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Original Research Report

Antitrypanosomal activity of essential oil from *Carica papaya* seed on rat infected with *Trypanosoma brucei brucei*

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Abstract

Trypanosoma brucei brucei is one of the main causative agents of African animal trypanosomosis in sub-Saharan Africa. Its infection causes death in cattle while the current treatment poses serious toxicity problem. But natural products can be used to overcome this problem associated with parasitic diseases including *Trypanosoma brucei brucei. Carica papaya* seed extracts were evaluated phytochemically for its constituents and anti-trypanosomal potential against *Trypanosoma brucei brucei.* The composition and the functional groups of the essential oils of the *carica papaya* seed were analyzed by GCMS and FTIR respectively. The *in vivo* anti-trypanosomal efficacies of the extracts were evaluated in Wister albino rats infected with *Trypanosoma brucei brucei*. The rats were divided into 3 groups (A - C) of 3 rats each. They were dosed intraperitoneally (IP) once with 500, 2000 and 5000 mg/kg body weight of the extract, respectively. The rats were observed for 24 hours for signs of toxicity like changes in behaviour or death. The extracts had a lethal dose greater than 2000 mg/kg. The essential oils were active on the parasite motility. Significant reduction in motility of parasites were observed at 0.5 mg/ml (30 minutes), 0.05mg/ml (30 minutes) and at 0.005mg/ml (90 minutes) of both crude methanol extract, fractions of n-hexane, ethyl acetate and n-butanol of *carica papaya* seed which was taken as a measure of the anti-trypanosomal effect of the crude methanol extract of *carica papaya* seed and fractions of n-hexane, ethyl acetate and n-butanol. And they were compared with the control which shows active motility even at 105minutes intervals. Results of this study indicated that the aqueous/methanolic and different fractions of *carica papaya* seed have anti-parasitic activity against *Trypanosoma brucei brucei.* In general, the results obtained revealed ethno-pharmacological application of the extracts and necessitate further studies to be carried on isolated active substances of the plant.

Keywords: *Trypanosoma brucei brucei,* Anti-trypanosomal activity*, Carica papaya.*

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1. Introduction

Trypanosomiasis remains a major health hazard to humans, animals and livestock populations in developing and under developed countries. It is one of the deadliest diseases caused by the flagellated haemoprotozoan parasite (Trypanosoma) which is transmitted by infected tse-tse fly *Glossina* species [1]. Sleeping sickness threatens millions of people in 36 countries in sub-Saharan Africa. The disease affects many populations live in remote rural areas with limited access to adequate health services [2]. Two trypanosome subspecies affect people: *T. b. gambiense* (found in western and central Africa) and *T. b. rhodesiense* (in eastern and southern Africa). The disease caused by *T. b. gambiense* is more abundant and accounts for more than 98% of all infections of patients. Infected persons go through two stages of the disease. The first or hemolymphatic phase causes fever, itching, and headache. In the second or neurological stage, the blood-brain barrier is crossed by the parasite and this affect central nervous system [3]. The economic importance of the disease is often characterized by increasing anemia, induce oxidative stress, inflammation, suppress immune system, poor reproductive performance, poor lactation, weakness, extreme emaciation and eventually death [4]. Chemotherapy is the most widely used means of controlling and treating trypanosomiasis. The few registered trypanocides are toxic and often associated with severe side effects [5]. Suramin and pentamidine are used for the first stage of the sleeping sicknesses caused by *T. b. rhodesiense* and *T. b. gambiense*, respectively. Melarsoprol is registered for treatment of the

neurological stage. Suramin and pentamidine were first synthesized more than 70 years ago, and resistance to those two drugs seems to have been established [6]. Eflornithine is a drug which is less toxic than melarsoprol but is effective only against *T. b. gambiense* [7]. Since only a few drugs, all of which exhibit severe side effects, are registered for treatment of African trypanosomiasis and since resistance to those drugs is emerging, new therapeutic strategies are urgently required [8;9]. The use of herbal medicine has always been part of human culture for decades because it contains some therapeutic properties for healing humans and animal diseases [10].About 80% of individual from developed countries use medicinal plants which contains compound drive from medicinal plants [11]. Secondary metabolites also represent an interesting alternative to synthetic antiparasitic drugs and many medicinal plants and isolated natural products have been screened, some of which show a high degree of trypanocidal activity that has been discovered [3].Bioactive compounds presence from plant materials provide unlimited opportunities for new drug discoveries and their presence in foods and dietary supplements have been implicated in the prevention of pathologic conditions as well as promoting general wellness [12]. In this study extract of *Carica papaya* were evaluated following the chemotaxonomic approach. The plant belongs to the class of magnoliops family of *caricaceae* genus of *Carica* and species of *Carica papaya* [13]. Papaya plants come in three sexes viz "male,""female," and "hermaphrodite." The male produces only pollen, never fruit. The female will produce small, inedible fruits unless pollinated. The hermaphrodite can self-pollinate since its flowers contain both male stamens and female ovaries. Almost all commercial papaya orchards contain only hermaphrodites [14]. In some parts of the world, papaya leaves are made into tea as a treatment for malaria. Antimalarial and anti-plasmodial activity has been noted in some preparations of the plant, but the mechanism is not understood and no treatment method based on these results has been scientifically proven [15].

2. Materials and Methods

2.1 Materials

2.1.1 Equipment

Centrifuge (MSB020.C×1.5), Ultra Shield Bruker DPX-400. Vet-automatic analyzer (Mindray-BC-2800, China), Spectrophotometer (Model-300 frequency 50/60H_z1A, serial number. 323127 optimal, Japan), Incubator (Model-INE-400,temperature range 37^{0}c to 70^{0}c Germany), Nikon Eclipse Microscope, Rotary Microtome (Microm HM340E, Thermo Scientific, Germany), GC-MS model 7265 Agilent technologies, FTIR model M530 bucks scientific, Soxhlet apparatus glass Pyrex England Automatic pipette, 48-well micro titre plate and micro plate absorbance reader (Austria). Beckmann Ultra sphere ODS reverse phase column (250 × 10 mm i.d)

2.1.2 Chemicals

Methanol, Chloroform, Xylene, Absolute Ethanol, Hexane, Ferric chloride, Glacial acetic acid, Sodium Hydroxide, Ammonia solution, acetic anhydride, ethyl acetate, n-butanol Dichloromethane, Sulfanilic acid and conc. Hydrochloric acid of analytic grade were procured from (Sigma, St. Louis, MO, USA).

