity than DFMO against *T. brucei* trypomastigotes. Several studies have highlighted the activity of plant extracts and fractions against leishmaniasis and trypanosomiasis (Jain *et al*., 2016; Obbo *et al*., 2019). In a study carried out by Mesia *et al*. (2008), the hydro-ethanolic leaf extract of *A. cordifolia* exhibited

strong activity against *Trypanosoma brucei brucei* (Tbb) with IC_{50} of 0.7 µg/mL and moderate activity against *Trypanosoma cruzi* (IC_{50} value of 34 µg/mL). In addition, the ethanol leaf extract was active against multi-resistant strains of *Trypanosoma congolense* with LD_{50} of 68.06 - 68.9 µg/mL, Adewunmi *et al*. (2001). The aqueous leaf extract was equally shown to be active against *Trypanosoma brucei brucei*, *T. cruzi* and *Leishmania infantum* with IC_{50} values of 6.67, 36.27 and 32.46 µg/mL, respectively (Musuyu-Muganza *et al*., 2012).

Antimicrobial Activity

The antimicrobial activities of the extracts against the different test organisms are as shown in Table 3.4. Except for AC-CHL and AC-HEX, all extracts exhibited good inhibitory activity against *Cryptococcus neoformans* with an IC_{50} range between 32.258 – 161.855 µg/mL.

In contrast, only the aqueous extract (AC-AQ) was active against methicillin-resistant *staphylococcus aureus*, IC_{50} 199.054 µg/mL. The other extracts were not active against the test organisms at a 200 µg/mL concentration. A careful examination of our results revealed that the polar extracts exhibited better antimicrobial activity.

Table 4: *In vitro* Antimicrobial effects of crude extracts of *A. cordifolia* leaves.

Extracts	CA IC_{50}	AF IC_{50}	CN IC_{50}	MRS IC_{50}	EC IC_{50}	PA IC_{50}	KP IC_{50}	VRE IC_{50}
AC-CHL	>200	>200	>200	>200	>200	>200	>200	>200
AC-AQ	>200	>200	32.258	199.054	>200	>200	>200	>200
AC-EAT	>200	>200	161.855	>200	>200	>200	>200	>200
AC-ETH	>200	>200	38.953	>200	>200	>200	>200	>200
AC-HEX	>200	>200	>200	>200	>200	>200	>200	>200
AC-MET	>200	>200	32.434	>200	>200	>200	>200	>200
FLU*	<0.1	>100	0.519	>100	>100	>100	>100	>100
AMB*	0.133	0.35	0.153	>100	>100	>100	>100	>100
CIPRO*	>10	>10	>10	9.235	<0.01	0.419	>100	>100

*Test concentration of positive control agents was 100 – 4 µg/mL, except Ciprofloxacin (10 – 0.4 µg/mL)
FLU = Fluconazole, AMB = Amphotericin B, CIPRO = Ciprofloxacin CA= *C. albicans*, AF= *A. fumigatus*, CN= *C. neoformans*, MRS= methicillin-resistant *S. aureus*, EC= *E. coli*, PA=*P. aeruginosa*, KP= *K. pneumoniae*, VRE=Vancomycin resistant enterococcus

The results of our study are in agreement with the findings of Bitchagno *et al*. (2015) and Gasting *et al*. (2008). The authors have reported that the polar fractions of *A. cordifolia* (aqueous, methanol, ethanol, ethyl acetate and acetone) exhibited strong and significant activity against different pathogenic microorganisms, with zones of inhibition ranging from 13 to 26 mm. The studies further stated that the antimicrobial activity might be due to the polarity of the solvent of extraction. There is a possibility that the active antimicrobial constituents in the plant may be polar since activity against the microorganisms is highest in the aqueous extract.

CONCLUSION

The antiprotozoal and antimicrobial activities of the crude extracts of *Alchornea cordifolia* leaves have been highlighted in this study. It reveals that the plant possessed important secondary metabolites responsible for the observed antiplasmodial, antileishmanial, antitrypanosomal and antimicrobial activities. Thus the leaves of *A. cordifolia* could serve as a veritable source of bioactive compounds that can further be explored as leads for the development of potent drugs to treat malaria and other neglected diseases. To the best of our knowledge, our study is the first comparative study of the effects of different crude extracts on the antimalarial activity of the leaves of *A. cordifolia*.

ACKNOWLEDGEMENTS

We acknowledge the grant [FPM/APU/IBR/67/XXX] to one of the authors by the TEFUND, Nigeria, the National Center for Natural Products Research (NCNPR), University of Mississippi, the United States of America, for using their laboratory for part of this work and finally the Natural Product Research Laboratory under Prof. Abiodun Falodun (PI), University of Benin, Nigeria.

