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Phytochemical Screening and in Vivo Antitrypanosomal Activity of Methanol Extracts of Peristrophe Bicalyculata in Mice Infected With Trypanosoma Evansi

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ABSTRACT

There is the urgent need for therapeutic agents that are more effective, accessible and affordable to Africans who bears the burden of most diseases since chemotherapy of African trypanosomiasis remains far from being satisfactory. The qualitative and quantitative analysis of the Methanol extracts of *Peristrophe bicalyculata* showed the presence of oxalate, phytate, saponins, cyanogenic glycosides, alkaloids, and flavonoids, with 0.12mg/100g, 59.5mg/100g, 2%, 960mg/100g, 15%, 0.42% compositions respectively. The LD_{50} was determined to be below 1500mg/kg body weight. The methanol extract at doses of 400mg/kg body weight and 500mg/kg body weight were found to have some curative properties and at doses of 300mg/kg body weight and 400mg/kg body weight were found to have some suppressive properties in mice infected with *Trypanosoma evansi* . This potency of the extract when compared to the standard drug (Diaminazine aceterate) was less effective but significant reduction was observed when compared with the control (untreated).

Keywords: Trypanosomiasis, chemotherapy, *Trypanosoma evansi*, *Peristrophe bicalyculata*, curative, suppressive.

I. INTRODUCTION

Trypanosomiasis is a complex debilitating, zoonotic protozoan disease of man and animal [26], a major factor limiting livestock production in large areas of humid and sub-humid Africa [13]. Tsetse fly transmitted African trypanosomiasis is found between latitude 14^0N and 29^0S covering 10 million Km^2 stretching across over 37 countries in Africa [15, 26, and 23]. *Trypanosoma* is a genus of kinetoplastids, a monophyletic group of obligate, unicellular parasitic flagellate protozoa. The most important trypanosome species in Nigeria are *Trypanosoma brucei*, *T. congolense*, *T. vivax*, and *T. evansi* in livestock and *T. gambiense* infect human [22]. *Trypanosoma evansi* is responsible for a disease known as 'surra', and is the most widespread pathogenic trypanosome globally [13 and 4].

Medicinal plants have been used for centuries as remedies for human diseases and other animals because they contain certain components of therapeutic value. There are more than 35, 000 plant species being used in various human cultures around the world for medicinal purposes [10]. According to World Health Organization [26], medicinal plants will be the best choice to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. The plant *Peristrophe bicalyculata* belongs to the family Acanthaceae. The leaves of the plant decoction were used traditionally as analgesic, antipyretic, anti-inflammatory, sedative, stomach ache, anticancer, fertility, diuretics, diarrhea and other skin related infection. It is also used as an antidote for snake poison when macerated in an infusion of rice, as insect repellant and as green manure [7]. With this knowledge about the plant, it became very necessary to subject this plant to detailed scientific screening and also its validation in the treatment of some of the important diseases such as surra. It was for this reason the study was designed to evaluate the efficacy of the plant extract in the treatment of *Trypanosoma evansi* infection.

II. LITERATURE REVIEW

Surra is endemic in great parts of South-East Asia, but has been found in Africa, America and Europe. Different mammalian species such as water buffaloes, cattle, camels, and pigs are susceptible to this disease [27]. All blood sucking flies can transmit the disease, all though the 'horse' flies, *Tabanus*, stable flies, *Stomoxys* are vectors in South-East Asia and increased time between blood meals reduces the survival of the parasite [27] while the tsetse flies are the most common vector in different countries in Africa and south and central America, vampire bats (*Desmodus rotondus*) may serve as vector and reservoir of the parasite [28 and 27].

Infection with *T. evansi* has a negative effect on fertility, growth rates and feed conversion, also the immune system is depressed [27]. Surra as an acute disease is not a problem for the pigs but the endemic state of the disease causes immunosuppressive effects. The parasite is covered by a thick layer of glycoprotein (or variable surface antigen) that is the primary immunogen eliciting antibodies formation; periodically, the organism alters the glycoprotein coating and evades the host's defensive responses [29]. This characteristic

has made development of vaccine against this organism to be difficult, because the parasite has the ability to vary antigens during the course of infection[27].

