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Antitrypanosomal screening and cytotoxic effects of selected medicinal plants

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Abstract. *Trypanosoma evansi*, the causative agent of "surra", infects many species of wild and domestic animals worldwide. In the current study, the aqueous and ethanolic extracts of six medicinal plants, namely, *Aquilaria malaccensis*, *Derris elliptica*, *Garcinia hombroniana*, *Goniothalamus umbrosus*, *Nigella sativa*, and *Strobilanthes crispus* were screened *in vitro* for activity against *T. evansi*. The cytotoxic activity of the extracts was evaluated on green monkey kidney (Vero) cells using MTT-cell proliferation assay. The median inhibitory concentrations (IC_{50}) of the extracts ranged between 2.30 and 800.97 µg/ml and the median cytotoxic concentrations (CC_{50}) ranged between 29.10 µg/ml and 14.53 mg/ml. The aqueous extract of *G. hombroniana* exhibited the highest selectivity index (SI) value of 616.36, followed by *A. malaccensis* aqueous extract (47.38). Phytochemical screening of the *G. hombroniana* aqueous extract revealed the presence of flavonoids, phenols, tannins, and saponins. It is demonstrated here that the aqueous extract of *G. hombroniana* has potential antitrypanosomal activity with a high SI, and may be considered as a potential source for the development of new antitrypanosomal compounds.

INTRODUCTION

Trypanosomes are hemoflagellate parasites of many classes of vertebrate animals including humans, and are transmitted by many species of hematophagous invertebrates (Stephen, 1986). These parasites are able to cause a debilitating disease in man, and in sub-Saharan Africa alone it is estimated that 50,000 – 70,000 individuals are infected with *Trypanosoma brucei* (Brun *et al.*, 2010). *Trypanosoma cruzi*, the causative agent of Chagas disease or American trypanosomiasis, infects approximately 10 million people, mainly in Latin America (World Health Organisation, 2012). *Trypanosoma evansi* is a widespread member of the genus that infects a wide variety of domestic and wild animals causing a wasting disease known as "surra" (Stephen, 1986).

The control of trypanosomiasis depends solely on chemotherapy as there is no vaccine available for immunization against the infection (Brun *et al.*, 2010). Currently, only a number of drugs are available for the treatment of trypanosomiasis, but these are laden with many challenges such as toxicity, ineffectiveness and emergence of resistant trypanosome strains (El Rayah *et al.*, 1999; Zhou *et al.*, 2004; Steverding, 2010). *Trypanosoma evansi* which is prevalent in Asia has produced resistance to some of the present commercial drugs such as diminazene, suramin, and quinapyramine (Ross & Barns, 1996; Witola *et al.*, 2005; Gillingwater *et al.*, 2009). This has spurred the exploration on the potential usage of

traditional remedies and herbal medicines for treatment of this disease (Freiburghaus *et al.*, 1996; Bawm *et al.*, 2010; Dua *et al.*, 2011; Abiodun *et al.*, 2012). Plants are still considered as the most important natural sources of new chemical compounds as a large proportion of new drugs are derivatives of compounds from plant origin (Harvey, 2008). To date, research on alternative ethnobotanical remedies for trypanosomiasis has been restricted to botanicals found in Africa (Atindehou *et al.*, 2004; Wurochekke & Nok, 2004; Bizimana *et al.*, 2006; Abiodun *et al.*, 2012) and South America (Muelas-Serrano *et al.*, 2000) in which trypanosomiasis is prevalent. Malaysia is a country with a vast floral diversity and ethnobotanical heritage, of which many traditional plants have been proven to contain active compounds effective in treating various diseases (Lin, 2005).

The current study investigates the *in vitro* antitrypanosomal potential and cytotoxic effects of 12 extracts derived from six medicinal plants. It is envisaged that this preliminary screening will pave the way for more in depth investigations on the activity of specific fractions and their trypanocidal mode of action.

MATERIALS AND METHODS

Preparation of plant extracts

Leaves of *Aquilaria malaccensis*, *Derris elliptica*, *Garcinia hombroniana*, *Goniothalamus umbrosus*, and *Strobilanthes crispus* were collected freshly from Agricultural Conservatory Park, Institute of Bioscience (IBS), Universiti Putra Malaysia and seeds of *Nigella sativa* were purchased from a local market in Selangor. Voucher specimens were deposited at the Biodiversity Unit, IBS.

