UJMR, Conference Special Issue Vol. 9 No. 3. June, 2024, pp. 560 - 566

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

https://doi.org/10.47430/ujmr.2493.061

Received: 12 March 2024 **Accepted:** 24 June 2024

In vitro Anti-trypanosomal Activity of Ethanolic and n-Hexane Extracts of *Hymenocardia acida* Stem Bark against *Trypanosoma brucei brucei* and *Trypanosoma congolense*

***[1,2]Lawal, S. M., [2]Inabo, H. I., [2]Ella, E. E. and [3]Okubanjo, O. O.**

[1]Department of Microbiology, Faculty of Science, Kaduna State University, Kaduna, Nigeria
[2]Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria
[3]Department of Parasitology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria
*Correspondence author: lawalshitu2550@gail.com,

Abstract

The development of resistance by trypanosomes to existing trypanocidal drugs necessitates the need to search for safer and more effective alternative drugs with a broad spectrum of activity. Hence, this study was carried out to evaluate the in vitro anti-trypanosomal activity of ethanolic and n-hexane extracts of Hymenocardia acida stem bark against Trypanosoma congolense and Trypanosoma brucei brucei. The stem bark of H. acida was collected, identified, dried and then ground into fine powder. The powdered H. acida stem bark was extracted successively using n-hexane and ethanol to obtain n-hexane and ethanolic extracts, respectively. Phytochemical screening of the extracts was carried out using standard procedure. In vitro, anti-trypanosomal activity of different concentrations of the stem bark extracts (2.5 mg/mL to 40.0 mg/mL) were determined using motility assay with diminazeneaceturate (100 µg/mL) and phosphate buffered saline used as positive and negative controls, respectively. Results of the phytochemical screening revealed that flavonoids, alkaloids, saponins, and tannins were present in the ethanolic and n-hexane extracts. In vitro, the anti-trypanosomal activity of ethanolic and n-hexane extracts was observed against T. brucei brucei and T. congolense. Cessation of trypanosome motility was observed after 30 and 50 minutes of exposure to 2.5 mg/mL of ethanolic extract and n-hexane extract, respectively, for both T. brucei brucei and T. congolense. In conclusion, ethanolic and n-hexane extracts of H. acida exhibited anti-trypanosomal activity against Trypanosoma brucei brucei and Trypanosoma congolense. Hence, the plant could serve as a source of new trypanocidal drugs.

Keywords: Anti-trypanosomal activity, Extracts, H. acida, T. brucei brucei, T. congolense

INTRODUCTION

Trypanosomiasis is a protozoan parasitic disease caused by species of trypanosomes, which are blood and tissue-dwelling unicellular protozoa. Trypanosomes have a complex life cycle that alternates between the insect vector (tsetse fly) and the host (mammals) (Ogbadoyi *et al.*, 2011; Sani *et al.*, 2018). *Trypanosoma brucei brucei* and *Trypanosoma congolense* are protozoa that cause trypanosomiasis in cattle and other domestic animals. It is transmitted by tsetse flies belonging to the family Glossinidae and Genus *Glossina*. In cyclical transmission, the trypanosomes multiply actively in the tsetse flies (Sani *et al.*, 2018).

Trypanosomiasis mostly affects poor populations that live in the remote areas of Africa. Trypanosomiasis is usually fatal especially when not treated. Travelers are at risk of being infected when they travel in tsetse fly prevalent regions. The disease is generally not commonly found in urban areas. However, there have been reports of some cases in suburban areas, especially in trypanosomiasis-endemic countries (Simarro *et al.*, 2011). Occurrence of sleeping sickness is restricted to only 36 countries in sub-Saharan Africa where tsetse flies that can transmit trypanosomiasis are found. Estimates by the WHO Expert Committee in 1995 project that 60 million individuals were at risk, and there are approximately 300,000 new cases annually in Africa, with less than 30,000 diagnosed and treated cases (WHO, 2012; Madaki *et al.*, 2016).

Plants have served as a remedy for illnesses since pre-historical times, and the use of medicinal plants as phytomedicines is increasing globally. Plants are vital in pharmacological

research as well as in drug development. They serve as a reservoir of bioactive compounds; hence, they can directly serve as therapeutic agents; so also, plants serve as starting materials in the synthesis of drugs and as models for pharmacologically bioactive compounds (Deepika *et al.*, 2016).