Trypanosoma evansi is a monomorphic haemoflagellate protozoan that has an elongated nucleus and a small circular terminal kinetoplast, which is sometimes missing in mutated wild strains that have followed drug treatment. This flagellate is long and slender, it has length that varies between 15-34µm and a width between 1.5 to 2.2µm; like all parasitic trypanosomes, *T. evansi* is covered by a dense protein layer consisting of a single protein called the variable surface glycoprotein (VSG). This acts as a major immunogen and elicits the formation of specific antibodies. The parasites are able to evade the consequences of these immune reactions by switching the VSG, a phenomenon known as antigenic variation [30].

It was initially suspected that transmission of trypanosomes were purely mechanical (as in the case of *T. evansi*, which is transferred by biting flies or when carnivores bite their prey and get wounded in the mouth). In 1909, Kleine carried out transmission experiments from human patients to apes and monkeys. He showed that infected tsetse flies were vectors and that the parasite had to be present in the fly for a minimum period before it could cause infection. He subsequently showed that only the metacyclic forms in the salivary glands of the insects were infectious. When tsetse fly bites an infected human patient, it can ingest the parasites. After their arrival in the insect's stomach, many (99%) die because of interactions with specific lectins in the insect stomach; lectins are proteins that bind specifically to certain saccharides, such are present on glycoproteins [31].

In man, the parasite multiply outside the cells, namely in blood, Lymph and cerebrospinal fluid. Others can survive in certain areas like the plexus choroideus in the brain, from whence they cause flare-ups [32].

III. MATERIALS AND METHOD

Plant materials: Fresh plant of *Peristrophe bicalyculata* was obtained from Karau-karau village of Giwa Local Government Area of Kaduna state, Nigeria. It was transported to the Herbarium section of the Department of Biological Sciences of Ahmadu Bello University, Zaria for identification. The plant was dried under laboratory conditions for one week thereafter pulverized using mortar and pestle.

Preparation of crude extracts: The method of extraction employed was maceration by means of separating funnel. 250g of the pulverized plant was weighed into a one-liter conical flask and one liter of 70% methanol was transferred and allowed for 72 hours. The solution was decanted and the residue was rinsed twice using the same concentration of methanol thereafter transferred into an evaporating dish and allowed on a water bath at temperatures range of 40-50^{0}C for 24 hours for evaporation of the methanol content. The residue was scrapped using sharp spatula and the weight recorded.

IV. QUALITATIVE ANALYSIS

Test for alkaloids [20]

To 1ml of the test solution, 1ml of HCL and 3 drops of Wegner's reagent was added. The formation of a brown precipitate indicates the presence of alkaloids.

Test for Glycosides [9]

To 1ml of the test solution, 2 drops of Conc. Sulphuric acid was added and placed in water bath for about 15 minutes. 20% KOH will be added to make the solution alkaline. To this solution, few drops of $FeCl_2$ were added. The formation brick red precipitate indicates the presence of glycosides.

Test for Saponins [9]

To 1ml of dissolve solution, 3ml of water was added, shaken and allowed for about 3 minutes. The presence of persistent froth denotes the presence of saponins.

Test for Anthraquinones[9]

To 1ml of solution, 5ml of 10% HCL was added and allowed to stay for 5 minutes. The solution was filtered. The filtrate was decanted into a test tube and shaken with 5ml of benzene. The upper benzene layer was pipette off and transferred into test tube containing 5ml of 10% ammonium hydroxide. Production of pink, red or violet colouration in the lower ammonia layer revealed the presence of combined anthraquinones.

Test for Tannins

To 1ml of the test solution, 5ml of 1% gelatin containing NaCl was added. Formation of a yellow precipitate denotes the presence of tannins.

Test for Sterols and Terpenes [9]

To 1ml of test solution, few drops of concentrated sulphuric acid in a slant position was added and left standing for an hour. The formation of brown ring at interphase indicates the presence of sterols and terpenes

Test for flavonoids [5]

To 1ml of the test solution, 3mls of 10% sodium hydroxide was added followed by 3mls of 10% HCL. The formation of a

yellow colour on addition of sodium hydroxide, which disappeared on addition of the HCL, indicates the presence of flavonoids.

