

Comparative Phytochemical and Anti-Trypanosomal Efficacy of Stem Bark and Leaf of *Erythrina Senegalensis*

Amadi¹ .O.D., Prof. J.Yisa² ., Dr. Mann .A³ ., Prof. E.O. Ogbadoyi⁴ ., Busari. M⁵ .

Department of chemistry Federal University of Technology Minna Niger Sate Nigeria,
Center for Genetic Engineering and Biotechnology, Federal University of Technology Minna

Abstract: *Erythrina senegalensis* is a common medicinal plant found in Nigeria and some other parts of African. A quantitative and qualitative phytochemical analysis of the stem bark and leaves were carried out and compared. Likewise, the bioactivity test of the crude extracts of both sample were carried out against *Trypanosoma brucei*. The results revealed the presence of alkaloids, tannins, flavonoids, saponins, phenols, steroids and terpenoids in both the stem bark and leaves of *E. senegalensis*. Higher values of alkaloids (6.94±1.68mg/100g), tannins (8.04±1.78mg/100g), flavonoids (12.00±9.04mg/100g), saponins (5.29±1.27mg/100g), steroids (3.00±3.20mg/100g) and glycoside (0.91±0.97mg/100g) was observed in the stem bark compared to the leaves with values of 3.38±2.45mg/100g for alkaloids, 4.07±1.78mg/100g tannins, 7.63±3.45mg/100g flavonoids, 3.61±0.09mg/100g saponins, 2.01±2.00mg/100g steroids and 0.82±0.80mg/100g glycoside. Although total phenols were found to be high in the leaves (10.06±8.01mg/100g) compared to the stem bark (4.77±2.02). As for the anti-trypanosomal activities, the dose 300mg/kg bodyweight of the stem bark extract showed more activities against *T. brucei* as the parasites was cleared within 17th days of post treatment. However, there was no pronounced activity recorded when various doses of the leaf extract were administered. Therefore, the stem bark extract of *Erythrina senegalensis* contain higher quantities of phytochemicals with high efficacy of anti-trypanosomal activities when compared to the leaf extract.

Key words: *Erythrina Senegalensis*, Stem bark, Leaves, Phytochemical, *Trypanosoma brucei*.

I. INTRODUCTION

African trypanosomiasis is one of major factor retarding the growth of the livestock industry. The disease has undergone a dramatic and devastating resurgence in recent years especially in sub-Saharan Africa (Welburn *et al.*, 2001) and thus an important priority for biomedical and public agencies, agricultural sector and the scientific community (Aksoy, 2003).

In Nigeria it has become one of the economically most important diseases of farm animals affecting livestock health and economy even in some tropical countries. The current methods of controlling the disease include the use of trypanotolerant cattle vector control and drug therapy. Four drugs (suramin, pentamidine, melarsoprol and eflornithine) are currently available to treat trypanosomiasis with only melarsoprol and eflornithine being effective against the meningoencephalitis that develops in the late stages of the disease. However, due to side effect, toxicity and high resistant of these chemical drug, there is a need to search for newer drug from plants that can control and cure this epidemical disease.

In Nigeria the indigenous people are exploiting a variety of herbs for effective curing of various ailments. Many researchers targeted finding new anti-trypanosomal agents to combat the trypanosomiasis by screening extracts of African plants. *Erythrina senegalensis* DC (Fabaceae) is a thorny shrub or small tree with common names that include coral tree (English) and minjirya (Hausa, Nigeria). The stem and root bark are used by traditional healers to cure wide range of illnesses (Togola *et al.*, 2008; Kone *et al.*, 2011). The leaves are used to treat malaria, gastrointestinal disorders, fever, dizziness, secondary sterility, diarrhea, jaundice, nose bleeding and pain (Togola *et al.*, 2008).

The stem bark extract has been shown to have antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, (Doughari, 2010).

In this study, the activity of stem bark and leave of *Erythrina senegalensis* crude extract against *trypanosome brucei* was investigated and evaluated for better solution to the disease.

