Journal of Biological Sciences and Bioconservation
Volume 7, Number 1, 2015
ISSN: 2277-0143

PHYTOCHEMISTRY, ACUTE TOXICITY AND *INVITRO* ANTITRYPANOSOMAL STUDIES ON AQUEOUS EXTRACT OF *GOSSYPIUM HERBACEUM* (LINN) LEAF

[1]Biu, A.A., [2]Buratai, L. B., [3]Lawal, J.R., [3]Yakaka, W. [1]Zango, M.K., [4]Mustapha, F.B. and [2]Ali, B.J.

[1]Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria
[2] Department of Biochemistry, Faculty of Science, University of Maiduguri, Nigeria.
[3]Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria
[4]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Maiduguri
E-mail: biuvet@yahoo.com

ABSTRACT

The phytochemical components, median lethal dose (LD_{50}) in albino rats and *In vitro* efficacy of aqueous extract of *Gossypium herbaceum* leaf on *Trypanosoma brucei* was investigated in this study. Tannins had a high score in the extract, alkaloids, saponins, flavonoids and reducing sugars indicated moderate scores, while glycosides and phlabotannins showed low scores. The calculated medium lethal dose (LD_{50}) in albino rats was 500mg/kg body weight, with early clinical signs of sluggishness, awkward posture, loss of appetite, starry hair coat and terminal death within 24 hours. All clinical signs were dose dependent. *In vitro* efficacy of *Gossypium herbaceum* leaf aqueous extract had a positive correlation with the extract dose, but negatively correlated with the time of exposure, showing a 100% inhibition of trypanosomes at the concentration of 5.0mg/kg. In conclusion *Gossypium herbaceum* leaf aqueous extract could be described to have bioactive components, moderately toxic and could inhibit trypanosomes *In vitro*.

Keywords: *Gossypium Herbaceum*, Aqueous Extract, Albino Rats, *Trypanosoma Brucei*

INTRODUCTION

Trypanosomosis a disease of man (Solano *et al.,* 2003), domestic animals (Abu *et al.*, 2009) and wild animals (Abenga and Anosa, 2006) is caused by trypanosomes and transmitted by the tsetse fly (Abu *et al.*, 2009). The disease is currently epidemic in about thirty-six countries in sub-Saharan Africa threatening more than sixty million lives on daily basis (Steverding and Tyler, 2005; Vanhollebeke *et al.*, 2006). Meanwhile, the disease has spread beyond its original distribution

in the sub- Saharan Africa and is now present in South America, North America and large parts of Asia (Vanhollebeke *et al.*, 2006). The pathology and pathogenesis of the disease have been reported by Anosa and Kaneko, (1983) and Mbaya *et al.*, (2009). Generally, its eradication and control is principally based on chemotherapy and chemoprophylaxis by trypanocides using the salts of isometamidium, homidium and diminazene (Anene, *et. al.* 2001; Brian *et al.,* 2010; Ajakaiye *et al.,* 2013; Ameen, 2013). Unfortunately the use of these trypanocides is beset by numerous limitations and drawbacks that include the lengthy parenteral administration, unaffordability of the drugs by low resource poor rural farmers, development of resistance to the drugs by the parasite, toxicity to the hosts due to long period of treatment (McDermott *et al.* 2000; Maikai *et al.* 2007; Eze *et al.* 2013; Lawal *et al.* 2013) and exhibition of antigenic variation which hampers vaccine production making drug therapy the only viable management (Anene, *et. al.* 2001; Prathalingham *et al.* 2007; Samdi *et al.* 2010).
However, it has been observed that natural plant products offer novel possibilities against trypanosomes (Atawodi *et al.* 2002; Hoet *et al.* 2004; Shuaibu *et al.* 2008; Oluyomi *et al.* 2011; Lawal *et al.* 2013). In spite of the possible role of medicinal plants as trypanocides some of the secondary metabolites in the extracts are toxic in nature (Nok, 2002; Mbaya *et al.* 2007; 2009; 2010). *Gossypium herbaceum* has been reported to be used for the treatment of diarrhea, dysmenorrhea, dysentery, gout, sexual debility, bronchial asthma and skin disease (Gupta and Sharma, 2006; Singh *et al.* 2011; Khalid *et al.* 2012; Sultan *et al.* 2010). It has also been used to stop bleeding from wound and help to induce abortion, augmentation of labour and retention of placenta (Fertosa *et al.* 2011; Singh *et al.* 2011; Khalid *et al.* 2012; Velmurugan *et al.* 2012). This study was carried out to determine the phytochemical components of the aqueous extract of *Gossypium herbaceum* leaf, its median lethal dose (LD_{50}) using albino rats and its efficacy against *Trypanosoma brucei In vitro*.