V. QUANTITATIVE ANALYSIS

Determination of cyanogenic glycoside [18]:

20 gram of the sample was made into a paste and the paste dissolved in 50ml of distilled water. The extract was filtered and the filtrate was used for cyanide determination. To 1ml of the filtrate, 4ml of alkaline picrate was added and absorbance was recorded at 550nm and cyanide content will be determined from the cyanide standard curve.

$$\text{Cyanide (mg/g)} = \frac{\text{Absorbance x GF x DF}}{\text{Sample weight}}$$

Where GF= gradient factor and DF= dilution factor

Alkaloid determination [11]

5g of the sample was weighed into 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hrs. This was filtered and the extract was concentrated on the water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation is complete. The whole solution was allowed to settle and the precipitate collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and then weighed.

Saponin Determination [16]

The sample was grounded and 20g of each were put into a conical flask and 100cm^3 of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4hrs with continuous stirring at about 55^{0}C. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts will be reduced to 40ml over ether bath at about 90^{0}C. The concentrate was transferred into a 250ml separator funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer discarded. The purification process was repeated. 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples was dried in the oven to a constant weight; the saponin content was calculated as percentage.

Flavonoid Determination [3]

10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whitman filter paper. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Oxalate Determination [18]

1 gram of the sample was weighed into 100ml conical flask and 75ml 3N H_2SO_4 was added and stirred intermittently with a negative stirrer for 1hr. it was then filtered using whatmann filter paper. From the filtrate, 25ml was taken and titrated while hot 80-90^0 against 0.1N $KMnO_4$ solution until a faint pink colour persisted for at least 30secs. The overall reaction is:

$$MnO_4^- + Cr_2O_4^{2-} + 8H^+ \text{ ---------} \rightarrow Mn^{2+} + 4H_2O + 2CO_2$$

$$\text{Oxalate (mg/100g)} = \frac{\text{T x Vme x DF}}{\text{Me x Ms (g)}}$$

Where T is the titer value of $KMnO_4$ (ml), Vme is the volume mass equivalent. DF is the dilution factor (Vt/A) where Vt is the total volume of titrate (filtrate) and A is the aliquot used, Me is the molar equivalent of $KMnO_4$ in oxalate.

Animals: Healthy Swiss albino mice of either sex, about 4-6 weeks old was obtained from the Department of Pharmaceutical Sciences of Ahmadu Bello University, Zaria and used for the research. These animals were allowed for two weeks to acclimate in the laboratory. During this period, the rodents were fed with commercial pellets diets and water *ad libitum.*

Parasites: *Trypanosoma evansi* was obtained from the Department of Parasitology in Faculty of Veterinary Medicine of Ahmadu Bello University, Zaria and was maintained in the laboratory by serial passaging in mice.

Antitrypanosomal screening and Safe Dose and Acute Toxicity (LD_{50}): Lethal dosage test was carried out as described in **[6].** Mice will be pre-screened by microscopy of wet mount, thin and thick tail tip blood smears to exclude the possibility of test animals harboring rodent *Trypanosoma evansi* species.

Suppressive Test

This involves treatment with the extract 24 hours post inoculation of the mice (early infection). Sixteen mice of both sexes were divided into four groups of four each. The passage mouse infected with the *T. evansi* was anaesthetized with chloroform and its blood collected by cardiac puncture with sterilized syringe and needle and diluted using normal saline. Each of the sixteen mice was inoculated intraperitoneally with

0.2ml diluted blood containing about 1.59 x 10^6 trypanosomes per ml of blood [12]. The extracts at dose levels of 300mg/kg body weight (T_2) and 400mg/kg body weight (T_3) dosage was administered intraperitonially (i.p.) once daily for twelve days. A parallel test with diaminazine aceterate (5mg/kg body weight) (T_4) in the fourth group served as reference. The first group was given normal saline and serve as control (T_1). Wet mount, thick and thin blood films were made at three days interval by puncturing the tail tip and rapid marching method for estimating host parasitaemia at x400 magnification was employed as described by [12].