II. MATERIALS AND METHODS MATERIALS

Plant materials

Fresh leaves and stem bark of *Erythrina senegalensis* was collected from Bida Niger Sate Nigeria. The plant was identified and confirmed in Forest Research Institute of Ibadan, Nigeria. The samples were washed and dried at room temperature. The dried samples were ground into fine powder using electric grinder and stored in an airtight container for further analysis.

Experimental animals

Albino mice, used for screening, were purchased from the Biochemistry and Chemotherapy division of the National Institute for Trypanosomiasis and Onchocerciasis Research, Vom, Plateau State, Nigeria. The animals were acclimatized in the Department of Biochemistry laboratory, Federal University of Technology, Minna for minimum of two weeks prior to study. All experiments involving the animals were conducted in compliance with the internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care guidelines on animal use protocol review (1997) and as also described by Adam *et al.* (2010).

Trypanosomabrucei

Trypanosomabrucei was obtained from the National Institute for Trypanosomiasis and Onchocerciasis Research (NITER), Kaduna, Kaduna State, Nigeria and subsequently maintained in the laboratory of the Biochemistry Department, Federal University of Technology, Minna, Niger State, Nigeria, by serial passage in mice.

III. METHODS

Preparation of plant extract

The powder samples were extracted with methanol by cold maceration for 48 hrs to obtain the methanol extract. The extract was filtered using cheese cloth and solvent was removed using rotary evaporator under reduced pressure. The dry extract was transferred into a sterile sample bottle and store in a refrigerator until required for use.

Phytochemical Screening

The methanolic extracts of the samples were used for the phytochemical screening. Tannins and Steroids was determined by the methods of Hassan *et al.*, (2004), Flavonoid, Saponins, Alkaloids, total phenol was determined following the methods of Edeoga *et al.*, (2005), while Anthraquinones and Glycoside was done by the methods of Abbas *et al.*, (2012); Amir *et al.*, (2011).

Phytochemical quantitative analysis

The secondary metabolites were determined quantitatively using standard method of analysis as describe by some researchers. Tannins, Total phenol by the methods of Edeoga *et al.*, (2005), Oxalate, Phytate as describe by Antia *et al.*, (2006), Flavonoid, Steroid (Anhwange *et al.*, 2006). Saponins by the methods of Obadoni and Ochuko, (2001).

Infection of animals

The animals were inoculated using the method described by Ogbadoy *et al.* (2007). Blood from a highly infected mouse was obtained by cardiac puncture and collected with EDTA-coated syringe. The blood was appropriately diluted with physiological saline to serve as inoculums. Healthy mice of weight range 25-35 g were infected intraperitoneally with 0.1 ml of the inoculums containing about 1×10^3 trypanosomes.

Preparation of stock solution of extract

The stock solution was prepared just before use by dissolving 1g of the aqueous extract in 10ml physiological saline.

Administration of crude extract and monitoring the course of parasitemia

“Rapid Matching” method of Herbert and Lumsden (1976); as described by Atawodi *et al* 2003 was used to estimate parasite in the blood of the infected animals. The method involves a matching technique where the microscopic fields were compared with a range of standard logarithm values. Counting of parasites per field in blood approximately diluted with physiological saline. A drop of blood was obtained on a slide by pinching the tip of the pre-sterilized tail with a sterile needle, immediately covered with a cover slip and the wet mount observed under the microscope at X40 magnification. The number of trypanosomes per microscopic field was compared with the table of logarithmic values. The logarithmic values which matched the microscopic observation were then converted to antilogarithm, from where the absolute number of trypanosomes per ml of blood was obtained.

Treatment and evaluation

The crude extracts of leaf and stern bark of *E.senegalensis* and administered at doses of 50mg/kgbw, 100mg/kgbw, 300mg/kgbw, 500mg/kgbw representing group A-D respectively, with each consisting of three infected mice. The extracts were administered intraperitoneally for 14 consecutive days. Group E involved the uninfected mice but treated with 1000mg/kgbw of each extracts in order to affirm the safety of the extract. The group F was treated with a single dose of 3.5mg/kgbw of standard drug (Berenil), while group G served as negative control as they were infected but not treated.

