

Research Article

Evaluation of In Vitro Activity of Essential Oils against *Trypanosoma brucei brucei* and *Trypanosoma evansi*

Nathan Habila,¹ Abel S. Agbaji,¹ Zakari Ladan,¹ Isaac A. Bello,² Emmanuel Haruna,³ Monday A. Dakare,¹ and Taofiq O. Atolagbe³

¹Biochemistry Division, National Research Institute for Chemical Technology, P.M.B 1052 Zaria, Nigeria

²Department of Chemistry, Ahmadu Bello University, P.M.B 1045 Zaria, Nigeria

³Department of Biochemistry, Ahmadu Bello University, P.M.B. 1045 Zaria, Nigeria

Correspondence should be addressed to Nathan Habila, nathanhabila@yahoo.com

Received 10 July 2009; Revised 13 January 2010; Accepted 13 January 2010

Academic Editor: Dave Chadee

Copyright © 2010 Nathan Habila et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Essential oils (EOs) from *Cymbopogon citratus* (CC), *Eucalyptus citriodora* (EC), *Eucalyptus camaldulensis* (ED), and *Citrus sinensis* (CS) were obtained by hydrodistillation process. The EOs were evaluated in vitro for activity against *Trypanosoma brucei brucei* (Tbb) and *Trypanosoma evansi* (*T. evansi*). The EOs were found to possess antitrypanosomal activity in vitro in a dose-dependent pattern in a short period of time. The drop in number of parasite over time was achieved doses of 0.4 g/ml, 0.2 g/mL, and 0.1 g/mL for all the EOs. The concentration of 0.4 g/mL CC was more potent at 3 minutes and 2 minutes for Tbb and *T. evansi*, respectively. The GC-MS analysis of the EOs revealed presence of Cyclobutane (96.09%) in CS, 6-octenal (77.11%) in EC, Eucalyptol (75%) in ED, and Citral (38.32%) in CC among several other organic compounds. The results are discussed in relation to trypanosome chemotherapy.

1. Introduction

African Trypanosomosis is a disease caused by several trypanosomes of the Trypanosomatidae family, which affect humans or animals. *Trypanosoma evansi* (*T. evansi*) is the most widely distributed of the pathogenic African trypanosomes, affecting domestic livestock and wildlife [1] and causes African trypanosomosis, a disease which affects humans and animals [2–4]. It is also well known that *T. evansi* is the etiological agent of a disease called Surra which is a major cause of livestock morbidity and mortality [5, 6] and it has been reported to occur in a variety of hosts the most economically important being horses, buffalo and cattle [3, 6, 7]. *Trypanosoma brucei brucei* (Tbb) also causes African Trypanosomosis along with several other species of trypanosomes although Tbb has morphological similarities with *T. evansi*, the latter differs from related species by the absence of kinetoplast DNA minicircle [8].

Currently, 35 million people and 25 million cattle in Africa are at risk of contracting the disease which is fatal if

untreated [9]. Ironically, some registered trypanocides are frequently toxic, require lengthy administration, lack efficacy and are sometimes unaffordable for most of the patients [10].

In many African countries, plants have traditionally been used for centuries and are still being widely used to treat this illness and other parasitic diseases which may be due to limited availability and affordability of pharmaceutical products. Essential oils (EOs) are odoriferous constituents produced by plants and have been traditionally used for respiratory tract infections and are known to possess antimicrobial properties [11, 12]. It is also well known that some EOs have some chemical components with insecticidal properties [13] and some EOs have characteristic array of compounds in different variations of which the most abundant types of chemical compounds present are Terpenoids and Phenyl compounds [14].

The present study, investigates the evidence of in vitro antitrypanosomal activity of EOs from *Cymbopogon citratus*, *Eucalyptus citriodora*, *Eucalyptus camaldulensis* and *Citrus*

sinensis against Tbb and *T. evansi* in an attempt to explore natural products that may be more accessible sources of trypanocides Vis-a-vis species difference and the fact that current available treatments are far from being ideal.