Curative test: The curative test is similar to that of the suppressive test but the treatment in this case begins from the sixth to seventh day post inoculation. However, the dosages of extracts administered in the curative test were 400mg/kg body weight (T_2) and 500mg/kg body weight (T_3). A total of sixteen mice were used for the curative test, four groups of four mice each. Administration of the drugs and the extracts were intraperitoneal and it continues on daily basis for 5 days. The parasitaemic level was monitored on daily basis by preparing wet mount and thin slide smear. The slides were mounted on the microscope and the level of parasitaemia was monitored daily at x400 magnification as described by [12].

VI. RESULTS AND DISCUSSION

The result of the qualitative and quantitative bioassay reveal the presence of oxalate, phytate, saponins, cyanogenic glycosides, alkaloids and flavonoids with compositions of 0.12mg/100g, 59.5mg/100g, 2%, 960mg/100g, 15% and 0.42% respectively as shown in the table below:

Table 1: Showing the qualitative and quantitative analysis of the methanol extract of *Peristrophe bicalyculata*

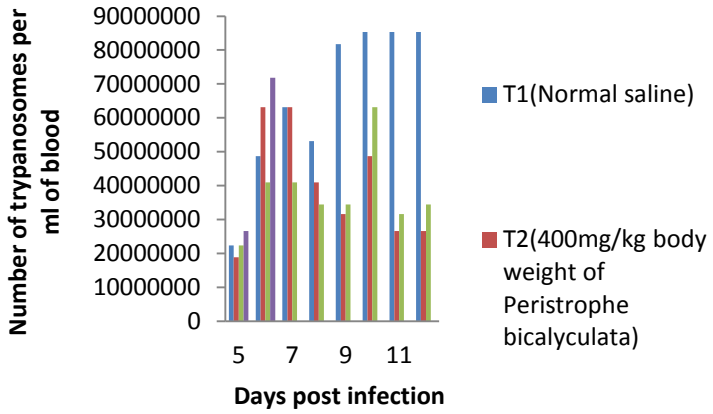
Secondary metabolites	Qualitative test	Quantitative test
Oxalate	+	0.12mg/100g
Phytate	+	59.5mg/kg
Saponins	+	2%
Cyanogenic glycosides	+	960mg/100g
Alkaloids	+	15%
Flavonoids	+	0.42%

The result shown above is in line with that reported by [8] except for the presence of cyanogenic glycosides. The absence of anthraquinones is in agreement with the report of [1] who reported similar observations. These variations in the composition of the secondary metabolites could be attributed to the different solvent used in the extraction process. These secondary metabolites act in synergy thereby contributing in varying degrees to their curative properties of varying ailments [24]. For instance, flavonoids have been shown to have antibacterial, anti-inflammatory, anti-allergic, antiviral, antineoplastic activity [2]. Alkaloids contributes to plants fitness for survival, they often have pharmaceutical effects and are used as medication and recreational drugs [21]. Many of these functions have been linked to their known functions as strong anti-oxidant, free radical scavengers and metal chellators [18].

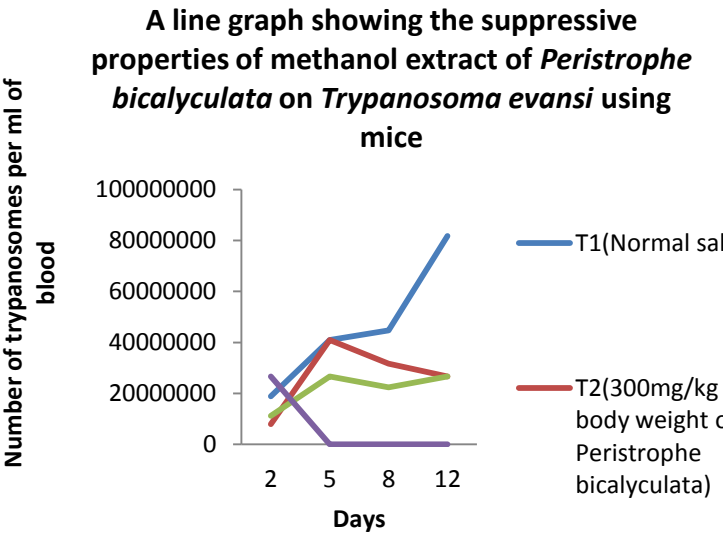
The lethal dosage test showed the LD_{50} was less than 1500mg/kg body weight. This means that the extract is safe for the bioassay.

