Effect of Biochemical Constituents of *Detarium senegalensis* (root) and *Eucalyptus camaldulensis* (leaf) mixed Methanolic Extracts on *Trypanosome brucei-brucei* using Mice Model.

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ABSTRACT
Medicinal plants are considered to be of great importance to human because they contain active constituents which can be used to treat different diseases. Methanol extracts of *Detarium senegalensis*(root) and *Eucalyptus camaldulensis* (leaf) were screened for trypanocidal activity. Mice infected with *Trypanosoma brucei-brucei* were treated with methanol extracts of root of D. *senegalensis* and leaf of *E. camaldulensis*. The combination of the extracts in different ratios (1:1,2:1,3:1,1:0 and 0:1) were administered intraperitoneally at a combined dose level of 200mg/Kg body weight/day respectively. Parasitemia was found to increase in all animals with a short relapse and an increase that proceeded till death, except for the ones treated with 2:1(group B) and 0:1(group E) which survived for 26days and 30days respectively. In the repeat screening with 2:1 and 0:1 doses, parasites were cleared from the blood but resurfaced two days later while treatment was on. Only animals treated with 0:1 dose survived for 19 days. This study indicates the possibility of exploiting *Eucalyptus* ***camaldulens*** for the treatment of Trypanosomiasis since its activity was more pronounced.
Key words**: Diseases, Medicinal plants, Parasitemia and Relaps**

INTRODUCTION
Nigeria's biodiversity is rich in medicinal plants. Over 25% of our common medicines contain at least some compounds obtained from plants (Mann and Ogbadoyi, 2012). The World Health Organization reported that 70–90% of the world's population relies chiefly on traditional medicine and a major part of the traditional therapies involve the use of plant extracts or their active constituents (Kumar and Navaratnam, 2013). The local use of natural plants as primary health remedies is due to their pharmacological properties (Robinson et al., 2011). Many plant extracts owe their potency to the presence of metabolites. These metabolites are usually found in various parts of the plants like roots, leaf, shoots and bark. Many plants have therefore become sources of important drugs and as such the pharmaceutical industries have exploited traditional medicine as a source of bioactive agents that can be used in the preparation of synthetic medicine (Kennedy and Wightman, 2011). Natural products play important roles in drug discovery and development process, particularly in the field of infectious diseases, where 75% of these drugs are of natural origin (Song et al., 2014).

Trypanosomiasis, a disease of major importance in human and animals has continued to threaten human health and economical development (Grant et al., 2015). T. b. gambiense and T. b. rhodesiense as the etiological agents of trypanosomiasis affect millions of people in sub-Saharan Africa and are responsible for the death of about half a million patients per year (Rodgers et al., 2011)

Therefore, the present study aimed to investigate the *in vivo* antitrypanosomal activity of *Detarium senegalensis* (root) and *Eucalyptus camaldulensis* (leaf) mix methanolic extracts

MATERIALS AND METHODS
Experimental animals

The experimental animals used were healthy adult albino mice. The mice were obtained from VOM, Plateau State. They were kept in safe cages and were adequately fed. The underlying saw dust in the cage containing the mice were changed regularly and allowed free access to water.

Trypanosomes

Trypanosome bruceibrucei was obtained from the Biochemistry and Chemotherapy Division, National Institute for Trypanosomiasis Research (NITR) VOM, Plateau State.

Plant materials

Eucalyptus camaldulensis leaf was obtained from Old Secretariat, Minna, Niger State. The picture of the tree is shown in plate 1. The leaf were rinsed, air dried and grounded into powder using pestle and mortar.

The *D. senegalensis* root was obtained from Suleja, Niger State. The root was rinsed, air dried and grounded into fine granules using mortar and pestle as well.

