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Determination of the Bioactive Components and Antitrypanosome Activity of the Leaves and Stem Barks Extract of *Olea Hochstetteri* Bak (Oleaceae)

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Abstract

Trypanosomiasis is one of the most infectious diseases caused by Trypanosoma parasites. With the raising resistance to conventional antitrypanosomal drugs, increasing resistance of vector to insecticides, lack of effective vaccines and adverse effects of the existing antitrypanosomal drugs justify the urgent need for affordable, tolerable and effective drug. The method of cold maceration was used in the extraction. Phytochemical analysis of ethanolic extract of *Olea hochstetteri* leaves EEOHL, ethanolic extract of *Olea hochstetteri* stem bark EEOHSB, aqueous extract of *Olea hochstetteri* leaves AEOHL and aqueous extract of *Olea hochstetteri* stem bark AEOHSB showed the presence of flavonoids, glycosides, saponins, terpenoids, steroids, reducing sugar, alkaloids, tannins, and phenol at various concentration, quantitative determination of some secondary metabolites indicates that extracts contained flavonoids saponins and phenol and alkaloid. Two compounds AEOHL and EEOHSB were tested for antitrypanosomal activities against *Trypanosoma evansi* field isolate using *in vitro* and *in vivo* techniques. Parasite load, packed cell volume (PVC) and body weight in Mice were evaluated. *In vitro* test was performed in triplicates to detect any motile trypanosomes in a 96 well microplates. Twenty microliters of blood containing about 16 - 32 organisms per field were mixed with 5 μ L of the test substance at concentration of 2.5, 5, 10 and 20 mg/mL to produce test concentration of 0.5, 1, 2 and 4 mg/mL, respectively. AEOHL inhibited parasite motility and eliminated the organism within 60 min at 4mg/mL concentration and within 80 min at 2mg/mL, the EEOHSB inhibited parasite motility within 80 min at 4 and 2mg/mL concentration respectively. The extracts also reduces parasitemia and prevented drop in Packed Cell Volume and body weight significantly ($p < 0.05$) at 400 and 200 mg/kg dose, as compared to control. Fourier Transform Infrared (FTIR) analysis of isolated compound revealed the presence of characteristic functional group of esters (1729 cm^{-1}), ethers (1077 cm^{-1}) absorption band at, 1259 cm^{-1} (C-O), 2922 cm^{-1} . GC-MS of the methanol extract of leaves of *O. hochstetteri* showed an active compound ethyl octadecanoate ethyl ester as one most abundance constituent with peak areas 12351016.76, height 3966129.27 and retention time 16.3 and base peak 88.1.

Keywords

Olea Hochstetteri,
Phytochemicals,
Flavonoids,
Alkaloids,
Antitrypanosomal,
parasitemia,
Packed cell volume ethyl

INTRODUCTION

Human African trypanosomiasis (HAT) is also known as sleeping sickness, a parasitic infection caused by single - celled protozoan parasites of the species *Trypanosoma brucei* (Pacheco 2017, Paul et al., 2012) that are mainly transmitted by the bite of infected tsetse flies. Species responsible for the disease complex, commonly known as Nagana, souma and surra in livestock include *Trypanosoma brucei*: *T. evansi*, *T. congolense* and *T. vivax*. Human pathogens that cause sleeping sickness include *T. gambiense*, *T. cruzi* and *T. rhodesiense* (Maikai et al., 2010). African animal trypanosomiasis (AAT) is a group of disease caused by different trypanosome species mostly of ruminants, equines, camels, swines and carnivores (Brun et al., 1998, Swallow BM 2000).

The economy of Africa is negatively affected by both human and animal trypanosomiasis, they weaken human health and livestock production (John et al., 2012). The ruminants-fly-human transmission cycle (trypanosomiasis) between species is high in areas where there is a high density of human, ruminant and fly (Ruiz et al., 2015). The HAT and AAT is a zoonotic disease of public health and economic importance (Ruiz et al., 2015; Okelo and Webarn 2014)

African trypanosomiasis is one tropical disease caused by different species of

trypanoses as it affects both human and livestock (Auwal et al., 2014). It is one of the major causes of death in sub-Saharan Africa and poses a health and economic set back in these regions with about 60 million people at risk of contracting this disease, which is fatal if left untreated (Pacheco 2017).

Management and treatment of trypanosomiasis is a major obstacle to livestock production in Africa since it is expensive. However, only a small group of chemoprophylactic, and chemotherapeutic trypanocidal compounds are currently in use which resistance has developed (Delespaulx et al., 2008). The registered trypanocides are confronted with the following drawbacks: frequent toxicity, lengthy parenteral administration, lack of efficacy and unaffordable price (Legros et al., 2002). There is an urgent need for a more reliable, readily available, less costly and less toxic herbal therapeutic agent to combat trypanosomiasis (Umar et al., 2010).

Majority of people from less developed countries, including Nigeria depend on medicinal plants for the treatment of various diseases, due to the high cost of synthetic drugs (Balogun et al., 2017). It has been observed that natural products derived from plants offer novel possibilities to obtain new drugs that are active against trypanosomes. Plants

identified with antitrypanocidal activity against *T. brucei brucei* including *Carissa spinarum* (Geyid et al., 2005), *Anchomanes difformis* (Atawodi et al., 2003) and *Saba florida* (Omale and Omajali 2010), *Peristrophe bicalyculata* (Yineger et al., 2007), *Artemisia abyssinica* (Atawodi et al., 2003, Yineger et al., 2007). Atawodi (Atawodi et al., 2003) noted that the significance of trypanomiasis to human health, nutrition and economy is enormous, causing continuous research for better way of combating and eliminating the disease.

The *in vitro* antitrypanosomal activity and cytotoxicity of the plant, *Artemisia* species against drug sensitive *T. brucei* was reported by Nibret and Wink (Nibret and Wink 2010). Therefore, the objective of this study are: qualitative and quantitative determination of secondary metabolites from the leaves and stem bark of *O. hochstetteri*; determine the antitrypanosomiasis of the extracts; characterization and identification of the purified components by using spectroscopic techniques.

2. Methods

2.1 Plant Collection and Authentication. Fresh leaves and stem bark of *Olea hochstetteri* was collected from Tafawa Balewa local government area of Bauchi state in Nigeria and authenticated by a taxonomist in the department of

biological sciences Abubakar Tafawa Balewa University Bauchi State. Leaves and stem bark will be examined and those attacked by insects or microbes were removed. Harvested leaves and stem bark were air-dried for about one week. The well-dried samples were pulverized to fine powder using a wooden Mortar and pestle. The powder of each sample was weighed using analytical balance and kept at room temperature until used (Ibrahim, et al., 2012).