Plants were cleaned, washed, oven dried (45°C) and pulverized using a laboratory grinder. A measured amount of each plant powder was soaked in absolute ethanol and sterile Type-I+ purified water in a tightly closed container. The volume of the solvent was 10 times the amount of the plant powder (wt/vol). The container was placed in an orbital incubator shaker for 3-4 h and the solution was filtered using a filter paper (Whatman no. 4). A rotary evaporator was used to separate the ethanol in the ethanolic extracts and the aqueous extracts were dried using a freeze dryer. The extracts were stored in tightly sealed containers at -28°C until subsequent analyses.

Trypanosome stock

Trypanosoma evansi strain Te7 (isolated from cattle in Selangor, Malaysia) was used for the experiment. Trypanosomes were cultured in 25cm^2 ventilated tissue culture flasks with HMI-9 medium, supplemented with 10% fetal bovine serum (FBS), 60 µg/ml streptomycin sulfate and 50 U/ml penicillin-G potassium. Culture flasks were incubated at 37°C, 5% CO_2 and 85% relative humidity. Trypanosomes were cultured and maintained for a period of two months before commencement of the assay in order to ensure their consistent growth rate.

Antitrypanosomal screening of plant extracts

Antitrypanosomal screening of plant extracts was performed *in vitro* using 24-well microtiter plates. Aqueous plant extracts were dissolved in HMI-9 medium while ethanolic extracts were dissolved first in dimethyl sulfoxide (DMSO) and diluted using sterile Type-I+ purified water. The volume of DMSO used for the assays did not exceed 1%. Ethanol extracts were diluted with equal volume of doubled concentration HMI-9 (2×HMI-9) medium in order to make a balanced concentration of the culture medium in every well.

The extracts were added into each well in a three-fold serial dilution pattern, covering a concentration range from 2 mg/ml to 2.27 µg/ml for the aqueous extracts, and 250 to 1 µg/ml for the ethanolic extracts. An equal volume of HMI-9 medium, containing 1×10^5 trypanosomes was added into each well making a final volume of 800 µl. Negative control wells containing trypanosome cultures were included and diminazene aceturate (Berenil®) was used as positive control. Each test was performed in triplicate. The plates were incubated at 37°C, 5% CO_2

and 85% relative humidity for a period of 72 h. The trypanosome concentration in each well was measured using a Neubauer counting chamber after 2, 4, 8, 24, 48 and 72 h of incubation. Trypanosomal growth curves were constructed and the median inhibitory concentration (IC_{50}), the concentration that gives half maximal growth of trypanosomes, of each plant extract and diminazene aceturate was calculated using linear regression analysis (Microsoft excel 2007 program).

Cytotoxicity assay of plant extracts

Cytotoxicity of the plant extracts was tested on green monkey kidney epithelial (Vero) cells using a MTT-cell proliferation assay kit (Cayman Chemical Company, USA). One hundred microliters of RPMI-1640 medium (Gibco), supplemented with 10% FBS and containing 2×10^5 cell/ml, was added into each well of the 96-well microtiter plate. The plate was incubated for 24 h at 37°C, 5% CO_2 and 85% relative humidity. Following incubation, the medium was carefully removed in order to prevent disruption of the monolayer of cells and new medium containing experimental concentrations of the extracts was added into each well in a three-fold serial dilution manner, covering a concentration range from 15 mg/ml to 1 µg/ml for the aqueous extracts and 500 to 1 µg/ml for the ethanolic extracts. The assay was then conducted according to the manufacturer's instructions and the intensity of colour change from yellow to blue was read using a microplate reader at a wave length of 590 nm. Negative control wells were also included and diminazene aceturate was used as a positive control. Each extract was tested in triplicate and the median cytotoxic concentration (CC_{50}), the concentration giving half maximal growth of cells, was calculated using linear regression analysis (Microsoft excel 2007 software).

