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GC-MS Analysis of Bioactive Fractions of *Terminalia avicennoides*, *Bombax buopodezense* Barks and Lipid Profile of *Trypanosoma brucei* Infected Wistar Rats

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ABSTRACT: *Trypanosoma brucei*, a causative agent of trypanosomiasis in livestock also possesses the ability to reoccur in disease conditions thereby increasing the trouble of ill health and if not treated on time could cause great losses to man. A comparative study was carried out to investigate the efficacy of different fractions of the extracts of *Terminalia avicennoides* and *Bombax buopodezense* on *Trypanosoma brucei brucei* infected wistar rats. The plants extract were fractionated using different solvents by chromatography technique. Seventy two male wistar albino rats weighing between 120-140gm were randomly distributed into fourteen (14) groups (A-N) of five rats each. The effect of treatment on parasitemia, packed cell volume (PCV), organ-body weight ratio, Lipid profile indices and extension in the days of survival were monitored. The infection caused a gradual decline in the values of packed cell volume (PCV) in infected groups; treatments with bioassay-guided fractions of the two plants resulted in reduction in parasitaemia, but were not curative. The group treated with crude extract of *Terminalia avicennoides* significantly ($P<0.05$) resulted in suppression of parasitemia, and prolongation of lifespan. The fractions of the plants showed reduced values of serum cholesterol, VLDL, HDL cholesterol and LDL, which suggest a low risk of cardiovascular disease. The GC-MS chromatogram of the aqueous extract of *Terminalia avicennoides* showed 30 peaks indicating the presence of thirty phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library the thirty phytoconstituents were characterized and identified. Similarly the GC-MS chromatogram of the aqueous extract of *Bombax buopodezense* showed 20 peaks indicating the presence of twenty phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library the twenty phytoconstituents were characterized and identified.

Keywords: Lipid profile, GC-MS analysis, Bioactive fractions, *Terminalia avicennoides, Bombax buopodezense*.

Introduction

African trypanosomiasis is a parasitic disease caused by a protozoan of the genus *Trypanosoma*. *Trypanosoma vivax* (*T. vivax*), *Trypanosoma congolense* (*T. congolense*) and to a lesser extent *Trypanosoma brucei brucei* (*T. b. brucei*) are the main species responsible for African animal

trypanosomosis (AAT) called nagana in West Africa while *T. b. rhodesiense* and *T. b. gambiense* cause human sleeping sickness (human African trypanosomiasis, HAT). Surra and Dourine are caused by the other trypanosome species *T. evansi* and *T. equiperdum* respectively. The disease is transmitted by a bite of the vector-tsetse fly (*Glossina* species) (D'Archivio *et al*., 2011).

African Trypanosomiasis, also known as African sleeping sickness is one of the neglected diseases in about thirty-six countries of sub-Saharan Africa threatening more than sixty million lives on daily basis (Steverding and Tyler, 2005). Trypanosomes are the causative agent of sleeping sickness in sub-Saharan Africa. The current chemotherapy of the human trypanosomiasis relies on only six drugs (Suramin, Pentamidine, Melarsoprol Eflorinithine, Arsobal and Mel B), five of which were developed 30 years ago. Furthermore, these drugs display undesirable toxic side effects (Steverding and Tyler, 2005) and studies shown the emergence of drug-resistant trypanosomes (Perez-Morga, 2007). Therefore, the development of cost effective new drugs in the treatment of sleeping sickness is urgently required in order to control the disease. However, it has also been observed that natural products derived from plants offer novel possibilities to obtain new drugs that are active against *trypanosomes* (Hoet *et al*., 2004). Many investigators targeted finding new anti-trypanosomal agents to combat the trypanosomomiasis by screening extracts of African plants (Adewumi *et al*., 2001; Ekanem *et al*., 2006; Akanji *et al*., 2009; Sulaiman *et al*., 2011).

For long, medicinal plants have been used traditionally to treat various kinds of diseases (Malekzadeh *et al.*, 2001) and today about 80% of the world's population relies mainly on traditional medicine for their primary health care needs (Akerele, 1993). Hence, it seems rather justified that the research for new and improved plants-derived drugs for the treatment of trypanosomosis should be further intensified. *Terminalia avicennioide* is a tropical plant with extensive medicinal applications (Adewunmi and Sofowora, 1980; Abdullahi *et al.*, 2001). It is called 'baushe' in the Hausa language of Northern Nigeria. A survey carried out in 2002 (Atawodi *et al.*, 2002) revealed that the aqueous extract of its stem bark is traditionally employed to treat sleeping sickness. Subsequent screening of the methanol extract of this plant suggested potent anti-trypanosomal activity (Atawodi *et al.*, 2003). Therefore, this work was designed to further explore the *in vivo* anti-trypanosomal potential and identify the major classes of phytochemicals present in *Terminalia avicennioides.*

Bombax buonopozense, commonly known as the Gold Coast *Bombax* or Red-flowered Silk Cotton Tree, is a tree in the mallow family. It is also known in the Dagbani language as *Vabga*. Many parts of the plant are utilised for medicinal and traditional purposes. In Ghana, where it is native, the leaves are commonly used as fodder for domestic livestock. The bark is burnt to produce a smoke that is believed to drive away evil spirits called *alizini* in Dagbani. The abundant thorns present on the bark are burnt and the resulting charcoal is mixed with butter to treat swelling. Dried gum produced from the tree is used as incense (Blench and Roger, 2006).

Material and Methods

***Plant Sample*:** *Terminalia avicennioides and Bombax buopodezense* stem bark were collected from a village in Ilorin, Kwara State, Nigeria. The plant's identity was confirmed at the Herbarium Section of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria, where a specimen with Voucher Number UIH003/899 for *Bombax buopodezense* and UIH002/756 *Terminalia avicennioides* was deposited respectively.

***Animals*:** Seventy (70) white albino rats weighing 100 -150g were obtained from the Animal house unit, department of Biochemistry, University of Ilorin, Kwara State, Nigeria. The animals were fed *ad libitum* on tap water and diet specially prepared from chick grower's mash (Pfizer Ltd, Lagos, Nigeria).

***Test Parasite*:** The parasite (*Trypanosoma brucei brucei*) was obtained from the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Ibadan, Oyo state, Nigeria. The parasite was isolated from infected cattle and until the time of this experiment, it was maintained in laboratory rats by continuous passage.