Plants are considered valuable sources of natural therapeutic agents with promising potential for the treatment of infectious diseases and with fewer side effects compared to synthetic drugs. In the past three decades, several studies have been conducted focusing on the antimicrobial activity of plants. Research on medicinal plants is a re-emerging health aid and is fueled by the increasing cost, adverse drug reactions, serious side effects, and toxicity of synthetic drugs, as well as the prospect of novel drugs derived from plants (Ehiagbonare and Onyibe, 2008).

The fight against trypanosomiasis relies majorly on vector control and chemotherapies. The effectiveness of the chemotherapies and their safety are a source of concern because of the side effects of the drugs and the resistance developed by trypanosomes to the drugs (Yusuf *et al.*, 2012; Madaki *et al.*, 2016). These dilemmas call for continued efforts to develop novel bioactive drugs for the treatment of trypanosomiasis.

Hymenocardia acida has been used in traditional medicine to treat various illnesses in Nigeria and some African countries. It is distributed widely within the savanna region of Nigeria. It is known as "Heartfruit" in English, "*janyaro*" among Hausas, "*yawasatoje*" among Fulanis, "*ikalaga*" among Igbos, "*Orunpa*" among Yorubas and "*Enache*" among Idomas (Haruna *et al.*, 2017; Sabo *et al.*, 2017), "*ii-kwarto*" in Tiv, "*emela*" in Etulo, "*Uchuo*" in Igede (Agishi *et al.*, 2004), "*enanche*" in Idoma (Abu and Uchendu, 2011). Decoction of its stem bark or powders of its roots is used in the treatment of fever, diarrhoea, jaundice, dysentery, muscle pains, and sexual incapacity (Sabo *et al.*, 2017).

In Nigeria, trypanosomiasis is considered a re-emerging disease, causing a major, clinically important disease in small ruminants and spreading to zones designated previously as tsetse-free (Majekodunmi *et al.*, 2013; Nwodo *et al.*, 2015).

The chemotherapy of trypanosomiasis is confronted with problems of unavailability of drugs, resistance to available anti-trypanosomal agents, unacceptable toxicity, and long treatment protocols. The rural poor in Africa are the worst hit by the disease, and without adequate treatment, it is fatal (Ogbadoyi *et al.*, 2011).

Despite major advances in drug development in recent decades, essential medicines used in the treatment of trypanosomiasis and other diseases that affect the world's poor are either too expensive, no longer produced, highly toxic, or ineffective, hence the need to develop new treatments for these diseases (Johnson and Omoniwa, 2014). *Hymenocardia acida* has been reported to contain a variety of phytochemicals and possess antimicrobial activity. Hence, this study aimed to assess the anti-trypanosomal activity of *H. acida* extracts.

MATERIALS AND METHODS

Collection of *Hymenocardia acida* Stem Bark Samples

Stem bark samples of *Hymenocardia acida* were collected within the environs of Zaria, Kaduna State, Nigeria, and identified at the herbarium, Department of Botany, Ahmadu Bello University, Zaria. The leaves and stems were used for identification, while the stem bark was used in the extraction and bioassay for *in vitro* anti-trypanosomal activity.

Extraction of *Hymenocardia acida* stem bark

The stem bark of *Hymenocardia acida* was cleaned, washed, air dried at ambient room temperature (28 °C – 30°C) for seven days, pulverized, ground to fine powder, and stored at room temperature in air-tight containers until required. The powdered stem bark of *Hymenocardia acida* was macerated with n-hexane and ethanol to obtain n-hexane and ethanolic extracts, respectively. One hundred grams (100 g) of the powdered material was then macerated with 1000 mL of n-hexane for 72 hours, filtered, and the filtrate was evaporated using a rotary evaporator to dryness in a water bath set 50 °C to obtain n-hexane extract. The residue was macerated with 1000 mL of ethanol for 72 hours, filtered, and the filtrate was evaporated using a rotary to dryness in a water bath set at 50 °C to obtain ethanolic extract (Simorangkir *et al.*, 2019).

Qualitative phytochemical screening of *Hymenocardia acida* stem bark extracts.

The phytochemical constituents of the extracts were determined as described by Yadav and Agarwala (2011), Wadood *et al*. (2013), and Chechet *et al*. (2018). The extracts were screened for the presence of flavonoids, alkaloids, terpenoids, tannins, steroids, saponins, and anthraquinones.

