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Anti-trypanosomal Potential of Eucalyptus Camaldulensis

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Abstract: Chemotherapy of African trypanosomiasis in both the human and animal forms has been confronted with multidimensional problems that include paucity of drugs, resistance, high cost, prolonged treatment protocol and adverse side effects. The main objective of this study was to explore alternative sources of antitrypanosomal agents from the leaves, stem and root barks of *Eucalyptus camaldulensis*. The plant parts were sequentially extracted with hexane, ethyl acetate, methanol and water; and the extracts screened for antitrypanosomal activity. Mice infected with *Trypanosoma brucei brucei* were administered intraperitoneally doses ranging from 200-600 mg/kg body weight/day of the extracts for 21 consecutive days. One control group was treated with 3.5mg/kg bodyweight of berenil while the other control group was left untreated. The methanol extract of *E. camaldulensis* (leaf) produced complete cure for the animals in the different dose groups and survived as long as those treated with the standard drug, berenil, although the clearance time was faster for the standard drug. Sub inoculation of healthy mice with the blood and Cerebrospinal Fluid (CSF) of the cured mice did not result in infection, thus indicating a complete and permanent cure. Acute toxicity studies of the methanol extract of *E. camaldulensis* (leaf) confirmed the safety of the extract because no mortality was recorded even at 5000 mg/kg bodyweight. However, the extract had no prophylactic activity. Bioassay-guided fractionation of the crude methanol extract of *E. camaldulensis* leaf gave 10 fractions, with only fractions 8 and 9 exhibiting minimal antitrypanosomal activities that were not comparable to those of the crude extract and the standard drug ($p \leq 0.05$). Phytochemical screening revealed the presence of terpenes, steroids, saponins, tannins, alkaloids and fatty acids in both the crude extract and fraction 9; while fraction 8 contained only terpenes, steroids and fatty acids. Data from GC-MS analysis of the two fractions indicated likely components to be methyl esters, amides, long chain alkenes and alcohols. The result of this study shows that the methanol extract of *E. camaldulensis* (leaf) has immense potential for the development of drugs against African trypanosomiasis.

Keywords: Antitrypanosomal, cerebrospinal fluid, *Eucalyptus camaldulensis*, methanol extract, Trypanosomiasis

INTRODUCTION

Human African trypanosomiasis, also known as sleeping sickness, is a vector-borne parasitic disease. The parasites concerned are protozoa belonging to the *Trypanosoma* genus. They are transmitted to humans by tsetse fly (*Glossina* genus) bites which have acquired their infection from human beings or from animals harboring the human pathogenic parasites.

The disease affects mostly poor populations living in remote rural areas of Africa. Untreated, it is usually fatal. Travellers also risk becoming infected if they venture through regions where the insect is common. Generally, the disease is not found in urban areas, although some cases have been reported in suburban areas of big cities in some disease endemic countries (Simarro *et al*., 2011).

Sleeping sickness occurs only in 36 sub-Saharan Africa countries where there are tsetse flies that can transmit the disease. In 1995, WHO Expert Committee estimated that 60 million people were at risk with an estimated 300 000 new cases per year in Africa, with fewer than 30,000 cases diagnosed and treated (WHO, 2012).

After continued control efforts, the number of cases reported in 2009 has dropped below 10 000 for first time in 50 years. This trend has been maintained in 2010 with 7139 new cases reported (WHO, 2012).

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Diagnosis and treatment of the disease is complex and requires specifically skilled staff.

Most of the drugs in use were developed decades ago and show variable efficacy, serious side effects, can require long-term treatment and many have poor activity in immuno-suppressed patients. These picture/prospects clearly indicate the necessity to search for new chemotherapeutic agents (Legros *et al*., 2002).

Relapse rate with melarsoprol in Northern Uganda, Southern Sudan and Northern Angola was as much as 30% (Ogbadoyi *et al*., 2007) and most of the available drugs are highly toxic with about 5% of those treated with melarsoprol dying as a result of high toxicity of the drug (Ogbadoyi *et al*., 2007). In addition to these drawbacks is the problem of paucity of the drugs in rural communities where the burden of the disease is more manifest and when the drugs are available, the cost is prohibitive. Despite efforts made by the World Health Organization (WHO) to help find new treatments, there have been few new drugs in recent times. Anti-trypanosomiasis vaccination still remains the best theoretical option in the fight against a disease that is continuously hovering between its wildlife reservoir and its reservoir in man and livestock. While antigenic variation of the parasite surface coat has been considered the major obstacle in the development of a functional vaccine, recent research into the biology of B cells has indicated that the problems might go further than that (Magez *et al*., 2010). As a consequence of these apparent setbacks and the non accessibility and affordability of the few available trypanocidal drugs, majority of the world's population especially in Africa depend on traditional medical remedies for this infection and it is estimated that some 20,000 species of higher plants are used medicinally throughout the world (Phillipson and Wright, 1991).