2.1.3 Collection of Plant Materials

Fresh mature seed of *Carica papaya*, was collected in Vom, Jos south local government area of Plateau state. Nigeria. The plant specimen was identified and authenticated in the herbarium unit at Federal College of Forestry, Jos, Nigeria.

2.2 Methods

2.2.1 Preparation of Plants Materials

Seed of *Carica papaya,* was washed thoroughly with tap water in order to remove the dust and soil particles, it was then air dried under the shade to prevent ultra-violet rays from inactivating the chemical constituents and was pulverized (ground into powder form) separately, using pestle and mortar [16].

2.2.2 Aqueous extraction of seed of Carica papaya

Six hundred grams (600g) of the individual pulverized seed of *Carica papaya* was macerated with 30000mls of distilled water for 72 hours with daily shaking, at the end of the extraction the mixture was sieved and filtered, using Whatman filter No. 1 Paper. The filtered was concentrated by drying at room temperature at 27^0 C. The dried extract was stored at 4^{0}C until needed for used[17].

2.2.3 Methanolic extraction of seed of Carica papaya.

The pulverized seed of *Carica papaya,* (600g) was extracted with methanol. The maceration process was performed at room temperature by adding 600g of the power form to 2600mls of methanol it was then extracted by cold maceration with daily shaking for three days and was filtered using Whatman filter No.1 Paper. The filtrated was air dried. It was harvested and weight, the dried extracts was preserved in a desicator[18].

2.3 Qualitative Phytochemical Determinations of aqueous and methanolic extracts of seed of Carica papaya

Phytochemical analysis was carried out on methanolic extract seed of *carica papaya* using the standard methods. Each of the concentrated extract was subjected to qualitative tests for the identification of its various phytochemical constituents as per standard procedures [19] and also by characteristics colour changes as described by Sofowora[20].

2.3.1 Test for Anthraquinone (Borntrager's test)

0.5 g portion of seed of aqueous and methanolic extract of *carica papaya* was shook with 5 ml of chloroform. The chloroform layer was filtered and 5.0 cm3 of 10 % ammonia solution was added to the filtrate. The mixture was shaken thoroughly and the formation of a

pink/violet or red, yellow colour in the ammoniacal phase indicates the presence of Anthraquinones

2.3.2 Test for Alkaloids (Mayer' and Wagner's test).

A 0.5g portion of seed of aqueous and methanolic extract was stirred with 5cm^3 of 1% aqueous HCl on a steam bath. Few drops of picric acid solution were added to the extract. Formation of a reddish-brown precipitate was taken as a preliminary evidence for the presence of alkaloid. 2 drops of Mayer's reagent was added along sides of the test of the test tube respectively. A white creamy precipitate indicated the presence of alkaloid [19].

2.3.3 Test for Cardiac Glycosides (Keller-Killiani test)

About 5 ml portion of seed of aqueous and methanolic extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution, followed by the addition of 1 ml of concentrated sulphuric acid. Brown ring formed at the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the thin layer [21].

2.3.4 Test for Flavonoids

Few grams of seed of aqueous and methanolc extract were heated with 10 ml of ethyl acetate in a test tube over a steam bath for 3 minutes. The `mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Yellow coloration was observed that indicated the presence of Flavonoids [22].

2.3.5 Test for Phlobatannins

0.5g of Aqueous and methanolic extract of seed of *carica papaya* was boiled with 1% aqueous hydrochloric acid; the formation of red precipitate thus indicated the presence of Phlobatannins [22].

2.3.6 Test for Saponins (Frothing's test)

One gram of the aqueous and methanolic extracts of seed of *Carica papaya,* were added in 10 ml of distilled water respectively and dissolved, shake vigorously for 30 seconds and was allowed to stand for 30 minutes. A honey comb formed which persisted for 30 minutes indicating the presence of Saponins [23].

Foam Test: This served as confirmatory test for Saponins. 0.5 gm of each of the extracts was shaken with 2 ml of water. The production of foam persisted for ten minutes, which confirmed the presence of Saponins.

2.3.7 Test for Tannins, Steroids and Triterpenes

A. Ferric chloride test

To 0.1 g of aqueous and methanolic extracts of seed of *Carica papaya* respectively, 10 ml of distilled water was added, filtered, follow by 4 drops of ferric chloride solution. Formation of a green precipitate indicated presence of tannins.

B. Lieberman-Burchards' test

Three ml of acetic anhydride was added to 8 ml of the extracts in a test tube, and 1 ml of concentrated sulphuric acid was added down side of the test tube. Red and blue-green colour changed was observed immediately and after 3 minutes respectively indicating the presence of steroids and triterpenes respectively.

2.4 Quantitative Phytochemical Determinations of aqueous and methanolic extracts of seed of *Carica papaya*

The quantitative analysis for the determination of the total phytochemicals of aqueous and methanolic extracts of seed of *Carica papaya,* was carried out using standard operational procedures (SOP) (with some modifications). A spectrophotometer was used to determine the quantity of Tannins and the Phenol contents on the basis of UV spetra through absorption maxima at individual wavelength of every bio component (The total Phenol contents was estimated using standard calibration curve).

2.4.1 Total phenol content

One gram of powder from each aqueous and methanolic extract of *papaya papaya* seed*,* were soaked in 40 ml of methanol (50%) for 18 h with occasional shaking it was centrifuged at 3,000 rpm for 20 minutes. The residue was extracted with 40 ml of acetone/water (4:1) agitated and centrifuged. The supernatant was passed through Whatman No. 5A filter paper. The total phenolic content of each sample was determined using spectrophotometric method, with modifications (Inglett *et al*., 2011 (22). Briefly, 9 ml of de-ionized water and 1 ml of Folin-Ciocalteu reagent (F9252, Sigma, St. Louis, MO, USA) was added to 100 ml of extract, and mixed in a vortex mixer. After 15 min, 1.5 ml of Na_2CO_3 (1.85 M) was added and incubated for 120 min at room temperature, 200 µl of each extracts and standard solution was pipetted into 96-well plates. The absorbance at 734 nm was measured using a microplate absorbance reader (Tecan Group, Austria). The results were expressed as mg tannic acid equivalents g^{-1}.

2.4.2 Determination of the quantity of Alkaloids using Harborne[21] method:

Two grams each of aqueous and methanolic extract seed of *Carica papaya* were put in a 250ml beaker; 80ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. It was then filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium was added in drops to the extracts until the precipitation was completed. The whole solution was collected and allowed to settle and then was precipitate, collected and washed with dilute ammonium hydroxide and was filtered. The residue which is the alkaloid was dried and weighed.