Phytochemical screening

Garcinia hombroniana leaves aqueous extract was screened for the presence of alkaloids, flavonoids, phenols, saponins, tannins, and terpenoids according to protocols established by Obadoni & Ochuko (2002), Edeoga *et al.* (2005) and Mariita *et al.* (2011). The aqueous extract of this plant was chosen for phytochemical screening because this extract scored the highest SI value.

RESULTS

Plant extract yields

Plants used in the current study, their families, part of plant used and percentage of extract yield are presented in Table 1. The yield percentage of the plant extracts ranged from 0.92%, for *G. umbrosus* ethanolic extract, to 20.40%, for *N. sativa* ethanolic extract.

Antitrypanosomal screening of plant extracts

The antitrypanosomal screening of the plant extracts revealed that one of the tested extracts scored IC_{50} below 10 µg/ml, which was the ethanolic extract of *G. umbrosus* (IC_{50} of 2.30 µg/ml), five plant extracts scored IC_{50} values from 10 to 50 µg/ml, and six extracts scored IC_{50} values higher than 50 µg/ml (Table 2). The trypanosomal growth curve of *T. evansi*, cultured in HMI-9 medium with different concentrations of *G. hombroniana* aqueous extract, is shown in Figure 1. The inhibitory effect of *G. hombroniana* was concentration-dependent; higher concentrations of the extract led to reduced growth of *T. evansi*. A concentration of 222.22 µg/ml of the extract resulted in complete inhibition of *T. evansi* growth, while the IC_{50} was 23.58 µg/ml. The IC_{50} of diminazene aceturate was 15.30 ng/ml (Table 2).

Cytotoxicity assay and selectivity index of plant extracts

The CC_{50} values of the plant extracts and diminazene aceturate is shown in Table 2. The ethanolic extract of *G. umbrosus* scored the highest toxicity as the CC_{50} value of the extract was 29.10 µg/ml. *Garcinia hombroniana* aqueous extract was the safest among the plants as the CC_{50} of the extract was 14533.87 µg/ml. The CC_{50} of diminazene aceturate was 31.80 µg/ml.

Table 1. Plants used in the current study, family names, voucher numbers, parts used and percentage of extract yield from each plant

Plant	Family	Voucher number	Plant part	Extract type	Yield (% wt/wt)*
Aquilaria malaccensis	Thymelaeaceae	Acp 0137	Leaves	Aqueous Ethanolic	1.42 4.95
Derris elliptica	Fabaceae	Acp 0045	Leaves	Aqueous Ethanolic	1.88 3.09
Garcinia hombroniana	Clusiaceae	Acp 0188	Leaves	Aqueous Ethanolic	4.54 4.00
Goniothalamus umbrosus	Annonaceae	Acp 0098	Leaves	Aqueous Ethanolic	4.15 0.92
Nigella sativa	Ranunculaceae	SK 1722/10	Seeds	Aqueous Ethanolic	6.40 20.40
Strobilanthes crispus	Acanthaceae	Acp 0130	Leaves	Aqueous Ethanolic	4.67 1.06

* Yield percentage is calculated as the percentage of extract yielded in grams over the amount of dried plant powder used for extraction