Preparation of extracts

***Eucalyptus camaldulensis* Leaf**

150 mg of the *Eucalyptus* leaf was grounded and weighed using a measuring cylinder, put in a beaker and 300ml of petroleum ether was added. This was left for 24 hours and sieved with a cheese cloth to obtain the leaf powder back. The leaf was dried and reweighed again. The petroleum ether was used to remove the fat components present in the leaf. Afterwards, 100g of the dried leaf was reweighed and 400 mL of absolute methanol was added into distillation flask and extracted at 65 ^{o}C by reflux heating for 2hours. After 2 hours, the solution was transferred into a rotary evaporator flask to evaporation until a considerable amount of methanol was obtained. The filtrate was evaporated to dryness with the aid of steam bath and the extract was obtained. The dried extract was transferred into sample bottles and stored in a refrigerator.

***Detariumsenegalensis* Root**

100 g of grounded *D. senegalensis* root was weighed using a weighing balance, put into a beaker and 300 ml of petroleum ether was added also to remove fat components. The beaker was covered and left for 24 hours. The solution was sieved and root dried again. 50 g of *D. senegalensis* was reweighed afterwards, and extracted at 65 ^{o}C by reflux heating using 300ml of absolute methanol for 2 hours. The solution was into a rotary evaporator and evaporated until a considerable amount of methanol (pure) has been extracted.

The extract was obtained when the filtrate was evaporated to dryness using a steam bath. The dried extract was transferred into a sample bottle and kept in the refrigerator for storage.

Experimental design

The animals were divided into two (2) phases. The phase one of the experiment consists of six (6) groups while the second phase consists of five (5) groups.

Phase one (1) – Six groups, each group consisting of three mice.

Group A Infected, treated with equal ratio of *D. senegalensis* and *E.camaldulensis*1:1
Group B Infected, treated with *D. senegalensis and E. camaldulensis*2:1
Group C Infected, treated with *D. senegalensis and E. camaldulensis*3:1
Group D Infected, treated with *D. senegalansis*
Group E Infected, treated with *E. camaldulensis*

Group F Infected not treated (control)
Phase two (2), there are five groups
Group A Infected, treated with *D.senegalensis* and *E camaldulensis* in ratio 2:1
Group B Infected, treated with *E. camaldulensis*
Group C Infected, treated with Berenil
Group D Infected not treated
Group E Infected, not treated
All groups in both phases are treated with 200 mg/kgbw. The extracts of *Detarium senegalensis* and *Eucalyptus camaldulensis* were mixed in different ratios for different groups.

Infections of animals
All the experimental animals except Group E of phase two were infected (inoculated) intraperitonially with 0.1ml of diluted blood containing $1x10^3$ *T. bruceibrucei*. Using an anticoagulant with 1ml syringe,

Checking and monitoring of the parasite level
Blood smears from animals were observed under the microscope starting from the second day post infection. This is to confirm the presence of parasites and to know the number of parasites per field.
The anti-trypanosomal activity of the methanolic extract was monitored by observation and counting the number of parasites in the blood smear taken from each animal under the microscope. This was done every three days. The blood smear was prepared by piercing the tail of the experimental animal and collecting a drop of the blood on a slide and covered immediately with cover slip. The number of parasites present in the blood smears was counted for 6 different fields and the average parasites count per field was recorded (Herbert and Lumsden, 1946).

Extract Administration and Dosage
The dose of the extracts was prepared in different ratio/dosage as follows;

Groups	**Ratio**		**Dosage (mg/kgbw)**
	D. senegalensis*: *E. camadulensis		
A	1	1	200
B	2	1	200
C	3	1	200

D	1	0	200
E	0	1	200
F	Negative control		

Groups	**Ratio**	**Dosage mg/kgbw**
	D. senegalensis : E. camaldulensis	
A	2 : 1	200
B	0 : 1	200
C	Berenil	infected, treated with Berenil
D	Negative control	Infected, treated
E	Positive control	Not infected, not treated

Methods for Phytochemical Screening of Plant Extract

Test for Anthraquinones: 0.5g of plant extract was dissolved in 10ml of Benzene, it was shaken and filtered. To the filtrate, 5ml 0f 10% NH_3 (ammonia solution) was added and shaken.

Test for Flavonoids: (Sodium hydroxide test) Few portion of extract was dissolved in 2ml of water, aqeous sodium hydroxide was added. A yellow color indicates the presence of flavonoids (Sofowora, 1982).