2.4.3 Determination of the quantity of Saponins

Two grams each of aqueous and methanolic extract of C*arica papaya* seed was added into a conical flask and 20cm of 20% aqueous ethanol was added. It was then heated over the water bath for 4 hours with continuous stirring at 55°C. The mixture was filtered and the residue

was re-extracted with another 200ml of 20% ethanol. The extract was reduced to 40ml over water bath at about 90°C. These concentrates were transferred into a 250ml separator funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60ml of n-butanol was added. The n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in the water bath. After evaporation the samples were dried in the oven to a constant weight to give the Saponins which were then calculated [24].

2.4.4 Determination of the quantity of Tannins

Five hundred milligrams each of aqueous and methanolic extract of *Carica papaya* seed were weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken vigorously or in a mechanical shaker for one hour. This will then be filtered into a 50ml volumetric flask and made up to the mark. 5ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120nm within 10 minutes to get the total test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120nm within 10 minutes to get the total Tannins [25].

2.4.5 Determination of the quantity of Flavonoids:

Two grams each of aqueous and methanolic extract *Carica papaya* seed wee extracted repeatedly with 20ml of 80% aqueous methanol at room temperature. The whole solution was filtered using filter paper No 42 (125mm). The filtrated was transferred into a crucible and evaporated to dryness over a water bath and was weighed to a constant weight, and the weight was calculated.

2.5 Fractionation of crude plant extracts method of Chandel *et al.*, [26]

Principle: Vacuum Liquid Chromatography

Procedure:

Fractionation (Vacuum Liquid Chromatography)

The methanolic *Carica papaya* seed (with the highest trypanocidal activity) was fractionated using a short column or a Buchner filter funnel fitted with glass frit (10-20µm, porosity D or porosity 2). It was dry and packed with sorbet (silica gel of TLC grade 10-40µm). The sorbet was allowed to settle by gentle tapping under gravity. Then vacuum was applied via the three ways stop cork and the sorbet compressed a hard layer by pressing with a rubber stopper and tapping. The vacuum was released, solvent of low polarity (n-hexane) was poured quickly onto the surface of the adsorbent, the vacuum was reapplied, the sample was pre-absorbed on celite. The column was developed with appropriate solvent mixtures starting with solvent of low polarity and gradually increasing the polarity, pulling the column dry between each fraction collected. Fractions was collected in a suitable separating funnel and finally into collection bottles. All the solvents used were distilled before the commencement of the work. The elutes were dried at room temperature and kept at 4°C until required [26].

2.6 GC-MS analysis

The GC-MS analysis of the methanolic fractions of seeds of *Carica papaya* was performed using GC-MS. The analysis was done by using a Perkin Elmer Clarus 600 GC system equipped with a fused silica gel column (30 m×0.25mm ID, film thickness 0.25 µm) coupled with a PerkinElmer Clarus 600C MS. The detection of data or spectra was done using an electron ionization system with ionization energy of 70 eV. Inert helium gas (99.999%) was used as a carrier gas at a constant flow rate of ±1 mL/min. Mass transfer line and injector temperatures was at 220 and 290 °C, respectively. The temperature programmed for oven was from 60 °C (hold 2 min) to 270 °C at 4 °C/min, and was held in isothermal for 20 min and was raised to 300 °C at 10 °C/min. The tested fractions were diluted with methanol (1/100, v/v, in methanol) [27]. The tested fractions were filtered with 0.45 µm Millex membrane filter paper (Millipore, France) to remove any dust particles. One microliter filtered test fractions was injected in the split mode. The split ratio was in ration of 120:1. The percentage (%) of the fractions from leaves and seeds of *Carica papaya,* was expressed as percentage by peak area. The whole process was carried out carefully from the light and heat [28].

2.7 Fourier Transform Infrared (FT-IR) Spectrometry

Infrared spectrometry of the Essential Oils of *Carica papaya* was carried out with a Buck scientific Infrared Spectrophotometer. The Essential Oils sample were placed directly on the surface of pair of rectangular sodium chloride plate at room temperature and the measurement were performed in the IR region at 4000-600 cm^{-1}. Two scans were performed at a scan speed of 3 cm/s for each essential oil and an air spectrum was used as reference [29].

2.8 Experimental Design

Albino rats weighing between 80-120 grams of either sex were used for the study. The albino rat was kept in clean wire meshed cages under standard animal condition in accordance with the recommendations in Guide for the Care and Use of Laboratory Animal (DHHS, NIH).The was given standard feed diet and water *ad libitum* during the entire period of the experiment.

2.8.1 Test organism

Trypasonoma brucei brucei (Federestrian) used for this work was obtained from Nigeria Institute for Trypanosomiasis Research (NITR), Vom Plateau State, Nigeria. The parasite was maintained in their laboratory by continuous passage in albino rats, until commencement of the work.

2.8.2 Preparation of test materials for in vitro assays

The first stock solutions of crude extracts and fractions of *Caraca papaya* seed were prepared in distilled water for the water-soluble samples at 10mg/ml or in DMSO (at 1 mg/100 µl) for the water-insoluble samples. For the extracts, the solutions were further diluted in 60minimum essential medium (MEM) to give 8, 4, 2 and 1 mg/ml stock solutions. These were further diluted various concentrations of 0.005, 0.05, 0.5 and 5µg/ml of solutions for the *in vitro* study

2.8.3 Acute Toxicity Studies of the Seed of the Extracts method of Lorke 1983 Modefied by OECD [30].

9 albino rats were used for this study. The rats were divided into3 groups (A - C) of 3 rat each. They were dosed intraperitoneally (IP) once with 500, 2000 and5000 mg /kg body weight of the extract, respectively. The rats were observed for 24 hours for signs of toxicity like changes in behavior or death.

Then the LD_{50} is calculated by the formula:

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality

Dosage Selection

The selection of dosage was based on the toxicity study (0.005mg/ml, 0.05mg/ml, 0.5mg/ml and 5mg/ml).

***2.8.4 In vitro* Evaluation of the fraction**

2ml of blood was collected in bijou bottles containing the anticoagulant Ethylene Diamine Tetra-acetic Acid (EDTA) from an albino rat heavily infected with *Trypanosoma brucei brucei for* trypanosome suspension preparation. A suspension of trypanosomes was prepared in normal saline and the concentration was adjusted to about 1 x 10^6 organisms per ml (Lumdsen) 0.5 ml of the suspension of Ringer's solution and 0.5 ml of suspension of trypanosomes were dispensed into 5 tubes (1-5). 0.5 ml of the extract concentrations 0.005mg/ml, 0.05 mg/ml, 0.5 mg/ml and 5mg/ml were added to the first 5 tubes (1-5). The sixth tube was an untreated control (no extract added). The tubes were then incubated at 37˚C. The contents of the tubes were each examined at time 0 minutes and subsequently observed at intervals of 15 minutes for 105 minutes by aspirating small amounts using a Pasteur pipette onto clean slides then covered with cover slips and checking for the presence and motility of the parasites under the microscope (X 40 objective lens). These were taken as a measure of anti-trypanosomal activity.