Assessment of *in vitro* anti-trypanosomal activity of the extracts

Five different concentrations (2.5, 5.0, 10.0, 20.0and 40.0 mg/mL) of the ethanolic and n-hexane stem bark extracts were prepared using phosphate-buffered saline (PBS) as diluent. Ten microliters (10µL) of each concentration was mixed with 60 µL of trypanosome-infected blood in wells of microtitre plates, and then the mixture was incubated for 10 minutes at 37°C. Ten microliters (10 µL) of PBS mixed with 60 µL of trypanosome-infected blood served as negative, while for positive controls, 10 µLof diminazene aceturate (100 µg/mL) was used. The trypanosomes were then observed microscopically (× 400magnification) for drop-in motility or cessation of motility at 5-minute intervals for 60 minutes (Abu *et al*., 2009).

RESULTS

Results of the phytochemical screening reveal the presence of flavonoids, alkaloids, terpenoids, saponins, tannins, steroids, anthraquinones, carbohydrates, and cardiac glycoside in the ethanolic extract of *H. acida* stem bark. However, terpenoids, steroids, anthraquinones, and cardiac glycoside were not detected in the n-Hexane extract of *H. acida* stem bark (Table 1).

In vitro* Anti-trypanosomal Activity of *Hymenocardia acida* Extracts against *Trypanosoma brucei brucei

Table 2 shows the *in vitro* anti-trypanosomal activity of *Hymenocardia acida* ethanolic and n-hexane extracts against *Trypanosoma brucei brucei*. The ethanolic extract exhibited higher activity against *T. brucei brucei,* with all the parasites dead after 30 minutes of exposure. Parasite exposure to n-hexane extract was still active after 30 minutes of exposure. Diminazeneaceturate at 100 µg/mL resulted in cessation of motility within 10 minutes of exposure. Trypanosomes exposed to phosphate-buffered saline were active even after 60 minutes of exposure.

In vitro* Anti-trypanosomal Activity of *Hymenocardia acida* Extracts against *Trypanosoma congolense

In vitro anti-trypanosomal activity of *Hymenocardia acida* extracts against *Trypanosoma congolense* is shown in Table 3. At 2.5 mg/mL, ethanolic extract and n-hexane killed all the trypanosomes after 30 and 50 minutes of exposure, respectively. Exposure of the trypanosomes to Diminazeneaceturate at 100 µg/mL resulted in cessation of motility within 10 minutes of exposure. Trypanosomes exposure to phosphate-buffered saline did not result in motility cessation even after 60 minutes of exposure.

Table 1: Phytochemical Constituents of Ethanolic and n-Hexane Extracts of *Hymenocardia acida* stem bark

Phytochemical Constituents	Ethanolic Extract	n-Hexane Extract
Flavonoids	+	+
Alkaloids	+	+
Terpenoids	+	-
Tannins	+	+
Steroids	+	-
Saponins	+	+
Anthraquinones	+	-
Carbohydrate	+	+
Cardiac glycoside	+	-

Key: + = present
- = absent

Table 2: *In vitro* anti-trypanosomal activity of *Hymenocardia acida* extracts against *Trypanosoma brucei brucei*

Extracts	Concentrations (mg/mL)	Time of Exposure (Minutes) / Motility											
		5	10	15	20	25	30	35	40	45	50	55	60
-ve control (PBS)		5+	5+	5+	5+	5+	5+	4+	4+	4+	4+	4+	3+
+ve control DA(100µg/mL)		2+	*										
Ethanolic	2.5	5+	4+	4+	3+	2+	*	-	-	-	-	-	-
	5.0	5+	4+	4+	3+	2+	*	-	-	-	-	-	-
	10.0	4+	3+	2+	3+	*	-	-	-	-	-	-	-
	20.0	4+	3+	2+	1+	*	-	-	-	-	-	-	-
	40.0	4+	2+	1+	1+	-	-	-	-	-	-	-	-
n-Hexane	2.5	5+	5+	4+	4+	4+	4+	4+	4+	4+	3+	2+	2+
	5.0	5+	5+	4+	4+	4+	4+	4+	4+	3+	3+	2+	2+
	10.0	5+	5+	4+	4+	4+	4+	4+	4+	3+	3+	2+	2+
	20.0	5+	5+	4+	4+	4+	4+	4+	4+	3+	3+	2+	2+
	40.0	5+	5+	4+	4+	4+	4+	4+	4+	3+	3+	2+	2+