MATERIALS AND METHODS

Plant Collection and Identification

Fresh leaves of *Gossypium herbaceum* were collected from Bolori ward in Maiduguri, Borno State, Northeastern Nigeria on the 17th of January, 2013. The plant was authenticated by a botanist of the Department of Biological Sciences, University of Maiduguri, Nigeria

Plant Preparation and Extraction

The fresh leaves were rinsed in clean tap water to remove dirt and later shade dried for a period of 8 days. The dried leaves were then pounded into fine powder with pestle and mortar, and then sieved to remove debris and coarse plant materials. A fine powder of 1000 grams weight was obtained, which was soaked in 300ml of distilled water, and left to stand for 24 hours with frequent shaking after every 6 hours. This solution was then filtered through Whatman® filter paper and the filtrate concentrated on a water bath at 40^0 – 50^0 C and evaporated by drying using a hot plate at temperature of 50^0 – 60^0C. The residue obtained was ground into powder to weigh 44.89g and stored at room temperature until used. The % yield was determined as 4.49%.

Phytochemical Analysis

The phytochemical screening of *Gossypium herbaceum* leaf was carried out using standard procedure to identify the constituents as described by Sofowara (1993). Test for tannins, alkaloids, glycosides, steroids, reducing sugars (Fehling's test), monosaccharides (Benedict's test), anthraquinones and phlabotannins were carried out as previously described by Evans (2000), while the test for flavonoids was carried out as previously described by Harbone (1989).

Determination of LD_{50}

Twelve adult albino rats of both sexes weighing between 56.3 – 211.8g were used for the determination of LD_{50}. They were grouped into 4 groups (A – D) of three rats each and kept within ambient conditions (temperature: 27 ± 1^0C., photoperiod: 12 hours natural light and 12 hour dark, humidity 40±5%). They were fed with standard feed (Grand Cereal Ltd, UAC Nigeria PlC, Jos, Nigeria) and portable water *ad libitum* for 4 weeks to acclimatize to the laboratory conditions before the toxicity study. All experimental albino rats were handled according to the international guiding principles for biomedical research involving animal use and care (C.I.O.M.S, 1985). Using group A – D the rats were intraperitoneally treated with different doses of 100, 200, 400 and 800mg/kg body weight, concentration of the *Gossypium herbaceum* leaf aqueous extract respectively. The rats were observed for 24 hours for clinical signs and death, LD_{50} of *Gossypium herbaceum* was calculated using the modified arithmetic mean of Karber (Aliu and Nwude, 1982)

Trypanosoma brucei Stock

The *Trypanosome brucei* that was used in this study was obtained from Nigeria Institute of Trypanosomiasis Research (NITR), Vom, Plateau State, Nigeria. *Trypanosome brucei* viability was maintained by subsequent passage into other clean (parasite free) albino rats.

In vitro Experiment

Serial dilutions of the extract were prepared in phosphate buffered saline to give concentrations of 40, 20, 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078 and 0 mg/kg into test tubes of 8 replicates each, two drops of infected blood obtained by albino rat tail puncture was diluted in glucose solution and added into each test tube and incubated at 37^0C. Neubauers chamber was used to count the number of parasites per field and antitrypanosomal activity was determined based on cessation of motility. The parasite count was carried out at intervals of 30, 60 90,and 120 minutes, after which percentage inhibition of the parasite for each extract solution was calculated using the expression described by Atawodi and Ogunbusola (2009).

$$\text{Percentage inhibition} = \frac{\text{Parasite count in control untreated (Pc)} - \text{Parasite count in extract treated (Pe)}}{\text{Parasite count in control untreated (Pc)}} \times 100\%$$

Statistical Analysis

In vitro data were expressed as mean ± standard deviation (SD), variation and range.