2.9 Infectivity Test

About 0.2 ml of 0.005mg/ml, 0.05mg/ml, 0.5mg/ml, 5mg/ml of various concentration of the mixture of extract, ranger's solution and the blood containing the parasite were taken into a clean 1 ml insulin syringe with a 25 gauge needle and was inoculated into 3 rats each not previously infected with the trypanosome. Control was also treated same way, but without extract. The rats were observed daily for development of parasitemia for a period of 90 days.

3. Results and Discussion

3.1 Preliminary phytochemical analysis

Table 1 shows the percentage yield of methanol extract of *Carica papaya* seeds was found to be 47.0 %, while the hexane fraction was 12.5%, ethyl acetate fraction gave the yield of 14.9%, butanol fraction was 20.1% and the residue was 52.5%. The methanol extracts gave the highest percentage yield while hexane fraction has the lowest percentage. This finding supports the work of [31] which stated that solvents used in extraction of plant materials influence the phytochemicals composition and also affect the polarity of the chemicals constituents. Table 2 shows the preliminary qualitative phytochemical screening of extract and fractions which revealed the presence of alkaloids, flavonoids, cardiac glycosides, tannins, terpenoids, saponins and steroids, while anthraquinones was not detected in both the extracts but was detected in n-butanol fractions. These phytochemical screening agreed with the findings of other studies [32-34] The absence of anthraquinone in this study is in accordance with the research done by Ajani *et al*., [35], Isela *et al*., [36] and Kinjir *et al*., [37] which reported that the presence or absence of metabolites maybe due to different in polarity of solvent used in the extraction.

The result of the quantitative analysis of phytochemical constituents of *Carica papaya* seed is shown in table 3. The *C. papaya* extract using methanol which gave highest concentration of alkaloids, cardiac glycosides, flavonoids, phenols, saponins and tannin, follow by n-butanol then ethyl acetate and lastly n hexane. These findings confirmed the work of Kanadi *et al.* [31] which observed that the most non-polar of all the solvents had the lest quantity and percentage yield.

Table 1: Percentage Yield of *Carica papaya* seed Methanol Extract and Fractions

Solvent	Yield (%)	Colour
Methanol extract	47.0	Brown
Hexane Fraction	12.5	Yellow
Ethyl acetate Fraction	14.9	Yellow
N- Butanol Fraction	20.1	Brown
Residue	52.5	Brown

Table 2: Qualitative Phytochemical Components of Aqueous, Methanolic Extracts, n-hexane, ethyl acetate and n-butanol fraction of *Carica papaya* Seed

Parameters	Aqueous extract	Methanolic extract	n-hexane fraction	Ethyl acetate fraction	n-butanol fraction
Alkaloids	-	-	-	-	+
Anthraquinones	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+
Flavonoids	+	+	+	+	+
Tannins	+	+	+	+	+
Terpenoids	+	+	+	+	+
Phenols	+	+	+	+	+
Saponins	+	+	+	+	+
Steroids	+	+	+	+	+

Key: + Detected and - Not detected

Table 3: Quantitative phytochemical components of Aqueous, Methanolic Extracts, n-hexane, ethyl acetate and n-butanol fraction of *Carica papaya* Seed

Parameters	Aqueous	Methanolic	n-hexane	ethyl acetate	n-butanol
Alkaloids	2.10 ± 0.40*	2.87 ± 0.50	2.45 ± 0-50	3.11 ± 0-40	3.16 ± 0-60*
Anthraquinones	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.50*
Cardiac gly.	3.18 ± 0.05*	2.15 ± 0.05*	2.18 ± 0.05	3.05 ± 0.01	3.97 ± 0.05*
Flavonoids	3.14 ± 0.15	3.78 ± 0.67	3.08 ± 0.17	3.55 ± 0.17	7.96 ± 0.05
Tannins	1.64 ± 0.11*	2.69 ± 0.32	2.19 ± 0.02	2.62 ± 0.50	2.09 ± 0.02
Terpenoids	7.18 ± 0.21	7.15 ± 0.10	6.65 ± 0.05	6.11 ± 0.03*	8.65 ± 0.05*
Phenols	5.04 ± 0.05*	5.70 ± 0.52	6.40 ± 0.01	7.50 ± 0.05	8.70 ± 0.02*
Saponins	7.69 ± 0.18*	6.12 ± 0.08	1.11 ± 0.10*	2.28 ± 0.05*	6.28 ± 0.05*
Steroid	5.54 ± 0.14	6.65 ± 0.11*	4.12 ± 0.08*	5.23 ± 0.01	6.18 ± 0.01

Values are Mean ± SEM for the determinations n = 3, *Significantly values (P<0.05)

3.2 The mean lethal dose (LD_{50}) of methanolic extracts the fractions of *Carica papaya* seed.

Lethal dose of *C. papaya* was estimate to be greater than two thousand milligrams per body weight ≥ 2000 mg/kg (v/v) for acute oral toxicity test because all the rats survived at the end of 14-days observation period. This implies that the plant exact were relatively safe in rats following oral acute exposure. These finding is in agreement with Kanadi *et al.,*[31] findings which showed LD_{50} of C. *papaya seed* above 5000mg/kg of body weight of rat. Biu[38]also reported the lethal dose of aqueous extract *C. papaya leaf* to be safe at 1000mg/kg.