Key: 5+ = Extremely active; 4+ = very active; 3+ = slowly active; 2+ = sluggish; + = slow ;* = motility ceases;-ve control = negative control; +ve control = positive control; PBS = phosphate buffered saline; DA = Diminazene aceturate

Table 3: *In vitro* anti-trypanosomal activity of *Hymenocardia acida* extracts against *Trypanosoma congolense*

Extracts	Concentrations (mg/mL)	Time of Exposure (Minutes) / Motility											
		5	10	15	20	25	30	35	40	45	50	55	60
-ve control (PBS)		5+	5+	5+	5+	5+	5+	4+	4+	4+	4+	4+	3+
+ve control DA(100µg/mL)		2+	*										
Ethanolic	2.5	3+	2+	2+	2+	1+	*	-	-	-	-	-	-
	5.0	3+	2+	2+	2+	1+	*	-	-	-	-	-	-
	10.0	3+	2+	2+	1+	*	-	-	-	-	-	-	-
	20.0	2+	2+	2+	1+	*	-	-	-	-	-	-	-
	40.0	2+	2+	1+	*	-	-	-	-	-	-	-	-
n-Hexane	2.5	5+	5+	4+	4+	3+	3+	2+	1+	1+	*	-	-
	5.0	5+	5+	4+	3+	3+	3+	2+	1+	1+	*	-	-
	10.0	4+	4+	3+	3+	3+	3+	1+	1+	*	-	-	-
	20.0	3+	3+	3+	2+	2+	2+	1+	1+	*	-	-	-
	40.0	2+	2+	2+	2+	1+	1+	*	-	-	-	-	-

Key: 5+ = Extremely active; 4+ = very active; 3+ = slowly active; 2+ = sluggish; + = slow ; * = motility ceases; -ve control = negative control; +ve control = positive control; PBS = phosphate buffered saline; DA = Diminazeneaceturate

DISCUSSION

Phytochemical compounds detected in the ethanolic extract of *H. acida* stem bark were saponins, tannins, flavonoids, terpenoids, steroids, anthraquinones, carbohydrates, cardiac glycoside, and alkaloids. Terpenoids, steroids, anthraquinones, and cardiac glycoside were absent in the n-hexane extract. The variation observed in the phytochemical constituents of the extracts might be linked to differences in the polarity of the solvents used in the extraction. These phytochemicals have been reported to exhibit trypanocidal or trypanosomatic activity either as individual or in synergy (Mergia *et al.*, 2014), and they act on a single site or multiple sites that are associated with the physiological process trypanosomes (Maikai, 2011). The anti-trypanosomal activity of flavonoids might be linked to their ability to serve as a scavenger of free radicals and metal chelators (Mergia *et al.*,2014). Terpenoids exert their anti-trypanosomal activity by reacting with sulfur-containing cellular components, leading to the production of aldehyde-thiol adducts and subsequently decreasing the buffering agents, leading to the creation of oxidative stress in the trypanosome (Nibret and Wink, 2010). Saponins

exert their anti-trypanosomal activity through stimulation of cell death (Johnson and Omoniwa, 2014).

Similar to this observation, the presence or absence of anthraquinones and cardiac glycosides in stem bark extract of *Hymenocardia acida* have been observed to vary based on the solvent of extraction. Usman *et al.*(2021) reported that anthraquinones and cardiac glycosides were absent in the methanol extract of *H. acida* stem bark. However, the presence of glycosides and the absence of anthraquinones in the hydroethanolic stem bark of *Hymenocardia acida* was reported by Abu *et al.* (2011). Anthraquinones and cardiac glycosides were found in the stem bark of *Hymenocardia acida* by Iyadi *et al.* (2003).

In vitro anti-trypanosomal activity of the extracts observed against *T. brucei brucei* and *T. congolense* in this study implies that the extracts contain compounds with trypanocidal activity. This was demonstrated by complete cessation or reduction in motility of trypanosome when exposed to different concentrations of the extracts. The motility of trypanosomes has been reported to constitute a reliable indicator of trypanosome viability, where complete cessation or reduction in trypanosome motility compared to the control serves as an index of trypanocidal activity (Abdeta *et al.*, 2020). Inhibition or killing of the trypanosomes when exposed to extracts is indicated by loss of motility. This suggests that the extract interferes with essential trypanosome functions or processes.

