



## **GC–MS Analysis and Antimalarial Activity of Methanolic Leaf Extract of *Carica papaya* against *Plasmodium berghei* NK65 Infection in Swiss Mice**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author JOM designed the methodology and study, wrote the protocol and the first draft of the manuscript and contributed to the discussions and corrections. Author OAD explained the interpretation of the AAS, GC-MS and the medicinal functions of the phytocomponents found in the plant. Authors JOM and OMO carried out the laboratory analyses. Author JOM was involved in the collation of data and statistical analysis. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aim:** The study determines the GC-MS and the anti-malarial activity of methanolic leaf extract of *Carica papaya* in Swiss mice infected with *Plasmodium berghei* NK65.

**Study Design:** The experimental study lasted for five weeks.

**Place of Study:** Department of Chemical Sciences (Biochemistry Unit) and animal House unit in Department of Biological Sciences (Environmental Biology Unit), School of Pure and Applied

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**Study Design and Methodology:** AAS, GC-MS and phytochemical analyses were determined in the plant extract using standard procedures. Thirty-six Swiss mice of both sexes (26–32g) were divided into six groups of six mice each. Group A (normal control) was untreated and uninfected. Groups B–F were intraperitoneally inoculated with *P. berghei* NK65, while group B (disease control) was untreated-infected group, group C and D (standard drugs) received standard drugs, chloroquine (10 mg/kg B.WT) and artesunate (10 mg/kg B.WT); groups E and F received methanolic leaf extract of *C. papaya* at 400 and 600 mg/kg B.WT respectively. WBC, HCT and HGB were determined in the whole blood using BC-3200 Auto Hematology Analyzer. MDA, TP, SOD % inhibition, SOD unit, CAT and GSH were all determined in the liver homogenate using standard procedures.

**Results:** The AAS analysis shows that the extract contains minerals like: potassium, calcium, magnesium, iron and sodium. Twenty-six compounds were identified to be present in the extract using GC-MS analysis. The active compounds with their retention time, molecular weight, molecular formula, peak area and activities were predicted. The three major prevailing compounds and their percentage abundance are: squalene (27.28%), neophytadiene (12.71%) and phytol (10.16%) respectively. The phytochemical analysis indicates the presence of tannins, saponins, alkaloids, phenolic compounds etc. The *C. papaya* extract caused 56.76% and 75.53% significant ( $P < 0.05$ ) reduction in parasitemia at 400 and 600 mg/kg body weight respectively while chloroquine exerted 92.86% and artesunate exerted 90.67% reduction at 10 mg/kg body weight respectively carried out during curative test. The extract significantly ( $P < 0.05$ ) reduced WBC count and increase HGB and HCT concentration in the treated mice compared to the infected untreated mice. There were significant ( $p < 0.05$ ) increase in the TP, SOD % inhibition, SOD unit, GSH and CAT levels in the liver homogenate of animals treated with chloroquine, artesunate and extract of *C. papaya* compared to the untreated mice. MDA level was significantly decreased in the malaria treated mice compared to the untreated mice.

**Conclusions:** The study shows that methanolic leaf extract of *Carica papaya* possess antimalarial activity in Swiss mice infected with *Plasmodium berghei* NK 65.

**Keywords:** *Carica papaya*; anti-malarial activity; GC-MS; *Plasmodium berghei* NK 65; Swiss mice; biochemical parameters.

## 1. INTRODUCTION

Malaria is an infectious disease, caused by different protozoan parasites of the genus *Plasmodium* [1]. Study has shown that *Plasmodium* are resistance to antimalarial drugs, which leads to drug failure, new drugs production or the use of drug combinations for the treatment of malaria infections using traditional medicinal plants [2]. Malaria is the leading cause of illness and death in sub-Saharan Africa with an annual mortality of approximately one million children under five [3].

*Carica papaya* Linn. (family: Caricaceae) is primarily cultivated for its fruit. Different parts of the plant have been used in traditional medicine to treat various diseases. The juice of the leaves is taken to treat malarial fever [4]. Ethyl acetate crude leaves extract of *C. papaya* showed high antiplasmodial activity against *Plasmodium falciparum* and *P. falciparum*-resistant strains [5]. The ethanolic extract of *C. papaya* leaves showed potential as a source of drugs against urinary tract microbes [6]. The aqueous extract of

the leaves exhibited the ability to inhibit tumor cell lines [7] and this activity was potentiated with phenolic compounds [8,9]. The aqueous extract of *C. papaya* also showed significant protective effect against alcohol induced oxidative damage to the gastric mucosa in rats [10], and it was effective in healing induced wound in rats [11]. The seed and fruit of *C. papaya* showed bacteriostatic activity against several enteropathogens in human [12]. The root of *C. papaya* plant is used to treat urinary disorders while the bark is used to treat toothache [13]. The present study indicate the GC–MS analysis and antimalarial activity of methanolic leaf extract of *Carica papaya* against *Plasmodium berghei* NK65 infection in Swiss mice

## 2. MATERIALS AND METHODS

### 2.1 Collection and Identification of Plant

The leaves of *Carica papaya* was obtained from Lagos State Polytechnic Ikorodu in Lagos, Nigeria. Authentication of the plant was done in the Department of Biological Sciences

(Environmental Biology Unit) School of Pure and Applied Sciences, Lagos State Polytechnic, Ikorodu, Lagos, Nigeria.