Many Traditional medical remedies have been used extensively and knowledge about them has been accrued by several generations of practitioners from experience, trial and error. Although formal toxicology studies are limited, most of the extensively used local remedies are unlikely to be severe toxins and are worthy of further evaluation for novel antiparasitic compounds (Swerdlow, 2000). Ethno medicine and ethno botany have long been of interest to medical researchers, physicians, the pharmaceutical industry, anthropologists and botanists (Phillipson and O, Neill, 1986; Phillipson and Wright, 1991). And because greater importance is now being attached to the use of locally available medicines as a means of reducing reliance on expensive imported drugs (Bodeker and Willcox, 2000), the need to investigate unexplored natural products for their medicinal properties with some urgency cannot be over-emphasized.

Eucalyptus camaldulensis (Myrtaceae), an Australian native, represented by around 700 species is a genus of tall, evergreen and magnificent trees cultivated the world over for its oil, gum, pulp, timber, medicine and aesthetic value. Among the various wood and non-wood products, essential oil found in its foliage is the most important one and finds extensive use in food, perfumery and pharmaceutical industry. In addition, the oil possesses a wide spectrum of biological activity including anti-microbial, fungicidal, insecticidal/insect repellent, herbicidal, acaricidal and nematicidal (Batish *et al*., 2008). *Eucalyptus* oil is readily steam distilled from the leaves and can be used for cleaning, deodorising and in very small quantities in food supplements; especially sweets, cough drops and decongestants. Eucalyptus oil has insect repellent properties and is an active ingredient in some commercial mosquito repellents (Doran and Brophy, 1990).

Based on the fore-going and the fact that *Eucalyptus camaldulensis* (Myrtaceae) leaves are used singly and in combination with some other plant parts to treat malaria and typhoid fevers in some Northern parts of Nigeria, this study was designed to evaluate the anti-trypanosomal potential of the plant.

MATERIALS AND METHODS

Materials:

Plant materials: The leaves of *Eucalyptus Camaldulensis* were collected from the Education Resource Center/Education Trust Fund ground in Minna, Niger State, Nigeria, between the months of June and July, 2009. The specimen was identified and allotted a voucher number (NIPRD/H/6263) at the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. All aspects of this study between 2009 and 2011 were carried out in the Biochemistry laboratory of Federal University of Technology, Minna, Nigeria, except for the fractionation that was done in the plant chemistry laboratory of National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.

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 bred in the Department of Biochemistry laboratory, Federal University of Technology, Minna, for subsequent use. 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 described by Adamu *et al*. (2010).

Trypanosoma brucei brucei: A stabilate of *Trypanosoma brucei brucei* (Lafia strain) was obtained

from the National Institute for Trypanosomiasis and Onchocerciasis Research, Vom, Plateau 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. The major part of this study was conducted in the Department of Biochemistry Laboratory, Federal University of Technology, Minna, while fractionation was done in the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria between 2010 and 2011.

Methods:

Preparation of plant samples: About 500 g of the leaves of the leaves of *E. camaldulensis* were removed fresh, washed with running tap water and dried at room temperature.

The dried leaves were blended into powdered form using mortar and pestle and subsequently stored in clean polythene bags until required for use.

Preparation of crude extracts: The extract was prepared and screening carried out using the method described by Adamu *et al*. (2010). In this method, fifty grams (50 g) of the dried powdered leaves was extracted under reflux with 400 ml of methanol for 2 h. The extract was filtered hot using cheese cloth and solvent was removed using rotary evaporator followed by drying on steam bath. The extract, now in a relatively dry form, was transferred into a sterile sample bottle for storage in the refrigerator until required for use.

Infection of animals: The animals were inoculated using the method described by Mann *et al*. (2011). 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 inoculum. Healthy mice of weight range 25-35 g were infected intraperitoneally with 0.1 ml of the inoculum containing about 10^3 trypanosomes.