Statistical Analysis

Data were analyzed using Analysis of variance (ANOVA) using SPSS-computer package. In all cases the level of statistical significance was considered at ($P < 0.05$).

IV. RESULTS

Table 1: Phytochemical Screening result of the samples

| Phytochemicals | Leaf | Stem bark |
|----------------|------|-----------|
| Alkaloids | + | ++ |
| Tannins | + | ++ |
| Saponins | + | + |
| Flavonoids | + | + |
| Phenols | + | + |
| Steroids | + | + |
| Terpenoids | + | + |
| Anthraquinone | + | - |

Key: ++ very strong positive + Positive - Negative

The phytochemical screening as reported in Table 1 review the presence of all the secondary metabolite in both samples, it was also observed that alkaloids and tannins are much in the stem bark compared to that of the leaves, while anthraquinones was not found in the stem bark but present in the leaf.

Table 2: Phytochemical quantitative result of the samples

| Phytochemicals mg/100g | Leaves | Stem Bark |
|------------------------|--|------------------------|
| Alkaloids | 3.38±2.45 ^b 6.94±1.68 ^a | |
| Tannins | 4.07±1.78 ^b 8.04±1.78 ^a | |
| Saponins | 3.61±0.529±1.27 ^b | |
| Phenols | 10.06±8.01 ^b 4.77±2.02 ^a | |
| Flavonoids | 7.63±3.45 ^b 12.00±9.04 ^a | |
| Steroids | 2.01±2.00 ^b | 3.00±3.20 ^a |
| Glycosides | 0.82±0.80 ^b | 0.91±0.97 ^b |
| Oxalates | 1.86±0.54 ^a 1.14±0.23 ^b | |

Values are Mean ± SD, n = 3. Values with different alphabetical superscripts at the same roll are significantly different at p < 0.05.

Table 2 shows quantitatively the amount of the phytochemical compounds present in both samples and it was observed that both the leaves and stem bark of *E. senegalensis* contains appreciable amount of the secondary metabolites. Although, some of the phytochemicals in the stem bark extract are significantly higher than the leaf extract