2. Materials and Methods

2.1. Collection of Plant Materials. Fresh *Citrus sinensis* (CS) peels were obtained from fresh oranges purchased in Zaria-Nigeria in April 2009. The Leaves of *Cymbopogon citratus* (CC) were harvested in the Research Garden of National Research Institute for Chemical Technology (NARICT) Zaria-Nigeria in March 2009, *Eucalyptus Camaldulensis* (ED) and *Eucalyptus citriodora* (EC) were harvested from state forestry Shika, Kaduna state—Nigeria in April 2009. The plants (CC, EC, and ED) were identified at department of Biological sciences, Ahmadu Bello University, Zaria-Nigeria.

2.2. Test Organisms. The parasites *Trypanosoma brucei brucei* and *Trypanosoma evansi* were used and obtained from stabulates maintained at the parasitology laboratory, Faculty of Veterinary medicine, Ahmadu Bello University, Zaria-Nigeria.

2.3. Extraction of Essential Oils. Briefly, 250 g fresh plant material of each plant was put in a round bottom flask and 1000 mL distilled water was added before subjecting to hydro distillation [15] for 6 hours. The EOs were recovered and dried over anhydrous sodium sulphate. Each EOs produced was diluted using pure vegetable oil obtained from Grand cereals and oil mills limited, United African Company, Lagos-Nigeria. The different concentrations of EOs were prepared at 0.4 g/mL, 0.2 g/mL and 0.1 g/mL in the diluent constituted into 50%, 25%, and 1%, respectively.

2.4. Determination of Parasitaemia. The parasites were maintained in the laboratory by continuous passage in rats until it is required. The passage was done when parasitaemia was in the range of 16–32 parasites per field with infected blood containing 1×10^3 parasites was introduced intraperitoneally into healthy rats in 0.1–0.2 mL blood/physiological saline solution. The numbers of parasites were determined microscopically at magnification $\times 400$ and Parasitaemia was monitored daily.

2.5. In Vitro Test. The Drug Incubation Infectivity Test [16] with Wet and thick blood films method [17] to detect any motile trypanosomes was done in triplicates in a 96 well microtitre plate with 50 μ L of infected blood was incubated with 20 μ L of each EOs from 50%, 25% and 1% (previously prepared) giving a final concentration of 114 mg/mL, 57 mg/mL and 28.6 mg/mL, respectively. Parasitaemia was monitored between 1–20 minutes of incubation at 30°C. About 0.5 μ L–1 μ L of test mixtures were observed every 1 minute under a microscope $\times 400$. A set of Positive Control (PC), Negative Control (NC) and Diluent Control (DC) were set up to stand for 4hrs with the infected blood containing the parasites. The PC contained 25 mg/mL

of DIMINAVETO^R (1.05 g of Diminazene diacetate and 1.31 g Antipyrine), the NC contained only the infected blood suspended in heparin and Phosphate Buffer Saline Glucose (pH 7.2) and the DC contained only the pure vegetable oil (100%).

2.6. Gas Chromatography-Mass Spectroscopy (GC-MS). This was carried out as described by Maciel et al. [13] using EOs obtained from CS, CC, EC, and ED in GC-MS QP2010 SHIMADZU, Japan. The Column oven temperature was 50°C, Injection temperature was 200°C, Pressure was 53.2 kPa., Linear velocity was 36.2 cm/sec and Total flow was 53.8 mL/min. The Compounds were identified by GC retention time (RT) and by comparison with those present in the National Institute for Standard Technology computer data bank (NIST; 05 s. LIB).

3. Results and Discussion

The results in Figures 1 and 2 show the in vitro activity of EC, CS, ED and CC on *T. evansi* and Tbb. In the results presented in Figures 1(a), 1(b), 1(c) and 1(d), it was observed that the dose of 0.4 g/mL concentration of each of the EOs used caused death of the parasite in 3 minutes (except for CC which was in 2 minutes) against *T. evansi*. When the concentrations of the EOs were 0.2 g/mL and 0.1 g/mL, the time of parasite death for CS was 7 minutes and 17 minutes, ED was 4 minutes and 15 minutes, EC was 3 minutes and 19 minutes, and CC was 2 minutes and 10 minutes, respectively. Figures 2(a), 2(b), 2(c), and 2(d) states the in vitro Antitrypanosomal activity of the four species of EOs against Tbb where 0.4 g/mL concentration induced death of Tbb in 5 minutes, 4 minutes, and 3 minutes for CS, ED/EC and CC. When the concentrations of the EOs were 0.2 g/mL and 0.1 g/mL, the time of parasite death for CS was 6 minutes and 15 minutes, ED was 8 minutes and 17 minutes, EC was 8 minutes and 22 minutes, and CC was 4 minutes and 11 minutes, respectively. This indicates a dose and time-dependent pattern of parasite death in both *T. evansi* and Tbb. The result shows that CC had a higher effect in decreasing cell number more than ED, EC, and CS against both *T. evansi* and Tbb. All EOs showed activity on both parasites even at 0.1 g/mL concentration but there was variation in the decreased in cell number of the candidates EO.