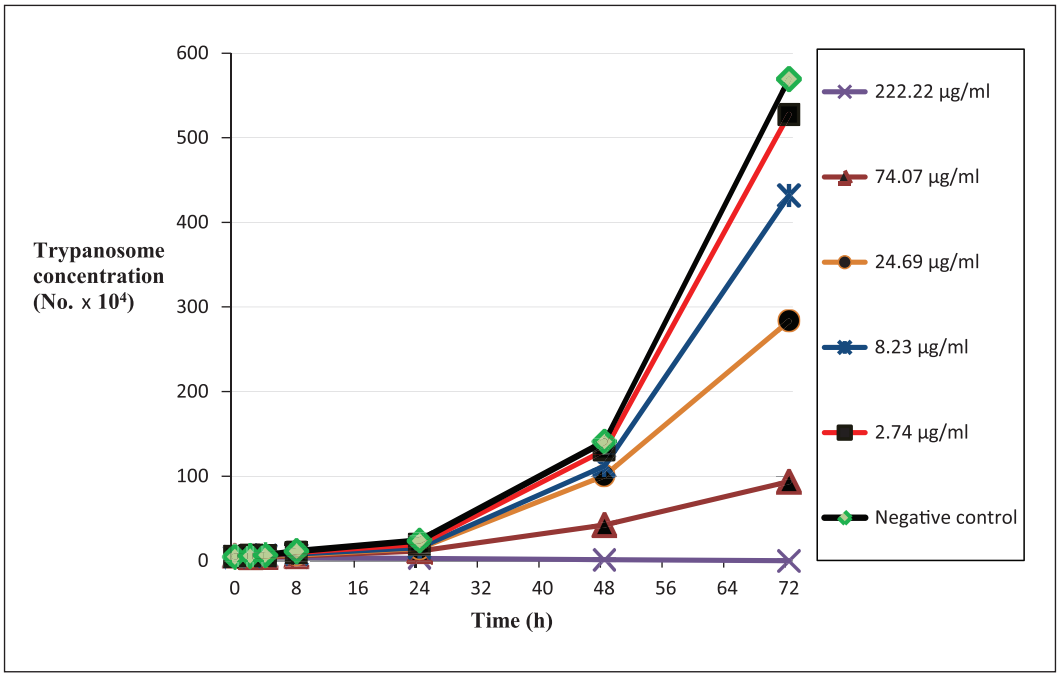

Figure 1. Growth curve of *T. evansi* cultured for 72 h in HMI-9 medium with different concentrations of *G. hombroniana* aqueous extract. The extract was added in three-fold serial dilutions, covering a concentration range from 2.27 µg/ml to 2000 µg/ml. The starting concentration of *T. evansi* was 5×10^4 cells/ml. Negative control contains *T. evansi* cultured on HMI-9 medium

Table 2. Antitrypanosomal, cytotoxicity and selectivity index values of plant extracts

Plant or drug	Extract type	IC_{50} on *T. evansi* (µg/ml)	CC_{50} on Vero cells (µg/ml)	SI*
Aquilaria malaccensis	Aqueous	36.29 ± 1.32	1719.42 ± 203.22	47.38
	Ethanolic	128.63 ± 6.70	259.78 ± 52.60	2.02
Derris elliptica	Aqueous	138.66 ± 5.92	2206.10 ± 49.60	15.91
	Ethanolic	17.79 ± 0.76	331.90 ± 62.20	18.66
Garcinia hombroniana	Aqueous	23.58 ± 2.39	14533.87 ± 296.86	616.36
	Ethanolic	10.17 ± 0.78	103.44 ± 4.48	10.17
Goniothalamus umbrosus	Aqueous	25.49 ± 4.33	81.04 ± 2.43	3.18
	Ethanolic	2.30 ± 0.90	29.10 ± 7.36	12.65
Nigella sativa	Aqueous	581.43 ± 30.36	382.02 ± 69.30	0.66
	Ethanolic	291.72 ± 41.60	381.59 ± 53.44	1.31
Strobilanthes crispus	Aqueous	800.97 ± 278.33	6451.96 ± 363.77	8.06
	Ethanolic	52.54 ± 1.05	355.21 ± 8.77	6.76
Diminazene aceturate		0.0153 ± 0.0029	31.80 ± 8.13	2078.43

* Selectivity index (SI) is calculated as the CC_{50} of the drug or extract on Vero cells divided by its IC_{50} on *T. evansi*.
Values are presented as mean of three tests ± SD

The aqueous extract of *G. hombroniana* exhibited a highly selective antitrypanosomal activity, as the SI of this extract was 616.36. The plant with the second highest SI was *A. malaccensis* with a SI value of the plant's aqueous extract of 47.38, while the SI of the standard antitrypanosomal drug diminazene aceturate was 2078.4 (Table 2).

Phytochemical screening of *G. hombroniana* aqueous extract

The phytochemical testing of constituents in the aqueous extract of *G. hombroniana* revealed the presence of flavonoids, phenols, saponins, and tannins, while alkaloids and terpenoids were not present (Table 3).