The curative bioassay of the methanol extract of *Peristrophe bicalyculata* reveals the action of the plant against *Trypanosoma evansi* to be significant at ($P < 0.05$). However, the standard drug used for the bioassay was more effective as presented in the figure below.

Figure 1: A chart showing the curative properties of the methanol extract of *Peristrophe bicalyculata* on mice infected with *Trypanosoma evansi*



The suppressive bioassay using the extract of Peristrophe bicalyculata in mice infected with trypanosoma evansi shows a significant ($P < 0.05$) progressive suppressive effect. However, the group treated with trypanosoma evansi was more effective as presented in the table below.



The suppressive effect was observed to be dose-dependent. The synergistic effects of the secondary metabolites found in the methanol extract interplay to bring about effective suppressive and curative properties against some ailments as reported by [25]. The group without treated with normal saline show a progressive increase in the level of parasitaemia while the group treated with the standard drug showed a complete eradication of the parasite in the host.

In conclusion, it is hoped that *Peristrophe bicalyculata* has certain bioactive principles and medicinal values against *Trypanosoma evansi*. The chemical compounds present in this plant form the characteristic nature of its medicinal uses. Thus, it leads to the establishment of new compounds which is used to formulate more potent antitrypanosomal drugs of natural origin with fewer side effects.

REFERENCES

[1]. Abdulazeez, A. M., Awasun, C. A., Dogo, Y. S., Abiayi, P. N. (2010). Effect of *Peristrophe bicalyculata* on blood pressure, kidney and liver functions of the two kidney one clip (2K1C) hypertensive Rats.

[2]. Alan L. and Miller N. D. (1996). Antioxidant flavonoids: structure, function and chemical usage. Alternative Medicine Revolution. 1:103-111

[3]. Boham B. A., Kocipai-Abyazan R. (1974). Flavonoids and condensed tannins from leaves of *Hawaiian vaccinium vaticulatum* and *V. calycinium*. *Pacific science*. 48: 458-463

[4]. Cadioli, F. A., Marques, L. C., Machado, R. Z., Alessi, A. C., Aquino, L. P. C. T., Barnade, P. A. (2006). Experimental *Trypanosome evansi* infection in donkeys' haematological, biochemical and hispathological changes. *Medical Veterinary Zootechnology*, 58 (5): 746-756

[5]. Dermarderosian, A. and Liberti, L. E. (1988). Natural products medicine: A scientific guide to foods, drugs and cosmetics, 1st Edition, Philadelphia, USA: Geo. F. Stickley Co., Pp 46, 61, 63-66

[6]. Lorke, D. (1983). A New Approach to Practical Acute toxicity Testing. *Archives of toxicity*, 54: 275-287

[7]. Dwivedi S. (2008). Ethnomedicinal uses of some plants species by ethnic and rural peoples of indore district of Madhya Pradesh India (online). Available from: http://pharmainfo.net

[8]. Esenovo, G. J., Sam, S. M. Bala, D. N., Ekpo, B. A. and Edung, E. M. (2010). Phytochemical screening and the haematological effect of *Peristrophe bicalyculata*.

[9]. Evans, W.C. (1996). *Trease and Evans' Pharmacognosy*, 14th Edition. W.B. Saunders Ltd., Pp.105-107, 415, 443, 546, 766.