Table 1 shows the various compounds present in the EOs and the chemical compositions were found to be different for each EO. For example, Citral (38.32%) was present in CC, Beta-myrcene (1.81%) and Cyclobutane (98.08%) was present in CS, Eucalyptol (75.04%) was present in ED and 6-Octenal (77.11%) was present in EC. Most of these compounds like, Beta-myrcene, Eucalyptol and Citral are known to have biological activity [13, 18, 19] which are also raw materials against plants and human pathogens [20]. Some reports have also shown that CC play a role in peroxidation by inhibiting free radical attacks on biomembranes [21, 22] especially the red blood cells, and are not likely to pose any health risk [15]. The activity of EOs could also be due to

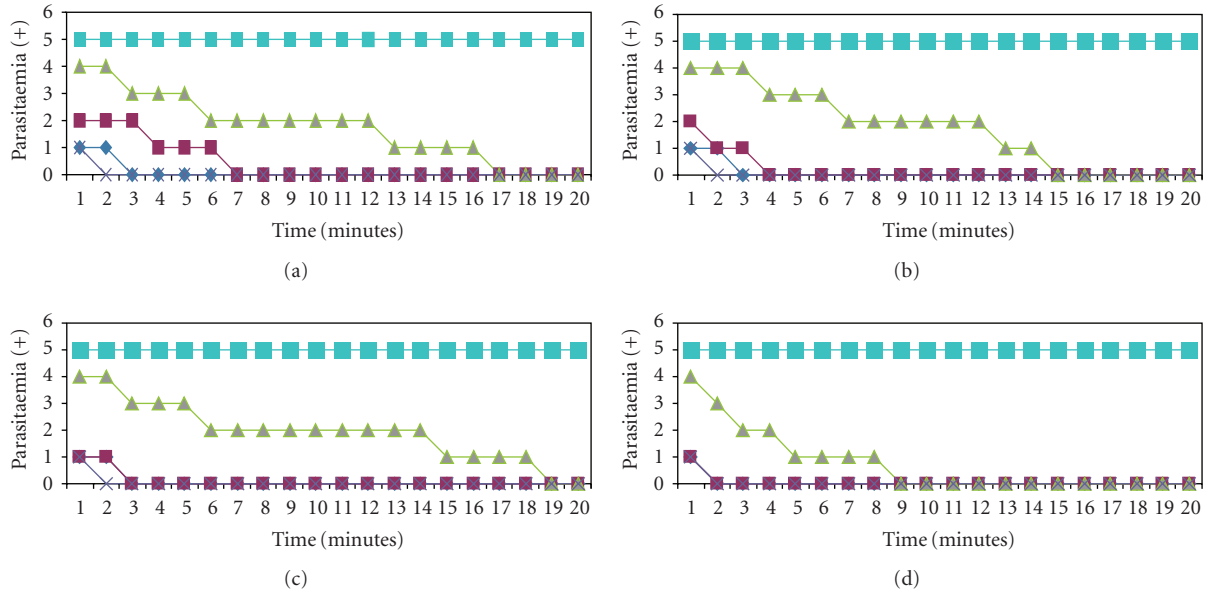


FIGURE 1: Effect of CS (a), ED (b), EC, (c) and CC (d) essential oils (EO) on *T. evansi* blood forms. The EOs were diluted at 0.4 g/mL (dark blue), 0.2 g/mL (red) and 0.1 g/mL (green) in pure vegetable oil as diluent and then added to the cultures, as described previously. Treatment of the parasites with Diminaveto (Positive control, purple) induced total lysis after 2 minutes. The number of parasites remained constant in negative controls and diluent control (light blue).