2.2 Preparation of Plant Extracts.

Air-dried powder of the plant material was macerated in an Erlenmeyer flask with absolute ethanol at room temperature. The extracts of the leaves and stem bark were prepared by soaking 100 g of each in 250 ml absolute ethanol for a period of 72 hours, two filtrations and the filtrate was concentrated by evaporation in a water bath, dried and weighed. The procedure was repeated on the plant samples using distilled water. The dried extracts were packed in glass bottles with proper labeling for future use (Odey et al., 2012)

2.3 Preliminary Phytochemical Screening of the Crude Extracts. Qualitative analysis of the crude extract was carried out on the ethanol and aqueous extracts of *Olea hochstetteri* (leaves and stem bark).

2.3.1 Test for alkaloids. About 0.5 g of each extract was stirred with about 5.0 cm³ of each filtrate, a few drops of Mayer's reagents were added, and to the thirds portion, few drops of Drangendoff's reagent were added. A creamy white precipitate (Meyer's reagent), a reddish brown precipitate (Wagnur's reagent) indicates the presence of alkaloids (Talukdar & Chaudhary, 2010).

2.3.2 Test for glycosides. The extracts were hydrolyzed with HCL solution and neutralized with NaOH solution. Few drops of Fehling's solution A and B were added. Red precipitate indicates the presence of glycosides (Alamzeb et al., 2013).

3.3.3 Test for tannins 2 ml of 5% FeCl₃ was added to 1 ml of plant extract samples. Appearance of greenish black or dark color confirmed the existence of tannins in the test sample (Archana et al., 2012).

2.3.4 Test for anthraquinones. 0.5 g of each of the plants extracts was shaken with benzene (2.0 cm³) and filtered were necessary. 10 % of ammonia solution (4.4 cm³) was then added to the filtrate. The resulting mixture was shaken and the presence of a pink colour in the ammonia solution phase (lower layers) indicates the

presence of anthraquinones (Archana et al., 2012).

2.3.5 Test for steroids. 2.0 ml of acetic anhydride was added to 0.5 g of the extract in 2 ml of H₂SO₄. The colour changed from violet to blue or green indicates the presence of steroids. 0.5 g of each extract was dissolved in 2.0 cm³ of chloroform. To the resulting solution, ferric chloride solution was added. The formation of bluish-green or blue coloured solution indicates the presence of steroids (Talukdar & Chaudhary, 2010),

3.3.6 Test for Reducing Sugars. The extracts were shaken with distilled water and filtered. The filtrates were boiled with drops of fehling's solution A and B for few presences of reducing sugars (Thusa & Mulmi, 2017).

2.3.7 Test for saponins. 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins (Alamzeb et al., 2013).

2.3.8 Test for flavonoids. Few drops of FeCl₃ solution ws added to 1 ml of crude extract samples. Blackish red precipitate revealed the existence of flavonoids in the test samples (Archana et al., 2012).

2.3.9 Test for Terpenoids (Salkowskitest). Solution of each of the extract was made by dissolved 0.5 g of the extract in 2.0 cm³ of chloroform and concentrated H₂SO₄. A reddish-brown colouration at the interface formed indicated positives results for the presence of terpenoids (Alamzeb et al., 2013)

2.3.10 Test for cardiac glycosides. 5 ml of plant extract was treated with 2ml of glacial acetic acid with one drop of FeCl₃ solution. A violet ring may appear or a greenish ring may form just which showed the presence of cardiac glycosides. (Talukdar & Chaudhary, 2010)

2.4 Phytochemical Screening for Secondary Metabolites. Standard screening tests of the extract were carried out for secondary metabolite according to the method described by Harbone 1973.

2.5 Test organisms and Experimental animals. The stocks of *T. evansi* that were originally isolated from a pure natural infection of cattle were obtained from National Institute of Trypanosomiasis Research (NITR) in Vom, Plateau state. The organism was maintained by serial passages in mice until required as used and described by different workers Maikai (2011). Mice (20 - 76 g) of both sexes aged 8 - 12 weeks, obtained from the breeding

colony of National Veterinary Research Institute (NVRI) Vom. Protocols for this experiment were in accordance with the guidelines on care and wellbeing of research animals (ILAR 1996) and were approved by research and ethics committee of the institute.

2.6 Experimental Procedures

2.6.1 Evaluation of In Vitro Antitrypanosomal Activity. In vitro test was performed in triplicates to detect any motile trypanosomes in a 96 - well microplate. Twenty microliters of blood containing about 16 - 32 organisms per field were mixed with 5µL of the test substance at concentration of 2.5, 5, 10 and 20 mg/mL to produce test concentration of 0.5, 1, 2 and 4.0 mg/mL, respectively. Phosphate buffer saline (PH 7.2) standard trypanocidal drug diminazene aceturate (DA), were used to serve as untreated control and treated controls. The mixtures were incubated at 37°C for up to 3h. The motility of the parasites was checked in a 20 -min interval, about 2µL of test mixtures was placed on a microscope slide and covered with cover slips, and the parasites were observed for reduced motility (Ogoti et al., 2016).

2.6.2 Evaluation of In vitro Antitrypanosomal Activity. The isolates were first inoculated heavily (10⁴ trypanosomers / mice) to donor mice. After establishment of infection (11-

12 days post-inoculation), the donor mice were then subjected to cardiac puncture and blood was collected and immediately diluted with PBS for subsequent *in vivo* test. A total of thirty mice of either sex were randomly grouped into seven (A, B, C, D, E, F and G) groups. Group A - D contained 6 animals per group, group E - G contained 2 animals per group. Group A, B, C and D were administered 100, 200 and 400 mg/kg of the aqueous and ethanol leaves and stem barks extracts body weight respectively for 7 consecutive days per oral administration from the 7th day of parasite inoculation.

All experimental groups of mice (except group G) were then infected intraperitoneally with 2000 trypanosome / mouse in 0.2 ml of blood as described and used by Jigamet *et al.*, 2017. Dose of extracts were selected based on the results of acute toxicity. The extracts were dissolved and reconstituted in 2 % Tween - 80 in distilled water. Group E received 3.5 mg / kg (intraperitoneal single dose) of standard drug, diminazene aceturate, respectively. Group F consisted of the negative control which were infected with the parasite and not treated (negative control). The group G included uninfected - untreated mice (positive control) for reference (Ogoti *et al.*, 2009).

2.6.3 Determination of Parasitaemia and Body Weight Change.

The degree of

parasitaemia was determined using the method of Herbert and Lumsden (Herbert and Lumsden 1976 and Yusuf *et al.*, 2010) was adopted. It involved the microscopic counting of parasite per field in blood smears from the peripheral blood obtained from tail vein of each mice the blood was collected into sample bottles and left for fifteen minutes to clot, and then centrifuged at 3000 rpm for 15 minutes in order to get the serum.