3.3 The result of *in vitro* evaluation of the effect of extracts and fractions of *Carica papaya* seed against *Trypanosoma brucei brucei*

The *in vitro* tests were performed in duplicates. In this *in vitro* studies, decreased and total inhibitor of the parasite motility and complete disintegration of parasite morphology was observed at 30 minutes intervals, at a concentration of 5mg/ml. Drastic reduction in motility of trypanosomes were observed after 30 minutes at 0.5 mg/ml and 0.05mg/ml, after 90 minutes interval at 0.005mg/ml of both crude methanol extract, fractions of n-hexane, ethyl acetate and n-butanol of *C. papaya* seed which was taken as a measure of the anti-trypanosomal effect of the crude methanol extract of C. papaya seed and fractions of n-hexane, ethyl acetate and n-butanol, these were compared with the control which shows active motility even at 105minutes intervals. These observation that shown activity even at a very low concentration of 0.005 mg/ml are in agreement with the works of Ogbole[39] and other workers which stated that natural products from medicinal possess biochemical structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase which is very sensitive to alterations in redox balance. The *in vitro* antitrypanosomal activity of the methanol crude extract could be attributed either to the solubility of the active ingredient(s) responsible for the observed *in vitro* activity or the variations in the types of phytochemicals compound as revealed by the result of phytochemicals screening. Accumulated evidences suggested that many natural products exhibited their anti-trypanosomal activity by virtue of their interference with the redox balance of the parasites acting either on the respiratory chain or on the cellular defenses against oxidative stress [37,39,40]. Alkaloid was also among the phytochemical compound detected in *Carica papaya* seed, which was reported by Wink *et al.,*[41] to interact with neuroreceptors, DNA and other molecular target and inhibit protein biosynthesis. The complete cessation of in motility of trypanosomes in all the fractions could be as a result of the analysis of the composition and results obtained from GC–MS allowed identifying 22 constituents, representing 69.57% of the oil from n-hexane, ethyl acetate and n-butanol fractions. The two main constituents of the essential oil, n-hexadecanoic acid and Oleic Acid may have interfered with glycolysis, these observations confirmed the

work of Bero[42] which stated that anti-trypanosomal activities on procyclic forms (Tbb PF) Compounds which were more active on bloodstream forms (BSF) than procyclic forms (PF) of Tbb may act on glycolysis. Indeed, bloodstream forms which have no Krebs cycle or mitochondrial respiratory chain coupled to ATP synthesis are exclusively dependent on glycolysis for their ATP formation. The higher concentration in phenolic content could attributed the inhibitory and disintegration of the morphology of the parasite, this research also in agreement with the work of Amisigo *et al*.[43] which stated that the phenolic acid which have been link to the numbers and position of hydroxyl groups of the benzene ring inhibitory and altering the morphology of the parasite by forming reactive oxygen intermediate in the parasites.

Table 4: *In vitro* motility effect of methanolic, n-butanol, ethyl acetate and n-hexane fractions of *Carica papaya* seed on *Trypanosoma brucei brucei*

Fractions	Concentration	0	15	30	45	60	75	90	105 (mins.)
Methanolic	Control	20/1	20/1	20/1	20/1	20/1	19/1	19/1	19/1
	0.005mg/ml	20/1	20/1	18/1	12/1	8/1	1/5	1/10	0/10
	0.05mg/ml	20/1	10/1	1/1	1/10	0/10	0/10	0/10	0/10
	0.5mg/ml	20/1	2/1	1/1	0/1	0/10	0/10	0/10	0/10
	5mg/ml	20/0	1/1	0/10	0/10	0/10	0/10	0/10	0/10
N-Butanol	Control	20/1	20/1	20/1	20/1	20/1	19/1	19/1	19/1
	0.005mg/ml	20/1	20/1	18/1	12/1	8/1	1/5	1/10	0/10
	0.05mg/ml	20/1	10/1	1/1	1/10	0/10	0/10	0/10	0/10
	0.5mg/ml	20/1	2/1	1/1	0/1	0/10	0/10	0/10	0/10
	5mg/ml	20/0	1/1	0/10	0/10	0/10	0/10	0/10	0/10
Ethyl acetate	Control	20/1	20/1	20/1	20/1	20/1	19/1	19/1	19/1
	0.005mg/ml	20/1	20/1	18/1	12/1	8/1	1/5	1/10	0/10
	0.05mg/ml	20/1	10/1	1/1	1/10	0/10	0/10	0/10	0/10
	0.5mg/ml	20/1	5/1	1/1	0/1	0/10	0/10	0/10	0/10
	5mg/ml	20/0	1/1	0/10	0/10	0/10	0/10	0/10	0/10
N-Hexane	Control	20/1	20/1	20/1	20/1	20/1	19/1	19/1	19/1
	0.005mg/ml	20/1	20/1	18/1	12/1	8/1	1/5	1/10	0/10
	0.05mg/ml	20/1	10/1	5/1	1/10	0/10	0/10	0/10	0/10
	0.5mg/ml	20/1	5/1	1/1	0/1	0/10	0/10	0/10	0/10
	5mg/ml	20/0	1/1	0/10	0/10	0/10	0/10	0/10	0/10

Key: 20/1 twenty parasites per field, 5/1 five per one field, 1/1 one parasite per field, 0/1 zero parasite per field, 0/10 zero parasite per ten.

3.4 Gas Chromatography-Mass Spectrometer Analysis of the n-hexane, ethyl acetate and n- butanol fractions of *Carica papaya* seed.

Molecular formula, molecular structures and the functional groups of bioactive compounds of the *Carica papaya* seed extracts were determined using Gas Chromatography-Mass Spectrometer (GC-MS). The GC-MS analysis conducted on the hexane, ethyl acetate and butanol fraction of *C. papaya* seed revealed the presence of some useful compounds with their retention time, most of these compound are fatty acids such as methyl ester, cis-9-octadecanoic acid (oleic acid), phenol (hydroxybenzene) and hexadecanoic acid (palmitic acid) The GC - MS chromatogram of n-hexane fraction of *Carica papaya seed* shown the highest peak with retention time of (9.440 min) with the named compound 11-octadecenoic acid methyl ester at the area of 25.61 with formula of $C_{19}H_{36}O_2$ and molecular weight of 296.5, followed by peck of (8.59 min) and the compound named hexadecanoic acid, methyl ester with formula of $C_{17}H_{34}O_2$ and molecular weight of 270.450 and the next peck with retention time of (9.74 min) compound name is 5-Eicosene, (E)-, with of formula of $C_{20}H_{40}$ and molecular weight of 280.54, benzeneacetamide (7.05 min) and area peak of 7.54 with molecular formula of C_8H_9NO and molecular weight of 135.166 was also in detected while the lest peck with retention time of (10.33 min) with area peak of 1.33 and the named is oleic acid which have molecular formula of $C_{18}H_{34}O_2$ and molecular weight of 1.33. The GC-MS chromatogram of ethyl acetate fraction of *Carica papaya seed* reveled the following peak with retention time of (7.05 min) of Benzeneacetamide with formula of C_8H_9NO and molecular weight of 135.166, (5.89 min) of 2,3,4-Trimethyllevoglucosan with molecular formula of $C_9H_{16}0_5$ molecular weight of 204.22, (7.01 min) of oleic acid with molecular weight of 296.5, (8.30 min) of Bis(2-ethylhexyl) phthalate with molecular formula of $C_{24}H_{38}0_4$ and molecular weight of 394.588, (9.68 min) of Bicyclo[3.2.1]octan-4-one-1-carbon with molecular formula of $C_{24}H_{44}O_4$ and molecular weight of 396.604 and (11.03 min) of beta-Sitosterol with molecular formula of $C_{28}H_{50}O$ molecular weight of 414.71. The GC-MS chromatogram of n-butanol fraction of *Carica papaya seed* reveled the following peak with retention time of 4.717with the named Pent-2-ynal, 4,4-dimethyl with molecular formula of C_7H_{14} and molecular weight of 98.19, next is retention time of 5.332 named phenol with molecular formula of C_6H_5OH and molecular weight of 94.11, next is retention time of 6.080 name Benzene, 1,2,3,5- tetrachloro-