REFERENCES

Adewunmi, C. O., Agbedahunsi, J. M., Adebajo, A. C., Aladesanmi, A. J., Murphy, N., and Wando, J. (2001). Ethno-veterinary medicine: screening of Nigerian medicinal plants for trypanocidal properties. *Journal of Ethnopharmacology*, *77*(1), 19-24.

Amos-Tautua, B. M. W., Angaye, S. S., and Jonathan, G. (2011). Phytochemical screening and antimicrobial activity of the methanol and chloroform extracts of Alchornea cordifolia. *Journal of Emerging Trends in Engineering and Applied Sciences*, *2*(3), 445-447.

Atouguia, J., and Costa, J. (1999). Therapy of human African trypanosomiasis: current situation. *Memórias do Instituto Oswaldo Cruz*, *94*(2), 221-224.

Banzouzi, J. T., Prado, R., Menan, H., Valentin, A., Roumestan, C., Mallie, M., ... and Blache, Y. (2002). In vitro antiplasmodial activity of extracts of Alchornea cordifolia and identification of an active constituent: ellagic acid. *Journal of Ethnopharmacology*, *81*(3), 399-401.

Bitchagno, G. T. M., Fonkeng, L. S., Kopa, T. K., Tala, M. F., Wabo, H. K., Tume, C. B., ... and Kuiate, J. R. (2015). Antibacterial activity of ethanolic extract and compounds from fruits of Tectona grandis (Verbenaceae). *BMC complementary and alternative medicine*, *15*(1), 1-6.

Boniface, P. K., Ferreira, S. B., and Kaiser, C. R. (2016). Recent trends in phytochemistry, ethnobotany and pharmacological significance of Alchornea cordifolia (Schumach. and Thonn.) Muell. Arg. *Journal of Ethnopharmacology*, *191*, 216-244.

CLSI (2008). Reference method for broth dilution, Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard, 3th ed., 28(14), 0–13.

Evans, W.C. (2008). Trease and Evans Pharmacognosy textbook, 15th edition, Elsevier, 333-350.

Gatsing, D., Moudji, S. T., Kuiate, J. R., Nji-Nkah, B. F., Fodouop, S. P., Njateng, G. S., ... and Tchouanguep, F. M. (2008). In vitro antibacterial activity of Alchornea cordifolia bark extract against Salmonella species causing typhoid fevers. *Ethiop Pharm J*, *26*, 83-94.

Igoli, J. O., Ogaji, O. G., Tor-Ayiin, T. A., and Igoli, N. P. (2005). Traditional medicine practice amongst the Igede people of Nigeria. Part II. *African Journal of Traditional, Complementary and Alternative Medicines*, *2*(2), 134-152.

Imieje, V., Zaki, A. A., Fasinu, P. S., Ali, Z., Khan, I. A., Tekwani, B., ... and Falodun, A. (2017). Antiprotozoal and cytotoxicity studies of fractions and compounds from enantia chlorantha. *Tropical Journal of Natural Product Research*, *1*(2), 89 – 94.

Ishola, I. O., Ashorobi, R. B., and Adeoluwa, O. (2012). Evaluation of the antinociceptive activities of the aqueous root extract of Alchornea cordifolia (Schumach and Thonn) Müll. Arg.(Euphorbiaceace). *Int J Appl Res Nat Prod*, *5*, 37-42.

Jain, S., Jacob, M., Walker, L., and Tekwani, B. (2016). Screening North American plant extracts in vitro against Trypanosoma brucei for discovery of new antitrypanosomal drug leads. *BMC complementary and alternative medicine*, *16*(1), 1-6.

Jain, S. K., Sahu, R., Walker, L. A., and Tekwani, B. L. (2012). A parasite rescue and transformation assay for antileishmanial screening against intracellular Leishmania donovani amastigotes in THP1 human acute monocytic leukemia cell line. *Journal of visualized experiments: JoVE*, (70):1 – 14. 10.3791/4054

Kayode, J., and Kayode, G. M. (2008). Ethnomedicinal survey of botanicals used in treating sexually transmitted diseases in Ekiti State, Nigeria. *Ethnobotanical leaflets*, 12: 44 – 55.

Makler, M. T., Ries, J. M., Williams, J. A., Bancroft, J. E., Piper, R. C., Gibbins, B. L., and Hinrichs, D. J. (1993). Parasite lactate dehydrogenase as an assay for Plasmodium falciparum drug sensitivity. *The American journal of tropical medicine and hygiene*, *48*(6), 739-741.

Mavar-Manga, H., Chapon, D., Hoet, S., Block, S., De Pauw-Gillet, M. C., and Quetin-Leclercq, J. (2006). N1, N2, N3-Trisisopentenyl guanidine and N1, N2-diisopentenyl guanidine, two cytotoxic alkaloids from Alchornea cordifolia (Schumach. and Thonn.) Müll. Arg.(Euphorbiaceae) root barks. *Natural product communications*, *1*(12), 1934578X0600101205.