RESULTS

Table 1 shows the result of the phytochemical screening of bioactive substance. Tannin had high scores (3+), alkaloids, saponins, flavonoids and reducing sugars had moderate scores (2+), while glycosides, monosaccharides and phlabotannins showed low scores (1+). Table 2 shows the calculated lethal dose of *Gossypium herbaceum* leaf aqueous extract. The dose of the extract that produces 100% mortality rate was 800mg/kg. The calculated LD_{50} was 500mg/kg, and doses of 100 and 200mg/kg did not cause mortality. Clinical signs observed were sluggishness, awkward posture, loss of appetite, starry hair coat and terminal death within 24hours. Table 3 shows the effect of grade doses of *Gossypium herbaceum* leaf aqueous extract on parasite motility (% inhibition). There was 100% inhibition of *T. brucei* at the extract concentration of 5.0mg/ml, meanwhile the various extract concentration generally had a positive correlation with parasite inhibition.

Table 1: Phytochemical Components of *Gossypium Herbaceum* Leaf Aqueous Extract

Component	Test	Observation	Scoring
Tannins	Ferric chloride	None	3+
Alkaloids	Dragendorff's test	Orange – red precipitate	2+
Saponins	Frothing	None	2+
Glycosides	Salkowski's test	Red precipitate	1+
Steroids	Chloroform	Green – ring colour	Nil
Flavonoids	Pevil's test	None	2+
Reducing sugars	Fehling's test	Orange precipitate	2+
Monosaccharides	Benedict's test	Red precipitate	1+
Anthraquinones	Borntrigger's test	None	Nil
Ketones	Standard	None	Nil
Phlabotannins	Hydrogen chloride	Red precipitate	1+

Keys:
Nil = not detected
1+ = low concentration
2+ = moderate concentration
3+ = high concentration

Table 2: Median Lethal Dose (LD_{50}) of *Gossypium Herbaceum* Leaf Aqueous Extract

Group (n= 3)	Plant extract (mg/kg body weight)	Dose difference (DD)	Dead rats	Mean dead (MD)	DDxMD
A	100		0		
		B – A = 100		0	0
B	200		0		
		C – B = 200		0.5	100
C	400		1		
		D – C = 400		2	800
D	800		3		
Total					**900**

$$LD_{50} = LD_{100} - \frac{DD \times MD}{n} = 800 - \frac{900}{3}$$
$$= 800 - 300$$
$$= 500\text{mg/kg body weight}$$

Table 3: Effects of Graded Doses of *Gossypium herbaceum* Leaf Aqueous extract on Parasite Motility (% Inhibition)

Concentration of Extract (mg/kg)	% Inhibition MPI (Mean ± SD: Range)			
	30	60	90	120
PSS (control)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.078	60.8 ± 3.4 (57.7 – 64.6)	67.8 ±2.7 (65.6 – 69.6)	74.4 ± 3.6 (71.2 – 77.8)	83.1 ± 4.1 (70.9 – 88.1)
0.156	67.4 ± 2.5 (64.0 – 70.0)	72.4 ± 1.7 (71.8 – 73.6)	77.9 ± 1.4 (77.0 – 80.0)	88.3 ± 2.4 (84.6 – 89.7)
0.313	74.1 ± 3.9 (69.3 – 78.7)	79.7 ± 2.8 (76.3 – 82.4)	83.6 ± 1.7 (81.6 – 85.4)	90.2 ± 1.7 (87.7 – 91.6)
0.625	0.30 ± 0.1 (70.1 – 80.0)	80.8 ± 5.2 (74.1 – 86.3)	87.5 ± 3.7 (82.1 – 90.0)	93.3 – 1.4 (91.9 – 95.2)
1.25	91.8 ± 2.7 (88.2 – 94.4)	97.3 ± 0.8 (96.2– 97.8)	98.6 ± 1.5 (96.6 – 100.0)	99.4 ± 0.7 (98.3 – 100.0)
2.5	93.3 ± 1.6 (91.3 – 94.6)	96.2 ± 1.6 (93.9 – 97.7)	97.9 ± 1.3 (96.2 – 99.2)	99.9 ± 0.3 (99.5 – 100.0)
5.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0