Test for Alkaloids: 0.2kg of extract was stirred with 5ml of 1% aqueous hydrochloric acid in a steam bath and filtered. 1ml of the filtrate was treated with a few drops of Wagner's reagent. Turbidity will indicate the presence of alkaloids (Trease and Evans, 1992).

Test for Reducing compounds (Fehlings's Test): 0.2g of plant extract was dissolved in 5ml of distilled water, followed by 5ml of Fehling's solution A and B, and was boiled. Red precipitate indicates the presence of reducing compound (Trease and Evans, 1992).

Test for Tannins (Ferric chloride test): Few portion of plant extract was dissolved in 10ml, filtered. And to the filtrate two drops of freshly prepared $FeCl_3$ was added. A dark green colour indicates the presence of tannins.

Test for Steroids/Triterpenes (Salkoworki test): Few portion of plant extract was dissolved in 2ml chloroform; conc. sulphuric acid was added to form a lower layer. A reddish brown colour at the interphase indicates the presence of steroid (Sofowora, 1982).

Test for Higher Fatty Acids: Few portion of plant extract was made alkaline with 25% ammonia and was exhaustively extracted with ether, and acidified with concentrated HCl acid. It was shaken with ethyl ether in the separating funnel and was evaporated to dryness. The acidic aqueous solution is then shaken with ethyl ether in a separating funnel then the ether is evaporated to dryness if the residue is oily, it indicates the presence of fatty acids.

Test for Saponins (Frothing test): 0.2g of plant extract was shaken with distilled water in a test tube and heated. If it produced frothy which persists on heating it indicates the presence of Saponins (Sofowora, 1982).

Results and Discussion

The result obtained with this study shows that *E. camaldulensis*methanolic leaf extract shows anti-trypanosomal activity. The parasitemic level observed animals receiving different doses of *E. camaldulensis*shows suppressived effects as compared with the negative control (infected but not treated) figure 1.