Mavar-Manga, H., Haddad, M., Pieters, L., Baccelli, C., Penge, A., and Quetin-Leclercq, J. (2008). Anti-inflammatory compounds from leaves and root bark of Alchornea cordifolia (Schumach. and Thonn.) Müll. Arg. *Journal of ethnopharmacology*, *115*(1), 25-29.

Mesia, G. K., Tona, G. L., Nanga, T. H., Cimanga, R. K., Apers, S., Cos, P., ... and Vlietinck, A. J. (2008). Antiprotozoal and cytotoxic screening of 45 plant extracts from Democratic Republic of Congo. *Journal of Ethnopharmacology*, *115*(3), 409-415.

Mikus, J., and Steverding, D. (2000). A simple colorimetric method to screen drug cytotoxicity against Leishmania using the dye Alamar Blue®. *Parasitology international*, *48*(3), 265-269.

Muganza, D. M., Fruth, B. I., Lami, J. N., Mesia, G. K., Kambu, O. K., Tona, G. L., ... and Pieters, L. (2012). In vitro antiprotozoal and cytotoxic activity of 33 ethonopharmacologically selected medicinal plants from Democratic Republic of Congo. *Journal of ethnopharmacology*, *141*(1), 301-308.

Nnamdi, A., Ettebong, E., and Davis, K. (2017). Antiplasmodial and antioxidant activities of methanolic leaf extract and fractions of Alchornea cordifolia. *Journal of HerbMed Pharmacology*, *6*(4), 171-177.

Obbo, C. J. D., Kariuki, S. T., Gathirwa, J. W., Olaho-Mukani, W., Cheplogoi, P. K., and Mwangi, E. M. (2019). In vitro antiplasmodial, antitrypanosomal and antileishmanial activities of selected medicinal plants from Ugandan flora: refocusing into multi-component potentials. *Journal of ethnopharmacology*, *229*, 127-136.

Ogungbamila, F. O., and Samuelsson, G. (1990). Smooth muscle relaxing flavonoids from Alchornea cordifolia. *Acta Pharmaceutica Nordica*, *2*(6), 421-422.

Okeke, I. N., Ogundaini, A. O., Ogungbamila, F. O., and Lamikanra, A. (1999). Antimicrobial spectrum of Alchornea cordifolia leaf extract. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *13*(1), 67-69.

Oliveira, L. F., Schubach, A. O., Martins, M. M., Passos, S. L., Oliveira, R. V., Marzochi, M. C., and Andrade, C. A. (2011). Systematic review of the adverse effects of cutaneous leishmaniasis treatment in the New World. *Acta tropica*, *118*(2), 87-96.

Osadebe, P. O., and Okoye, F. B. C. (2003). Anti-inflammatory effects of crude methanolic extract and fractions of Alchornea cordifolia leaves. *Journal of ethnopharmacology*, *89*(1), 19-24.

Osadebe, P. O., Okoye, F. B., Uzor, P. F., Nnamani, N. R., Adiele, I. E., and Obiano, N. C. (2012). Phytochemical analysis, hepatoprotective and antioxidant activity of Alchornea cordifolia methanol leaf extract on carbon tetrachloride-induced hepatic damage in rats. *Asian Pacific journal of tropical medicine*, *5*(4), 289-293.

Oseghale, I. O., Imieje, V. O., Erharuyi, O., and Falodun, A. (2020). Determination of physico-chemical parameters, acute toxicity and heavy metals profile of Eleusine coracana seeds. *Scientia Africana*, *19*(2), 29-40.

Samoylenko, V., Jacob, M. R., Khan, S. I., Zhao, J., Tekwani, B. L., Midiwo, J. O., ... and Muhammad, I. (2009). Antimicrobial, antiparasitic and cytotoxic spermine alkaloids from Albizia schimperiana. *Natural product communications*, *4*(6), 1934578X0900400611.

Sofowora, A. (1993). Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, 191-289.

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological research*, *50*(6), 537-546.

Tona, L., Kambu, K., Ngimbi, N., Mesia, K., Penge, O., Lusakibanza, M., ... and Vlietinck, A. J. (2000). Antiamoebic and spasmolytic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasa, Congo. *Phytomedicine*, *7*(1), 31-38.

Volpedo, G., Pacheco-Fernandez, T., Holcomb, E. A., Cipriano, N., Cox, B., and Satoskar, A. R. (2021). Mechanisms of Immunopathogenesis in Cutaneous Leishmaniasis And Post Kala-azar Dermal Leishmaniasis (PKDL). *Frontiers in Cellular and Infection Microbiology*, *11*, 512.

WHO. (2021). Antimicrobial resistance. Accessed 14th December, 2021.

WHO. (2020).World Malaria Report, https://www.who.int/publications/i/item/9789240015791.