Keys:
PSS = Physiological saline solution
MPI = Minutes post inoculation

DISCUSSION

Although the present study did not involve detailed characterization of different compounds that could be responsible for the observed activities, preliminary phytochemical screening of *Gossypium herbaceum* leaf has shown the presence of bioactive components using various tests. Tannins had high concentrations, alkaloids, saponins, flavonoids and reducing sugars were found in moderate concentrations, while glycosides, monosaccharides and phlabotannins were found in low concentrations. This plant extract no doubt have medicinal properties. This agrees with the findings of Wurochekke *et al.*, (2014) who reported that medicinal plants are those plants that contain potential phytochemical constituents used in the treatment and management of diseases.The trypanocidal property of the *Gossypium herbaceum* extract may be due to the action of one or more of these secondary metabolites present. Several authors have either identified or isolated tannins and phenolic compounds (Shuaibu *et al.* 2008; Mbaya *et al.* 2010), flavonoids (Ambrozin *et al.* 2004; Umar *et al.*, 2014) and alkaloids (MerschJohann *et al.* 2001) in plants that showed trypanocidal activities. The LD_{50} of the leaf extract was calculated at

500mg/kg, with mortality and some clinical symptoms observed in all the groups tested. It has been previously reported that scientific evaluation of toxicities by determining lethal dosages (LD_{50}) usually preceded *In vivo* trypanocidal efficacy trials (Mbaya *et al.* 2010). During *In vitro* studies cytotoxicity in animal cell cultures has been documented (Camacho *et al.* 2003; Sara *et al.* 2004). It has also been previously reported by Biu *et al.,* (2010) that toxicity of plant extracts and clinical signs that were dose dependent are related to the effects of the extract on the liver, kidneys and other functional organs of tested animals. There was a 100% inhibition on *T. brucei* motility *In vitro* at 5.0mg/kg concentration of *Gossypium herbaceum* extract on *T. brucei.* However, there was a positive correlation between extract concentrations and inhibition of *T. brucei* activity. This report agrees with findings previously reported by Sepulveda-Boza and Cassel (1996), however, this anti – trypanosomal property of *Gossypium herbaceum* leaf observed in this study have a lesser activity when compared with that previously reported by Igweh *et al.,* (2002) and Shaba *et al.*, (2012). The mechanism of the extract trypanocidal action was not determined. However, Atawodi *et al.,* (2003) reported that many natural products exhibit their trypanocidal activity by virtue of their interference with the redox balance of the parasites acting either on the respiratory chain or on the cellular defenses against oxidative stress. This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to enzymes that are very sensitive to alteration in redox balance. It is also known that some agents also act by binding with the kinetoplast DNA of trypanosomes as reported by Alli *et al.,* (2011).

REFERENCES

Abenga, J. N. and Anosa V. O. (2006): Clinical studies on Vervet Monkeys (*Cercopethicusaethiopes*) infected with *Trypanosoma Brucei Gambiense*. *Vet. Arhiv*, 76(1): 11-18.

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.

Ajakaiye, J. J., Mazadu, R. M., Benjamin, M. S., Bizi, L. R., Shuaibu, Y., Kugu, B. A., Mohammed, B. and Muhammad, A. A. (2013). Effects of Dietary Vitamins C and E Oral Administration on Body Temperature, Body Weight and Haematological Parameters in Wistar Rats Infected with *T. brucei Brucei* (Federe strain) during the Hot Rainy Season. *Int. Res. J. Pharm. Pharmacol.*, 3(7): 105-111.

Aliu, O.Y. and Nwude, N. (1982). *Veterinary Pharmacology and Toxicology Experiments.* Baraka Press, Nigeria Ltd Zaria. 1st ed. Pp. 104-109.

Alli, L. A., Okochi, V. I. and Adesikan, A. A. (2011). Anti-trypanosome activity and Haematological effects of Aqueous extract of the Leaf of *Morinda Lucida* on *Trypanosome Brucei* Infected Rats. *Asian J. Pharm. Res. Sci*. 1 (3): 111 – 115.

Ambrozin, A. R. P., Vieira, P. C., Fernandes, J. B., Fernandes da Silva, M. F. and de'Albusquerque, S, (2004). Trypanocidal activity of Meliaceae and Rutaceae plant extracts. *Memoria Institute Oswaldo Cruz* 99(2): 227 – 231.

