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: Erythinasenegalensis 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 terpemoids 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±0.9mg/100gsaponins, 2.01±2.00mg/100gsteroids 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 Erythinasenegalensis contain higher quantities of phytochemicals with high efficacy of anti-trypanosomal activities when compared to the leaf extract.

Key words: ErythrinaSenegalensis, Stem bark, Leaves, Phytochemical, Trypanosomabrucei.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 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 epidemic 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. Erythinasenegalensis 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 leaf of Erythinasenegalensis crude extract against trypanosome brucie was investigated and evaluated for better solution to the disease.

II. MATERIALS AND METHODS MATERIALS

Plant materials

Fresh leaves and stem bark of Erythinasenegalensis 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.
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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).

Trypanosoma brucei
Trypanosoma brucei 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

Infection of animals
The animals were inoculated using the method described by Ogbadoyi 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 stem 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 intraperitoneallyfor 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

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Leaf</th>
<th>Stem bark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Leaves</th>
<th>Stem Bark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>3.38±2.45</td>
<td>6.94±1.68</td>
</tr>
<tr>
<td>Tannins</td>
<td>4.07±1.78</td>
<td>8.04±1.78</td>
</tr>
<tr>
<td>Saponins</td>
<td>3.61±0.529</td>
<td>1.27b</td>
</tr>
<tr>
<td>Phenols</td>
<td>10.06±8.01</td>
<td>4.77±2.02</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>7.63±3.45</td>
<td>12.00±9.04</td>
</tr>
<tr>
<td>Steroids</td>
<td>2.01±2.00</td>
<td>3.00±3.20</td>
</tr>
<tr>
<td>Glycosides</td>
<td>0.82±0.80</td>
<td>0.91±0.97</td>
</tr>
<tr>
<td>Oxalates</td>
<td>1.86±0.54</td>
<td>1.14±0.23</td>
</tr>
</tbody>
</table>

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.

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![Graph showing the effect of crude extract of *Erythrina senegalensis* stem bark on mice infected with *T. brucei*](image)

**Fig 1: Effect of crude extract of *Erythrina senegalensis* stem bark on mice infected with *T. brucei***
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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 27 days while all groups in the leaf extract irrespective of the doses treated live up to 7 days.

V. DISCUSSIONS

The values are comparable to the one reported in flaking bark of *Commiphora kerstingii* (Mann et al., 2013). But the stem bark of *Erythrina senegalensis* 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 et 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 *Erythrina senegalensis* 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 day), 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 *Khaya senegalensis* 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 et al., 2002).

Fig 2 shows the activity of *Erythrina senegalensis* 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 antitypansomal 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 anttrypanosomal drug.

REFERENCES