The sera were stored in the refrigerator at -20°C for subsequent analysis. The whole blood was also collected into EDTA bottles for hematological analysis. Wet smears were prepared in triplicates from each animal and the mean value of slide counts was taken per sample examined microscopically. Logarithm values of these counts were obtained by matching with the table given by Herbert and Lumsden. Parasitaemia was monitored every two days until the 21th day posttreatment initiation.

For the assessment of antitrypanosomal effect of the extracts, the level of parasitaemia (expressed as log of absolute number of parasites per ml of blood) in the treated animals was compared to that of the control animals. The body weight of each mouse in all group was recorded on the day of treatment

initiation and every 2 days for 14 days by a sensitive digital weighing balance (Nweze et al., 2011)

2.6.4 Determination of packed cell volume. The peripheral blood obtained from tail vein of each mice was used for determination of packed cell volume (PCV). PCV was determined using a microhematocrit centrifuge and microhematocrit tube reader. PCV was monitored on the day of treatment initiation and every 7 days until 21th day of post-treatment initiation (Ngalda et al., 2013).

2.7 Statistical analysis Values of the data obtained from the study were summarized and expressed as mean \pm standard error of the mean (SEM). Data analysis was performed using Statistical Package for Social Science (SPSS), version 21.0. Statistical significance of the results between groups was determined using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests (DMRT). Differences in mean were considered to be significant at $p < 0.05$.

2.8 Isolation and purification of the bioactive compounds

2.8.1 Thin layer chromatography (TLC). TLC plate preparation and checking of the components in the mixture was carried out by the method described by

Babu et al., 2016. The TLC plate prepared was used and observed for the separation of individual compounds and a single spot was trimmed and the position of the origin marked by a straight line.

2.9 Structural elucidation. Structures of the active isolated and purified compounds will be elucidated using Fourier transform-infrared (FT-IR) and gas chromatography-mass spectrometry (GC-MS).

3 Results

3.1 Qualitative Phytochemical Screening

The results of the various phytochemical screening are shown in Table 1. Tannin, terpenoid, flavonoid, steroid, alkaloid, cardiac glycoside, phenol, saponin, terpenoids and anthraquinone are the phytoconstituents found in the plants. According to the literature and the tally done with the obtained result, Flavonoid caused risk reduction mainly from cardiovascular diseases and cancer (Ballard and Marostica, 2019).

The presence of classes of phytochemicals as such: flavonoid, alkaloid, tannin, showed cytotoxic effect (Chowdhury et al., 2017). The color and aroma imparting flavonoids were stated to show anticancer properties. Additionally, cholesterol lowering, as well as cytotoxic qualities, antibacterial, anti-viral properties are credited to the presences

of saponin (Bailly and Vergoten, 2020). Tannin shows an inhibiting activity towards growth (Mazai et al., 2016). Plants containing a high number of flavonoids could be useful as ant-bacterial (Ballard and Marostica, 2019). So, the plant like *Olea hochstetteri* could be used as antibacterial, antiseptic agents. The phenolic compound, tannin, terpenoid, flavonoids possess an anti-helmintic property so the plant *Olea hochstetteri*

could be used to treat stomach problems (Nath and Yadov, 2016). The polyphenolic compounds, flavonoid, terpenoid found in *O. hochstetteri* is useful as an antioxidant, anti-inflammatory, antibacterial agent, likewise, they play an important role in reducing blood pressure, in preventing heart diseases (Tinky et al., 2020)

Table 1: Qualitative Photochemistry of aqueous and ethanol leave and stem bark extract of *Olea hochstetteri* Bak

Phytochemical Constituents	Aqueous Extract of leaves (AEL)	Aqueous Extract of Stem Bark (AESB)	Ethanol Extract of Leaves (EEL)	Ethanol Extract of Stem Bark (EESB)
Colour	Light Green	Brown	Green	Green
Alkaloids	+	+	++	++
Flavonoid	++	+	++	++
Glycoside	++	+	++	-
Phenols	+	++	++	+
Reducing Sugar	+	-	++	-
Saponin	+++	+	++	++
Steroids	++	+	+	-
Tannin	+++	+++	+++	+++
Terpenoids	+	+	++	+++
Anthraquinone	-	+	+	+

Key:+++ Heavily Present; ++ Slightly/ Moderately Present; + Present; - Absent

3.2 Quantitative Phytochemical Screening for Secondary Metabolites

The results of quantitative analysis on four major groups of phytochemical constituents in the plant are presented in Table 2. The result of the quantitative analysis have shown a significant presence of alkaloids, flavonoids, phenols and saponins in the major parts of the plants. And this have further confirmed the effective medicinal application as widely reported (Akinpelu et al., 2015)

Table 2: Table of quantitative phytochemicals of *Olea hochstetteri* Bak

Phytochemicals	Aqueous extract of leaves	Aqueous extract of stem bark	Ethanol extract of leaves	Ethanol extract of stem bark
Alkaloid %	7.40	8.04	9.04	11.72
Flavonoid %	12.20	2.16	10.08	7.16
Phenol %	3.29	3.25	5.10	1.94
Saponnin %	10.36	10.08	11.16	9.28

3.3 In Vitro Antitrypanosomal Activity.

Aqueous extract of leaves ceased motility of the trypanosomes within 60 min at 4 mg/mL concentration and 80 min at 2 mg/mL concentration. At 0.5 mg/mL of the aqueous extract of leaves of *O. hochstetteri* bak, the motility was maintained for 100 min after which

motility of the parasite is ceased. The motility of parasites stopped at 100 minutes for the aqueous leaves extract at a dose of 1 mg/mL. However, ethanol stem bark extract ceased motility of the trypanosomes within 80 min at 4 and 2 mg/mL concentration. The motility of parasites stopped at 120 minutes for the ethanol extract of stem bark at dose of 1 mg/mL (Table 3 and 4).