Ameen, S. A. (2013) Antitrypanosomal activities of Natural Honey in *Trypanosoma Congolense*- Infected Wistar Rats. *Vet .Res*. 6(1): 10-14.

Anene, B. M., Onah, D. N. and Nawa, Y. (2001). Drug Resistance in Pathogenic African Trypanosomes: What Hopes for the Future? *Veterinary Parasitology,* 96:83 – 100.

Anosa, V. O. and Kaneko J. J. (1983). Pathogenesis of *Trypanosoma Brucei* Infection in Deer Mice (*Cp manuculatus*). Light Electron Microscopic studies on Erythrolytes Pathological Changes and Phagocytosis. *Anim. J. Vet. Res*. 44: 645–651.

Atawodi, S. E., Ameh, D. A., Ibrahim, S., Andrew, J. N., Nzelibe, H. C., Onyike, E., Anigo, K. M. and Sallau, A. B. (2002) Indigenous Knowledge System for Treatment of Trypanosomiasis in Kaduna State of Nigeria. *J. Ethnopharmacol.,* 79: 279-282.

Atawodi, S. E., Bulus, T., Ibrahim, S., Ameh, D.A., Nok, A.J., Mamman, M., Galadima, M. (2003): *In-vitro* Trypanocidal effect of Methanolic extract of some Nigerian Savannah Plants. *African Journal of Biotechnology*, 2(9): 317-321.

Atawodi, S.E. and Ogunbusola, F. (2009). Evaluation of Antitrypanosomal Properties of Four Extracts of Leaves, Stem and Root Barks of *Prosopis Africana* in Laboratory Animals. *Biokemistri*, 21(2): 101-108.

Biu, A. A., Yusufu, S. D. and Rabo, J. S. (2010). Acute Toxicity study on Neem (*Azadirachta indica*, Juss) Leaf Aqueous extract in Chicken (*Gallus Gallus Domesticus*).*African Scientist* 11 (4): 241 – 244.

Brian, T. E., Melvin, D. D., Joann, M. T., Conrad, L. E. and David, M. E. (2010). Calflagin Inhibition Prolongs Host Survival and Suppresses parasitaemia in *Trypanosoma brucei* infection. *Eukaryot. Cell.*, 9(6): 934 - 942.

Camacho, M. R., Phillipson, J. D., Croft, S. L., Solis, P. N., Marshal, S. J. and Ghazanfar, S. A. (2003). Screening of Plant Extracts for Antiprotozoal and Cytotoxic Activities. *J. Ethnopharmacol,* 89: 185 – 191.

Council for International Organization for Medical Science (C.I.O.M.S.) (1985). International Guiding Principles for Biomedical Research involving Animals. Developed by Council for International Organization of Medical Sciences in Collaboration with World Health Organization(WHO),1211 Geneva, 27, Switzerland

Evans, C. W. (2000). *Trease and Evans Pharmacology*. 3rd edition, W. B. Saunders Company Ltd London, England, Pp. 224 – 239.

Eze, J. I., Okeke, M. C., Ngene, A. A., Omeje, J. N. and Abonyi, F. O. (2013). Effects of Dietary Selenium Supplementation on Parasitaemia, Anaemia and serum proteins of *Trypanosoma brucei brucei* infected rats. *Exptl. Parasitol.*, 135: 331-336.

Fertosa, C. M., Freitas, R. M., Luz, N. N. N., Bezerra, M. Z. B. and Trevisan, M. T. S. (2011). Acetylcholinesterase Inhibition by some Promising Brazilian Medicinal Plants. *Braz. J. Biol.* 71 (3): 783 – 789.

Gupta, R. S. and Sharma, R. A. (2006). Review on the Medicinal Plant Exhibiting Anti-Fertility activity in Males. *Natural Product Radiance;* 5 (5): 389 – 410.

Harborne, J. B. (1989). *Phytochemical Methods*: A Guide to Modern Techniques of Plant Analysis. London, Chapman and Hall Ltd, 1989.

Hoet, S., Opperdoes, F., Brun, R. and Quetin-Lectercq, J. (20004). Natural products active against African Trypanosomes: A Step Towards New Drugs. *Nat. Prod. Rep.*, 21, 353 – 364.