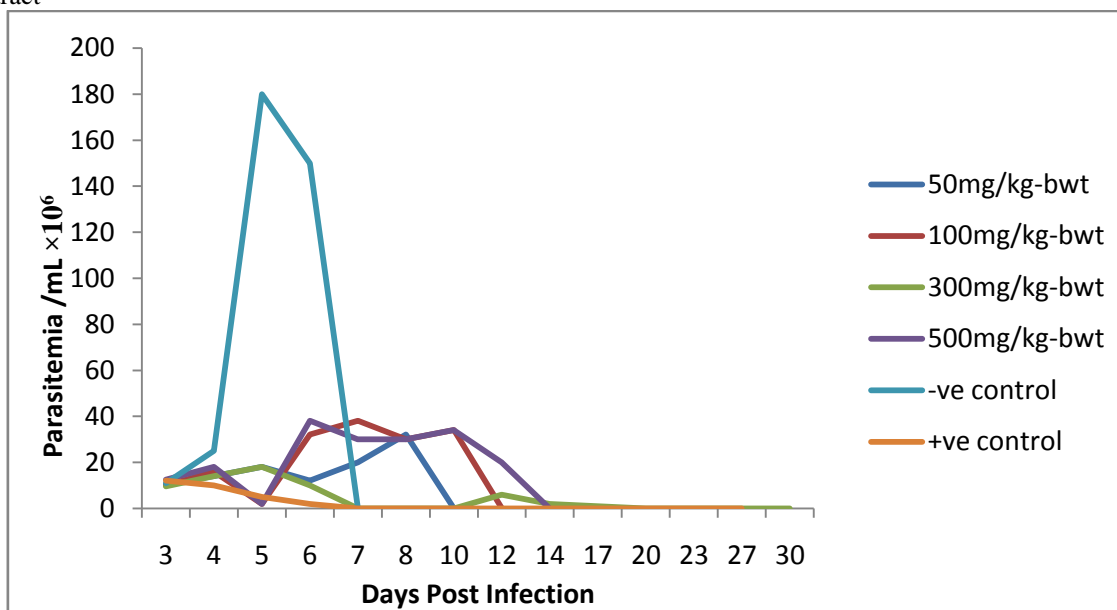


Fig 1: Effect of crude extract of *Erythrina senegalensis* stem bark on mice infected with *T. brucei*

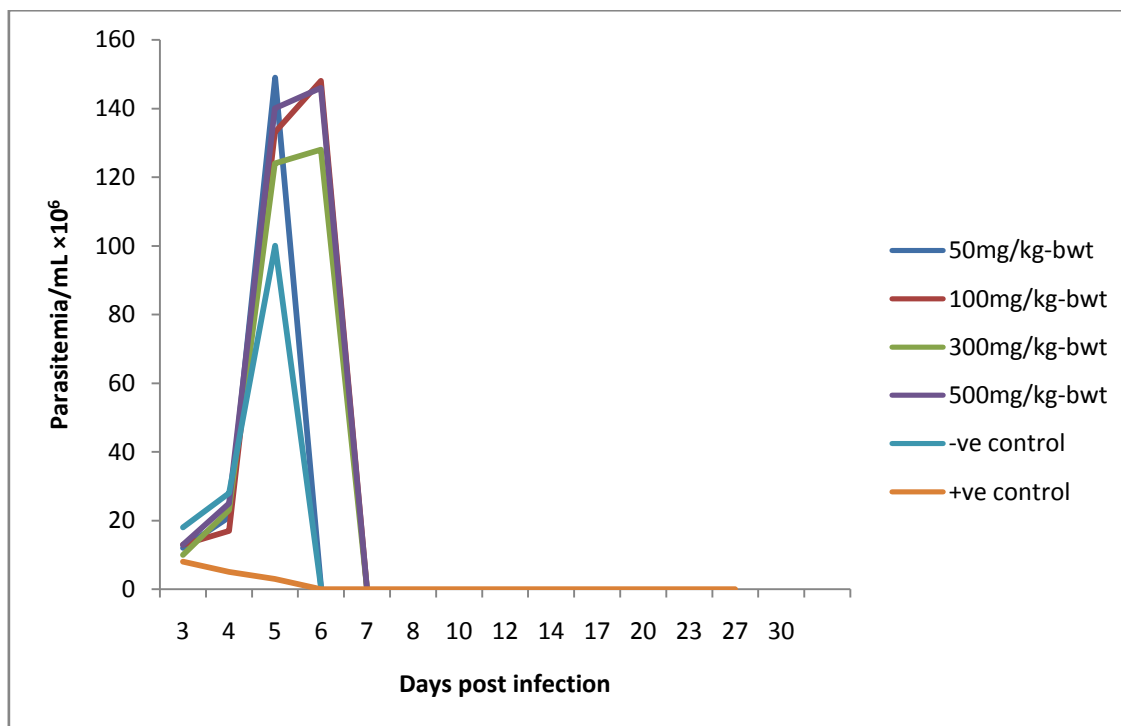


Fig 2: Effect of *E.senegalensis* leaf crude extract on mice infected with *T. brucei*

The anti-trypanosomal activities of the stem bark extract is highly pronounced compared to that of leaf extract (Fig. 1 and 2). The 300mg/kgbw group which is the most effective dose live up to 27days while all groups in the leaf extract irrespective of the doses treated live up to 7days

