Effect of Biochemical Constituents of *Detarium senegalensis* root (tallow tree) and *Eucalyptus camaldulensis* leaf (river red gum) 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 Trypanosomosis since its activity was more pronounced.

Key words: Diseases, Medicinal plants, Parasitemia, 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) mixed methanolic extracts

MATERIALS AND METHODS

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.

Trypanosome brucei-brucei was obtained from the Biochemistry and Chemotherapy Division, National Institute for Trypanosomiasis Research (NITR) VOM, Plateau State. *Eucalyptus camaldulensis* leaf was obtained from Old Secretariat, Minna, Niger State. 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. One hundred (100) g of the dried samples were weighed and 400 mL of 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.

Results and Discussion

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

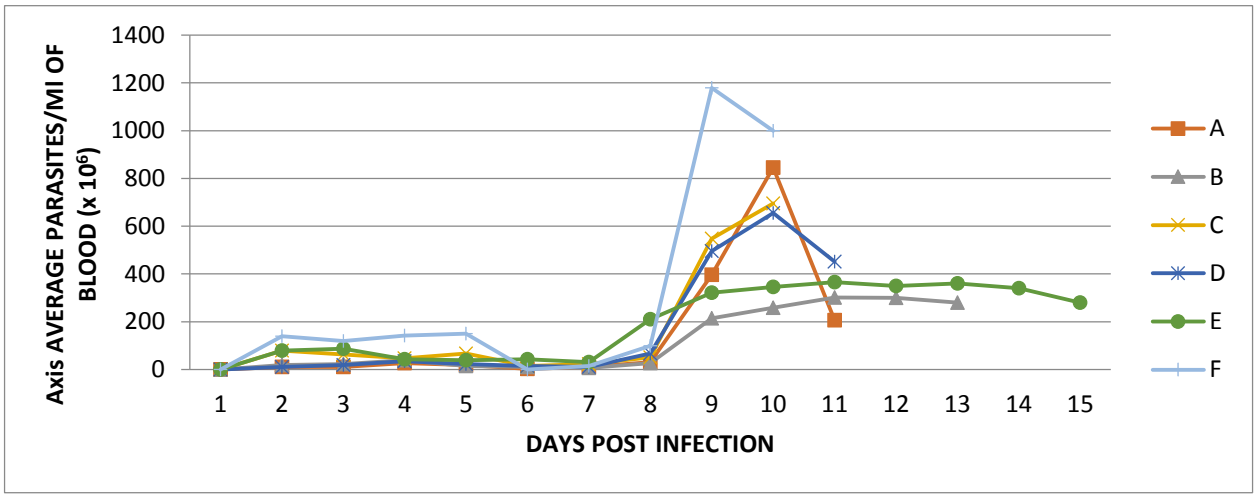

Fig 1: shows the result of antitrypanosomal activity of combined extracts of *D. senegalensis (*root) and *E. camaldulensisi (*leaf*)* in phase one of the experiments. Animals treated with extract ratio of 1:1, 3:1 and 1:0 could not survive post treatment. The animals showed high parasitemia level per microscopic field before death.