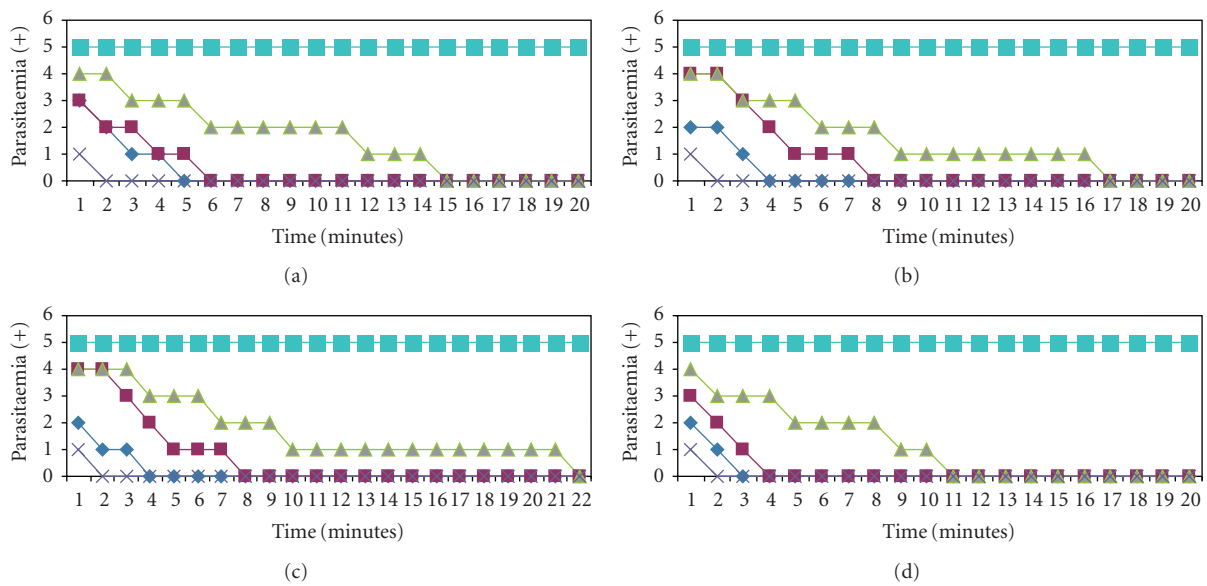


FIGURE 2: Effect of CS (a), ED (b), EC (c) and CC (d) essential oils (EO) on *Tbb* blood forms. The EOs were diluted at 0.4 g/mL (dark blue), 0.2 g/mL (red) or 0.1 g/mL (green) in pure vegetable oil as diluent and then added to the cultures, as described previously. Treatment of the parasites with Diminaveto (Positive control, purple) induced total lysis after 2 minutes. The number of parasites remained constant in negative controls and diluent control (light blue).

the hydrophobic nature of the cyclic hydrocarbons (Table 1), which allow EOs to interact with the Trypanosomes causing conformational changes in the parasite membrane structure resulting to loss in membrane stability [21].

The quick decrease in parasite number in the in vitro tests for both *Tbb* and *T. evansi* indicates that the EOs is in fact

killing the parasites, by an unknown mechanism although EOs can have multiple modes of action and multiple points of disruption [23] like inhibiting some key enzymes in the parasite glycolytic pathway. It is also known that some EOs components inhibit acetylcholinesterase activity and act on other vulnerable sites, such as cytochrome p450 [13]. This

TABLE 1: GC-MS analysis for essential oils from CS, ED, EC and CC.