## 2.2 Mineral Analysis of Methanolic Leaves Extract of *Carica papaya*

2 g of dried *C. papaya* leaves was digested with 10 ml of aqua regia (HNO<sub>3</sub> and HCl in the ratio 1:3) and the mixture was heated on porcelain crucible until the brown fumes disappeared leaving white fumes. It was later filtered with whatman filter paper into universal bottle. The mineral elements in the sample were determined by Atomic Absorption Spectrophotometer (Model Perkin Elmer AAnalyst 400).

## 2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Methanolic Leaf Extract of *Carica papaya*

GC-MS analysis of the plant extract was carried out on an Agilent technology 7890 GC system equipped with a mass spectrometric detector (MSD) as described by Momoh et al. [14]

## 2.4 Detection of Components

Analysis of mass spectrum GC-MS was conducted by the database of National Institute Standard and Technique (NIST) which contained more than 62,000 patterns. The spectrum of the unidentified compound was compared with the spectrum of the identified compounds stored in the National Institute Standard and Technique library. The names, molecular weight, structure of the compounds in the test material were ascertained.

## 2.5 Preparation of *Carica papaya* Methanolic Leaf Extract

The *Carica papaya* leaves were washed with water to remove dirt, air dried under shade in the laboratory, pulverised into coarse powder using industrial machine. Extraction was carried out by dispersing 200g of the grounded *C. papaya* leaves into 2 Litres of 80% methanol at room temperature (25 ± 2 °C) and shaken for 72 hours. The solution was filtered by passing through cotton and the resultant filtrate was concentrated with the help of rotary evaporator at a temperature not exceeding 40°C. The concentrated extract was later dried to complete

dryness in an aerated oven at 40°C for 48 hours. The concentrate was later stored in a refrigerator at 4°C.

## 2.6 Qualitative Phytochemical Analysis of *Carica papaya* Leaf Extract

Phytochemical analyses for phytochemical constituents were carried out on the *C. papaya* leaf extract using standard procedures [15,16].

## 2.7 Experimental Animals

All the Swiss mice of both sexes used have a weight ranging from 26 to 32 gram. They were obtained from University of Lagos, Nigeria. They were acclimatized for seven days to experimental laboratory condition of 23±2°C. They were kept in cages and fed with commercial rodent chow and supply with water *ad libitum*. The mice were used in accordance with NIH guide for the care and use of laboratory animals; NIH Publication revised, (1985) [17].

### 2.7.1 Malaria parasites

*Plasmodium berghei* NK65 is chloroquine-sensitive and was used to assess the antimalarial activity of *C. papaya* methanolic leaf extract. The parasite was obtained from National Institute for Medical Research (NIMR) Lagos, Nigeria. The parasites were maintained by continuous re-infestation in mice.

### 2.7.2 Inocula

Parasitized red blood cells were obtained from a donor infected mouse by ocular puncturing. This was prepared by determining percentage parasitemia and the red blood cells count of the donor mouse and diluting them with normal saline in proportions indicated by both determinations [18]. Each mouse was inoculated intraperitoneally with infected red blood suspension (0.2 mL) containing 1×10<sup>7</sup> *P. berghei* NK65 parasitized erythrocyte.

### 2.7.3 Grouping of animals

Group A (Normal control): Healthy Swiss mice

Group B (Disease control): mice infected with *P.berghei* NK65 received no treatment

Group C (Positive control I): Infected mice that received 10mg/kg B.WT of chloroquine (CQ)

Group D. (Positive control II): Infected mice that received 10mg/kg B.WT of Artesunate

Group E: Infected mice that received 400mg/kg B.WT of *C. papaya* methanolic leaf extract

Group F: Infected mice that received 600mg/kg B.WT of *C. papaya* methanolic leaf extract

#### 2.7.4 *In vivo* antiplasmodial activities Curative (Rane's) test

Thirty Swiss mice weighing between 26 and 32 gram were injected intraperitoneally with standard inocula of *P. berghei* NK65 containing  $1 \times 10^7$  infected red blood cells. Seventy-two hours later, the mice were randomly divided into 5 groups of 6 mice per group as shown above. Group A mice were not induced with *P. berghei* NK65. All treatments were administered orally. Treatment continued daily until the seventh day when thin films were made from the tail blood of each mouse. The films were fixed with methanol, stained with Giemsa and parasitemia density examined by microscopically counting the parasitized red blood cells on at least 1000 red blood cells in 10 different fields [19,20].