V. DISCUSSIONS

The values are comparable to the one reported in flaking bark of *Commiphorakerstingii*(Mannet *et al.*, 2013). But the stem bark of *Erythrinasenegalensis* contains more values of Flavonoids, Tannins, Alkaloids, Saponins and total Phenolic compounds compared to the leaves. The biological function of alkaloids and their derivatives are very important and are used in analgesic, antispasmodic and bactericidal activities (Iqbalet *al.*, 2011).The functions of flavonoids include protection against inflammatory allergies, free radical scavenging, ulcers, microbes, hepatoxins, platelets aggregation, viruses and tumors (Okwu and Omodamiro, 2005; Okwu and Josiah, 2006). Tannins decrease the bacterial proliferation by blocking key enzymes at microbial metabolism. Saponin is useful in medicine and pharmaceutical industry due to its foaming ability that produces frothy effect. (Okwu.,2003). It is beneficial in reducing heart disease by binding with plasma membrane and cholesterol.Thus flavonoids, alkaloids and tannins are found to have anti-trypanosomal activity.

The activities of *Erythrinasenegalensis* stem bark crude extract against the parasite was reported in Fig 1. All the animal in the group administered 50mg/kgbw died within ten days(10th days), and those in 100mg/kgbw died on the 12th day with increasing number of parasite, this may be that at these doses the chemical constituents present in the extracts are not enough to show strong activity on the parasite. The animals receiving 300mg/kgbw survived up to thirty days with the parasite cleared at the 17th day. This result is comparable to the one recorded in *Khayasenegalensis*stem bark as the parasite was shown to be cleared within 9-10th days of treatment (Umar *et al.*, 2010). The group administered 500mg/kg-bwt which is the highest dose survived up to 14 days and then died. This may be due to the hyperactivity marked by reduction in aggressiveness, locomotion, depressive effect on the nervous system and pain sensitivity induced by high dose of the extract on the animal which then reduce the efficacy of the immune system thereby causing the parasite to form resistant to the extract (Atawodiet *al.*, 2002).

Fig 2 shows the activity of *Erythrinasenegalensis* leaves crude extract against *trypanosome brucei* parasite. From the result, it was observed that all the animals in every group (50mg/kgbw, 100mg/kgbw, 300mg/kgbw, 500mg/kgbw) died within 7 days with increasing number of parasite.The failure of the crude extract of the leaves of *E. senegalensis* to show any trypanocidal action depicts that the anti-trypanosomes are lacking in the leaves.

VI. CONCLUSION

It was shown from this study that *E. senegalensis* stem bark possess more antitrypanosomal activities when compare to the leaf of the same plant. As such, the stem bark of *E. senegalensis* can serve as a source of lead compound that may lead tonew anti-trypanosomal drug.