Compound	RT	CS(%)	ED(%)	EC(%)	CC(%)
Beta-Myrcene	10.045	1.81	—	—	—
Cyclobutane	11.322	96.08	—	—	—
1, 6-octadien-3-ol	13.186	1.70	—	—	—
Decanal	15.355	0.41	—	—	—
6-octenal	14.405	—	—	77.11	—
Cyclohexanol	14.573	—	—	3.42	—
6-octen-1-ol	15.878	—	—	14.09	—
2, 6-Octadiene	17.790	—	—	2.58	—
Cyclohexanol	17.899	—	—	1.11	—
Bicyclo[7.2.0]undec-4-ene	18.968	—	—	1.29	—
1, 7-Nonadiene	25.464	—	—	0.40	—
Bicyclo[3.1.1]hept-2-ene	8.490	—	10.27	—	—
Benzene	11.129	—	6.23	—	—
Cyclohexene	11.217	—	4.92	—	—
Eucalyptol	11.452	—	75.04	—	—
p-meth-1-en-8-ol	15.261	—	1.65	—	—
2-Oxabicyclo[2.2.2]octan-6-ol	17.610	—	1.09	—	—
(2S, 4R)-P-metha-[1(7), 8]-diene-2-hydroperoxide	21.253	—	0.80	—	—
2, 6-octadien-1-ol	18.717	—	—	—	26.63
2, 6-octadienal	19.242	—	—	—	35.05
Citral	18.408	—	—	—	38.32

multicomponent nature of plant EOs is an advantage for several target sites on trypanosomes, which is especially relevant at a time of increasing emergence of multi-resistant pathogens.

We did not observe any resumption of flagellate movement or increase in cell number of Tbb and *T. evansi* after 4 hours incubation and observation with the PC, CC, ED, EC and CS. The NC and DC had no effect on the parasites or cell number (Figures 1 and 2) and this gives credence to the impetus of all the EOs species used. The observations from Figures 1 and 2 indicates that species difference of Trypanosomes may be responsible for the efficacy of Trypanocides in line with the fact that all the EOs differ in the way the parasite number decreases with time.

In this study, the evaluation of the four EOs species was able to decrease cell number of Tbb and *T. evansi* in a dose dependent pattern. The EOs from CC had a greater effect of decreasing parasite number more than EC, CS and ED but understanding the real mechanism of action will require further investigation. Sequel to these qualities of EO, it would be expedient to subject it for further trypanosome pathophysiology.

Acknowledgments

The authors appreciate the Benevolence of Professor Joseph Ajanusi and Mallam Bala of Department of Veterinary Parasitology, Ahmadu Bello University, Zaria-Nigeria for the kind release of the Test organisms. The authors also appreciate NARICT for research facilities.

References

- [1] A. Tamarit, C. Gutierrez, R. Arroyo, et al., “*Trypanosoma evansi* infection in mainland Spain,” *Veterinary Parasitology*, vol. 167, no. 1, pp. 74–76, 2010.
- [2] D. Fernández, B. González-Baradat, M. Eleizalde, E. González-Marcano, T. Perrone, and M. Mendoza, “*Trypanosoma evansi*: a comparison of PCR and parasitological diagnostic tests in experimentally infected mice,” *Experimental Parasitology*, vol. 121, no. 1, pp. 1–7, 2009.
- [3] B. Vanhollebeke, P. Truc, P. Poelvoorde, et al., “Human *Trypanosoma evansi* infection linked to a lack of apolipoprotein L-1,” *The New England Journal of Medicine*, vol. 355, no. 26, pp. 2752–2756, 2006.
- [4] R. M. Powar, V. R. Shegokar, P. P. Joshi, et al., “A rare case of human trypanosomiasis caused by *Trypanosoma evansi*,” *Indian Journal of Medical Microbiology*, vol. 24, no. 1, pp. 72–74, 2006.
- [5] R. E. Morty, R. Pellé, I. Vadász, G. L. Uzcanga, W. Seeger, and J. Bubis, “Oligopeptidase B from *Trypanosoma evansi*: a parasite peptidase that inactivates atrial natriuretic factor in the bloodstream of infected hosts,” *Journal of Biological Chemistry*, vol. 280, no. 12, pp. 10925–10937, 2005.
- [6] M. A. Saleh, M. B. Al-Salahy, and S. A. Sanousi, “Oxidative stress in blood of camels (*Camelus dromedaries*) naturally infected with *Trypanosoma evansi*,” *Veterinary Parasitology*, vol. 162, no. 3-4, pp. 192–199, 2009.
- [7] T. W. Jones, R. C. Payne, I. P. Sukanto, and S. Partoutomo, “*Trypanosoma evansi* in the Republic of Indonesia. FAO cooperate document repository,” in *Proceedings of the 1st Internet Conference on Salivarian Trypanosomes*, 1997.