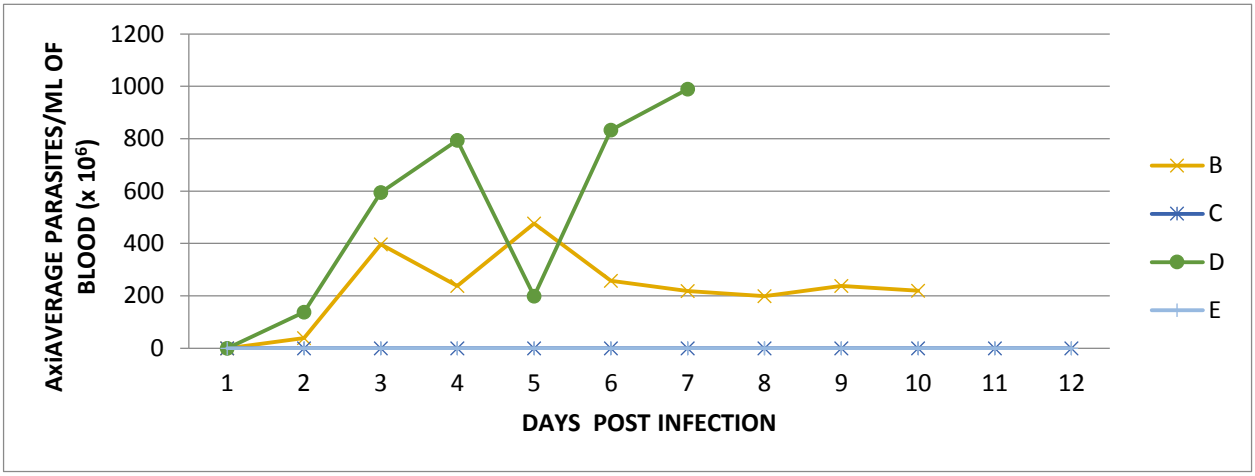

Fig 2: shows the result for the repeat screening for anti trypanosomal activity using effective combinations 2:1 and 0:1. It shows that the methanol extract of *E. camaldulensis* (leaf) is most effective against trypanosomosis. A 2:1, B 0:1, C Berenil (3.5 mg/kgbw), D Infected, not treated (negative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

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-trypansomal activity of different medicinal plants such as *Annona* senegalensis, bitter kola, *Azadirchta indica* (Neem), *Adansonia digitata*, 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). Natural products such as alkaloids, terpenes, quinones, and polyphenols found in these extracts have been shown to be potent growth inhibition of *T. cruzi* (da Silva, da Silva, & Correia, 2017). Triterpenoids and sterols from the plants are reported to possess anti trypanosomal activity (Ibrahim *et al.*, 2014). The anti trypanosomal activities of alkaloids like actinodaphine, dicentine, cassythine isolated from *Cassytha filiformis* (Andre *et al.*, 2007) are also found in *B. buonopozense* and several other alkaloids displayed significant *in vitro* anti trypanosomal activity (Andre *et al.*, 2007). The DNA intercalation in combination with portion biosynthesis inhibition is reported to be the mechanism of action responsible for the observed anti-trypanosomal effect of the active alkaloids (Merschjohann *et al.*, 2001). The trypanocidal activity of several flavonoids such as quercetagetin (Tasdemir *et al.*, 2006); hispidulin and santin (Muschietti *et al.*, 2013) has been previously reported. An azaanthraquinone early reported in *A. nilotica* was associated with the observed anti trypanosomal effects (Passalacqua *et al.*, 2015). 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

In this *in vivo* studies, with mice infected with *T. b. brucei*; the methanol extracts of *E.* camaldulensis and *D. senegalensis* were found to be significantly active. Therefore, the trypanocidal effects of extracts will require further experimentation after fractionation and characterization using chromatographic and spectroscopic techniques, a work that is currently in progress.

References

Adebayo, O. L., Akwasi, G. S., & Confidence, A. (2013). In vitro antibacterial potential of solvent fractions of aqueous and ethanolic leaf extracts of vernonia colorata against selected human pathogenic bacteria. *International Journal of Research in Ayurveda and Pharmacy*. https://doi.org/10.7897/2277-4343.04618

Andre Gustavo Tempone, Patricia Sartorelli, & Charles Mady. (2007). Natural Products to Anti-trypanosomal Drugs: An Overview of New Drug Prototypes for American Trypanosomiasis. *Cardiovascular & Hematological Agents in Medicinal Chemistry*. https://doi.org/10.2174/187152507781058726

Atawodi, S. E., Bulus, T., Ibrahim, S., A Ameh, D., J Nok, A., Mamman, M., & Galadima, M. (2004). *Full Length Research Paper - In vitro trypanocidal effect of methanolic extract of some Nigerian savannah plants*. *African Journal of Biotechnology (ISSN: 1684-5315) Vol 2 Num 9*. https://doi.org/10.5897/AJB2003.000-1065

da Silva, L. C. N., da Silva, M. V., & Correia, M. T. do. S. (2017). Editorial: New frontiers in the search of antimicrobials agents from natural products. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2017.00210

Grant, C., Anderson, N., & Machila, N. (2015). Stakeholder Narratives on Trypanosomiasis, Their Effect on Policy and the Scope for One Health. *PLoS Neglected Tropical Diseases*. https://doi.org/10.1371/journal.pntd.0004241

Halici, M., Kufrevioglu, O. I., Odabasoglu, F., Halici, Z., Cakir, A., & Aslan, A. (2011). The ethanol-water extract of ramalina capitata has gastroprotective and antioxidative properties: An experimental study in rats with indomethacin-induced gastric injuries. *Journal of Food Biochemistry*. https://doi.org/10.1111/j.1745-4514.2010.00363.x