4,6-difluoro with molecular formula of $C_6Cl_{14}F_2$ and molecular weight of 251.863, next is retention time of 7.539 named 2-Pyridinamine, 3,5-dibromowith molecular formula of $C_3H_3Br_2N_3O_2$ and molecular weight of 296.9 next is retention time of 8.732 named Erucic acid with molecular formula of $C_{22}H_{42}O_2$ and weight of 338.57 and lastly retention time of this 9.865 named cis-9-Hexadecenoic acid with molecular formula of $C_{23}H_{44}O_2$ and molecular weight of 352.60. Some of these compounds have high level of activity against trypanosomes, that's why there is drastically reduction and complete destruction of the parasite at various concentration of the extract and fractions.

These findings are consistent with other studies which described the presence of fatty acid in chloroform fraction of *C. papaya* seed, with *in vitro* activity of against protozoan *Trichomonas vaginalis* at a 5.6 ug/mL concentration also activity against *T. cruzi* and *T. brucei brucei* [37;44]. Oleic acid being the chief fatty acid is known for its therapeutic properties is believed to be good for human health in lowering the blood levels of cholesterol and have been shown to slow the development of heart disease and also promote the production of antioxidants. Moderate levels of palmitic acid in diets also display antioxidant properties [45]. Many report by researches have shown that 9-octadecenamide, 1-nonadecene, (*Z*)-9-eicosene, hexadecanol, 1 pentadecanol for fraction A; (Z)-9-Eicosene, penduletin, methyl hexadecanoate, methyl *cis*-9-octadecenoate, Methyl cis-9-octadecenoate and 1-heptadecanol from fraction B of aqueous extract from *Lophira lanceolata* (Ochnaceae) leaf and ethanol extract of *Gardenia erubescens* (Rubiaceae) stem had 100% activity *in vitro* against *T. b. brucei* and *T. congolense* and stated that trypanocidal activity of the compound increases with increase methylation of hydroxyl groups and further stated that lipophilicity increases permeability of molecule across membranes of the parasite [46;47 and 48].

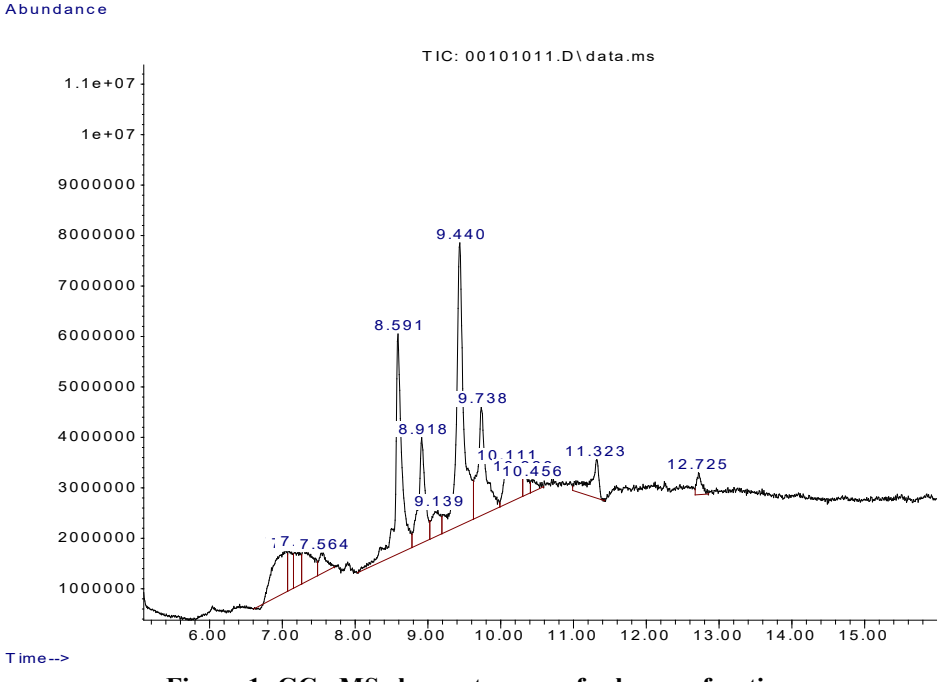

Figure 1: GC - MS chromatogram of n-hexane fraction

Table 5: Gas Chromatography-Mass Spectrometry of identified Component of n-hexane fraction of *Carica papaya* seed

R/Time	MW	Formula	Names of compound	% Area of peak
7.05	135.166	C_8H_9NO	Benzeneacetamide	7.54
7.09	120.151	C_8H_80	Phthalan	2.35
8.59	270.450	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	18.4
9.14	566.952	$C_{36}H_{70}O_4$	Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester	2.76
9.44	296.5	$C_{19}H_{36}O_2$	11-Octadecenoic acid, methyl ester	25.61
9.74	280.54	$C_{20}H_{40}$	5-Eicosene, (E)-	11.32
10.11	282.468	$C_{18}H_{34}O_2$	cis-Vaccenic acid	6.48
10.33	282.5	$C_{18}H_{34}O_2$	Oleic Acid	1.33
10.46	240.3386	$C_{14}H_{24}O_3$	Oxacyclotetradecane-2,11-dione, 13 -methyl	0.93
12.73	330.5026	$C_{19}H_{38}O_4$	Hydroxymethyl ethyl ester	1.41