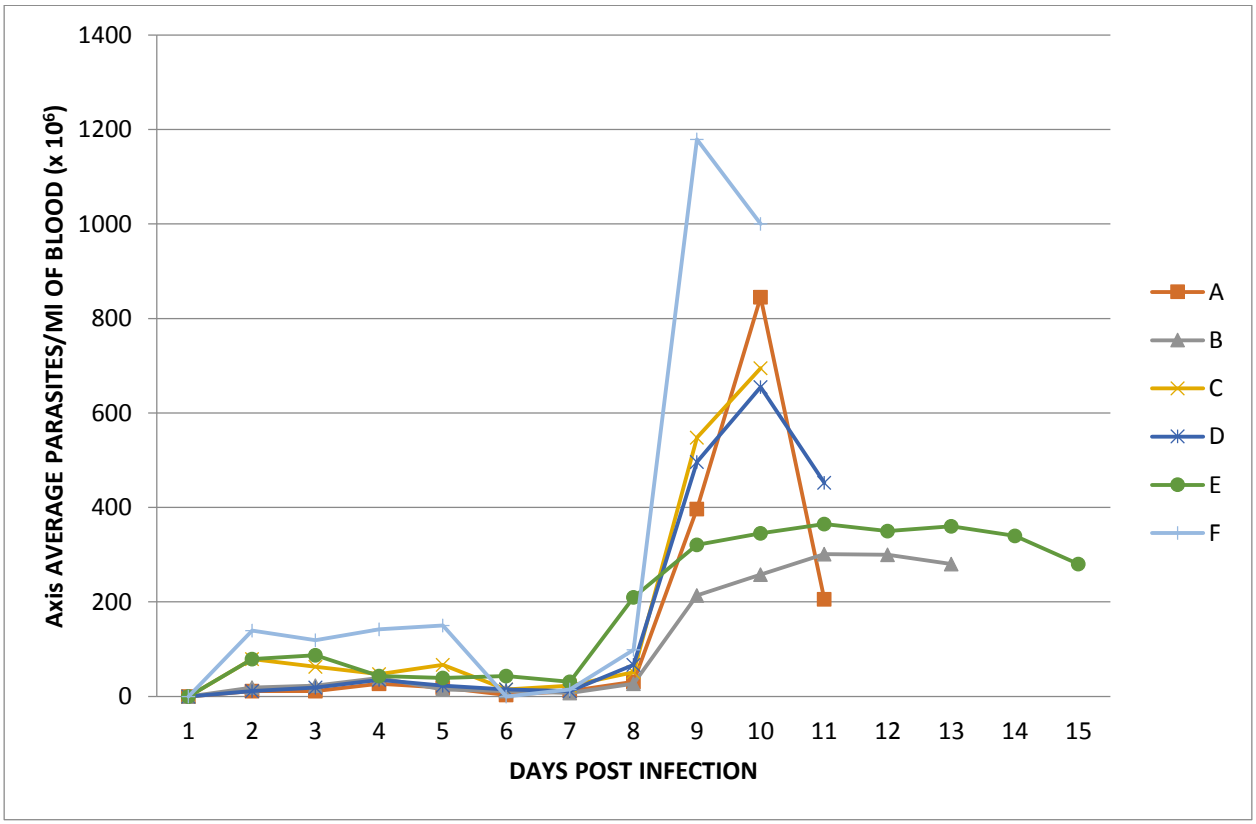

Fig 1: Figure 3 shows the result of antitrypanosomal activity of combined extracts of *D.senegalensis(*root) and *E.camaldulensisi(*leaf*)* inphase one of the experiment. Animals treated with extract ratio of 1:1, 3:1 and 1:0 could not survive post treatment. The animals showed high parasitemia level per microscopic field before death. This showed that the anti trypanosomal activity of those combined extracts were not effective. Extract ratios of 2:1 and 0:1 was the only groups that survive post treatment for 26 days and 30 days respectively. Parasitemic level was reduced as well as the PCV counts, appetite and weight

lost and eventually, death results. Extract ratios 2:1 and 0:1 was repeated for the second screening to know the best activity of the extracts.