***Plant Extraction*:** Fresh bark of *Terminalia avicennioides and Bombax buopodezense* were cut into small pieces and then dried under shade for about two (2) weeks, until they became brittle. Exactly 500g of dried sample was macerated in 5 liters of distilled water for 48 hrs. Thereafter, extract was filtered through muslin cloth and then with Whatman No. 1 filter paper. The filtrate was dried on a water bath at 40 ºC, and stored in a refrigerator at 4ºC until required.

***Bioassay-Guided Fractionation of T. avicennioides and B. buopodezense Stem Bark Extracts*:** The crude aqueous extracts of *T. avicennioides and B. buopodezense* stem bark was fractionated on silica gel packed into a 1.5x30 cm column. The column was eluted with four solvent-mixtures (ethylacetate/methanol, 19:1; benzene/methanol, 9:1; acetic acid/methanol, 1:1 and water/methanol, 1:1) in order of increasing polarity. The fractions were collected in separate beakers, labelled 1-4 and made to dry at 50 ^{o}C in water bath. The dried fractions were kept at 4^{o}C until required for administration into rats in groups G to N.

***Estimation of Trypanosome Parasite in Blood*:** A drop of blood obtained from the rats by tail snipping was used to make smears on the slides. Parasites were observed and estimated from each of the infected animals under the microscope as described by Herbert and Lumsden (1976). Parasitemia was monitored for twelve (12) days.

***In vivo Evaluation of the Anti-trypanosomal Activity of T. avicennioides and B. buopodezense Extracts*:** Seventy male albino rats were used for this study. The animals were separated into fourteen groups of 5 animals each. Animals in group A were left as positive control group; they were not infected with the parasite but were administered with only 0.3 ml distilled water. Animals in the remaining groups (B-N) were all infected with parasites. Group B was left as a negative control group (infected but not treated). Animals in groups C and D were each treated intraperitoneally with 0.3ml each of Deferoxamine and Inositol Hexaphosphate (Standard drugs) respectively, Animals in groups E and F were administered the crude aqueous extracts of *T. avicennioides* and *B. buopodezense* respectively and each rat was treated with 200 mg/kg weight of the animal. Animals in groups G-J were each treated intraperitoneally, with 200mg/kg of fractions 1-4 of *T. avicennioides* respectively while Animals in groups K-N were each treated with 200mg/kg rat body weight of fractions 1-4 of *B. buopodezense* respectively. The treatments were commenced four days post infection (when parasitemia was 10-12 per microscopic field) and lasted for twelve days. All the animals were monitored daily for parasitemia and for their weight throughout the course of the study. Blood samples were also obtained daily from tails of animal into capillary tubes for determination of Packed Cell Volume (PCV).

***Packed Cell Volume Determination*:** Blood samples, collected into heparinized capillary tubes with one end of each tube sealed with plasticine were spinned at X2000g for five minutes in a microhaematocrit centrifuge. The packed cell volumes (PCVs) were determined with the aid of a micro-haematocrit reader, and the values expressed as percentages (Barbara, 1980).

***Determination of Total Cholesterol, Triacylglycerol, HDL-Cholesterol and LDL-Cholesterol*:** The determination of serum levels of cholesterol in the animals was carried out using colorimetric enzymatic end point method. Serum triacylglycerol were analyzed using colorimetric method after enzymatic hydrolysis with lipases. HDL-cholesterol was determined using precipitant method. All these analyses were carried out using standard commercial test kits (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom), following strictly the instructions provided by the

manufacturers. LDL-cholesterol expressed in mmol/l was calculated from the values of total cholesterol, triacylglycerol and HDL-cholesterol using the formula described by Friedewald *et al*. (1972) as shown below:

LDL-Cholesterol = Total Cholesterol – Triacylglycerol /2.2 – HDL-Cholesterol.

***GCMS Analysis of Plants*:** Gas chromatography-mass spectrometry (GC-MS) analysis of the whole plant extracts of *T. avicennioides* and *B. buopodezense* were performed on a GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II.

***Statistical Analysis*:** Data generated were presented as means with standard deviation. The data were subjected to analysis of variance (ANOVA). Means were considered significant at $P < 0.05$ and the means separated using Duncan's multiple range tests.

Results

Daily average weights of experimental rats infected with *T. brucei*

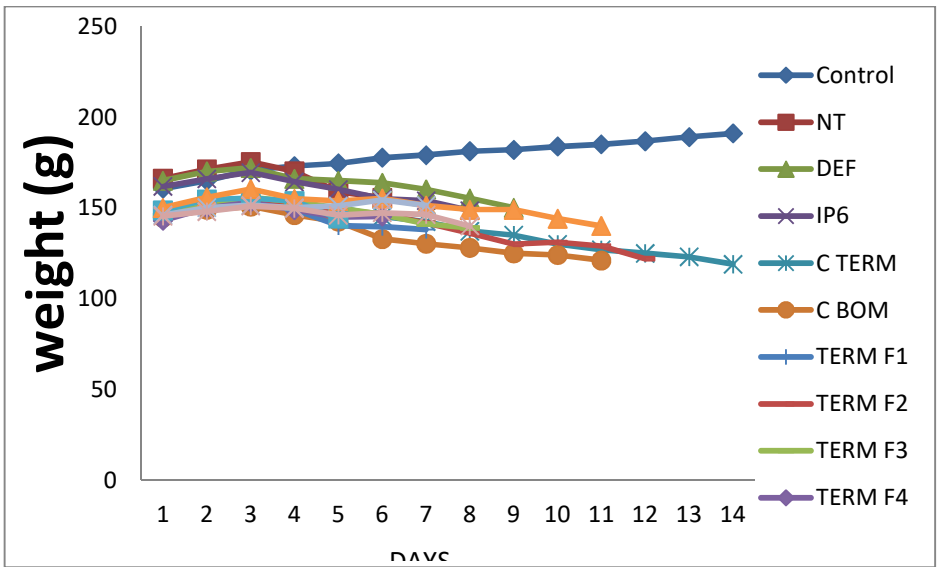


Fig 1: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on the daily average weight of experimental rats.

From the figure above, it was observed that there was a gradual decrease in the average weight of rats in each group across the two weeks of experiment when compared with the positive control group.