$$1. \% \text{ parasitemia} = \frac{\text{No. of parasitized RBC}}{\text{Total no. of RBC counted}} \times 100$$

2. Percentage suppression of parasitemia was calculated:

$$\frac{\text{Parasitemia of negative control} - \text{parasitemia of test}}{\text{Parasitemia of negative control}} \times 100$$

#### 2.7.5 Collection of blood samples

The experimental mice were sacrificed by cervical decapitation after 24 hours of fasting. Blood were collected from the animals by ocular puncture into EDTA tubes for the determination of hematological parameters. The animals were dissected while their livers were excised for biochemical examinations.

#### 2.7.6 Hematological analysis

The bloods in the EDTA tubes were assayed for hematological parameters using BC-3200 Auto Hematology Analyzer in Central Research Laboratory in Lagos University Teaching Hospital Lagos, Nigeria. The hematological parameters investigated were White blood cell count (WBC), Hemoglobin (HGB) and hematocrit (HCT) counts.

#### 2.7.7 Preparation of liver homogenate

The liver tissues of some of the sacrificed animals were removed and the liver samples were cut into pieces and homogenized with phosphate buffer saline (PBS) to give a 10% (w/v) liver homogenate. The liver homogenates were then centrifuged at 12,000 rpm for 60 minutes. The supernatant obtained was later used for the assay of total protein, catalase, MDA, GSH, SOD% inhibition and SOD unit.

#### 2.7.8 Determination of total protein

Total protein (TP) was determined in the liver homogenate using Randox diagnostic kits.

#### 2.7.9 Determination of catalase (CAT)

The liver homogenate was assayed for catalase colorimetrically at 620 nm and expressed as  $\mu\text{moles of H}_2\text{O}_2$  consumed/min/mg protein by the method of Rukkumani et al. [21]

#### 2.7.10 Determination of Lipid Peroxidative (LPO) indices

Lipid peroxidation as evidenced by the formation of TBARS was measured in the liver homogenate by the method of Jiang et al. [22]

#### 2.7.11 Determination of Superoxide Dismutase (SOD)

The SOD activity was estimated by its capacity of inhibiting pyrogallol autooxidation in alkaline medium. The liver homogenate was assayed for the presence of SOD% by utilizing the technique described by Zou et al. [23]

#### 2.7.12 Determination of Reduced Glutathione (GSH)

Reduced glutathione (GSH) was determined in the liver homogenate using the method of Rukkumani et al. [21].

### 2.8 Statistical Analysis

The data were expressed as mean  $\pm$  SD and parasitemia of the different groups were assessed by unpaired student t-test using Graphpad prism InStat Demo version 5.01. Comparisons were done using one way analysis of variance (ANOVA) complemented with Bonferroni's to compare all pair of columns. For

all the analyses, a  $P < 0.05$  was considered statistically significant.

### 3. RESULTS

#### 3.1 Mineral Analysis of *Carica papaya* Leaves

Table 1 below shows the percentage composition of minerals in the leaves of *C. papaya*

#### 3.2 GC-MS Analysis of Methanolic Leaf Extract of *C. papaya*

The GC-MS analysis of methanolic leaf extract of *C. papaya* showed that the extract contains 26 compounds with 3 major prominent compounds (Table 2).

#### 3.3 Phytochemical Result

The Phytochemicals shows the result of qualitative phytochemical composition of methanol leaf extract of *Carica papaya*. The results revealed the presence of alkaloids, cardiac glycosides anthraquinones, tannins, reducing sugar, flavonoids, saponins, terpenoids and phenolic compounds as shown in Table 3.

#### 3.4 Antiplasmodial (Curative) Test

The mean parasitemia density and percentage suppression of *Plasmodium berghei* NK65 in infected mice treated with standard drugs and methanolic leaf extract of *Carica papaya* are shown in Table 4. The percentage suppression of infected mice treated respectively with chloroquine, artesunate, 400 and 600 mg/kg body weight of leaf extract of *Carica papaya* significantly ( $P < 0.05$ ) reduced when compared with infected mice without treatment (group B). The result shows that the plant is more active at a dose of 600 mg/kg body weight by having a percentage suppression of 75.53%.

#### 3.5 Complete Blood Count (CBC)

The graph below shows that there were significant increase ( $P < 0.05$ ) in HGB concentration in the healthy and treated mice (group C-F) compared to the infected untreated mice (group B). HCT concentration was significantly lowered ( $P < 0.05$ ) in group B mice compared to mice in other groups. WBC count was significantly ( $P < 0.05$ ) high in the group B mice compared to mice in other groups.