Preparation of stock solution of extract: The stock solution was prepared just before use by dissolving the methanol extract in 10% Dimethylsulfoxide (DMSO).

Administration of crude extract and monitoring the course of parasitemia: The course of parasitemia was monitored using the method described by Adamu *et al*. (2010). The presence of parasites in experimental animals was monitored three times weekly by making a blood smear on a glass slide and observing under a microscope set at 40X magnification. The number of parasites in 1 ml of blood was then estimated using the method of Herbert and Lumsden (1976). The methanol extract of *E. camaldulensi* (leaf), was screened for anti-trypanosomal activity using five groups; with each consisting of three infected mice. Groups A, B and C were administered intraperitoneally 200, 400 and 600 mg/kg body weight per day of the extracts respectively for 21 consecutive days commencing 24 h after infection. Group D was infected but not treated to serve as negative control, while Group E was infected and administered a single dose of 3.5 mg/kg bodyweight of berenil to serve as positive control.

Determination of minimum curative dose of the methanol extract of *E. camaldulensis* leaf: The methanol extract of *E. camaldulensis* (leaf), based on the anti-trypanosomal potential exhibited in the preliminary screening, was subjected to determination of the minimum dose that will produce a complete cure for *T. b. brucei* - infected mice. Six groups of mice, each group consisting of three mice, were set up. Groups A, B, C and D were infected with *T. b. brucei* and treated with 50,100, 150 and 200 mg/kg bodyweight per day of the extract for 21 days. Group E was infected and left untreated, while Group F was infected and administered a single dose of 3.5 mg/kg body weight of berenil. Parasitemia was monitored for four weeks.

Blood and Cerebrospinal Fluid (CSF) infectivity tests: Both tests were carried out according to the method described by Ugwu *et al*. (2011). Blood from animals that survived after treatment with the methanol extract of *E. camaldulensis* leaf was used to inoculate healthy mice in order to rule out the possibility of infection and to ascertain that cured mice were completely cleared of parasites.

Blood infectivity test: One mouse from each of the dose groups treated and cured with crude methanol extract of *E. camaldulensis* (leaf) was sacrificed 12 weeks post-treatment and 0.02 ml of diluted blood obtained from the punctured heart of each mouse was sub-inoculated into clean parasite-free mice (two in each group) and parasitemia was monitored over a 2-month period.

Cerebrospinal Fluid (CSF) infectivity test: One mouse from each of the dose groups treated and cured with crude methanol extract of *E. camaldulensis* (leaf) had the hair on the back carefully shaved and was positioned such that the head touched the limbs. This made the vertebrae conspicuous for easy insertion of needle. The lumbar was then punctured by the insertion of a clean micro needle and syringe, into which a Clean, Clear and Transparent Fluid (CSF) flowed. This was done under a mild anaesthesia and gentle handling. For each of the mouse from the treated groups, two clean, parasite-free mice were sub-inoculated with 0.02ml of the CSF and parasitemia was monitored over a 2-month period.

Screening for prophylactic activity of extract: The methanol extract of *E. camaldulensis* leaf was screened for prophylactic activity using a modified method of

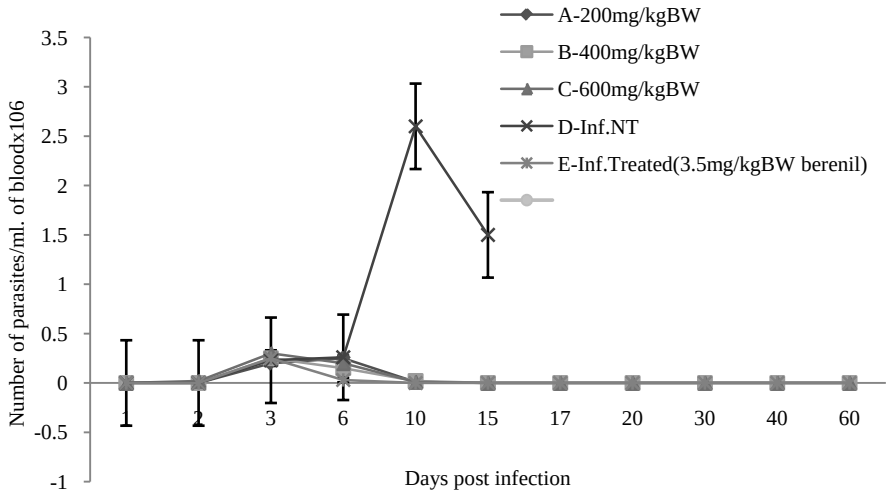

Fig. 1: Course of parasitemia in infected mice treated with crude methanol extract of *E. camaldulensis* (leaf), treatment with 200, 400 and 600 mg/kg Body Weight (BW) of the extract cleared parasites from infected mice 10 days into treatment as did the standard drug (berenil)