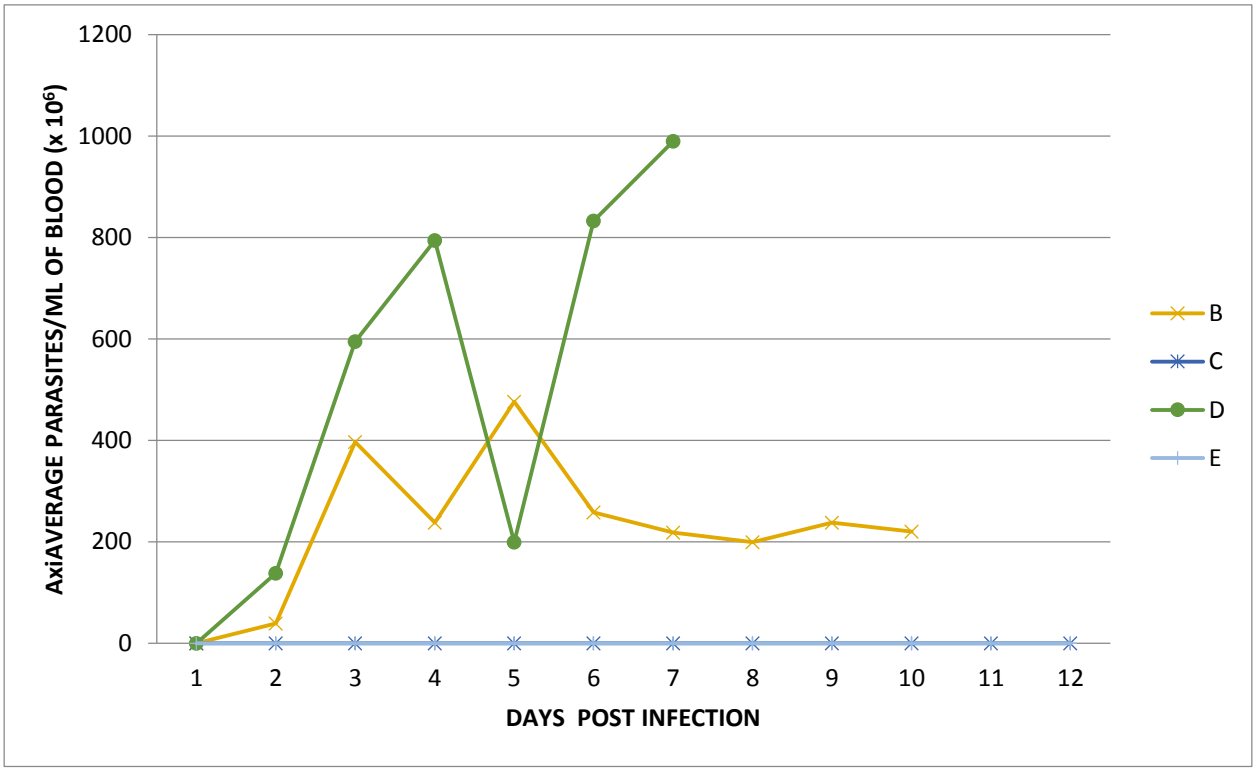

Fig 2: Figure 4 shows the result for the repeat screening for anti trypanosomal activity using effective combinations 2:1 and 0:1. It shows that the methanol extract of *E.camaldulensis*(leaf) is most effective against trypanosomiasis. A 2:1, B 0:1, C Berenil (3.5 mg/kgbw), D Infected, not treated (negative control) and E Not infected not treated (positive control).

Phytochemical study shows the primary constituents of *E.*camaldulensis and *D.senegalensis.*
The primary constituents of *Eucalyptus camaldulensis* includes essential oil, high level of phenolics and terpenoids which can be toxic, alkaloids, flavonoid, reducing compound, saponin and high fatty acids, and *Detariumsenegalensis* contains alkaloids, reducing compounds, steroids and triterpenes, fatty acids and saponins.

Plants of different families could possess potent trypanocidal activity. It is therefore, not surprising that the plants tested showed promising trypanocidal effects. Atawodi et al., (2004), reported that these plants drastically reduced motility of *T. b. brucei*, in vitro. This has prompted the in vivo study to verify the efficacy exhibited in vitro. Many researched have been done in anti-trypansome activity of different medicinal plants such as *Annona*senegalensis, bitter kola, *Azadirchtaindica* (Neem), *Adansoniadigitata*, and most plant extracts were found to show different level of experimental anti-trypanosomal activities (Rajendran et al., 2014).

The results obtained with methanolic extract of *Eucalyptus camaldulensis* agrees with the previous work done on *Annona senegalensis*. The extract of *Annona senegalensis* was used to treat mice orally and intra peritoneally, the parasites were cleared from circulation after few days of infection (Mustapha, 2013). It was observed that *Eucalyptus* extract to some extent showed same effect of anti-trypanosome activity as most parasites were cleared from circulation and the animal survived longer than the negative control group.

Eucalyptus leaf was reported to contain high level of phenolic and terpenoid (which can be toxic), Oleic acid, ascorbic acid, butilinic acid (Vuong et al., 2015). Ethanol extract of *Eucalyptus camaldulensis* leaf have been reported to show activity against bacteria and fungi (Mabona et al., 2013). Due to its spicy aroma, the leaf has been smoked in order to provide relief in bronchial, catarrh, asthma and other infections of the respiratory organs (Okmen et al, 2014). Although it is difficult to speculate the mechanism by which this extract exhibits their trypanocidal actions, however accumulated evidence suggests that many natural products exhibits their trypanocidal activity by virtue of their interference with the redox balance of the parasites acting on their respiratory chain or their cellular defenses against oxidative stress (Adebayo et al., 2013). This may be so because the natural products possess structures capable of generating radical; this may cause peroxidative damage to trypanothione reductase that is very sensitive to alterations in the redox balance. It is also known that some agent act by binding with the kinetoplast DNA of the parasite (Halici et al., 2011).

Conclusion and Recommendation

The result from this study shows that the methanolic extract of *E. camaldulensis* (leaf) at 200mg/Kgbw was more effective than the combination of extracts.

The result obtained in this research work suggests the possibility of further exploiting. *Eucalyptus camaldulensis* leaf for the treatment of Trypanosomiasis. More research is therefore recommended in this area for the benefit of humanity.

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