#### 3.6 Antioxidant and Oxidative Stress Parameters

Fig. 3 below shows that total protein and reduced glutathione were significantly ( $P < 0.05$ ) increased while MDA values are decreased in the healthy and treated group mice (group A, C-F) compared to the infected untreated mice (group B).

Fig. 4 shows that there were significant increase ( $P < 0.05$ ) in catalase, SOD % inhibition and SOD (unit) concentration in the healthy and treated mice compared to the infected untreated mice (group B).

### 4. DISCUSSION

Medicinal plants used in the treatment of infectious diseases contain active phytochemicals some of which are responsible for the plants' characteristic odors, color and pungencies while others are used as food and medicinal [44].

The data from Table 1 shows that *C. papaya* leaves contain elements like K, Na, Mg, Ca and Fe in various proportions ( $Ca > Mg > K > Fe > Na$ ). The result shows that Ca content is the highest in the leaves of *C. papaya*. Different research works have shown the present of minerals in *C. papaya* leaf [45,46]. Study has shown that high amount of potassium in the body increase iron utilization [46] and it helps to control hypertension through body fluid [46]. Sodium ion is present in outside cells and is one of the primary electrolytes responsible for maintaining fluid balance [46]. Magnesium is an essential element that is used by plants in relatively large amounts and its deficiency can affect virtually every organ system of the human body [45]. Calcium is essential for healthy teeth, bones and blood [45]. Deficiency of Fe causes anemia [45].

For the quantitative determination of compounds, gas chromatography with flame ionization detector (GC-FID) and GC-MS are usually preferred. The different compounds identified with their functions from each of the mass spectra fragmentation patterns are listed in Table 2. A total of 26 compounds were identified consisting of three (3) major compounds and twenty-three (23) minor compounds. The three prominent compounds constitute 50.15% of the *C. papaya* extract. The three major compounds and their percentage abundance are Squalene (27.28%), Neophytadiene (12.71%)

and Phytol (10.16%) respectively. These prominent compounds were represented by peaks 24, 7 and 16 with retention times of 31.010, 14.330 and 18.832 respectively. The activities of some of the compounds have been reported in Table 2. The most prominent compound in the extract of *C. papaya* was squalene. Squalene is a natural lipid that belongs to the terpenoid family and a precursor of cholesterol biosynthesis. It is extensively used as an excipient in pharmaceutical formulations for disease management and therapy [42]. Squalene acts as a protective agent and has been shown to decrease chemotherapy-induced side-effects and exhibits chemopreventive activity [42]. It is a weak inhibitor of tumor cell proliferation; it contributes to the treatment of cancer due to its potentiation effect. It enhances the immune response to various associated antigens. Squalene is well absorbed orally and has been used to improve the oral delivery of therapeutic molecules [42]. Ruba and Mohan, [41] study shows that squalene is a good antibacterial, cancer preventive, antioxidant, antitumor, chemo preventive, immunostimulant and lipoxygenase-inhibitor. Mostafa et al. [29] study shows that squalene is used for gastrin inhibitor, histamine release inhibitor, sugar-phosphatase inhibitor, anti-inflammatory, anthelmintic and antiparasitic. In another study carried out by Ezhilan and Neelamegam, [47], it was observed that squalene has anti-oxidant, anti-tumor, anti-cancer, chemo-preventive, pesticidal and sun-screen properties. Squalene is used to neutralize different xenobiotics, it has anti-inflammatory, anti-atherosclerotic and anti-neoplastic, it plays a role in skin aging and pathology and possess adjuvant activities [28]. The second most prominent compound in the extract was neophytadiene and it is used as antioxidant, analgesic, antimicrobial, antipyretic, and anti-inflammatory activities [28]. Mostafa et al. [29] study shows that neophytadiene is used for histamine release inhibitor, carminative, gastrin inhibitor, antiulcerative, antiprotozoal (Leishmania) and possess anti-parasitic properties. The third abundant compound in the methanolic leaf extract of *C. papaya* was phytol. Phytol is an acyclic diterpene alcohol it is used as a precursor for the manufacture of synthetic forms of Vitamin E and vitamin K. It has anticancer, antimicrobial, and anti-inflammatory activities. It is also used as diuretic [37]. Mostafa et al. [29] study shows that phytol is used as histamine release inhibitor, lipid metabolism regulator, anthelmintic, antiparasitic, antiprotozoal, (leishmania) and spasmolytic.

Phytol treatments decreases the autoimmune response and ameliorate both acute and chronic phases of arthritis [48]. In a study carried out by Venkata et al. [28], it was stated that phytol has antimicrobial, anticancer, anti-diuretic, anti-inflammatory, immunostimulatory and anti-diabetic properties.