Igweh, A. C., Aguiyi, J. C. and Okwuasaba, F. K. (2002). Antitrypanosomal effects of Aqueous extract of *Brassica Oleracea. Fitoterapia*, 7391: 17 – 21.

Khalid, M. S., Hasan, B. K., Suresh, D. H., Hasan, R., Saleen, M. N. and Farooqwi, Z. (2012). Anti – Ulcer Activity of Ethanolic extract of *Gossypium*

Herbacium Linn Flowers, *RGUHS. Journal of Pharmaceutical Sci.*, 1 (1): 79 – 84.

Lawal, M., Oboh, A. and Malann, Y. D. (2013). Antitrypanosomal activity of the Ethanolic Leaf extract of *Senna occidentalis* (Fabaceae) on *Tyrpanosoma brucei brucei*- Infected Mice. *Int. J. Basic. Appl. Sci.*, 2(1): 32-37.

Maikai, V. A., Salka, M. N., Adeiza, A. A. and Makeri, H. K. (2007). Assessment of Isometamidium Chloride and Diminazene Aceturate in Laboratory Mice infected with Field Isolates of *T. Congolense* from Naturally Infected Cattle. *Journal of Production Agriculture and Technology*, 3(1) 147 – 152.

Mbaya, A. W., Aliyu, M. M., Nwosu, C. O., Taiwo, V. O. and Ibrahim, U. I. (2009). Effects of Melarsamine Hydrochloride (Cymelarsan®) and Diaminazene aceturate (Berenil®) on the Pathology of Experimental *Trypanosoma brucei* Infection in Red Fronted Gazelles (*Gazella rufifrons*). *Veterinary Parasitology.,* 163 (1-2): 140-143.

Mbaya, A. W., Ibrahium, U. I., Onyiche, T. and Sanya, L. (2010). Toxicity and Potential Anti-Trypanosomal activity of Ethanolic extract of *Azadirachta indica* (*Maliacea*) stem bark: An *in-vivo* and *in-vitro* Approach using *Trypanosoma brucei*. *J. Ethnopharmacol*, 128: 495-500.

Mbaya, A. W., Nwosu, C. O. and Onyeyili, P. A. (2007). Toxicity and atitrypanosomal effect of ethanolic extract of *Butyrospermum paradoxum* (Sapotaceae) stem bark in rats infected with *Trypanosoma brucei brucei* and *Trypanosoma congolense. J. Ethnopharmacol,* 111: 526 – 530.

McDermott, J. J., Sidibe, I., Bauer, B., Diarra, B., Clausen P.H., Woitang, T., Ouedraogo,D.,Kamuanga, J.M.B., Peregrine, A.S., Eisler, M.C. and Mehlitz, D. (2000). Field studies on the Development and Impact of Drug Resistant Animal Trypanosomes in Market Oriented Production Systems in the Southern Guinea Zone of West Africa. Newsletter No2 of EU Concerted Action.

MerschJohann, K., Sporer, F., Steverding, D. and Wink, M, (2001). *In vitro* Effects of Alkaloids on Bloodstream forms of *Trypanosoma brucei* and *Trypanosoma Congolense*. *Planta Medica* 67 (7): 623 – 627.

Nok, A. J. (2002). Azanthraquinone Inhibits Respiration and *In vitro* growth of Long Slender Bloodstream forms of *T. Congolense*. *Cell Biochem. Func.* 20: 205 - 212.

Oluyomi, S. A., Melissa, L. S., Musbau, A. A. and Vicky, M. A. (2011). Anti-Trypanosoma and Cytotoxic activity of *Psidium Guajava* Leaves in Alamar Blue based Assays. Vet. Arhiv. 81 (5), 623-633.

Prathalingham, S. R., Wilkison, S. R., Horn, D. and Kelly, J. M. (2007). Deletion of the *Trypanosoma brucei* Superoxide Dismutase Gene sodb1 Increases Sensitivity to Nifurtimox and Benznidazole. *Antimicrob. Agents Chemother*, 51(2): 755-758.