Table 3: In Vitro activity of *O. hochstetteri* bak aqueous extract of leaf

<i>O. hochstetteri</i> bak extract motility						
Duration (min)	0.5mg/mL	1mg/mL	2 mg/mL	4 mg/mL	DA 3.5 mg/mL (motility)	Control (motility)
0	+	+	+	+	+	+
20	+	+	+	+	-	+
40	+	+	+	+	-	+
60	+	+	+	-	-	+
80	+	+	-	-	-	+
100	+	-	-	-	-	+
120	-	-	-	-	-	+
140	-	-	-	-	-	+
160	-	-	-	-	-	+

Table 4: In Vitro activity of *O. hochstetteri* bak ethanolic extract of stem bark

O. hochstetteri bak extract motility						
Duration (min)	0.5 mg/mL	1mg/mL	2 mg/mL	4 mg/mL	DA 3.5 mg/mL (motility)	Control (motility)
0	+	+	+	+	+	+
20	+	+	+	+	-	+
40	+	+	+	+	-	+
60	+	+	+	+	-	+
80	+	+	-	-	-	+
100	+	+	-	-	-	+
120	+	-	-	-	-	+
140	-	-	-	-	-	+
160	-	-	-	-	-	+

3.4 Effect on Parasitaemia of *T. evansi*-Infected Mice.

There were fluctuations in the level of parasitaemia of all the treated groups which were however, kept at relatively low or very low level relative to infected - untreated control especially at higher doses of all extracts. All dose of the extract including the standard drug suppressed parasitaemia ($P < 0.05$). Treatment with extracts at 200 mg/kg and 400 mg/kg and DA 3.5 mg/kg showed statistically significant ($P < 0.05$) reduction in parasitaemia compared to 100 mg/kg body weight (Figure 4.5a, 4.5b, 4.5c and 4.5d). The best results were obtained with aqueous leaves extract group C (figure 4.5e) at an optimum dose of 400 mg/kg, which was effective compared with 3.5 mg/kg of standard drug Diminazene aceturate (DA). Comparison with the negative control (Group F), revealed that the 400 mg/kg of aqueous leaves

extract (Group C) has kept parasitaemia at a significantly low level ($p < 0.05$ on day 9 through 14) and 200 mg/kg has considerably reduced parasitaemia ($p < 0.05$) on day 10 and 12 of treatment. The mice treated with 400 mg/kg of ethanol stem bark Figure 4.5d (Group D) had significantly reduced parasitaemia ($p < 0.05$ on day 10, 12 and 14 of treated). When compared with the infected - untreated group (Group F). The 3.5 mg/kg DA was able to clear the trypanosomes from all animal right from the beginning of treatment (Figure, 9, 10, 11 and 12). Comparison between the groups treated with the four types of extracts indicated that the aqueous leaves extract (group C) appeared to be superior to other extract in reducing the parasite burden. The result showed that mice treated with 400 mg/kg of aqueous leaves extract had continually reduced parasitaemia level from day 6 of treatment to day

14 which was further kept on average at lowest level up to the end of the monitoring period. Ethanol stem bark extract (Group D) was also able to reduced parasitaemia level at 400 mg/kg continually from day10 of treatment to day 14, the end of the monitoring period. There was parasitaemia reduction at higher dose, 400 and 200 mg/kg of other extracts significantly