REFERENCES

- [1]. Abbas .S.A, Rana .M.,Shahid .N., Mahmood . H., Hussain .M.(2012). Chemical evaluation of weed seeds mixed with wheat grains at harvest. *Journal of Ani. & plant Sci* 22(2)
- [2]. Adamu, Y.K., Aderonke, A.S. and Ogbadoyi, E.O. (2010) Therapeutic effects of *Annonasenegalensis* stem bark extracts in experimental African trypanosomiasis. *Int. J. Health Res.*, 3(1): 45-49.
- [3]. Amir .M.K, Rizwana .A.Q, Faizen .U, Syed .A.G, Muhammed .Y.L., (2011). Phytochemical
- [4]. analysis of selected medicinal plants of Margalla Hill and surroundings. *Journal of medi. Plant Research* vol. 5(25) 6017-6023
- [5]. Anhwange, B.A; Ajibola, V.O & Okibe, F.G; (2006). Nutritive value & Anti- Nutritional factors in *Hibiscus Sabdaripa*, *Journal of fisheries international* 1(2-4): 73-76.
- [6]. Antia, B.S., Akpan, E.J., Okon P.A & Umoren, I.U, (2006). Nutrition & nutritive Evaluation of sweet potatoes (pomoebabatas) Leaves. *Pakistan Journal nutrition* 5:166-168
- [7]. Togola . A, Ingvild . A, Annette .T, Drissa .D & Berit .S.P ;(2008). Ethnopharmacological
- [8]. uses of *Erythrasenegalensis*: a comparison of three areas in Mali, and a link between traditional knowledge and modern biological science. *J.Ethno.Ethnomed.* 1-9.
- [9]. Aksoy S (2003). Control of tsetse flies and trypanosomes using molecular genetics. *Vet. Parasitol.*, 115(2):125-145
- [10]. Atawodi, S.E., Ameh, D.A., Ibrahim, S., Andrew, J.N., Nze-libe, H.C., Onyike, E., Anigo,
- [11]. K.M., Abu, E.A., James, D.B., Njoku, G.C., Sallau, A.B., (2002), Indigenous knowledge system for treatment of trypanosomiasis in Kaduna state of Nigeria. *Journal of Ethnopharmacol.*, 79, 279-282
- [12]. Doughari J.H; (2010). Evaluation of antimicrobial potentials of stem bark extracts of *Erythrasenegalensis* DC. *Afr.J.Microbiol.Res.* 4 (17): 1836- 1841
- [13]. Edeoga, H.O., Okwu, D.E., Mbaebie, B.O. (2005). Phytochemical constituents of some
- [14]. Nigerian medicinal plants. *African Journal of Biotechnology*, 4:685-688
- [15]. Hassan, M.M; Oyewale, A.O; Ampitan, J.O; Abdullahi M.S Okonkwo, E.M; (2004). Preliminary photochemical and anti-bacterial investigation of crude extracts of the root bark of *Detarium microcapum*. *Journal of chemical society of Nigeria* vol 29:1
- [16]. Herbert, W.J. and Lumsden, W.H., (1976). *Trypanosomabrucei*: A rapid 'matching' method for estimating the host's parasitemia. *Exp. Parasitol.*, 40: 427-431.
- [17]. Kone W.M, Solange K.E, & Dosso M.(2011). Assessing Sub-saharian *Erythrina* for efficacy traditional uses, biological activities and phytochemistry. *Pak.J.Biological Sci.* 14(10):560-571
- [18]. Mann, A, Ogbadoyi .E.O., (2012). Evaluation of medicinal plants from Nupeland for their *in vivo* Antitrypanosomal activity. *American journal of Biochemistry* 2(1)1-6
- [19]. Mann .A, Andrew .O.L, Fadipe .L.A, Ibikunle .G.F, Yisa .J, Ogbadoyi .E.O (2013), Phytochemical screening of the extract of the Flaking bark of *Commiphora kirstingii* and its GC-MS analysis. Proceeding of 36th Annual International Conference of Chemical Society of Nigeria
- [20]. Obadoni .B.O and Ochuko P.O. (2001). Phytochemical studies and comparative efficacy of the crude extract of some homeostate plants in Edo and Delta States Nigeria. *Global Journal of pure Sci.* 8:208.
- [21]. Ogbadoyi, E.O., Akinsunbo, O.A., Theophilus, Z.A. and Okogun, J.I. (2007). *In vivo* trypanocidal activity of *Annonasenegalensis* Pers. Leaf against *Trypanosomabrucei* *brucei*. *J. Ethnopharmacol.*, 112: 85-89.
- [22]. Okwu .D.E., Josiah .C., (2006). Evaluation of the chemical composition of two Nigeria medicinal plants. *Afr. Journal of BioTec* 5(4) 357-361
- [23]. Umar. I.A, Ibrahim .M.A, Fari .N.A, Isah .S, Balogun .D.A., (2010) *In- Vitro* and *-Vivo antitrypanosomaevansi* activities of extract from different parts of *Khaya senegalensis*. *Journal of Cell & Animal Biology.* Vol4(6) pp 91-95
- [24]. Welburn SC, Coleman PG, Fevre E, Mandlin I (2001). Sleeping sickness- a tale of two diseases. *Trends Parasitol.*, 17: 19-24.