Samdi, S. M., Abenga, J. N., Attahir, A., Wayo, B. M., Sumayin, H. M., Haruna, M. K., Jijitar, A. M., Ogunwale, R. T., Ramatu, R. N. and Bizi, R. L. (2010). Constraints in the Control of African Trypanosomosis: The prevailing Factors in Kurmin - Kaduna, Northern Nigeria. *Int. J. Anim. Vet. Adv.,* 2(1): 31-36.

Sara, H., Frederik, O., Reto, B., Victor, A. and Joelle, Q. L. (2004). *In vitro* Antitrypanosomal Activity of Ethnopharmacologically Selected Beninese Plants. *J. Ethnopharmacol.* 91: 37 – 42.

Sepulveda-Boza, S. and Cassel, B.K. (1996). Plant Metabolites active against *Trypanosoma cruzi*. *Planta Med*. **62**:98-105

Shaba, P., Pendey, N. N., Sharma, O. P., Rao, J. R. and Singh, R. K. (2012). Anti – Trypanosomal effects of *Brassica Oleracea* (Cabbage) Fruits and Leaves. *Jour. Vet. Adv.* 2 (7): 304 – 401.

Shuaibu, M. N., Wuyep, P. T. A., Yanagi, T., Hirayama, K., Ichinose, A., Tanaka, T. and Kouno, I. (2008). Trypanocidal activity of Extracts and Compounds from the Stem Bark *Anogeissus Leiocarpus* and *Terminalia Avicennoides*. *PARASITOLOGY* 102(4): 697 –703.

Singh, P. K., Singh, S., Kumar, V. and Krishma, A. (2011). Ethnoveterinary Healthcare Practice in Marihan Sub-division of District Mirzapur, Uttra Predesh India. *Life Sciences Leaflets*; 16: 561 – 569.

Sofowora, A. (1993) *Medicinal Plants and Traditional Medicine in Africa*, Ibadan, Spectrum Books, Pp. 289.

Solano, P., Dela, R. S., Duvalet, G. (2003). Biodiversity of Trypanosomes Pathogenic for Cattle and their Epidemiological Importance. *Annals of Society.*, 68: 189-171.

Steverding, D. and Tyler, K. M. (2005), Novel Antitrypanosomal Agent. *Expert Opinion on Investigational Drug*, 14 (8): 939-955.

Sultan, A., Farman, U., Khan, I., Hussein, M., Murad, A., Khan, I. and Ullah, K. (2010). Evaluation of Chemical Analysis profile of *Citrullus Colocynthis* growing in Southern Area of Pakistan. *World Applied Science Journal,* 10(4): 402-405.

Umar, I. A., Ndidi, U. S., Mohammed, A., Anaedum, V. C., Zambuk, U. U., Umar, A. U. and Bello, M. A. (2014). *In vitro* Antitrypanosomal Activity, Antioxidant Property and Phytochemical Constituents of Aqueous extracts of Nine Nigerian Medicinal Plants. *Asian Pac. J. Trop. Dis.*, 4(5): 348-355.

Vanholebeke, B., Truc, P., Doelvoorde, P., Pays, A., Joshi, P. P., Kalti, R., Jannin, J. G. and Pays, E. (2006). Human *Trypanosoma evansi,* infection Linked to a lack of Apolipoprotein. *New Engl. J. Med.*, 355: 2756 - 2759.

Velmurugan, C. Venkatesh, S., Sandhya, K., Lakshmi, S. B., Vardhan, R. R. and Sravanthi, B. (2012). Wound Healing activity of Methanolic extract of Leaves of *Gossypium Herbaceum*. *Cent. Euro. J. Exp. Bio*. 1 (1): 7 – 10.

Wurochekke, A. U., Bello, H., Yusuf, H. B. and Mahmoud, S. J. (2014).*In-Vitro* Trypanocidal Activity of Aqueous Stem Bark of *Haematostaphisbarteri* against *Trypanosoma congolense* and *Trypanosoma brucei brucei*. *Sch. Acad. J. Pharm.,* 3 (1): 38-42.

Reference to this paper should be made as follows: Biu, A.A., *et al* (2015), Phytochemistry, Acute Toxicity and *Invitro* Antitrypanosomal Studies on Aqueous extract of *Gossypium Herbaceum* (Linn) Leaf. *J. of Biological Science and Bioconservation,* Vol. 7, No. 1, Pp. 1 – 12.