Mgbemena *et al*. (2010). Two groups of mice, each group consisting of five mice, were set up. Animals in Group A were administered 200 mg/kg bodyweight of methanol extract of *E. camaldulensis* while those in Group B were administered 0.1ml of physiological saline for five consecutive days. On the sixth day, animals in the two groups were inoculated with 0.1ml of diluted trypanosome - infected blood (approximately 10^3 trypanosomes/ml). Parasitemia was thereafter monitored twice weekly for two weeks.

Acute toxicity studies of methanol extract of *E. camaldulensis* (leaf): The acute toxicity of the crude methanol extract of *E. camaldulensis* leaf was evaluated using the method described by Ugwu *et al*. (2011). Six groups, consisting of three mice each, were administered single doses of 1000, 2000, 3000, 4000 and 5000 mg/kg body weight of the extract respectively. The control group was administered 0.4 mL of physiological saline. Signs of acute toxicity such as death, changes in physical appearance and behavioural change were monitored over a two-week period.

Bioassay-guided fractionation of methanol extract of *E. camaldulensis* leaf: Ten fractions obtained from column chromatography were screened for antitrypanosomal activity. For each of the fractions, three groups, consisting of three mice each, were set up. Doses of 50, 100 and 150 mg/kg body weight were administered on *T. b. brucei*-infected mice in groups 1, 2 and 3 respectively and parasitemia was monitored for two weeks.

Phytochemical analysis: The crude methanol extract of *E. camaldulensis* (leaf) and two moderately active fractions (8 and 9) were subjected to phytochemical analysis using standard analytical methods described by Ugwu *et al*. (2011).

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis: Fractions 8 and 9 were subjected to Gas Chromatography-Mass Spectroscopy (GC-MS) in order to identify likely structures present in the two fractions. The GC-MS spectrums were obtained using GCMS-QP2010 PLUS (SHIMADZU, JAPAN).

Statistical analysis of experimental data: Data obtained in this study were subjected to a one-way analysis of variance (ANOVA) to derive mean values of parasitemia which were compared with least significant difference. Mean values among the treated groups were deemed to be significantly different if the level of probability was ≤ 0.05.

RESULTS

Antitrypanosomal activity of crude methanol extract of *E.camaldulensis* (leaf): All animals in the three dose groups (200, 400 and 600mg/kg bodyweight/day) had parasites cleared from circulation about 2 weeks into the treatment period and this was maintained beyond 40 days (Fig. 1). The anti-trypanosomal activity of the extract was not significantly different from that of the standard drug, berenil ($p \leq 0.05$). Animals in the untreated group started dying 10 days post-infection with parasitemia rising to about 0.3 x 10^6/ml. of blood.

Minimum curative dose of methanol extract of *E. Camaldulensis* (leaf): The best anti- trypanosomal activity was observed in the group treated with 200mg/kg bodyweight indicating that it was the minimum dose that can clear blood stream trypanosomes in the test animal (Fig. 2).

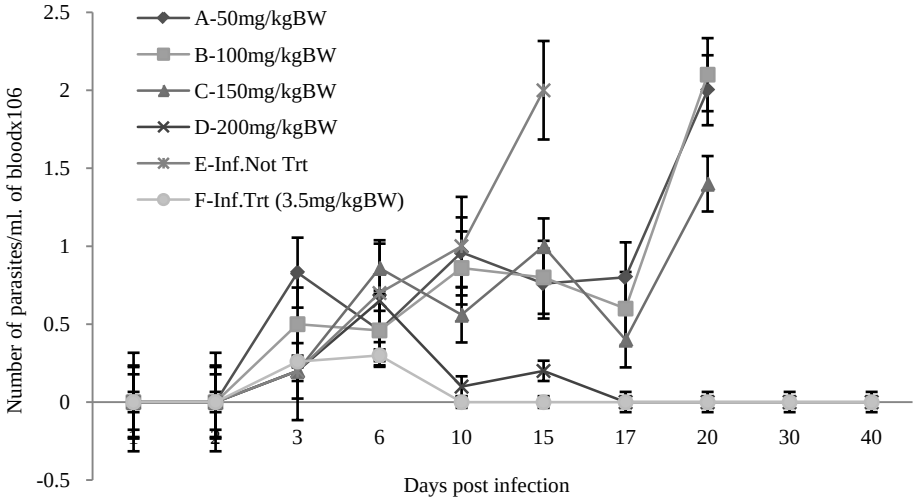

Fig. 2: Determination of minimum curative dose for crude methanol extract of *E. camaldulensis* (Leaf)