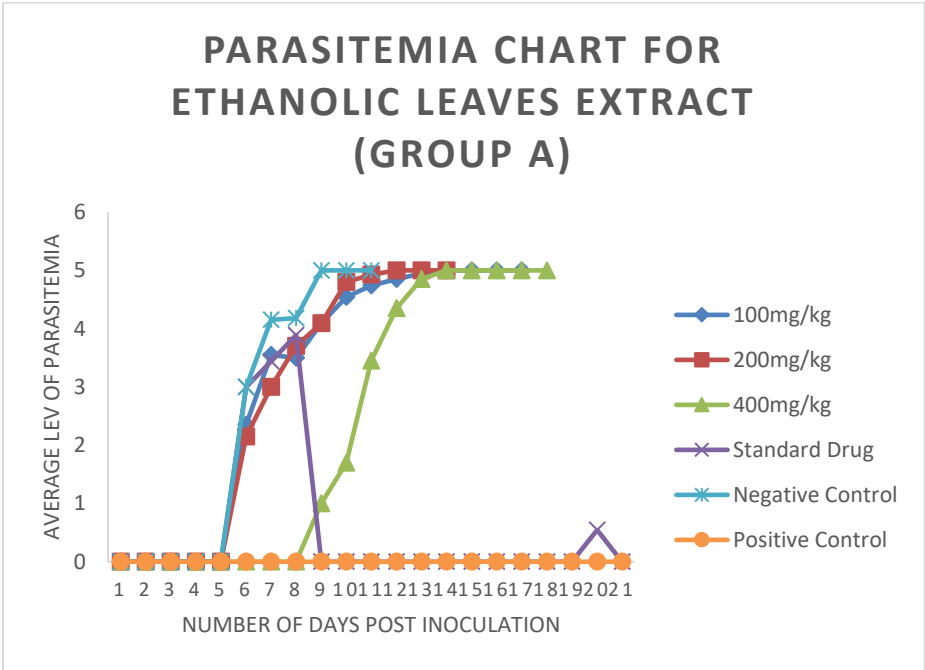


Figure 1: Effect of Parasitemia of *T. evansi* - Infected Mice

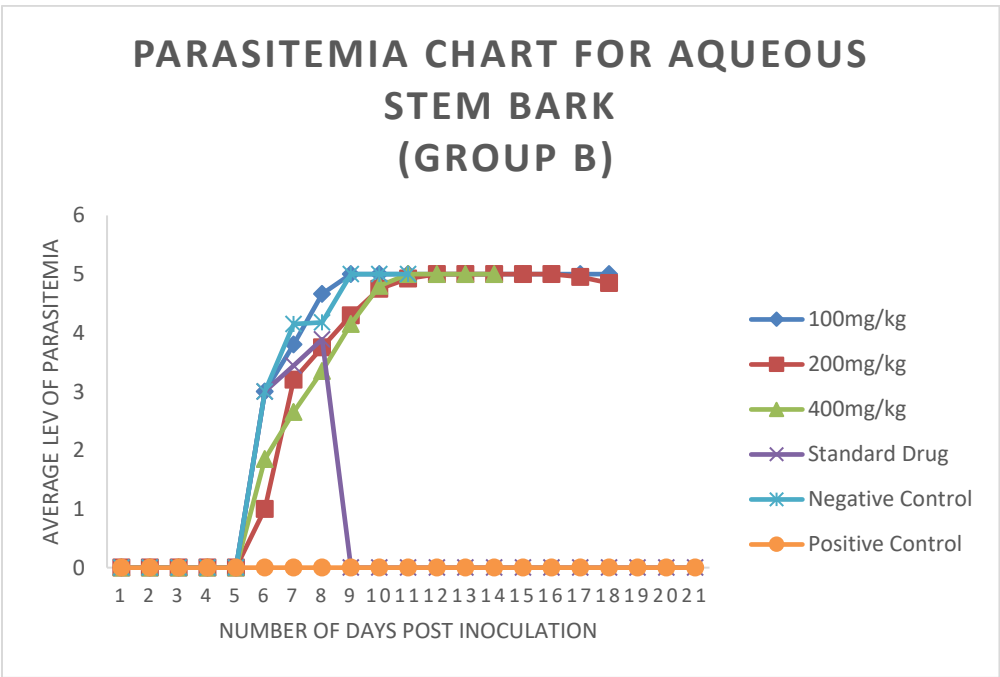
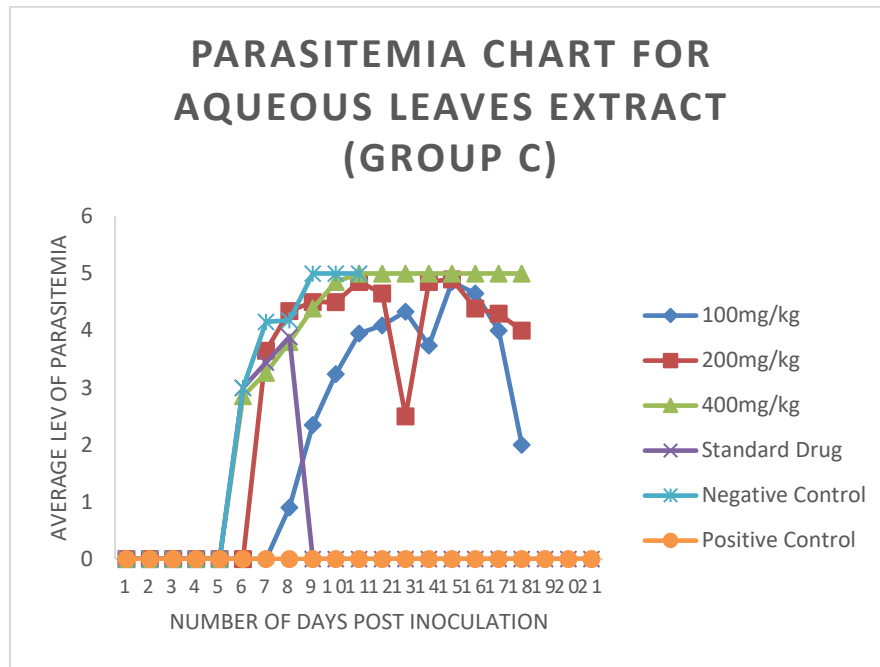
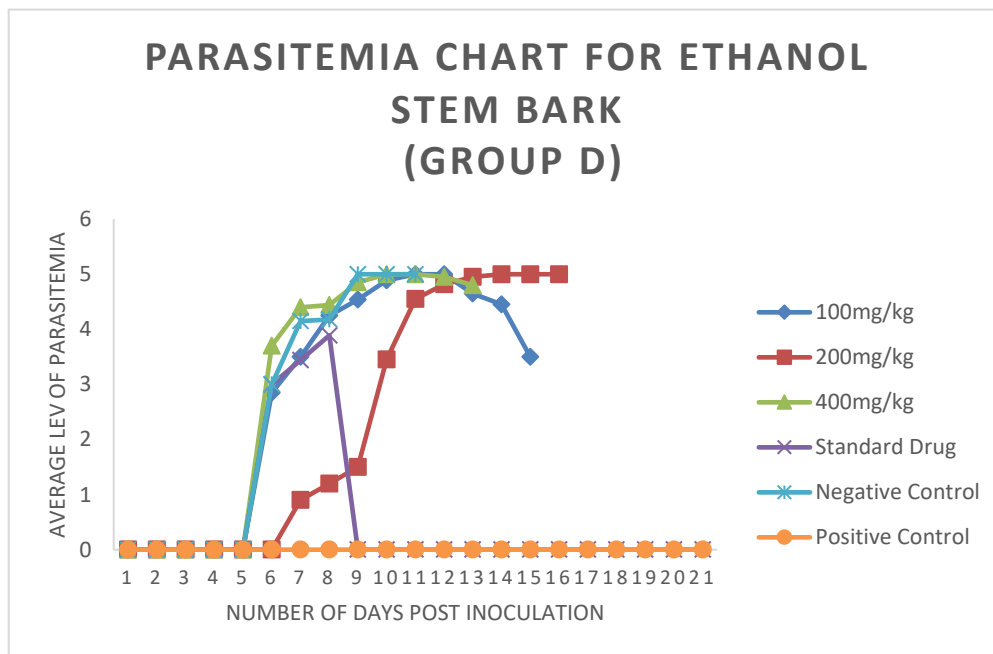


Figure 2: Effect of Parasitemia of *T. evansi* - Infected Mice

Figure 3: Effect of Parasitemia of *T. evansi* - Infected MiceFigure 4: Effect of Parasitemia of *T. evansi* - Infected Mice

4.5 Effect on Body Weight of *T. evansi*-Infected Mice

Treatment with the crude extracts prevented loss of weight associated with parasitaemia particularly at 200 and 400 mg/kg dose level of Group A, B, C and D compared to the negative control Group F (Figure 4.6a and

4.6b). There was detectable difference in preventing weight loss associated with parasitaemia between the extracts and standard (3.5 mg/kg) at these dose levels throughout the monitoring period. Considerable body weight improvement was seen by 3.5 mg/kg DA and group C extracts figure 4.6b, while there was considerable loss of weight by the negative control (group F).