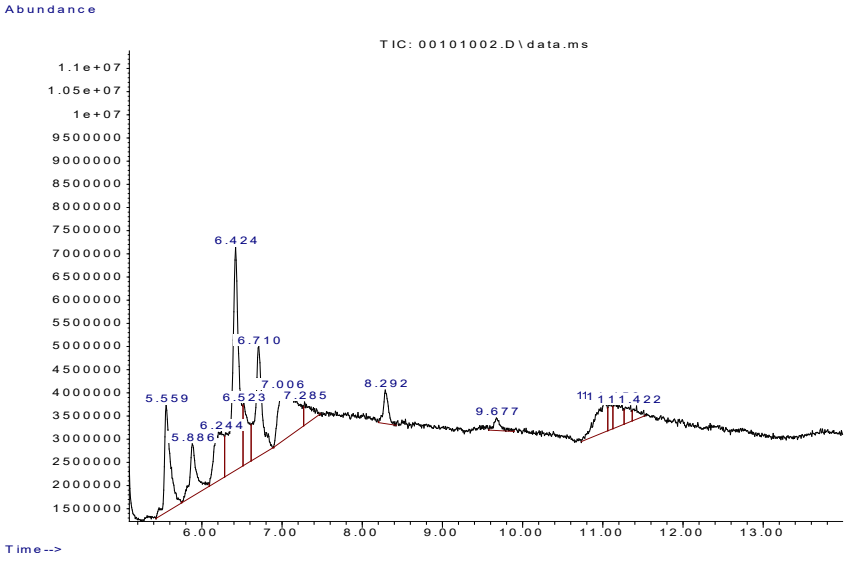

Figure 2: Gas chromatogram of ethyl acetate fraction

Table 6: Gas Chromatography-Mass Spectrometry of identified Component of ethyl acetate fraction of *Carica papaya* seed

R/Time	MW	Formula	Names of compound	% Area
5.56	270.4507	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	10.28
5.89	204.22	$C_9H_{16}0_5$	2,3,4-Trimethyllevoglucosan	5.94
6.42	296.4879	$C_{19}H_{36}0_2$	10-Octadecenoic acid, methyl ester	24.51
7.01	296.5	$C_{18}H_{36}O_2$	Oleic Acid	13.81
8.30	394.588	$C_{24}H_{38}0_4$	Bis(2-ethylhexyl) phthalate	2.68
9.68	396.604	$C_{24}H_{44}O_4$	Bicyclo[3.2.1]octan-4-one-1-carbon	1.42
11.03	414.71	$C_{28}H_{50}O$	beta.-Sitosterol	5.75

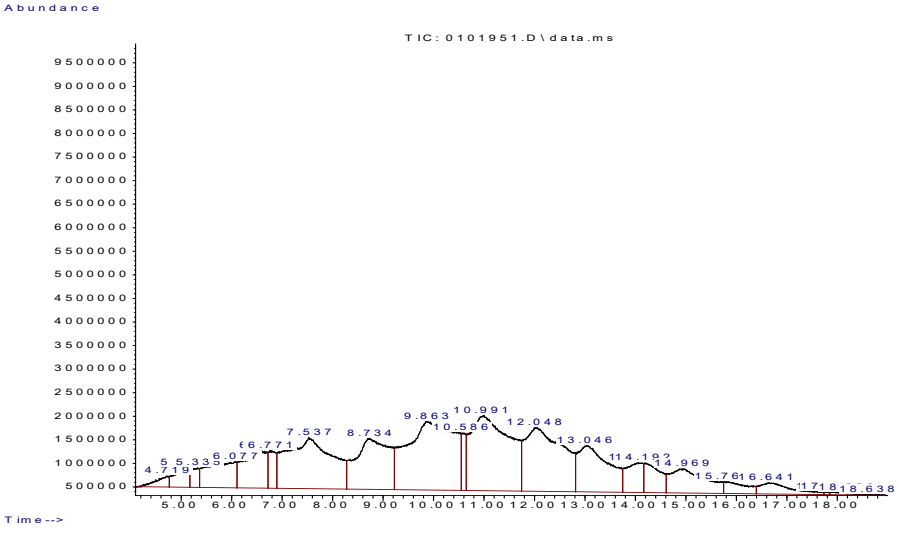

Figure 3: Gas chromatographic (GC) of n-butanol fraction

Table 7: Gas Chromatography-Mass Spectrometry (GC-MS) of identified Component of n-butanol fraction of *Carica papaya* seed

R/Time	MW	Formula	Names of Compound	% Area
4.717	98.19	C_7H_{14}	Pent-2-ynal, 4,4-dimethyl	0.72
5.332	94.11	C_6H_5OH	Phenol	0.80
6.080	251.863	$C_6Cl_{14}F_2$	Benzene, 1,2,3,5-tetrachloro-4,6-difluoro	3.81
7.539	296.9	$C_3H_3Br_2N_3O_2$	2-Pyridinamine, 3,5-dibromo	12.05
8.732	338.57	$C_{22}H_{42}O_2$	Erucic acid	8.81
9.865	352.60	$C_{23}H_{44}O_2$	cis-9-Hexadecenoic acid	16.90
10.583	236.40	$C_{16}H_{28}O$	7,11-Hexadecadienal	1.28
10.991	508.92	$C_{34}H_{68}O_2$	Heptadecanoic acid, heptadecyl ester	15.32
12.050	252.49	$C_{18}H_{36}$	1-Octadecene	12.53