The minor identified constituents in the plant extract also possess biological activity or may act in synergy to reduce parasite load. n-Hexadecanoic acid functions as inhibitor of phospholipase A2 [34]; hence, it is an anti-inflammatory compound used for the treatment of rheumatic symptoms in traditional medical system [34]. Delta.-Tocopherol is an isomer of vitamin E. Vitamin E is a family of eight natural compounds (four tocopherols ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ -) and four tocotrienols ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ -)) with different antioxidant and biological activities [43]. Vitamin E is the major lipid-soluble component in the cell antioxidant defence system and is entirely obtained from diet. The most active form of this vitamin is  $\alpha$ -tocopherol, which is widely distributed in nature and it is often used in vitamin supplements [43]. It has various important roles within the body because of its antioxidant activity. Oxidation reaction has been linked to various possible diseases conditions, including diabetes, cancer, ageing, asthma, arthritis and cataracts. Vitamin E has been shown to be effective in preventing or ameliorating the effect of these diseases [43]. It helps to reduce the production of prostaglandins such as thromboxane, which causes platelet clumping [43]. Mono (2-ethylhexyl) phthalate (MEHP) stimulated basal steroidogenesis associated with up-regulation of StAR protein expression with no effect on hCG-stimulated androgen production by LCs from CBA/Lac and C57BL/6j mouse genotypes [40]. MEHP attenuated ATP production and increased superoxide generation by both phenotypes of mouse LCs that indicated mitochondrial dysfunction induced by the monophthalate [40].

This study revealed the presence of important phytochemicals like saponins, reducing sugar, alkaloids, terpenoids, tannins, anthraquinones, flavonoids, cardiac glycosides and phenolic compounds in the methanolic leaf extract of *Carica papaya*. The study is in line with the reports of a number of authors that showed that active phytochemicals like: phenolics, alkaloids, steroids, saponins, phlabotannins, amino acids, flavonoids etc are present in the different extract of *Carica papaya* [49-51]. Studies have shown

**Table 1. Mineral constituents of *Carica papaya* leaves**

Plant	K (mg/100g)	Ca (mg/100g)	Mg (mg/100g)	Fe (mg/100g)	Na (mg/100g)
<i>C. papaya</i>	10.1531±0.0050	38.2392 ± 0.001	15.1608 ± 0.002	0.23218 ± 0.003	0.03286 ± 0.0050

**Table 2. Phytocomponents identified in the leaf of *Carica papaya* using GC-MS**

PK no	Retention time	Name of compound	Molecular formulae	Molecular weight (g/mol)	Peak area (%)	Activity
1	10.131	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro -6-hydroxy-4,4,7a-trimethyl-	C <sub>11</sub> H <sub>16</sub> O <sub>3</sub>	196.24	1.12	Antimicrobial [24].
2	10.909	3-Trifluoroacetoxypentadecane	C <sub>17</sub> H <sub>31</sub> F <sub>3</sub> O <sub>2</sub>	324.4	1.19	Anti-nephrotoxic and antioxidant activities [25].
3	11.555	cis-2,5-Dimethoxy-4-ethoxy-.beta.-methylstyrene	C <sub>13</sub> H <sub>18</sub> O <sub>3</sub>	222.28	0.85	NF
4	12.871	4-Heptafluorobutyryloxyhexadecane	C <sub>20</sub> H <sub>33</sub> F <sub>7</sub> O <sub>2</sub>	438.5	1.13	NF
5	13.644	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a -tetrahydrobenzofuran-2(4H)-one	C <sub>11</sub> H <sub>16</sub> O <sub>3</sub>	196.2429	2.53	Antidiabetes[26]. Anti-inflammatory response [27].
6	13.718	Cyclotetradecane	C <sub>14</sub> H <sub>28</sub>	196.37	2.20	NF
7	14.330	Neophytadiene	C <sub>20</sub> H <sub>38</sub>	278.5	12.71	Antioxidant, analgesic, antimicrobial, antipyretic, and anti-inflammatory activities [28]. Histamine release inhibitor, carminative, gastrin inhibitor, antiulcerative, antiprotozoal (Leishmania) and possess anti-parasitic properties [29].
8	14.433	2-Pentadecanone, 6,10,14-trimethyl	C <sub>18</sub> H <sub>36</sub> O	268.4778	2.15	Allelopathic and antibacterial [30], Antibacterial [31].
9	14.657	cis-5-Decen-1-yl acetate	C <sub>12</sub> H <sub>22</sub> O <sub>2</sub>	198.302	1.04	NF
10	14.891	1-Methoxy-3-(2hydroxyethyl)- nonane	C <sub>12</sub> H <sub>26</sub> O <sub>2</sub>	202.33	2.09	NF
11	15.452	Trans-Farnesol	C <sub>15</sub> H <sub>26</sub> O	222.3663	1.74	NF
12	15.544	Hexadecanoic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.451	6.14	Antioxidant, antimicrobial, decrease blood cholesterol and anti-inflammatory [31,32].
13	16.293	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	4.40	Antibacterial, antifungal activity and anti-inflammatory [33,34].
14	18.078	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.4721	2.08	Antiinflammatory, insectifuge, anticancer, hepatoprotective, antimicrobial, antieczemic,