[10]. Fidock, D.A., Rosenthal, P.J., Croft, S.L., Brun, R., Nwaka S. (2004). Antimalarial drug discovery: Efficacy Models for Compound Screening Supplementary Documents. *Trends in Parasitology.* **15:** 19-29

[11]. Harbourne J. B. (1973). *Phytochemical methods*, London. Chapman and Hall, Ltd. Pp 49-188

[12]. Herbert, W. J. and Lumsden, W. H. R. (1976). *Trypanosoma brucei*: A Rapid "Matching" Method for Estimating the Host's Parasitaemia. *Experimental Biology*. Pp. 427-431

[13]. Luckins, A. G. (1998). Epidemiology of surra unanswered question. *Journal of protozoology research*, 8: 106-119

[14]. Mihret, A. and Mano, G. (2007). Bovine trypanosomosis in three districts of east Gojjan zone bordering the blue, Nile River in Ethiopia. Journal of infected developing countries, 1(3): 321-325

[15]. Molumba, K. (2003). Socio-economic and agricultural factors in the research and control of Trypanosomiasis. PAAT technical and scientific series4. FAO, Rome.

[16]. Obadoni B. O., Ochuko P. O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta states of Nigeria. *Global Journal of pure applied sciences* 8b: 203-208.

[17]. Onwuka, B. (2005). Food analysis and instrumentation theory and practical. Naphtali prints, lagos, Nigeria. P. 148

[18]. Omale, J. Omajali, J. B. (2010). Studies on some nutritional characteristics of the fruits and leaf of saba florida (benth) from Ibaji forest. *International journal of nutrition metabolism*. Volume 2 (1): 11-26

[19]. Philip K., Sri Nurestri A. M., Wirakarnain S., Sim K. S. and Saravana K. (2009). Antibacterial activity of some medicinal plants from Malaysia. *American Journal of Applied sciences* **6:** 1613-1617

[20]. Rafauf R. (1970). Handbook of alkaloids and alkaloid containing plants. Wiley, New York USA: Academic Press, Pg 6.

[21]. Roger M. F. and Wink M. (1998). Alkaloids: Biochemistry, ecology and medicine applications. Plenum press, pp. 2-3.

[22]. Samdi, S. M., Abenga, J. N., Attahir, A., Haruna M. K., Wayo, B. M., Fajinmi, A. O., Sumayin, H. M., Usman, A. O., Hussaina, J. Z., Muhammed H., Yarnap J. E., Ovbagbedia R. P. and Abdullahi R. A. (2010). Impact of trypanosomiasis on food security in Nigeria: A Review in *International Journal of Animal and Veterinary Advances* 2(2): 47-50.

[23]. Seifert, H. S. H. (1996). Tropical animal Kluwer academic publishers. Dorrech Bonston, London, Pp: 53-260

[24]. Sofowora, E. S. (1993). Medicinal plants and traditional medicine in Africa. Spectrum books Ltd, Ibadan, pp. 55-71.

[25]. Janakiraman, N., Sahaya, S. S., Johnson, M. (2010). Anti-bacterial studies on *Peristrophe bicalyculata* (Retz.) Nees. *Asian Pacific Journal of Tropical Medicine*

[26]. World Health Organization (1998). Control and surveillance ofAfrican Trypanosomiasis. A report of WHO expert committee. WHO technical report series No. 881.

[27]. Womack, S. (2001). *Trypanosoma evansi* in Horses, University of Georgia Pp. 1-2.

[28]. Hoare, C. A. (1972). *Trypanosoma evansi*: In trypanosomes of mammals, a zoological monograph. Blackwell scientific publication Pp. 555-593

[29]. Luckins A. G. (2002). Surra. Office International Epizootics (OIE) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Pp. 33

[30]. Goday, V. G., Miller, D. M., Russo, T. A., and Malamy, M. H. (1993). A role for bacteriodes fragilis neuraminidase in bacterial growth in two model systems. *International Journal of Infection and immunlogy*, 61: 4415-4426

[31]. Holland, W. G. (2003). The diagnosis of *Trypanosoma evansi* and its immunosuppressive effect in water buffaloes and pigs, proefschrift, Pp 3-16

[32]. World Health Organisation (2012). Human African Trypanosomiasis (sleeping sickness).P. 445