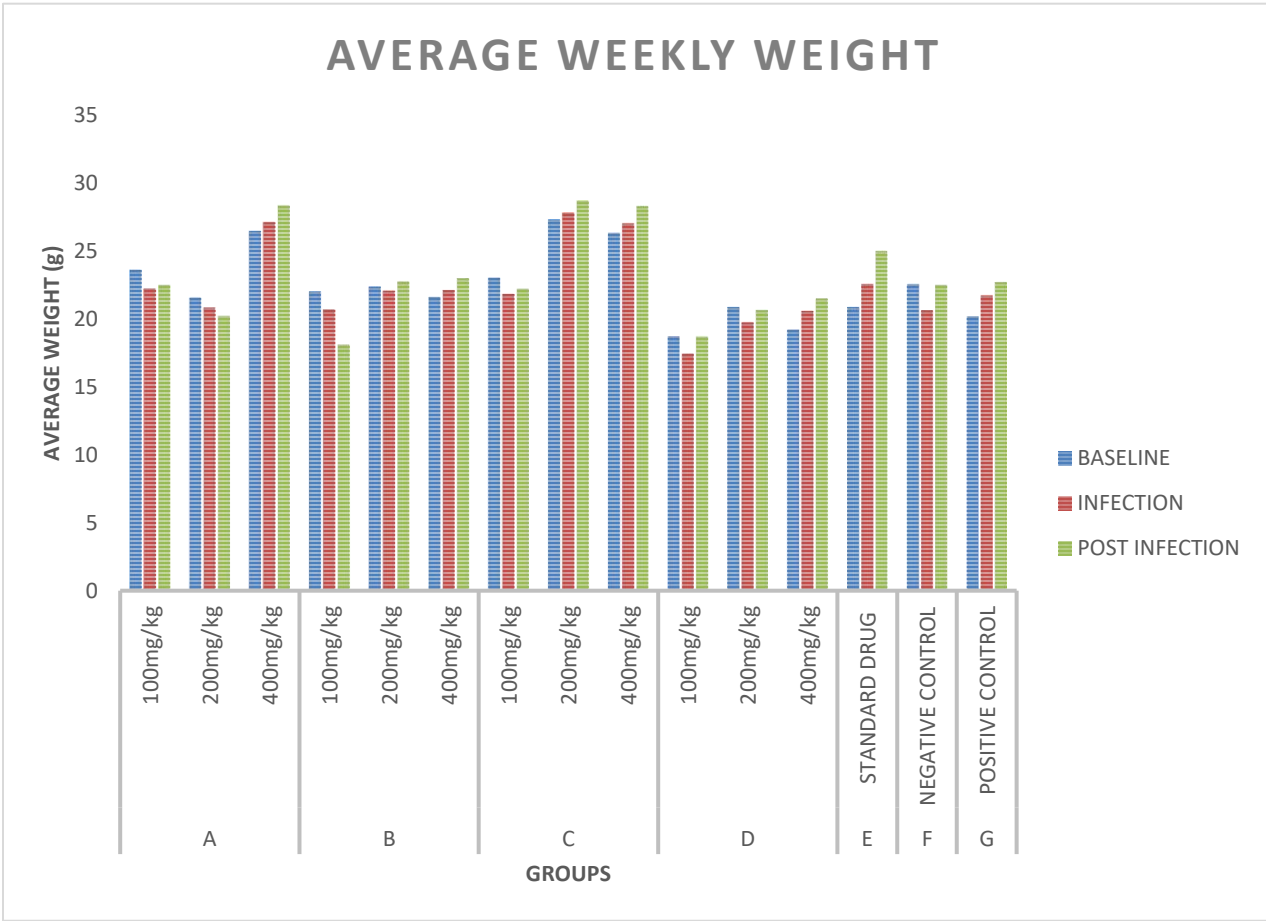


Figure 5: Effect of crude extracts on body weight of *T. evansi* - Infected mice. A=ethanol leaves extract; B=aqueous stem bark; C=aqueous leaves extract; D=ethanol stem bark; E=3.5 mg/kg DA; F=negative control; G= positive contro

4.6 Effect of crude extracts on packed cell volume (PCV)

As shown in figure 13 and 14, administration of the plant extracts to the infected group produced a substantial difference in PCV when

compared with the infected - untreated group (Group F). The prevention of PCV reduction observed at Group C over the infected - untreated group was comparable. These values were also comparable between these dose levels and to that caused by the standard at 3.5 mg/kg (group E) with statistically significant difference.

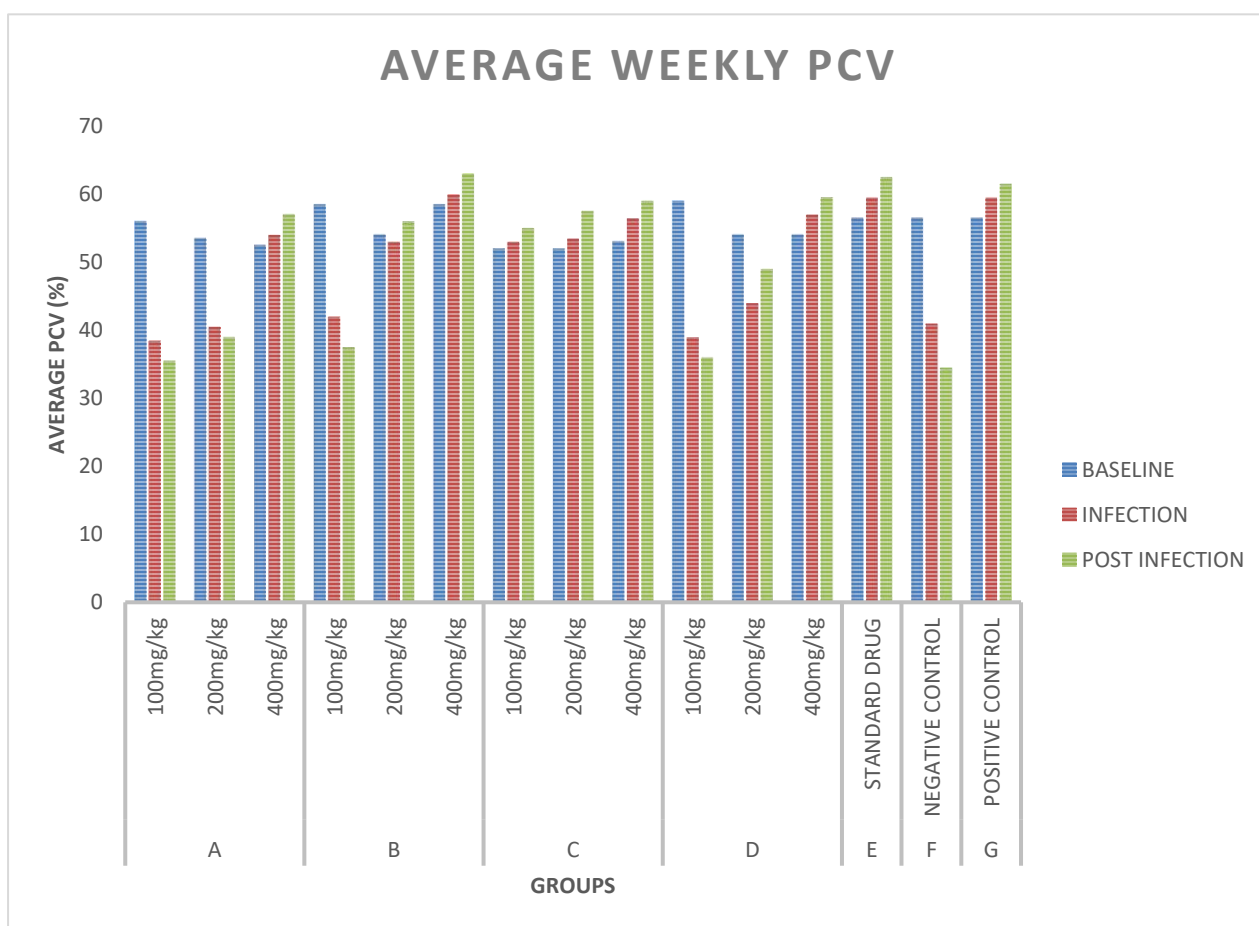


Figure 6: Effect of crude extracts on Packed Cell Volume of *T. evansi* - Infected Mice. A=ethanol leaves extract; B=aqueous stem bark; C=aqueous leaves extract; D=ethanol stem bark; E= 3.5 mg/kg DA; F =negative control; G= Positive control

4.7 Results of Column and Thin Layer Chromatographic (TLC) Fraction of Crude Extract

Table 5: Characteristics of column fractions of *O. hochstetteri*

Fraction	Quantity(g)	Rate of flow (RF)	TLC collection UV 254nm	Track/ spot number
OH F1	1.16	0.84	Gray	One
OHF2	1.78	0.62	Bluish brown	One
OH F3	2.09	0.59	Reddish brown	One
OHF4	0.06	-	-	-
OHF5	1.59	0.87	Light brown	One

Base on purification, two samples are obtained that are presumed to be pure; OHF1 and OHF5, with retention factor 0.919 and 0.923, respectively.