In vitro anti-trypanosomal activity of ethanolic extract of *H. acida* stem bark was observed against *T. brucei brucei* and *T. congolense*. Similar to this finding, *in vitro*antitrypanosomal activity of ethanolic extract of *H. acida* stem bark against *T. brucei brucei* at 40.0 mg/mL – 2.5 mg/mL concentrations was also reported by Abu *et al.* (2009).

Higher *in vitro* anti-trypanosomal activity was exhibited by ethanolic extract against *T. brucei brucei* and *T. congolense* compared to n-hexane extract. The difference observed in the anti-trypanosomal activity of the extracts might be due to differences in the phytochemical constituents of the extracts as well as the quantity of the phytochemicals.

CONCLUSION

Ethanolic and n-hexane extracts of *H. acida* stem bark exhibited *invitro* anti-trypanosomal activity against *Trypanosoma brucei brucei* and *Trypanosoma congolense*. These extracts can be exploited for novel anti-trypanosomal drugs.

REFERENCES

Abdeta, D., Kebede, N., Giday, M., Terefe, G. & Abay, S.M. (2020). *In vitro* and *in vivo* anti-trypanosomal activities of methanol extract of *Echinopskebericho* roots. *Evidence-Based Complementary and Alternative Medicine*, *2020*, 8146756. **[Crossref]**

Abu, A.H. & Uchendu, C.N. (2011).Effect of aqueous extract of *Hymenocardia acida* stem barkon oestrous cycle in albino rats.*Journal of Medical Plants Research*, *5*(8),1280-1283.

Abu, A.H., Uchendu, C.N. & Ofokwu, R. (2011). Sperm immobilisation properties of aqueous ethanolic extract of *Hymenocardia acida* stem bark. *Macedonian Journal of Medicinal Sciences*, *4*, 261-264. **[Crossref]**

Abu, A.H., Uchendu, C.N. and Ofukwu, R.A. (2009).*In vitro* anti-trypanosomal activity of crude extracts of some Nigerian medicinal plants. *Journal of Applied Biosciences,21*, 1277 - 1282

Agishi, E.G. (2004).Etulo, Igede, Idoma, Tiv and Hausa names of plants.Agitab Publishers Ltd, Makurdi. Pp 5, 32, and 42.

Chechet, G. D., Yahaya, J. &Nok, A. J. (2018).In *vitro* and *in vivo*anti-trypanosomal potentials of *Afrormosialaxiflora* and *Khaya senegalensis* against *Trypanosoma brucei brucei*. *Nigerian Veterinary Journal*, *39*(3), 269-284. **[Crossref]**

Deepika, K., Mahesh Kumar, M.V.S., Radhika, T.S.D. &Rajagopal, S.V. (2016). Studies on isolation, purification and structure elucidation of chemical constituents from methanolic flower extract of *Blepharis molluginifolia* pers. and their biological activities. *Internal Journal of Pharmaceutical Science Research*, *7*(12), 4893-4904. **[Crossref]**

Ehiagbonare, J.E. & Onyibe, H.I. (2008).Studies on raising and preparation of planting stock of three indigenous forest timber species in Nigeria.*International Journal of Biological and Chemical Sciences,2*(4), 573-578. **[Crossref]**

Haruna, A., Pateh, U.U., Sule, M.I., Musa, A.M., Sani, Y.M., Mohammed, M., Lawal, M.D

&Garba, M.A. (2017).Evaluation of anticonvulsant activity of methanol leaf extract of *Hymenocardia acida*, Tul (Euphorbiaceae) in laboratory animals.*Journal of Pharmaceutical and Allied Sciences*, *14*(5), 2675-2683.

Iyadi, K.C., Nia, R. & Antai, A.B. (2003). Phytochemical and anti-sickling properties of *Hymenocardia acida* (Tul).*Nigerian Journal of Physiological Sciences*, *18*(1-2), 82-86. **[Crossref]**

Johnson, T.O. & Omoniwa, B.P. (2014).*In vivo*trypanocidal activity of ethanolic crude extract and phytochemical fractions of *Garcinia kola* seeds. *Annual Research and Review in Biology, 4*(1), 212-222. **[Crossref]**

Madaki, F.M., Kabiru, A.Y., Mann, A., Abdulkadir, A., Agadi, J.N. and Akinyode, A.O. (2016).Phytochemical analysis and in-vitro anti-trypanosomal activity of selected medicinal plants in Niger State, Nigeria. *International Journal of Biochemistry Research and Review, 11*(3),1-7. **[Crossref]**