PK no	Retention time	Name of compound	Molecular formulae	Molecular weight (g/mol)	Peak area (%)	Activity
15	18.176	Cyclooctene, 3-ethenyl-	C <sub>10</sub> H <sub>16</sub>	136.23	5.74	antihistaminic, hypocholesterolemic, [35,36]. NF
16	18.382	Phytol	C <sub>20</sub> H <sub>40</sub> O	296.5	10.16	Anticancer [31], Antimicrobial, anticancer, anti-inflammatory, diuretic [37].
17	18.628	Heptadecanoic acid, 16-methyl-, methyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298.5038	1.98	Used against skin cancer protein [38].
18	19.040	9,12-Octadecadienoic acid (Z,Z)-	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280.44	2.16	hepatoprotective, hypocholesterolemic, anti-arthritic, anti-inflammatory, anti-histaminic and nematocidal activities [39].
19	22.496	4,8,12,16-Tetramethylheptadecan-4-olide	C <sub>21</sub> H <sub>40</sub> O <sub>2</sub>	324.5	2.00	NF
20	23.051	(2E,6E)-3,7,11-Trimethyldodeca-2,6,10-trienyl propionate	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	278.4296	1.15	NF
21	25.151	1-Chloroeicosane	C <sub>20</sub> H <sub>41</sub> Cl	317	1.76	NF
22	26.026	Mono(2-ethylhexyl) phthalate	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278.3435	0.87	Stimulates androgen production [40].
23	28.687	Ethanol, 2-(dodecyloxy)-	C <sub>14</sub> H <sub>30</sub> O <sub>2</sub>	230.3868	1.15	NF
24	31.010	Squalene	C <sub>30</sub> H <sub>50</sub>	410.7	27.28	Antioxidant, antibacterial, cancer preventive, antitumor, cancer preventive, chemo preventive, immunostimulant and lipoxygenase-inhibitor [41,42].
25	32.698	Carbonic acid, decyl tetradecyl ester	C <sub>25</sub> H <sub>50</sub> O <sub>3</sub>	398.6627	3.04	NF
26	33.699	Delta.-Tocopherol	C <sub>27</sub> H <sub>46</sub> O <sub>2</sub>	402.7	1.34	antiinflammatory, dermatologic, antisecretoric, antiacne, antiprotozoal (Leishmania) and Bone formation stimulant [29], Antioxidant activity, effective against asthma, allergies, diabetes, atherosclerosis, cancer, ageing, arthritis and cataracts [43].

*NF is not found*



that saponins, flavonoids, tannins and other secondary metabolites in plant may play some roles in the inhibition of malaria parasites in infected animals [52,53]. Flavonoids have been

reported to have significant *in-vitro* antimalarial activity against *P. falciparum* [54] and this may be responsible for the antimalarial activities exhibited by *C. papaya* methanolic leaf extract.