Parasite Count

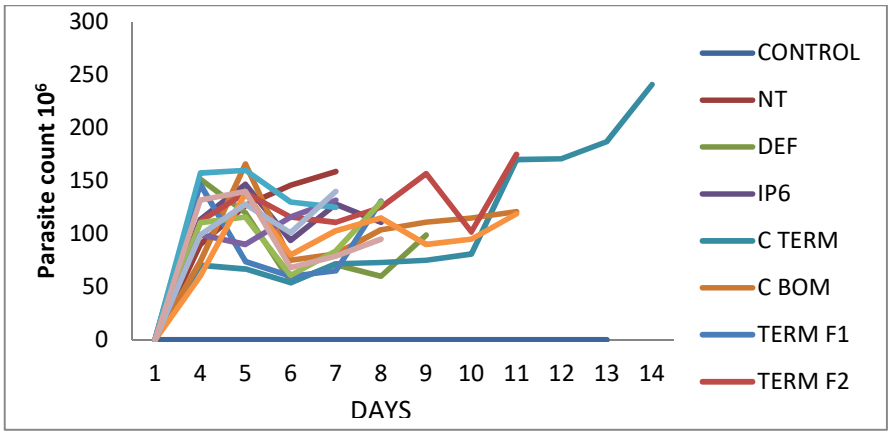


Fig 2: Parasitemia level of the fractions of *Terminalia avicennoides* and *Bombax buopodezense* on *Trypanosoma brucei* infected rats.

A gradual increase in parasitemia was observed across the various groups relative to the positive group, within whose blood, no parasite was counted.

Packed Cell Volume

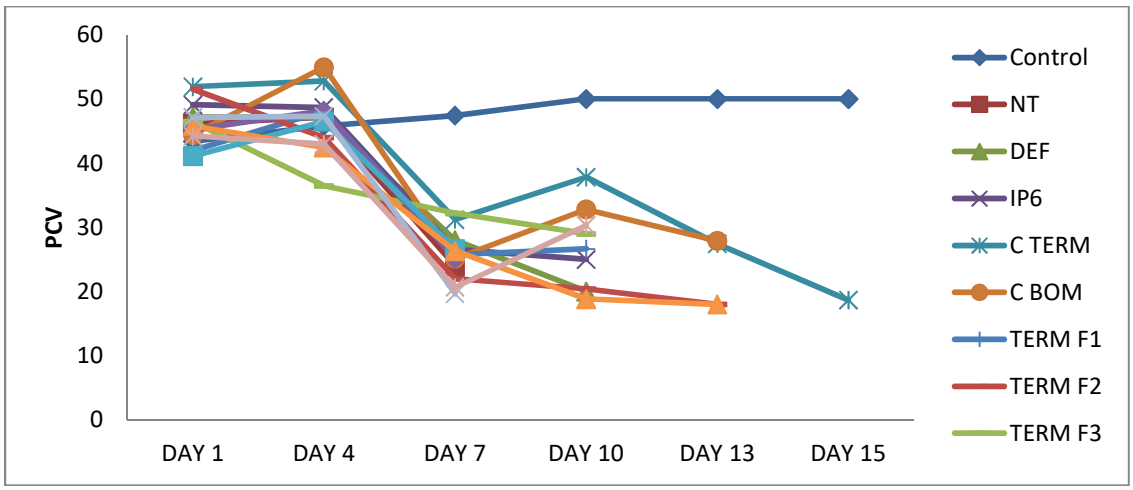


Fig 3: The packed cell volume of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on *Trypanosoma brucei* infected rats.

From the figure above, it was observed that there was a decrease in the packed cell volume of rats in each group across the two weeks of experiment when compared with the positive control group.

Lipid Profile

Cholesterol

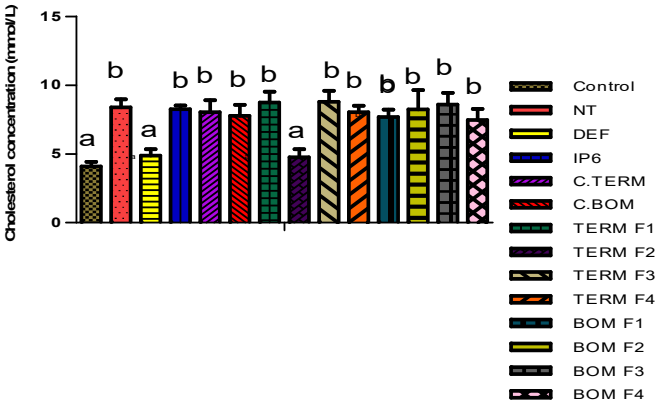


Fig 4: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on Liver Total Cholesterol of Rats infected with *T. brucei*

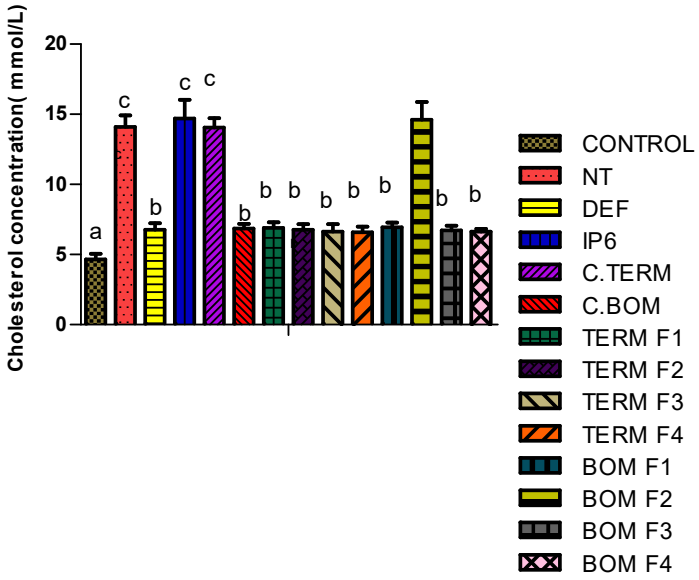


Fig 5: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on kidney total cholesterol of rats infected with *T. brucei*.