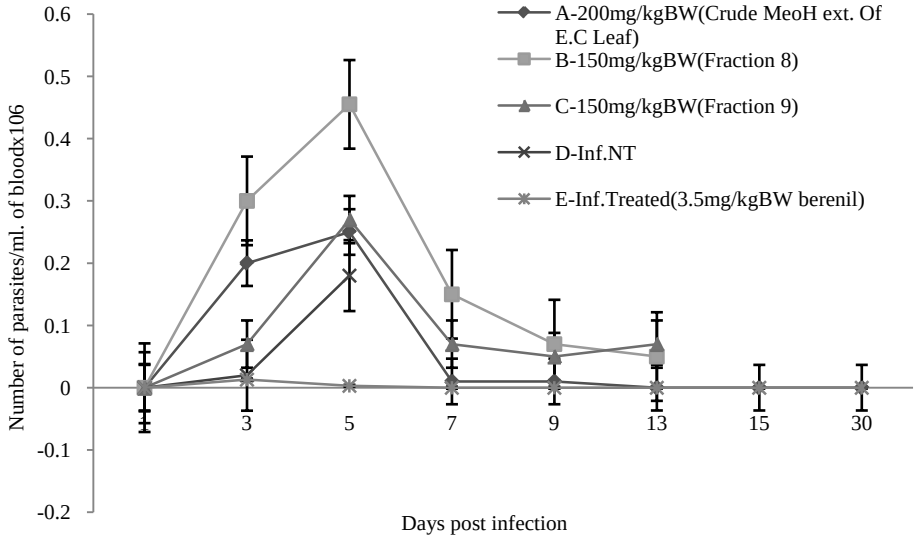

Fig. 3: Course of parasitemia in infected mice treated with crude methanol extract of *E. camaldulensis* (leaf), fractions 8, 9 and standard drug, berenil; the two fractions succeeded in reducing the parasitemia but did not completely clear parasites from circulation

Blood and cerebrospinal fluid infectivity tests: All animals sub-inoculated with blood and cerebrospinal fluid from cured mice failed to come down with *T. b. brucei* infection after two months of monitoring, thus ascertaining the complete clearance of parasites by the crude methanol extract of *E.camaldulensis* (leaf).

Prophylactic activity of crude methanol extract of *E.camaldulensis*: The extract had no prophylactic activity because infected mice exhibited high levels of parasitemia and died within one week.

Acute toxicity test: All animals administered 1000-5000 mg/kg bodyweight of crude methanol extract of *E.camaldulensis* (leaf) survived the study period with no record of death. Initial signs of weakness and difficulty in feeding were reversed on the same day .

Antitrypanosomal activity of Fractions: Only fractions 8 and 9 at a dose of 150 mg/kg bodyweight/day extended the lifespan of animals by 6 days beyond the untreated infected control and within the treatment period, parasitemia was reduced considerably, while the animals treated with the crude methanol extract of *E. camaldulensis* and the standard drug cleared parasites from circulation and survived longer (Fig. 3). There was no significant difference between the activities of the crude extract and the standard drug, but both were significantly different from the activities exhibited by the two fractions ($p \leq 0.05$).

Phytochemical compositions of crude methanol extract of *E. camaldulensis* (leaf) and fractions 8 and 9: Phytochemical analysis of the crude methanol extract of *E. camaldulensis* (leaf) revealed the presence of terpenes, steroids, saponins, tannins, alkaloids and fatty acids while fraction 8 contained terpenes, steroids and fatty acids and fraction 9 contained terpenes, steroids, saponins, flavonoids, tannins and fatty acids. Alkaloids were absent in both fractions.