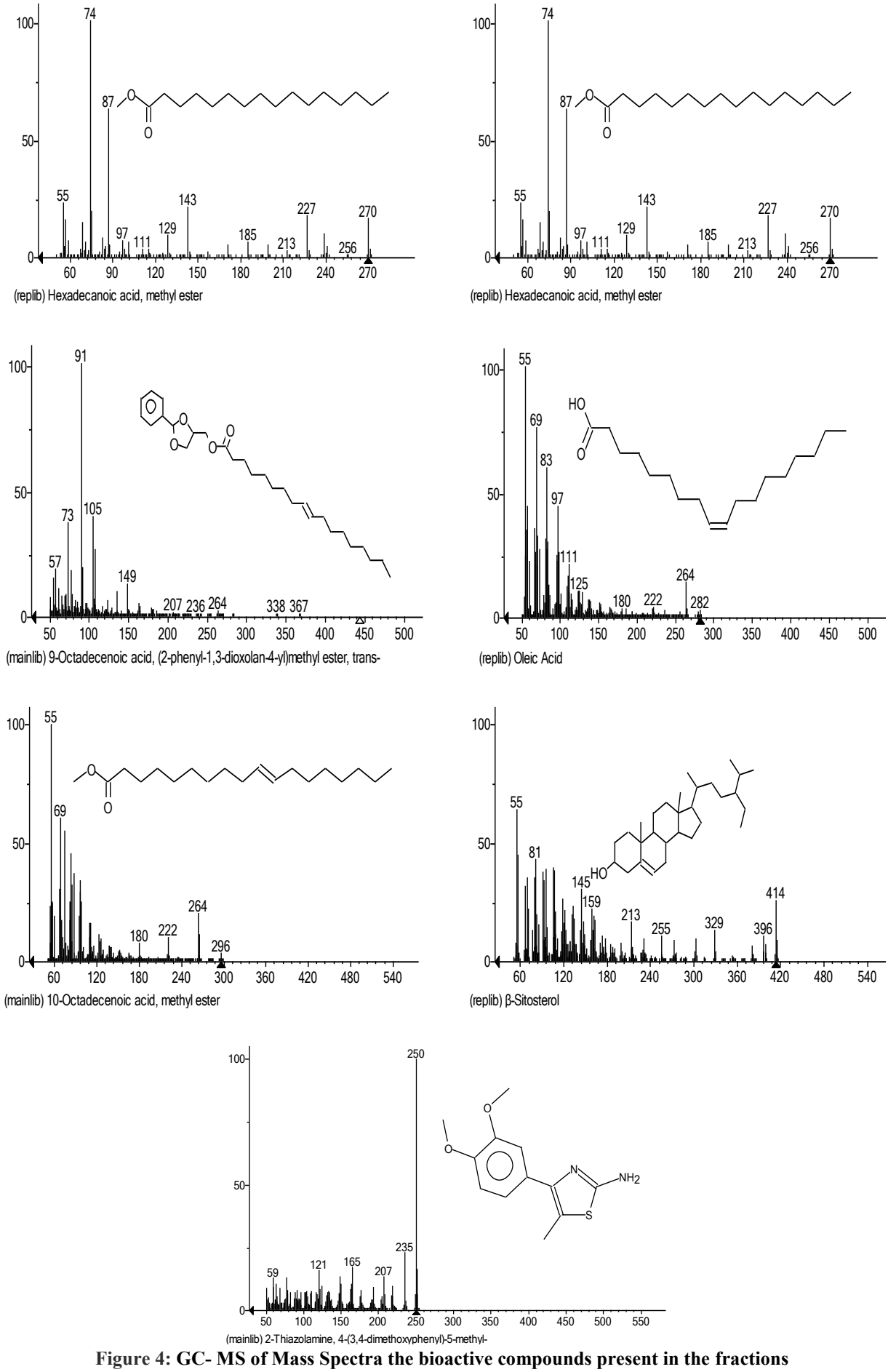

Figure 4: GC- MS of Mass Spectra the bioactive compounds present in the fractions

3.5 Fourier Transform-Infra-Ray Analysis of the n-hexane, ethyl acetate and n- butanol fractions of *Carica papaya* seed.

Table 8, 9, and 10 below, showed the FTIR bonding vibrations occurred due to each functional groups of hexane, ethyl acetate and butanol fraction of *C. papaya* seed. Fourier Transform-Infra-Ray (FT-IR) Performed on hexane, ethyl acetate and butanol fraction of *C. papaya* seed revealed the presence of some useful alcohols, phenols, aromatic bend, hydrocarbon, amines, alkenes, carboxylic acid stretch, methyl, enol, oximes and ammonium salts, most of these aromatic groups have anti-inflammatory and antioxidant effect, the reduction of parasites level could attribute to the presence of the aromatic compounds. The findings of the current study are consistent with those of Amisigo, *et al*. [43] who confirmed the presence of aromatic and phenol in garlic and later stated that trypanocidal activity of gallic acid could be due to the presence of the extra hydroxyl group and its ability to form reactive oxygen intermediates in the parasite. Dowson and Huc[49] stated that the aromatic amide 3-aminobenzamide, an inhibitor of nuclear poly- (ADP-ribose) polymerase, prevents the calcium-induced activation of endonucleases in rat liver nuclei.

Table 8: Infrared spectroscopy result showing functional groups present in n-hexane fraction of *Carica papaya* seed

Peak cm^{-1}	Bonds	Suspected functional groups
1080.43	C ─ H	Alcohols and Phenols
1168.43	C ─ N and C─ O	Amines, Ester, Ethers and Alcohol
1371.43	N ═ O	Nitro group
1453.93	C─ H	Methyl
1739.13	C ═ O Stretch	Esters
2861.34	C ─ H	Methyls, Methines and Methylenes
2929.43	H ─ C ─ H Stretch	Methyls, Methines and Methylenes
3341.69	─ O ─ H	Alcohols, Enol, Oximes and Phenols

Table 9: Showing the Infrared spectrum of ethyl acetate fraction of *Carica papaya* seed

Peak cm^{-1}	Bonds	Suspected functional groups
703.00	C ─ H Bend	Aromatics Hydrocarbon
843.99	C ─ H Bend	Aromatics and Mono subtited Benzene
1186.46	C ─ N	Amines
1442.98	H ─ C ─ H	Alkanes
1599.98	C ═ C	Aromatic Bend
1729.77	C ═ C	Carboxylic acid Stretch
2308.68	NH and C ═ C	Ammonium salts
2548.61	─ C ─H Bend	Acid and chelated ─ OH groups

Table 10: Showing the Infrared spectrum of n-butanol fraction of *Carica papaya* seed

Peak cm^{-1}	Bonds	Suspected functional groups
1046.42	C ─ N and C ─ O	Amines, Esters, Acetates, Epoxides/Ethers and Alcohols
1124.47	C ─ N and C ─ O	Amines, Esters, Acetates, Epoxides/Ethers and Alcohols
1241.49	C ─ N and C ─ O	Amines, Esters, Acetates, Epoxides/Ethers and Alcohols
1385.48	C ─ H	Methyls, Methylenes and Methines
1460.11	C ─ C Bend or Scissoring	Methyls, Methylenes and Methines
1628.22	C ─ N Bend and C ═ C Bend	Aromatic Bend
2361.54	NH and C ═ C	Ammonium salts
2864.81	C ─ H Stretch	Methyls, Methylenes and Methines
2934.13	C ─ H Stretch	Methyls, Methylenes and Methines
3347.50	N ─ H Stretch	Secondary Amine

4. Conclusions

In vitro anti-trypanosomal assays showed that methanol extract and fractions of *Carica papaya* seed had demonstrated highest activity at concentration of 5mg ml, 0.5mg/ml and 0.05mg/ml respectively, there is a clear indication that these extract and fraction are dosage's defendant. Thus, results of the current study laid down some significant groundwork for large-scale bioassay-guided isolation of key anti-trypanosomal constituents from the active extracts which could target mainly the major constituents. Consequently, the result of this study validated the anti-trypanosomal activity of *carica papaya* seed extract and fractions on trypanosomes even at leaser concentration the plants.

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