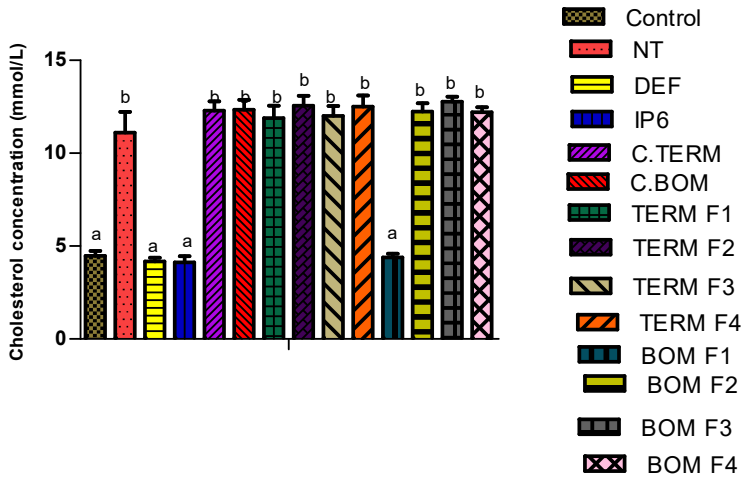


Fig 6: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on Heart Total Cholesterol of Rats infected with *T. brucei*.

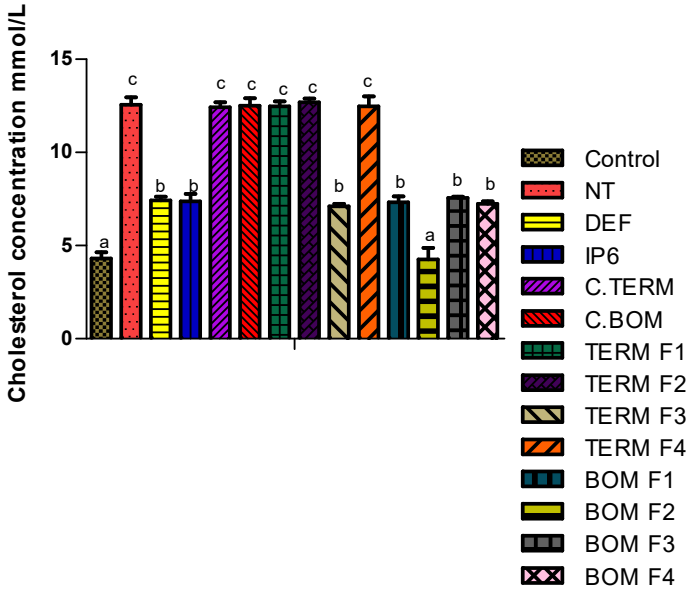


Fig 7: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on Serum Total Cholesterol of Rats infected with *T. brucei*. Bars are expressed as mean of three replicates ± S.E.M and bars with different superscripts are significantly different ($P<0.05$).

Triacylglycerol

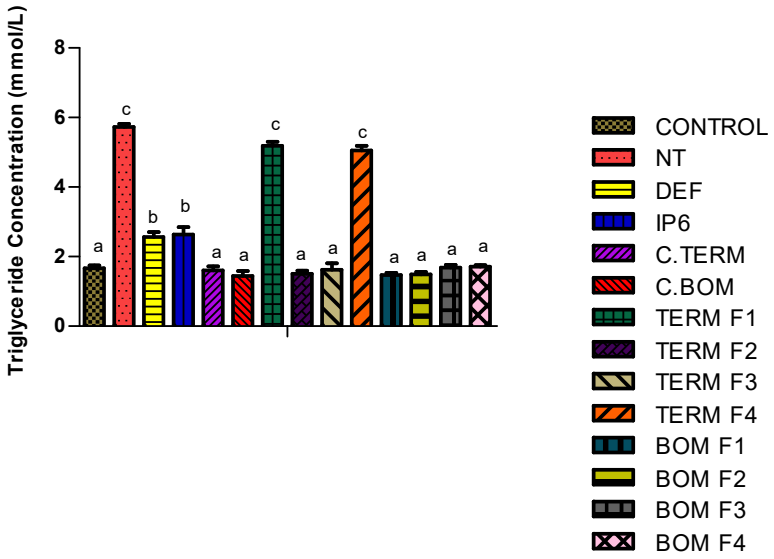


Fig 8: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on liver triacylglycerol of rats infected with *T. brucei*.

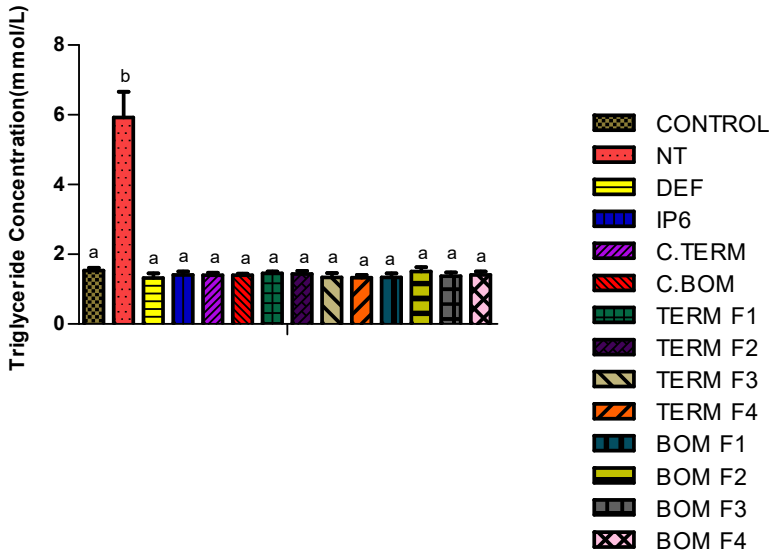


Fig 9: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on kidney triacylglycerol of rats infected with *T. brucei*.