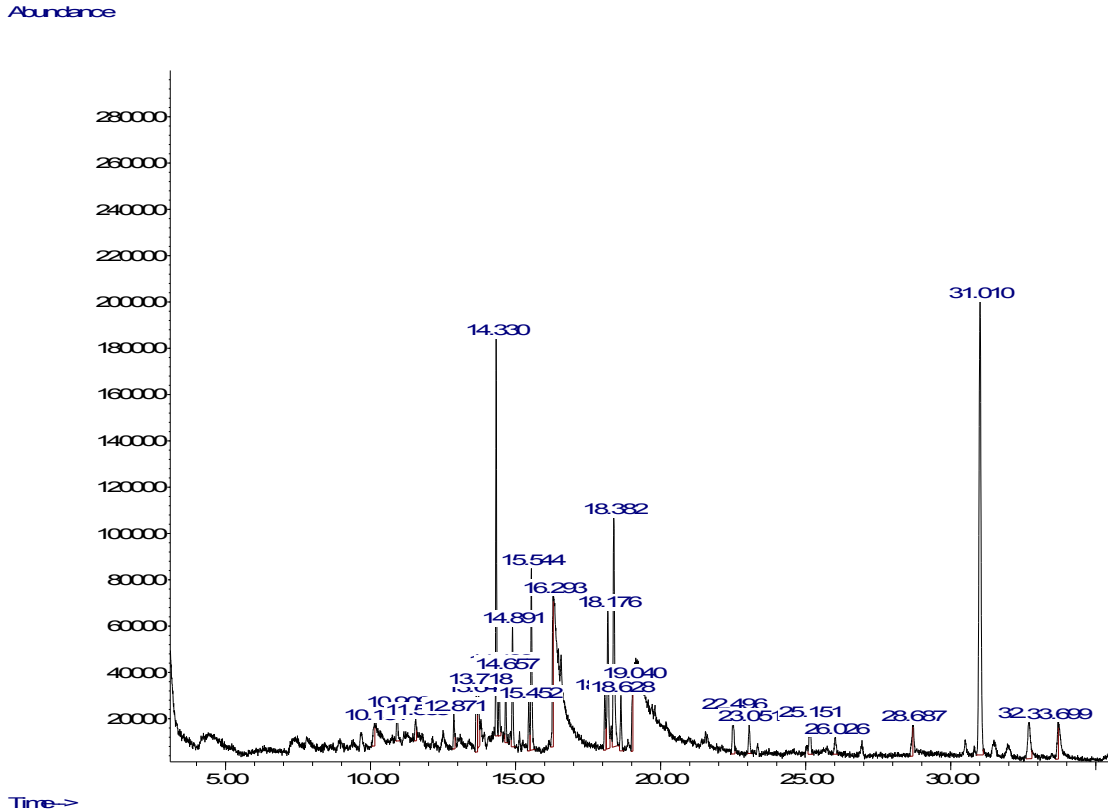


Fig. 1. Gas chromatography-mass spectrometry chromatogram of *Carica papaya* methanolic leaf extract

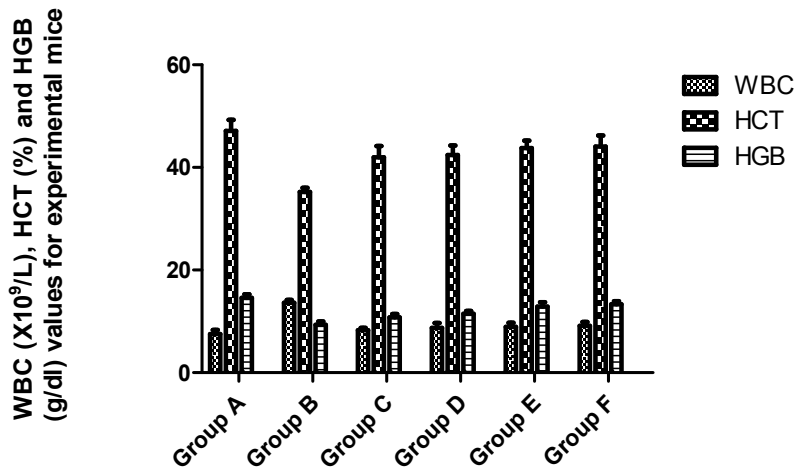


Fig. 2. Effect of methanolic leaf extract of *C.papaya* on WBC, HCT and HGB values in mice infected with *P.berghei* NK 65

**Table 3. The qualitative phytochemical constituents of methanolic leaf extract of *Carica papaya***

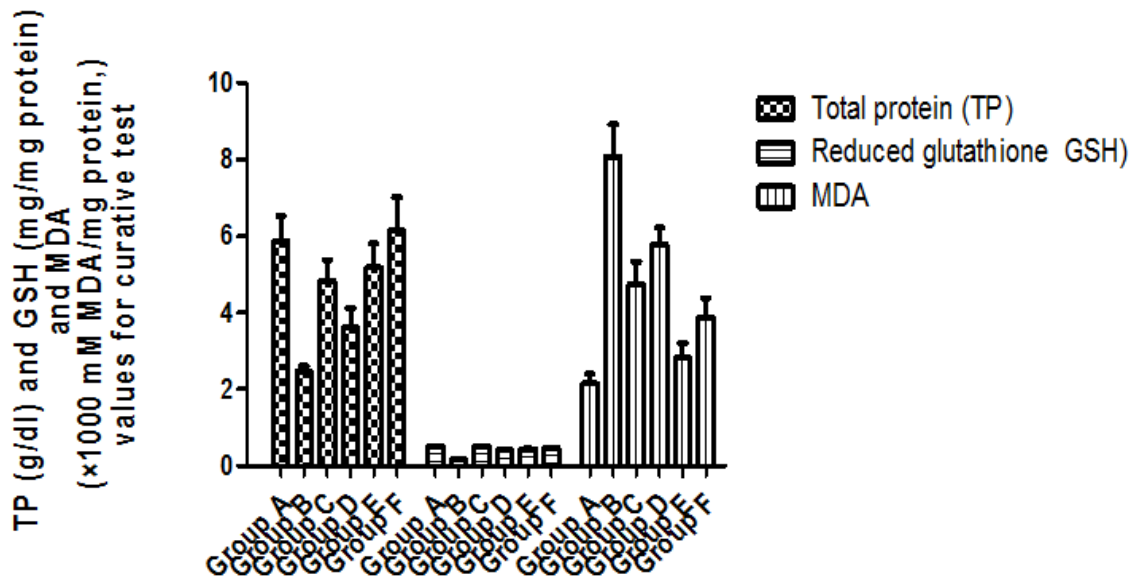
Phytochemical constituent	Inference
Tannins	+
Flavonoids	+
Alkaloids	+
Cardiac glycosides	+
Reducing sugar	+
Anthraquinone	+
Terpenoids	+
Saponins	+
Phenolic compounds	+