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis: Based on the spectroscopic data from GC-MS, the relative intensities, selectivity index and the peak values of components and deductions made from the National Institute of Science and Technology (NIST) library, GC-MS analysis indicated components that resembled 2-Chloro-N-(1, 3-thiozol-2-yl) acetamide, 9-Octadecenamide, 1-Nonadecene, (Z)-9-Eicosene, Hexadecanol, 1-pentadecanol for fraction 8; and Methylhexadecanoate, Methyl cis-9-octadecenoate, Methyl-n-octadecanoate, 1-heptadecanol for fraction 9.

DISCUSSION

The crude methanol extract of the *E. camaldulensis* (leaf) exhibited very significant activity against experimental *Trypanosoma brucei brucei* infection in mice. The extract when administered consecutively for three weeks completely cleared parasites from circulation and all animals in the different dose groups survived thereafter for more than three months until they were used for further studies.

The crude methanol extract of *E. camaldulensis* (leaf) cleared parasites completely from circulation only when it was administered 24 h after infection, but did not clear parasites completely when treatment was delayed beyond 24 h post-infection although some anti-trypanosomal activity was still observed.

The partially purified fractions (fractions 8 and 9) that exhibited minimal antitrypanosomal activities did not completely clear parasites from circulation and the animals died in less than 2 weeks. It was clear from these results that the crude extract as used was more efficacious than the single fractions. We can deduce three possible inferences from these observations which are that, the crude extract is trypanocidal, but only at the initial phase of *T. b. brucei* infection; secondly, fractionation of the crude extract must have resulted in a partial loss of activity and thirdly, there is the possibility of synergistic action between the bioactive components of the crude extract since the fractions, when administered individually, were not as active as the crude extract. It has been observed before now that the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (Shariff, 2001). The importance of these observations and inferences are that: first, the crude extract can be standardised and packaged as phytomedicine, just the way it is done in traditional medical practice. Secondly, the partially purified fractions or their components, though not as active as the crude extract could be subjected to chemical modification to potentiate their anti-trypanosomal activities.

Sub inoculation experiments using blood and CSF from cured mice did not result in infection thus indicating that the extract succeeded in clearing all blood stream parasites and there was ultimately no infiltration of parasites into the Central Nervous System (CNS). Therefore, this extract, based on the result obtained in this study, is a possible ethno medical candidate for the treatment of early stage trypanosome infection in animals. As for late stage infection, we cannot completely rule out the ability of the crude extract to cross the blood brain barrier (bbb) into the CNS because when it was administered 72 h post infection, a significant level of parasite clearance was observed ($p \leq 0.05$).

An overview of the identified components in fraction 8 shows that 1- Nonadecene and (Z)-9-Eicosene are sex pheromones in some insects, where they serve as attractants for males (El-Sayed, 2009); hexadecanol, is a commercial product used as a cosmetic ingredient, while excessive exposure to 1-pentadecanol produces some central nervous system depression and prolonged contact produces skin irritation. It is not clear what role they could be playing in the anti-trypanosomal activity of the crude methanol extract of *E. camaldulensis*. The case of the other two components, 2-Chloro-N-(1, 3-thiozol-2-yl) acetamide and 9-Octadecenamide is different because evidence from literature shows that some acetanamides are potent inhibitors of Histone Deacetylase (HDAC), an enzyme responsible for the deacetylation of N-acetyl lysine residues of histone and non-histone proteins (James and Henry, 2006). And there is growing evidence that the acetylation state of proteins and thus the HDAC enzyme plays a crucial role in the modulation of a number of biological processes, including transcription and cell cycle, which is the basis for their use in the treatment of cancer (Marks *et al*., 2000). On the basis of this, we may infer that the amides in fraction 8 could be playing the role of inhibitors of certain enzyme (s) unknown to us, but because their concentration in the fraction was small, they elicited a minimal effect on the trypanosomes.

Another aspect of the present study that could be exploited is the treatment of trypanosomiasis by administration of methylating agents. Some methylating agents have been found to be effective against *T. rhodesiense* and *T. gambiense*, which cause fatal