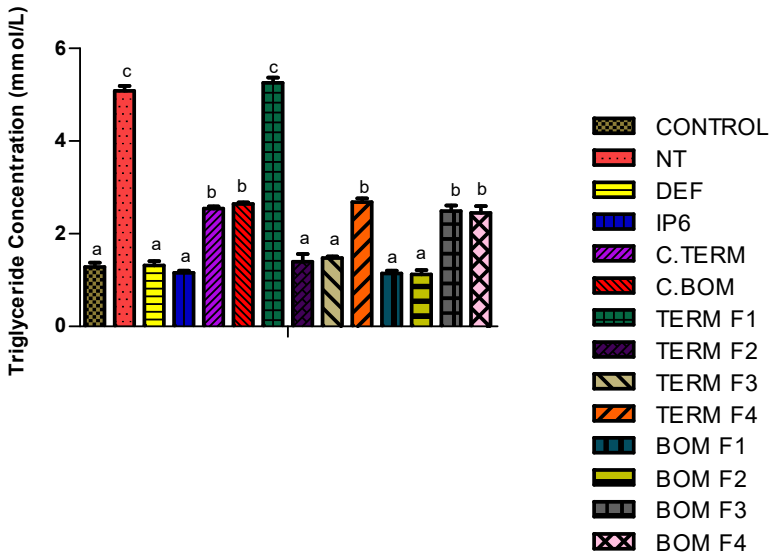


Fig 10: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on heart triacylglycerol of rats infected with *T. brucei*.

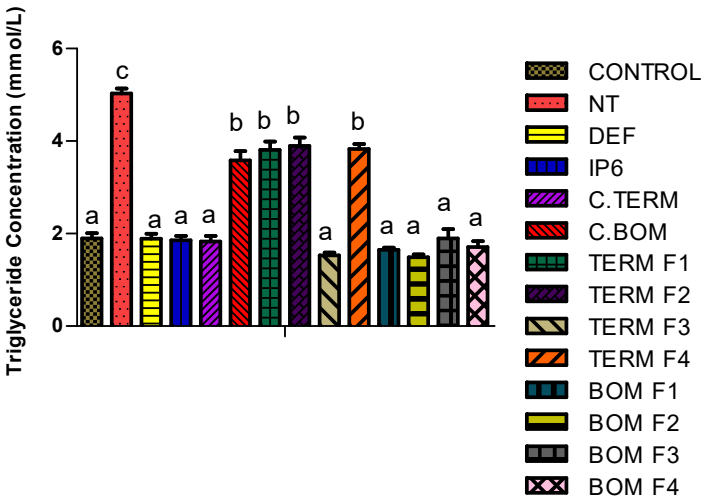


Fig 11: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on Serum Triacylglycerol of Rats infected with *T. brucei*. Bars are expressed as mean of three replicates ± S.E.M and bars with different superscripts are significantly different (P<0.05).

High Density Lipoprotein- Cholesterol

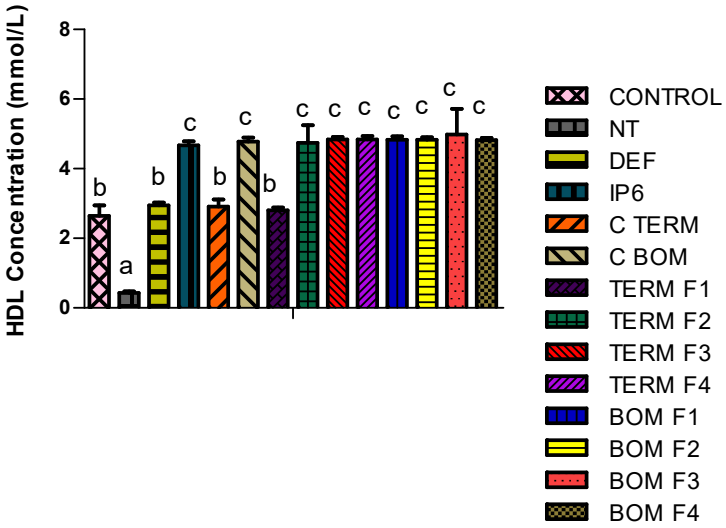


Fig 12: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on liver high density lipoprotein cholesterol of rats infected with *T. brucei*.

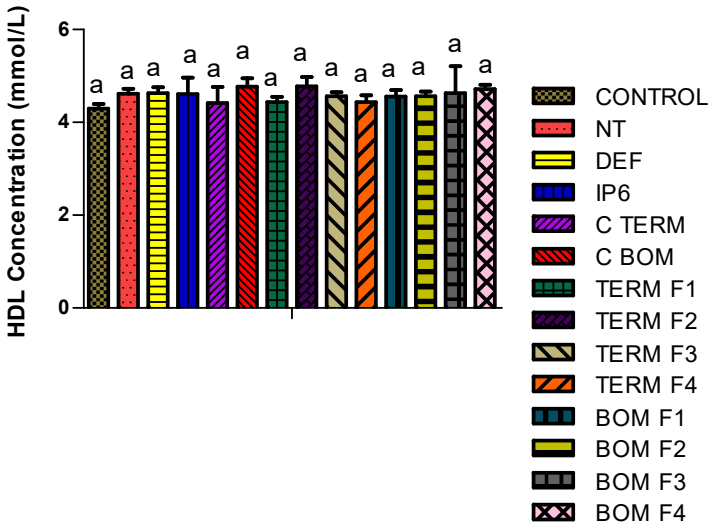


Fig 13: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on heart high density lipoprotein cholesterol of rats infected with *T. brucei*.

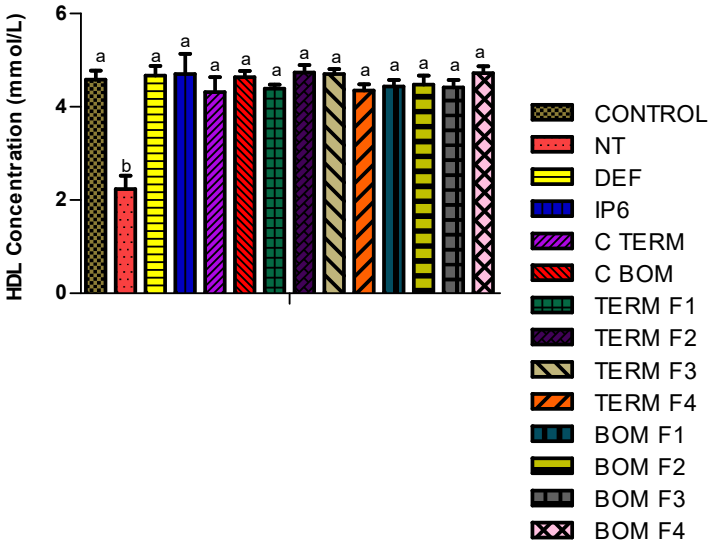


Fig 14: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on kidney high density lipoprotein cholesterol of rats infected with *T. brucei*.