Table 3. Phytochemical screening of the aqueous extract of *G. hombroniana*

Chemical group	Availability
Alkaloid	–
Flavonoid	++
Phenol	++
Saponin	+
Tannin	++
Terpenoid	–

– is no result
1+ is a weak reaction
2+ is a mild reaction
3+ is a strong reaction

DISCUSSION

Trypanosomiasis is considered one of the parasitic diseases that have been neglected for development of new drugs since it is a disease affecting people living in low income regions of the world (Renslo & McKerrow, 2006).

The traditional methods of drug discovery seem to be more effective in the development of new antitrypanosomal compounds as the rational methods of drug design based on a biological target have only led to the discovery of eflornithine in the 1970's as a treatment of *Trypanosoma brucei gambiense* (Steverding, 2010).

Plants and other natural products remain as important sources for the discovery of new

chemical compounds (Harvey, 2008). Plant extracts have been extensively screened for their antitrypanosomal activity in the recent years and some have shown promising antitrypanosomal potential (Talakal *et al.*, 1995; Bawm *et al.*, 2010; Habila *et al.*, 2011; Gressler *et al.*, 2012). Moreover, the continuous reports about emergence of resistant strains of *T. evansi* to the standard antitrypanosomal drug diminazene aceturate (El Rayah *et al.*, 1999; Zhou *et al.*, 2004) encourage the development of new antitrypanosomal compounds for the treatment of surra and other related trypanosomal diseases.

In the current study, the antitrypanosomal and cytotoxic properties of 12 extracts prepared from six plants were investigated. Selectivity index was used as a criterion for each extract as it gives a good indication about a drug or drug candidate's safety, i.e., the higher the SI, the safer is the extract to mammalian cells. The results have shown that the aqueous extract of *G. hombroniana*, which is commonly known as "seashore mangosteen", exhibited a considerably potent antitrypanosomal activity against *T. evansi* with the highest SI value (616.36). Although the SI value scored by diminazene aceturate (2078.43) was higher than that scored by the *G. hombroniana* extract, it is worthy to mention that the plant extract was a crude aqueous fraction. The SI value scored by the *G. hombroniana* aqueous extract is comparably higher than that obtained for other plant extracts screened for antitrypanosomal activity. Bawm *et al.* (2010) tested 71 plant extracts from 60 plant species for antitrypanosomal activity against *T. evansi* and cytotoxic activity against MRC-5 cells. Their results showed that the highest SI value was scored by the methanol extract of *Vitis repens* which was 24.4. Abiodun *et al.* (2012) screened the antitrypanosomal activity of ten plants against *Trypanosoma brucei rhodesiense* STIB 900 and the cytotoxicity against mammalian L6 cells. The highest SI (76.89) was scored by the methanol extract of *Ocimum gratissimum* leaves.

Phytochemical screening of *G. hombroniana* revealed the presence of flavonoids. Several flavonoid compounds were reported to exert antitrypanosomal activities (Camacho *et al.*, 2000; Tasdemir *et al.*, 2006). Plants belonging to the genus *Garcinia* have been known to contain high proportions of xanthone compounds (Sultanbawa, 1980; Elfita *et al.*, 2009), which exert antioxidant (Saputri & Jantan, 2012) and anticancer (Aisha *et al.*, 2012; Chitchumroonchokchai *et al.*, 2012) effects. Compounds showing anticancer activities are a good candidate to be tested for antitrypanosomal activity (Barrett *et al.*, 2004) as the only antitrypanosomal drug developed over the past 40 years was eflornithine, which was basically discovered as an anticancer agent. This drug was eventually used for the treatment of the chronic form of sleeping sickness caused by *T. brucei gambiense* (Steverding, 2010). Hence, isolation of xanthone compounds from different parts of *G. hombroniana* plant and screening of their antitrypanosomal and cytotoxic activities could probably result in the discovery of potent antitrypanosomal compounds with considerably high SI.

It can be concluded from the current study that aqueous extracts of *G. hombroniana* leaves possess anti-trypanosomal activity with a considerably high SI. This plant may present a potential source for the development of new antitrypanosomal compounds, especially at the current time when the development of new antitrypanosomal compounds which are safe, cheap and easy to administer is an urgent requirement.

REFERENCES

Abiodun, O.O., Gbotosho, G.O., Ajaiyeoba, E.O., Brun, R. & Oduola, A.M. (2012). Antitrypanosomal activity of some medicinal plants from Nigerian ethnomedicine. *Parasitology Research* **110**: 521-526.