- [8] C. A. Hoare, "Classification and the Salivaria (Subgenus Trypanozoon)," in *The Trypanosomes of Mammals*, A Zoological Monograph, pp. 60–79 and 476–604, Blackwell Scientific, Oxford, UK, 1972.
- [9] A. J. Nok, S. Williams, and P. C. Onyenekwe, "Allium sativum—induced death of African trypanosomes," *Parasitology Research*, vol. 82, no. 7, pp. 634–637, 1996.
- [10] S. Hoet, F. Opperdoes, R. Brun, V. Adjakidjé, and J. Quetin-Leclercq, "In vitro antitrypanosomal activity of ethnopharmacologically selected Beninese plants," *Journal of Ethnopharmacology*, vol. 91, no. 1, pp. 37–42, 2004.
- [11] S. Inouye, T. Takizawa, and H. Yamaguchi, "Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact," *Journal of Antimicrobial Chemotherapy*, vol. 47, no. 5, pp. 565–573, 2001.
- [12] R. B. Pedroso, T. Ueda-Nakamura, B. P. Dias Filho, et al., "Biological activities of essential oil obtained from *Cymbopogon citratus* on *Crithidia deanei*," *Acta Protozoologica*, vol. 45, no. 3, pp. 231–240, 2006.
- [13] M. V. Maciel, S. M. Morais, C. M. L. Bevilaqua, et al., "Chemical composition of Eucalyptus spp. essential oils and their insecticidal effects on *Lutzomyia longipalpis*," *Veterinary Parasitology*, vol. 167, no. 1, pp. 1–7, 2010.
- [14] R. O. B. Wijesekera, *Practical Manual on the Essential Oils Industry*, UNIDO, Vienna, Austria, 1978.
- [15] S. A. Bankole and A. O. Joda, "Effect of lemon grass (*Cymbopogon citratus* Stapf) powder and essential oil on mould deterioration and aflatoxin contamination of melon seeds (*Colocynthis citrullus* L.)," *African Journal of Biotechnology*, vol. 3, no. 1, pp. 52–59, 2004.
- [16] V. Delespaux, D. Geysen, P. Van den Bossche, and S. Geerts, "Molecular tools for the rapid detection of drug resistance in animal trypanosomes," *Trends in Parasitology*, vol. 24, no. 5, pp. 236–242, 2008.
- [17] F. Chappuis, L. Loutan, P. Simarro, V. Lejon, and P. Büscher, "Options for field diagnosis of human African trypanosomiasis," *Clinical Microbiology Reviews*, vol. 18, no. 1, pp. 133–146, 2005.
- [18] R. M. Peter, *Characterization of Essential Oils by Comprehensively Coupled Supercritical Fluid and Gas Chromatography*, University of Pretoria ETD, Makgane, South Africa, 2006.
- [19] F. M. McIntosh, P. Williams, R. Losa, R. J. Wallace, D. A. Beever, and C. J. Newbold, "Effects of essential oils on ruminal microorganisms and their protein metabolism," *Applied and Environmental Microbiology*, vol. 69, no. 8, pp. 5011–5014, 2003.
- [20] E. Lewinsohn, N. Dudai, Y. Tadmor, et al., "Histochemical localization of citral accumulation in lemongrass leaves (*Cymbopogon citratus* (DC.) Stapf., Poaceae)," *Annals of Botany*, vol. 81, no. 1, pp. 35–39, 1998.
- [21] S. Calsamiglia, M. Busquet, P. W. Cardozo, L. Castillejos, and A. Ferret, "Invited review: essential oils as modifiers of rumen microbial fermentation," *Journal of Dairy Science*, vol. 90, no. 6, pp. 2580–2595, 2007.
- [22] O. O. Ojo, F. R. Kabutu, M. Bello, and U. Babayo, "Inhibition of paracetamol-induced oxidative stress in rats by extracts of lemongrass (*Cymbopogon citratus*) and green tea (*Camellia sinensis*) in rats," *African Journal of Biotechnology*, vol. 5, no. 12, pp. 1227–1232, 2006.
- [23] A. Smith-Palmer, J. Stewart, and L. Fyfe, "Influence of subinhibitory concentrations of plant essential oils on the production of enterotoxins A and B and α -toxin by *Staphylococcus aureus*," *Journal of Medical Microbiology*, vol. 53, no. 10, pp. 1023–1027, 2004.