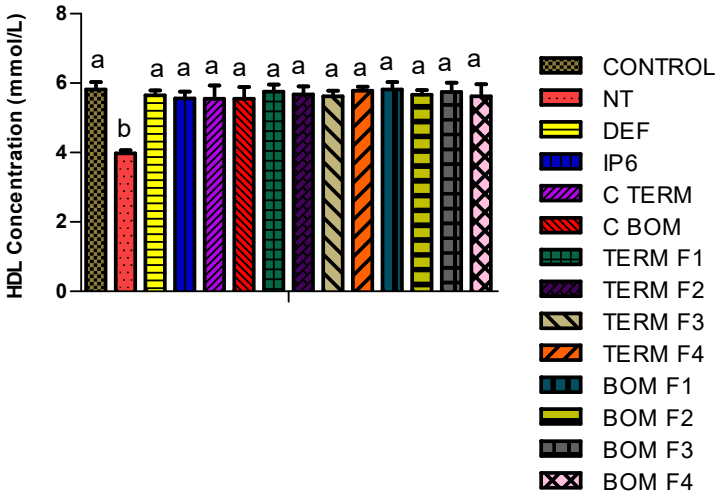


Fig 15: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on serum high density lipoprotein cholesterol of rats infected with *T. brucei*. Bars are expressed as mean of three replicates ± S.E.M and bars with different superscripts are significantly different (P<0.05)

Low Density Lipoprotein Cholesterol

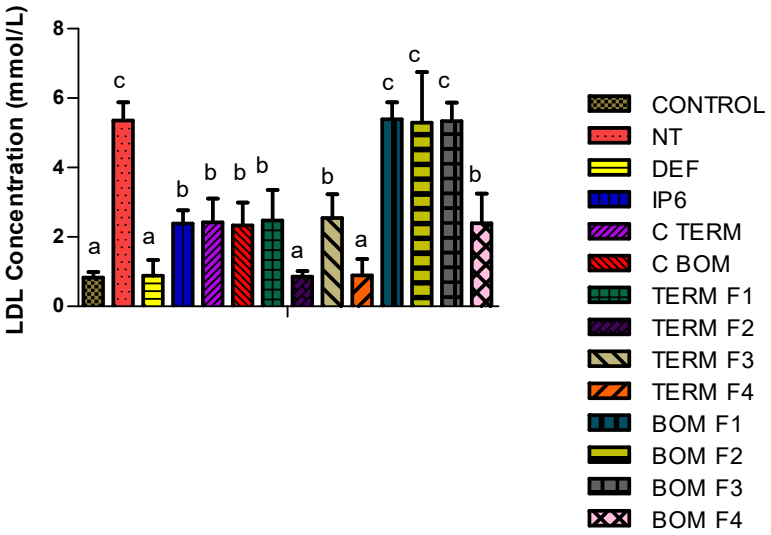


Fig 16: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on liver low density lipoprotein cholesterol of rats infected with *T. brucei*.

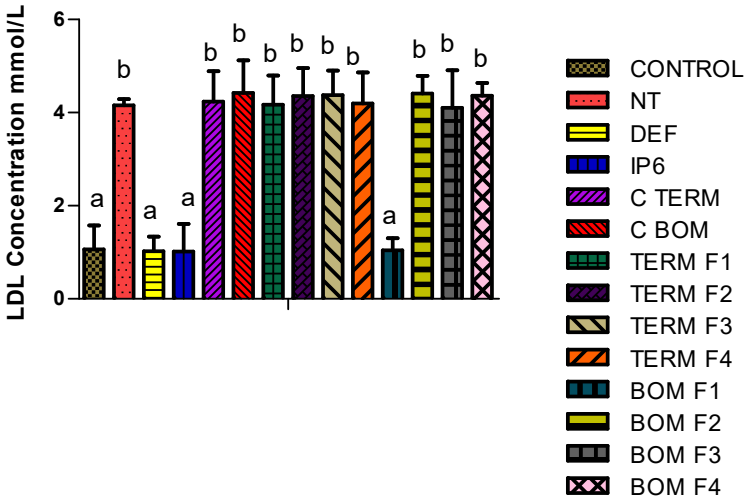


Fig 17: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on heart low density lipoprotein cholesterol of rats infected with *T. brucei*.

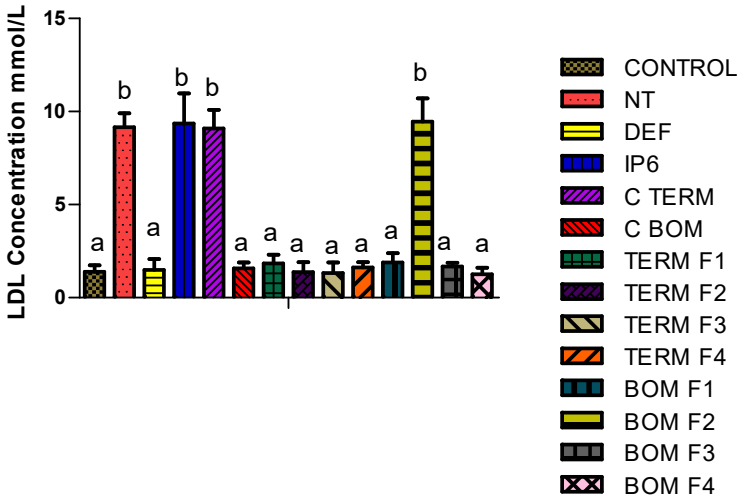


Fig 18: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on kidney low density lipoprotein cholesterol of rats infected with *T. brucei*.

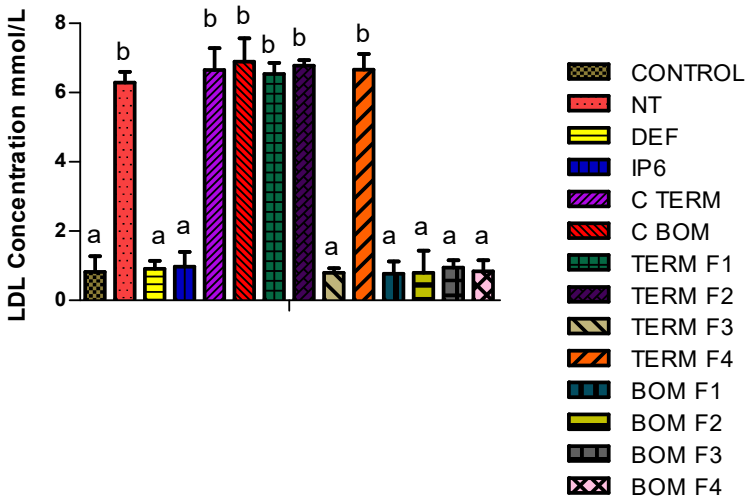


Fig 19: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on serum low density lipoprotein cholesterol of rats infected with *T. brucei*. Bars are expressed as mean of three replicates ± S.E.M and bars with different superscripts are significantly different (P<0.05).