Aisha, A.F., Abu-Salah, K.M., Ismail, Z. & Majid, A.M. (2012). *In vitro* and *in vivo* anti-colon cancer effects of *Garcinia mangostana* xanthones extract. *BMC Complementary and Alternative Medicine* **12**: 104.

Atindehou, K.K., Schmid, C., Brun, R., Koné, M.W. & Traore, D. (2004). Anti-trypanosomal and antiplasmodial activity of medicinal plants from Côte d'Ivoire. *Journal of Ethnopharmacology* **90**: 221-227.

Barrett, M.P., Coombs, G.H. & Mottram, J.C. (2004). Future Prospects in Chemotherapy for Trypanosomiasis. In: *The Trypanosomiases*, Maudling, I., Holmes, P.H. & Miles, M.A. Oxfordshire, UK: CABI Publishing, Pp. 445-460.

Bawm, S., Tiwananthagorn, S., Lin, K.S., Hirota, J., Irie, T., Htun, L.L., Maw, N.N., Myaing, T.T., Phay, N., Miyazaki, S., Sakurai, T., Oku, Y., Matsuura, H. & Katakura, K. (2010). Evaluation of Myanmar medicinal plant extracts for antitrypanosomal and cytotoxic activities. *The Journal of Veterinary Medical Science* **72**: 525-528.

Bizimana, N., Tietjen, U., Zessin, K.H., Diallo, D., Djibril, C., Melzig, M.F. & Clausen, P.H. (2006). Evaluation of medicinal plants from Mali for their *in vitro* and *in vivo* trypanocidal activity. *Journal of Ethnopharmacology* **103**: 350-356.

Brun, R., Blum, J., Chappuis, F. & Burri, C. (2010). Human African trypanosomiasis. *Lancet* **375**: 148-159.

Camacho, M.R., Mata, R., Castaneda, P., Kirby, G.C., Warhurst, D.C., Croft, S.L. & Phillipson, J.D. (2000). Bioactive compounds from *Celaenodendron mexicanum*. *Planta Medica* **66**: 463-468.

Chitchumroonchokchai, C., Thomas-Ahner, J.M., Li, J., Riedl, K.M., Nontakham, J., Suksumrarn, S., Clinton, S.K., Kinghorn, A.D. & Failla, M.L. (2012). Anti-tumorigenicity of dietary á-mangostin in an HT-29 colon cell xenograft model and the tissue distribution of xanthones and their phase II metabolites. *Molecular Nutrition and Food Research* **57**: 203-211.

Dua, V.K., Verma, G., Agarwal, D.D., Kaiser, M. & Brun, R. (2011). Antiprotozoal activities of traditional medicinal plants from the Garhwal region of North West Himalaya, India. *Journal of Ethnopharmacology* **136**: 123-128.

Edeoga, H.O., Okwu, D.E. & Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* **4**: 685-688.

El Rayah, I.E., Kaminsky, R., Schmid, C. & El Malik, K.H. (1999). Drug resistance in Sudanese *Trypanosoma evansi*. *Veterinary Parasitology* **80**: 281-287.

Elfita, E., Muharni, M., Latief, M., Darwati, D., Widiyantoro, A., Supriyatna, S., Bahti, H.H., Dachriyanus, D., Cos, P. & Maes, L. (2009). Antiplasmodial and other constituents from four Indonesian *Garcinia* spp. *Phytochemistry* **70**: 907-912.

Freiburghaus, F., Ogwal, E.N., Nkunya, M.H., Kaminsky, R. & Brun, R. (1996). *In vitro* antitrypanosomal activity of African plants used in traditional medicine in Uganda to treat sleeping sickness. *Tropical Medicine and International Health* **1**: 765-771.

Gillingwater, K., Kumar, A., Anbazhagan, M., Boykin, D.W., Tidwell, R.R. & Brun, R. (2009). *In vivo* investigations of selected diamidine compounds against *Trypanosoma evansi* using a mouse model. *Antimicrobial Agents and Chemotherapy* **53**: 5074-5079.