Ibrahim, M. A., Mohammed, A., Isah, M. B., & Aliyu, A. B. (2014). Anti-trypanosomal activity of African medicinal plants: A review update. *Journal of Ethnopharmacology*. https://doi.org/10.1016/j.jep.2014.04.012

Kennedy, D. O., & Wightman, E. L. (2011). Herbal Extracts and Phytochemicals: Plant Secondary Metabolites and the Enhancement of Human Brain Function. *Advances in Nutrition: An International Review Journal*. https://doi.org/10.3945/an.110.000117

Kumar, V. S., & Navaratnam, V. (2013). Neem (Azadirachta indica): Prehistory to contemporary medicinal uses to humankind. *Asian Pacific Journal of Tropical Biomedicine*. https://doi.org/10.1016/S2221-1691(13)60105-7

Mann, A., & O. Ogbadoyi, E. (2012). Evaluation of Medicinal Plants from Nupeland for Their in vivo Antitrypanosomal Activity. *American Journal of Biochemistry*. https://doi.org/10.5923/j.ajb.20120201.01

Merschjohann, K., Sporer, F., Steverding, D., & Wink, M. (2001). In vitro effect of alkaloids on bloodstream forms of Trypanosoma brucei and T. congolense. *Planta Medica*. https://doi.org/10.1055/s-2001-17351

Muschietti, L. V., Sülsen, V. P., & Martino, V. S. (2013). Bioprospection of potential trypanocidal drugs: A scientific literature survey over the period 2000-2010. *Studies in Natural Products Chemistry*. https://doi.org/10.1016/B978-0-444-62615-8.00009-6

Mustapha, A. A. (2013). Annona senegalensis Persoon : A Multipurpose shrub , its Phytotherapic , Phytopharmacological and Phytomedicinal Uses. *International Journal of Science and Technology*. https://doi.org/10.1016/j.jep.2004.09.055

Passalacqua, T. G., Dutra, L. A., De Almeida, L., Velásquez, A. M. A., Torres Esteves, F. A., Yamasaki, P. R., … Graminha, M. A. S. (2015). Synthesis and evaluation of novel prenylated chalcone derivatives as anti-leishmanial and anti-trypanosomal compounds. *Bioorganic and Medicinal Chemistry Letters*. https://doi.org/10.1016/j.bmcl.2015.05.072

Rajendran, P., Rengarajan, T., Nandakumar, N., Palaniswami, R., Nishigaki, Y., & Nishigaki, I. (2014). Kaempferol,

a potential cytostatic and cure for inflammatory disorders. *European Journal of Medicinal Chemistry*, *86*, 103–112. https://doi.org/10.1016/j.ejmech.2014.08.011

Robinson, Molly Meri, Zhang, X. (2011). the World Medicines Situation 2011 Traditional Medicines : Global Situation , Issues and Challenges. *World Health Organization*.

Rodgers, J., Jones, A., Gibaud, S., Bradley, B., McCabe, C., Barrett, M. P., … Kennedy, P. G. E. (2011). Melarsoprol cyclodextrin inclusion complexes as promising oral candidates for the treatment of human African trypanosomiasis. *PLoS Neglected Tropical Diseases*. https://doi.org/10.1371/journal.pntd.0001308

Song, Y.-H., Sun, H., Zhang, A.-H., Yan, G.-L., Han, Y., & Wang, X. (2014). Plant-derived natural products as leads to anti-cancer drugs. *Journal of Medicinal Plant and Herbal Therapy Research*. https://doi.org/10.14719/pst.2014.1.2.17

Tasdemir, D., Kaiser, M., Brun, R., Yardley, V., Schmidt, T. J., Tosun, F., & Rüedi, P. (2006). Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: In vitro, in vivo, structure-activity relationship, and quantitative structure-activity relationship studies. *Antimicrobial Agents and Chemotherapy*. https://doi.org/10.1128/AAC.50.4.1352-1364.2006

Vuong, Q. V., Hirun, S., Chuen, T. L. K., Goldsmith, C. D., Munro, B., Bowyer, M. C., … Scarlett, C. J. (2015). Physicochemical, antioxidant and anti-cancer activity of a Eucalyptus robusta (Sm.) leaf aqueous extract. *Industrial Crops and Products*. https://doi.org/10.1016/j.indcrop.2014.10.061