Atherogenic Index

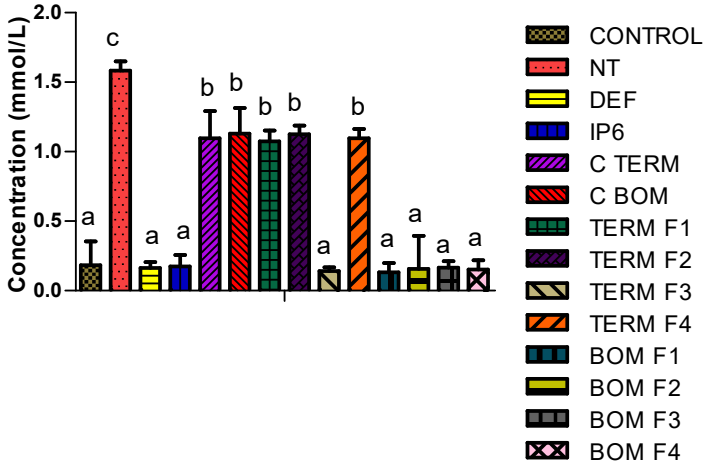


Fig 20: Effect of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on serum atherogenic index of rats infected with *T. brucei*. Bars are expressed as mean of three replicates ± S.E.M and bars with different superscripts are significantly different (P<0.05).

Bioactive Compounds in *Terminalia avicennoides and Bombax buopodezense*

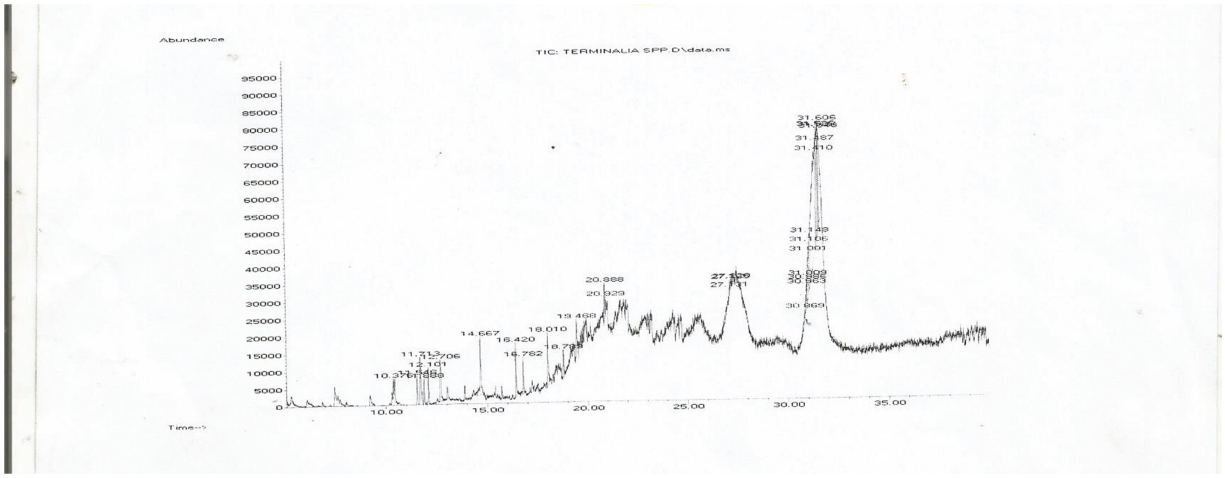
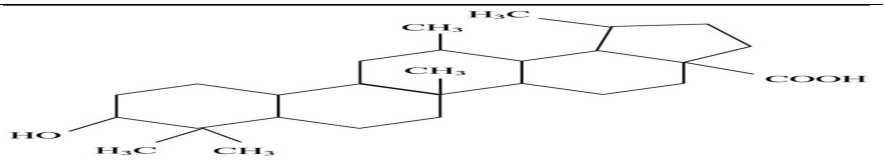
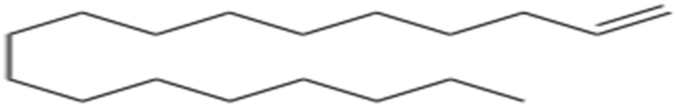
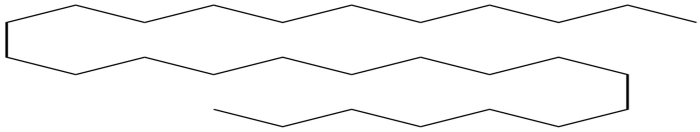
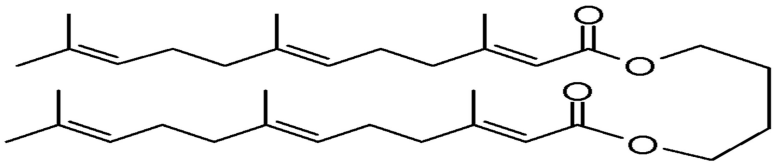


Fig 21: GC-MS chromatogram of aqueous extract of *Terminalia avicennoides*

GC-MS chromatogram of the aqueous extract of *Terminalia avicennoides* showed 30 peaks indicating the presence of thirty phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library the thirty phytoconstituents were characterized and identified.