diseases in man and also against *T. brucei*, *T. evansi* and *T. equiperdum*, which are of veterinary importance. That study concerned a method of treating trypanosomiasis in patients, e.g., warm-blooded animals, such as humans, horses, sheep, goats, swine, camels or cattle, by administering to such patients a trypanocidal effective amount of a methylating agent of the formula CH_3 N = NX', wherein X' is a leaving group, e.g., OH or SO_2 R'" and R'" an alkyl or an aryl, more particularly an unsubstituted or substituted alkyl having 1 to 10, preferably 1 to 6, carbon atoms or an unsubstituted or substituted aryl, including other species capable of generating methyl radicals ($CH_3^{\bullet}$), diazomethane (CH_2 N_2) or methyldiazonium (CH_3 N_2^+) (Shyam *et al*., 1987). It has also been demonstrated that repeated exposure of trypanosomes *in vitro* or *in vivo* to low concentrations of the methylating agent 1,2-bis (methylsulfonyl)-1-methylhydrazine induces a series of moderately synchronous morphological and biochemical changes. Cell division halts and the long-slender bloodstream forms transform to short-stumpy forms via larger intermediate-stage cells which contain approximately double the normal G_2 content of DNA (Philip *et al*., 1991).

Interestingly, fraction 9 contains compounds like Methyl hexadecanoate, Methyl-cis-9-octadecanoate and Methyl-n-octadecanoate, which might be likely methylating agents that could play positive roles in the therapy of African trypanosomiasis. Literature indicates that Methyl-cis-9-octadecenoate was first identified in the cerebrospinal fluid of sleep-deprived cats, rats and humans. It induced physiological sleep when injected into rats intraperitoneally at 5-50 mg doses (Cravatt *et al*., 1995). Methylating agents appear to have two major effects on trypanosomes, depending upon the dose level. At high levels, cytokinesis appears to be inhibited almost immediately and the cells are transformed into transitional forms containing multiple nuclei and kinetoplasts. These cells disappear from the bloodstream in 48 to 72 h. When administered at repetitive low doses, methylating agents induce the entire population to differentiate into short-stumpy forms (short-stumpy forms cannot differentiate further unless they are taken up by a feeding tsetse fly or placed in appropriate culture conditions), as judged by morphology, NADH diaphorase positivity and other biochemical and physiological criteria. Short-stumpy forms are non-dividing differentiated cells and are not infective to the mammalian host. The latter property may make these agents useful biochemical tools in the study of differentiation in trypanosomes, since, with these compounds, it is possible to induce the entire population of trypanosomes to differentiate in a moderately synchronous manner and through this approach early events in the differentiation process can be studied. Both single high dose regiments and repetitive low doses can result in cures of trypanosomiasis using a number of the methylating agents (Philip *et al*., 1991).

Difluoro Methyl Ornithine (DFMO) or eflornithine has also been shown to induce differentiation in *T. brucei* (Goldberg *et al*., 1997). This effect is generally attributed to the depletion of polyamines. The depletion of polyamines and trypanothione as a result of the DFMO treatment may potentiate the actions of SAM and DSAM as methylating agents by decreasing the levels of competing nucleophiles. Depletion of polyamines may also make the nucleic acids more susceptible to methylation (Fairlamb *et al*., 1983). SAM is also the methyl donor used by many methylases; therefore, enzymatically - mediated - methylation reactions may also be affected. Although methylating agents in general are mutagenic, in cases of multi-drug resistant trypanosomiasis which have failed to respond to existing therapies, these compounds may be extremely effective. The distinct advantages of methylating agents over existing trypanocides include:

- High therapeutic indices
- Oral activity
- Novel mechanism of action
- Broad spectrum antitrypanosomal activity
- Favourable pharmacokinetics which makes these compounds candidates for both agricultural and clinical development (Fairlamb *et al*., 1983).

From the foregoing, it can be deduced that the minimal antitrypanosomal activity exhibited by fraction 9 may be due to the presence of three potential methyl donors and the relatively low concentration of these agents prevented complete clearance of parasites from the circulation of infected mice.

The anti-trypanosomal potential of the methanol extract of *E. camaldulensis* has been demonstrated in this study and may give credence to the acclaimed therapeutic potency of *E. camaldulensis* (leaf) as an anaesthetic, antiseptic, astringent and as a folk remedy for colds, colic, coughs, diarrhoea, dysentery, haemorrhage, laryngalgia, laryngitis, pharyngitis, sore throat, spasm, trachalgia and wounds (Batish *et al*., 2008) and its use locally in combination with other plants as a remedy for fever in some Nigerian communities.

It could be concluded from the result obtained in this study that the crude methanol extract of *E. camaldulensis* (leaf) is a potential candidate for the treatment of African Animal Trypanosomiasis because it is effective, cheap and is not acutely toxic and the plant is readily available.

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