Gressler, L.T., Da Silva, A.S., Machado, G., Rosa, L.D., Dorneles, F., Gressler, L.T., Oliveira, M.S., Zanette, R.A., de Vargas, A.C.P. & Monteiro, S.G. (2012). Susceptibility of *Trypanosoma evansi* to propolis extract *in vitro* and in experimentally infected rats. *Research in Veterinary Science* **93**: 1314-1317.

Habila, N., Humphrey, N.C. & Abel, A.S. (2011). Trypanocidal potentials of *Azadirachta indica* seeds against *Trypanosoma evansi*. *Veterinary Parasitology* **180**: 173-178.

Harvey, A.L. (2008). Natural products in drug discovery. *Drug Discovery Today* **13**: 894-901.

Lin, K.W. (2005). Ethnobotanical study of medicinal plants used by the Jah Hut peoples in Malaysia. *Indian Journal of Medical Sciences* **59**: 156-161.

Mariita, R.M., Ogol, C.K.P.O., Oguge, N.O. & Okemo, P.O. (2011). Methanol extract of three medical plants from Samburu in Northern Kenya show significant antimycobacterial, antibacterial and antifungal properties. *Research Journal of Medicinal Plant* **5**: 54-64.

Muelas-Serrano, S., Nogal, J.J., Martínez-Díaz, R.A., Escario, J.A., Martínez-Fernández, A.R. & Gómez-Barrio, A. (2000). *In vitro* screening of American plant extracts on *Trypanosoma cruzi* and *Trichomonas vaginalis*. *Journal of Ethnopharmacology* **71**: 101-107.

Obadoni, B.O. & Ochuko, P.O. (2002). Phytochemical studies and comparative efficacy of the crude extract of some haemostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences* **8**: 203-208.

Renslo, A.R. & McKerrow, J.H. (2006). Drug discovery and development for neglected parasitic diseases. *Nature Chemical Biology* **2**: 701-710.

Ross, C.A. & Barns, A.M. (1996). Alteration to one of three adenosin transporters is associated with resistance to cymelarsan in *Trypanosoma evansi*. *Parasitology Research* **82**:183-188.

Saputri, F.C. & Jantan, I. (2012). Inhibitory activities of compounds from the twigs of *Garcinia hombroniana* Pierre on human low-density lipoprotein (LDL) oxidation and platelet aggregation. *Phytotherapy Research* **26**: 1845-1850.

Stephen, L.E. (1986). *Trypanosomiasis: A Veterinary Perspective*. Pergamon Press, Oxford, Pp. 184-215.

Steverding, D. (2010). The development of drugs for treatment of sleeping sickness: a historical review. *Parasites and Vectors* **3**: 15.

Sultanbawa, M.U.S. (1980). Xanthonoids of tropical plants. *Tetrahedron* **36**: 1465-1506.

Talakal, T.S., Dwivedi, S.K. & Sharma, S.R. (1995). *In vitro* and *in vivo* antitrypanosomal activity of *Xanthium strumarium* leaves. *Journal of Ethnopharmacology* **49**: 141-145.

Tasdemir, D., Kaiser, M., Brun, R., Yardley, V., Schmidt, T.J., Tosun, F. & Rüedi, P. (2006). Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: *in vitro*, *in vivo*, structure-activity relationship, and quantitative structure-activity relationship studies. *Antimicrobial Agents and Chemotherapy* **50**: 1352-1364.

Witola, W.H., Tsuda, A., Inoue, N., Ohashi, K. & Onuma, M. (2005). Acquired resistance to berenil in a cloned isolate of *Trypanosoma evansi* is associated with upregulation of a novel gene, TeDR40. *Parasitology* **131**: 635-646.

World Health Organisation. (2012). Chagas disease (American trypanosomiasis). Fact sheet N°340.

Wurochekke, A.U. & Nok, A.J. (2004). *In vitro* anti trypanosomal activity of some medicinal plants used in the treatment of trypanosomosis in Northern Nigeria. *African Journal of Biotechnology* **3**: 481-483.

Zhou, J., Shen, J., Liao, D., Zhou, Y. & Lin, J. (2004). Resistance to drug by different isolates *Trypanosoma evansi* in China. *Acta Tropica* **90**: 271-275.