4.8 FT-IR Spectrum Analysis of *Olea hochstetteri*

The concentrated fractions of *O. hochstetteri* extract were analyzed for the functional groups present, using Fourier Transformed Infrared (FT-IR) spectroscopy according to Huck 2015. The IR results for methanolic leaves extract for *O. hochstetteri* is presented in table 4.8. Table 4.8 shows band of absorption at different level. The spectrum revealed twelve peaks. A broad absorption band at 3384 cm^{-1} indicate the presence of hydroxyl (-OH) compound. A sharp peak at 2922 cm^{-1} showing aliphatic compounds, absorption band for long chain linear aliphatic compounds is identified at 2922 cm^{-1} . C-H stretching at 2855 cm^{-1} for asymmetric aliphatic compounds. Peak at 2109 cm^{-1} is absorption band of C=C alkyne. C=O stretching at 1729 cm^{-1} indicates the presence of some carbonyl double bond, which can be from

ketones, aldehydes or esters. Peaks at 1461 cm^{-1} bending and 1375 cm^{-1} (C-H) bending indicates methylene and methyl group respectively. C-O stretching at 1259 cm^{-1} indicates the presence of ester, C-O-C stretching at 1077 cm^{-1} informed the presence of ether.

4.9 GC -MS Analysis of the isolated compound

The GC - MS analysis of methanolic leaves extract of *Olea hochstetteri* was performed using a GC 7890B, MSD5977A, Agilent Tech and Mass detector. The GC - MS analysis of the isolated compound reveals a compound with peak area of 12351016.76 height 3966129.27 and retention time 16.3 and base peak is 88 m/z. according to the data base of National Institute Standard and Technology (NIST) spectral library collection

Table 6: MS Analysis of Methanolic leaves extract of *O. hochstetteri*

Mass of fragment ion	Proposed compound
283 (2%), 269(5%), 255(2%), 241(2%), 227(2%), 213(3%), 199(2%), 185(2%), 171(2%), 157(5%), 143(2%)	Ethyl octadecanoate ethyl ester
129(2%), 115(2%), 101(50%), 88 base peak (100%), 69(3%), 61(1%)	
55(5%), 43(6%)	

RT = 16.3, peak area = 12351016.76, height = 3966129.27 and base peak = 88 correspond to octadecanoic acid, ethyl ester compare with the data base of National Institute Standard and Technology (NIST).

4.9 Discussion

In the present study, antitrypanosomal activities of the *O. hochstetteri* aqueous and ethanolic extracts of leaves and stem bark revealed that the extract could contain trypanocidal constituents that are active in the *in vitro* and *in vivo* environments. Parasite motility constitutes a reliable sign of viability of most trypanosomes (Freighburghans et al., 1996), and a total elimination or reduction in the motility of trypanosomes when compared to the control could be taken as index of trypanocidal activity (Wurochekke and Nok 2004). Assessment of the extracts *in vivo* revealed a suppression of parasite load at 200 and 400 mg/kg.

The reason may be that the parasite is decreased in the blood could be as a result of access of the extract to the parasites. It reduced level of parasitaemia (Obah and Malann 2013) even if it may decline after relapse of parasites. The relapsing of parasitemia is related to low amounts of the active compound in the crude extract and high virulence of the test organism. The increased survival of treated mice in this study could be related to the action of the active compounds in the extract i.e antioxidant activity. The current finding is in accordance of Tadess et al., (2015) who reported that treatment with *D. abyssinica* extract resulted in decrease level of parasitemia and Kifleyohannes (Kifleyohannes et al., 2014) who reported animals treated with *A. absinthium* and *M. stenopetala* extracts reduced parasite count (Ene et al., 2009), and (Ngure et al., 2009) reported that active ingredients from *A.*

maciverae and *A. indica* significantly reduced parasitemia with a dose - dependent effect. The finding indicates that the *O. hochstetteri* bak extracts contain some trypanocidal agent that is active for both chemotherapeutics and chemoprophylaxis of the disease.

The result revealed that the extracts of the plant has the ability to improve the PCV. The mean PCV between different doses of the extract and standard drug (DA) were relatively comparable with the findings of Kifleyohannes et al. and Tadesse et al (Kifleyohannes et al., 2014. Tadesse et al., 2015). Reduction in mean PCV from untreated control may be as a result of anemia (Obah and Malann 2013) which is the most outstanding clinical and laboratory feature of African trypanosomiasis (Stijlemans et al., 2010).

The ability to improve PVC is the reason for the survival of treated mice. The result reveals that the ability of extracts to extend mice's lives may be attributed to the support of their antioxidant defense system. Packed cell volume usually gives an indication of the anemia and status of a trypanosome infected animal (Lelisa and Meharennet 2021). Hepatic injury induced by trypanosomiasis is one of the health challenges to both human being and domestic and wild animals (Dkhil et al., 2021).

The gradual increase in mean body weight for all of the extracts treated mice through day 0 to day 14 posttreatment for all treatment with a decrease for untreated control is in support with the finding of (Maikai 2011) who reported an

increase in body weight (Dkhil et al., 2021) and reported treatment with the crude extracts of *Z. officinale* prevented loss of weight associated with parasitaemia (Kifleyohannes et al., 2014). The weight in the untreated infected mice group started to decrease after 6 days' post-infection till the mice died by day 12 whereas those on standard drugs and extracts generally showed a gradual increase in mean weight until the end of the experimental period.

The extract of *Olea hochsteteri* bak was found to contain alkaloids, tannins, saponins, phenols, terpenes, flavonoids, glycosides and anthraquinones. Studies revealed that flavonoids are effective antitrypanosomal substances against different trypanosome species (Hoets et al., 2004). Phenolics and polyphenols are reported to have antitrypanosomal activity by inhibiting the trypanosomes alternative oxidase (Yabu et al., 2014). Alkaloids affect trypanosomes by DNA intercalation with the inhibition of protein synthesis (Hoets et al., 2004). The pharmacological action of the *O. hochsteteri* bak extract and other therapeutic uses are due to their therapeutically active constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds or secondary metabolites. The preliminary phytochemical analysis revealed pronounced importance because the crude drug possess varied composition of secondary metabolites (Meyer et al., 1997; Study showed that no toxicity or weight change was reported throughout the observation period at the dose of 2000 mg/kg which is in support with the results of Shifema et al and Toma et al. (Shifema et al., 2013, Toma et al., 2015).