Maikai, V.A. (2011). Anti-trypanosomal activity of flavonoid extracted from *Ximeniaamericana* stem bark. *International Journal of Biology,3*(1):1. **[Crossref]**

Majekodunmi, A. O., Fajinmi, A., Dongkum, C., Picozzi, K., Thrusfield, M. V., &Welburn, S. C. (2013). A longitudinal survey of African animal trypanosomiasis in domestic cattle on the Jos Plateau, Nigeria: prevalence, distribution and risk factors. *Parasites & vectors*, *6*(1), 239. **[Crossref]**

Mergia, E., Shibeshi, W., Terefe, G. &Teklehaymanot, T. (2014).Phytochemical screening and *in-vitro*antitrypanosomal activity of aqueous and methanol leaf extract of *Verbascumsinaiticum* (Scrophulariaceae) against *Trypanosomacongolense* field isolate.*Journal of Clinical and Experimental Pathology*, *4*:183. **[Crossref]**.

Nibret, E. & Wink, M. (2010) Volatile components of four Ethiopian *Artemisia* species extracts and their *in vitro*antitrypanosomal and cytotoxic activities.*Phytomedicine,17*(5), 369-374. **[Crossref]**

Nwodo, N.J., Ibezim, A., Ntie-Kang, F., Adikwu, M.U. &Mbah, C.J. (2015). Anti-trypanosomal activity of nigerian plants and their constituents. *Molecules*, *20*(5), 7750-7771. **[Crossref]**

Ogbadoyi, E.O., Kabiru, A.Y. &Omotosho, R.F. (2011). Preliminary studies of the anti-trypanosomal activity of *Garciniakola* nut extract in mice infected with *Trypanosoma brucei brucei*. *Journal of Medicine and Medical Sciences*, *2*(1), 628-631.

Sabo, S.Y., Otimenyin, S.O., Uguru, M.O. &Bukar, B.B. (2017). Ethnobotanical survey of medicinal plants used as aphrodisiacs in Bauchilocal government area. *Journal of Complementary and Alternative Medical Research*, *4*(4), 1-13. **[Crossref]**

Sani, A., Zakariyya, U.A., Mahe, A. & Singh, D. (2018).*In vitro*study of anti-trypanosomal activity of ethanolic leaf extract of *Garcinia kola* against *Trypanosoma brucei brucei*. *Asian Journal of Pharmaceutical and Clinical Research, 11*(6), 417-419. **[Crossref]**

Simarro, P. P., Diarra, A., Ruiz Postigo, J. A., Franco, J. R., &Jannin, J. G. (2011). The human African trypanosomiasis control and surveillance programme of the World Health Organization 2000-2009: the way forward. *PLoS neglected tropical diseases*, *5*(2), e1007. **[Crossref]**

Simorangkir, M., Nainggolan, B. &Silaban, S. (2019). Secondary metabolites phytochemical analysis of n-hexane, ethyl acetate and ethanol extracts of sarangbanua (*Clerodendrum fragram* Vent Wild) leaves. *Proceedings of the 5th Annual International Seminar on Trends in Science and Science Education*, AISTSSE 2018, Medan, Indonesia. **[Crossref]**

Usman, A.M., Danjuma, N.M., Ya'u, J., Ahmad, M.M., Alhassan, Z. &Abubakar, Y.M. (2021). Antidiarrhoeal potential of methanol extract of *Hymenocardia acida*Tul (Euphorbiaceae) in laboratory animals.*Bulletin of the National Research Center*, *45*(118), 118. **[Crossref]**

Wadood, A., Mehreen, G., Syed, B., J., Muhammad, N. &Ajmal, K. (2013).Phytochemical analysis of medicinal plants occurring in local area of Mardan. *Biochemistry Analytical Biochemistry2*, 144.

WHO (World Health Organization).Human African Trypanosomiasis (Sleeping Sickness). Fact Sheet No. 259; 2012.

Yadav, R.N.S. &Agarwala, M. (2011).Phytochemical analysis of some medicinal plants. *Journal of Phytology*, *3,*2075-6240.

Yusuf, A.B., Umar, I.A. &Nok, A.J. (2012). Effects of methanol extract of *Vernonia amygdalina* leaf on survival and some biochemical parameters in acute *Trypanosoma brucei brucei* infection. *African Journal of Biochemistry Research*, *6*(12), 150-158.