S/N	Name of Compound	Structures
1.	Tetratetracontane	
2.	L-Octadecene	
3.	Octacosane	
4.	Sulfurous acid, butyl tetradecyl ester	

Bombax buopodezense

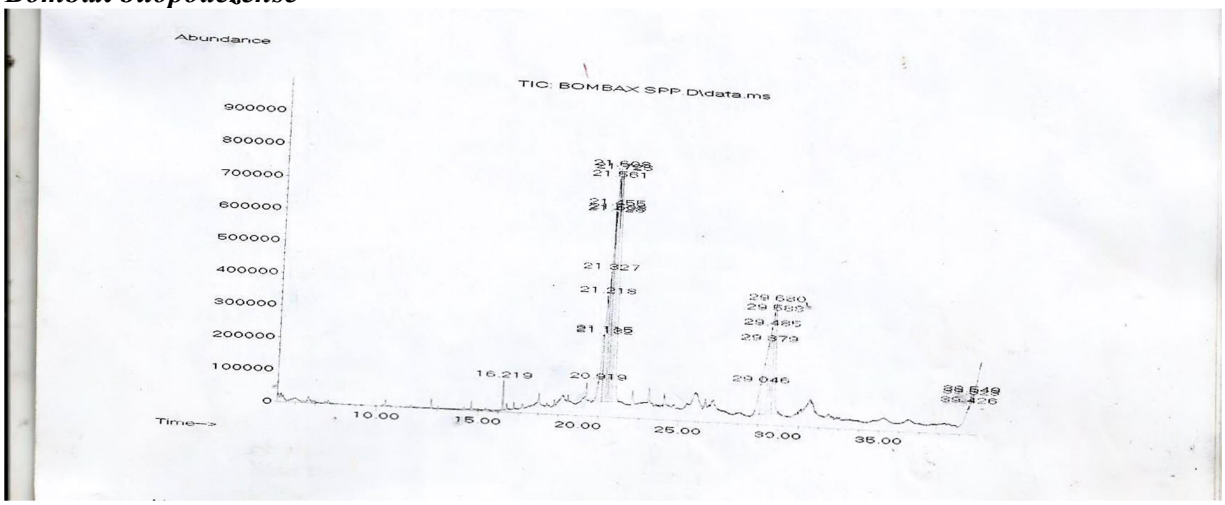
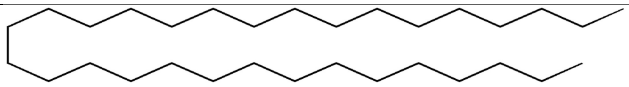


Fig 22: GC-MS chromatogram of aqueous extract of *Bombax buopodezense*

GC-MS chromatogram of the aqueous extract of *Bombax buopodezense* showed 20 peaks indicating the presence of twenty phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library the twenty phytoconstituents were characterized and identified.

S/N	Name of Compound	Structures
1.	Hentriacontane	
2.	Octadecane, 1-iodo	
3.	Hexadecane, 1-iodo	

Discussion

There was a gradual decline in the weight of the experimental rats across the two weeks of the experiments. It could be as a result of reduction in the quantity of food the rats ingested due to the complications usually associated with parasitic infections. Fraction 2 of both *Terminalia avicennoides* and *Bombax buopodezense* showed more potency in managing trypanosomiasis than all other fractions when compared with the untreated group, this fractions also prolonged the experimental rats life span till day 8 post treatment, compared to rats in other groups that were treated with other fractions which all died on day 4 and 5 post treatment. Some researchers have shown that treatment failure is possible in cases of massive parasitemia at the time of the therapeutic intervention (Tijani *et al*., 2009).

The level of anemia determined through PCV is one of the reliable indicators of trypanosome infection (Rowlands *et al*., 2001). In this study, the means of PCV were significantly higher in non-infected rats. Similar results were found by Quadeer *et al*. (2008), Sam-Wobo *et al*. (2010) in Nigeria and by Desquesnes and Dia (2003) in Burkina Fasso. Therefore, the decrease in total cholesterol concentration were recorded in some fractions of the plants administered to the rats, with the highest decrease at fraction 2 of *Bombax buopodezense,* may be attributed to their ability to increase the excretion of cholesterol.

Triglycerides and very low density lipoproteins have clinical values in assessing atherosclerosis diseases (Adebayo *et al.,* 2005). The fractions administered did not have adverse effect on the concentration of triglycerides and very low density lipoprotein, which is suggestive that it does not enhance the activity of any of the biosynthesis and catabolic enzymes of the triglyceride and very low density lipoprotein.

The decrease in concentration of LDL-cholesterol observed in some of the fractions of the plants administered, especially fraction 2 of *Bombax buopodezense* in the heart and the serum when compared with the control, might be due to the fractions making liver cells more efficient to remove LDL-cholesterol from the blood by increasing the LDL-cholesterol receptor densities in liver, heart, kidney and serum and by binding to apolipoproteins B.

High density lipoprotein cholesterol (HDL-C) mediates the removal of cellular cholesterol and its secretion into the bile by the liver. The fractions of the plants increase the HDL-C activity. Therefore, the fractions might be of great help in the reduction of atherosclerosis disease. All the decreased values for atherogenic index observed in the groups treated with fractions of the plants, when compared with the control, might be suggestive of the fractions of *Terminalia avicennoides* and *Bombax buopodezense* bark possessing anti-atherosclerotic potential and so do not predispose users to cardiovascular diseases.

The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The composition determined for this aqueous extract corresponds to 92.63% of the entire GC-MS chromatogram. GC-MS chromatogram of the aqueous extract of *Terminalia avicennoides* showed 30 peaks indicating the presence of thirty phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library the thirty phytoconstituents were characterized and identified. The four major phytochemical constituent's mass spectra out of thirty were identified as Tetratetracontane (27.544%), 1-octadecene (6.980%), octacosane (6.968%) and sulfurous acid, butyl tetradecyl ester (8.161%).

GC-MS chromatogram of the aqueous extract of *Bombax buopodezense* showed 20 peaks indicating the presence of twenty phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library the twenty phytoconstituents were characterized and identified. The three major phytochemical constituent's mass spectra out of twenty were identified as Hentriacontane (26.277%), octadecane, 1-iodo (9.666%) and hexadecane, 1-iodo (9.156%).

Conclusions

The present study evaluated the comparative study of the effects of fractions of *Terminalia avicennoides* and *Bombax buopodezense* on Lipid profile, PCV, and some selected parameters in rat liver, kidney, heart and serum.

- The results of the study suggest that some of the fractions of *Terminalia avicennoides* and *Bombax buopodezense* have trypanocidal effect by prolonging the life span of the experimental rats.
- Fraction 2 (Benzene fraction) of both plants exhibited more trypanocidal effect on the experimental rats than the other fractions.
- Bioactive compounds, which could be potent against trypanosome, were identified.
- The fractions exhibited a reduced risk of cardiovascular disease.

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