4.9.1 Melting point of Fraction. The result of the melting point showed that the methanol extract of leaves of *Olea hochsteteri* have high melting point ranging from 96-98°C, this could be due to high molecular weight of the compound or presence of impurities. The melting point of methanol extract of leaves of *O. hochsteteri* fraction is higher than that of the literature reviewed which is 69.4°C (156.90F) (Cappa et al., 2008).

4.9.2 The FTIR Spectrum. The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The outcomes of IR functional groups were represented in IR spectra of *Olea hochsteteri* extract of leaves and different functional groups peaks with different intensity were observed (table 9). The presence of a broad absorption band at 3384 cm⁻¹ which is followed by the presence of spectra at frequencies of 1259, 923 and 723 cm⁻¹ indicate the presence of hydroxyl (-OH) compound) (Kumar et al., 2017). Alkaloids, polyphenols and flavonoids are due to OH stretching 3384 cm⁻¹, terpenes due to C-H stretching 2855 and 2922 cm⁻¹ (Maobe and Nyarango, 2013).

Double bond can be as carbonyl (C=O), imino (C=N) and azo (N=N) groups, regarding the double bond region, a sharp peak was detected at 1729 cm⁻¹ and 1077 cm⁻¹. This inform some carbonyl double bond which can be from ketone, aldehyde or ester. Since there is no peak for aldehyde, there are two C-O single bonds at 1259 cm⁻¹ and 1077 cm⁻¹, the prospective peak for carbonyl should be from ester. The peak at 1461 cm⁻¹ corresponds to C-O-H bond. The peak at 2855 cm⁻¹ is attributed

to symmetric stretching of C-H (CH₂) vibration of lipids (Fung et al., 1996). C-H stretching mode of the methylene chains in membrane lipids (Fung et al., 1996). Peak at 2922 cm⁻¹ gives C-H stretching bands. The band at 3384 cm⁻¹ is attributed to the existence of hydroxyl group (O-H). Schulz and Baranska. (2007).

The presence of characteristic functional group of esters (1729 cm⁻¹), ethers (1077 cm⁻¹) absorption band at, 1259 cm⁻¹ (C-O), 2922 cm⁻¹ (C-H) stretching, and C-H bending at 1375 and 1461 cm⁻¹ could attest for the compound to be an ester compound.

4.9.3 GC-MS analysis. The Gas Chromatogram of methanolic extract of the leaves of *Olea hochstetteri* is shown in table 10. The constituent was identified by comparison with the NIST data based. The mass spectral data for the compound whose molecular weight was observed at 312 (table 11) showed prominent peak at 283(2%), 269(5%), 255(2%), 241(2%), 227(2%), 213(3%), 199(2%), 185(2%), 171(2%), 157(5%), 143(2%), 129(2%), 115(2%), 101(50%), 88 base peak(100%), 69(3%), 61(1%), 55(5%), 43(6%). With peak area (table 6) 12351016.75 and height 3966129.27, RT = 16.3, National Institute Standard and Technology (NIST) spectral library collection molecular weight base peak = 88.1 correspond to Ethyl octadecanoate ethyl ester compared with the data base.

The IR absorption spectrum of *O. hochstetteri* justify the identification of the named compound ethyl octadecanoate ethyl ester for there were characteristics absorption of an ether C-O-C stretching (1077 cm⁻¹), C=O stretching at 1729 cm⁻¹, C-H stretching at 2922 cm⁻¹, C-O stretching at

1259 cm⁻¹ and C-H bending at 1375 and 1461 cm⁻¹ respectively Asep et al., (2019). There is no change in the position of the CH₂ peak at 2922 cm⁻¹ and that is to be expected since this functional group does not participate in bonding. These finding also confirm the investigations of Basak et al. and de Assis et al. respectively, who found similar C=O stretching in cellulosic and banana fibres. The band at 1729 cm⁻¹ is assigned to the extension stretching vibration of the C=O bond due to esters group, vibrational stretching at 2885 cm⁻¹ (C-H) is attributed to the assymetric aliphatic compound (Asep et al., 2019; Al-Wathnani et al., 2012). Such compound also appeared in the extract of *Pseudogtochidion anamalayanum* (Gideon 2015).

The finding is in line with Kanimozhi and Bai (2012) that reported ethyl octadecanoate ethyl ester is the most abundance constituent in the ethanol extract of *Coriandrum sativum* with relative percentage of peak area 56.68%. Ethyl octadecanoate ethyl ester appeared to be one of the most abundance constituent, when compared to the other nine constituents of methanol extract of *Olea hochstetteri* with a relative peak area of 12351016.76 (Table 10). Ethyl octadecanoate have been reported to be effective in the treatment of infantile neuroaxonal destrophy (Adam et al., 2020) also it possesses an anti-inflammatory effect (Zhao et al., 2005). Ethyl ester is a fatty acid ester is safe for oral ingestion and is used as solvent for pharmaceutical drug preparation (Ory et al., 1993). The presence of various phytocompounds in the leaves of *O. hochstetteri* ought to justify the use of the plant in folk medicine for the treatment of various ailments

5.0 SUMMARY AND CONCLUSION

The result of the phytochemical screening of *O. hochstetteri* leaf and stem bark revealed some bioactive compounds of pharmacological importance such as flavonoids, alkaloids, tannins, saponins, steroids, glycoside, reducing sugar in both the ethanolic and aqueous extracts. These classes of phytochemicals have been reported to exert therapeutic and antioxidant properties and trypanocidal activity. Antitrypanosomal activities of the *O. hochstetteri* bark aqueous and ethanol extracts of leaves and stem bark revealed that the extract contained trypanocidal constituents that are active in the *invitro* and *invivo* environments.

Olea hochstetteri extracts was able to reduce level of parasitaemia, improve packed cell volume in mice, and increase mean body weight. In conclusion, the present study results obtained revealed that aqueous and ethanolic extracts of *Olea hochstetteri* bark possess antitrypanosomal activity against field isolates of *Trypanosoma evansi*. FTIR carried out on column, TLC purified extract revealed the presence of characteristic functional group that identify the compound as ester. The data obtained from the GC-MS analysis correspond to ethyl octadecanoate ethyl ester compared with the data base of National Institute Standard and Technology (NIST) spectral library collection.

Finding from this study buttressed the fact that the arrays of phytochemicals present in plant extracts (*Olea hochstetteri*) are arguably responsible for its antitrypanosomal and medicinal properties. Further investigation should be carried out to see whether the extract will have an improved effect or not

when administered by injection to minimize the negative impact of limited bioavailability from the gut.

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