+, mean present

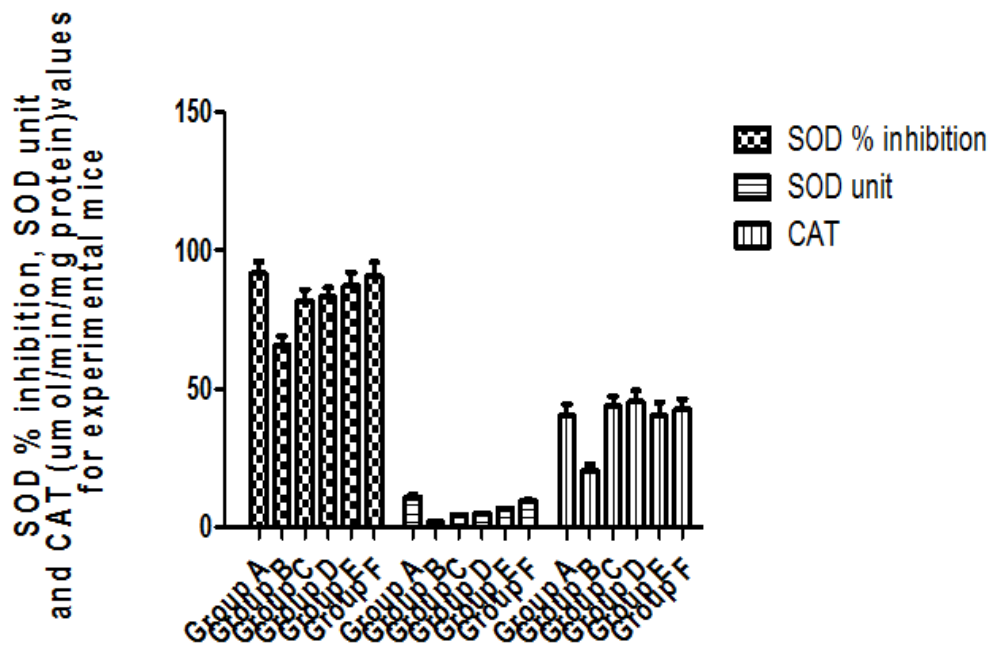
**Table 4. Curative test showing the effect of Chloroquine, Artesunate, and different concentration of methanolic leaf extract of *Carica papaya* against *Plasmodium berghei* NK65 induced Swiss mice**

Group	Dose (mg/kg)	Mean parasitemia density (D10)	% Suppression
A	-	-	NIL
B	-	12,571.63	Nil
C	10	897.52*	92.86*
D	10	1,172.73*	90.67*
E	400	5,436.31*	56.76*
F	600	3,076.82*	75.53*

The values are expressed in mean ± SD for six mice in each group. \*indicate significant difference from negative control at P<0.05



**Fig. 3. Effect of methanolic leaf extract of *C. papaya* on TP, GSH and MDA values in mice infected with *P. berghei* NK 65**



**Fig. 4. Effect of methanolic leaf extract of *C. papaya* on SOD % inhibition, SOD unit and CAT values in mice infected with *P. berghei* NK 65**

The study shows clearly that 400 and 600 mg/kg body weight of *C. papaya* methanolic leaf extracts showed a dose dependent and progressive reduction in parasitemia with time observed during curative test. Other studies showed similar antiplasmodial activities of the plant. Melariri et al. [5] study showed that antiplasmodial activity was observed in the ethyl acetate crude extract of *C. papaya* against *P. falciparum* [5]. Okpe et al. [55] study showed that individual administration of aqueous leaf extract of *C. papaya*, *V. amygdalina*, and the combination of both plants significantly ( $P < 0.05$ ) decreased parasite load in mice induce with *P. berghei* and enhanced their survival. Longdet and Emmanuel Adoga, [49] study shows that the average daily parasitemia of infected mice treated with chloroquine, 400 mg/kg and 200 mg/kg leaf extract of *Carica papaya* respectively significantly ( $P < 0.05$ ) reduced when compared with control group. However, they did not observe any significant ( $p > 0.05$ ) difference in the level of the parasitemia in infected mice treated with 100 mg/kg body weight of *Carica papaya* leaf extract when compared with the control group. Another study showed that methanolic extract of *C. papaya* at 200 mg/kg body weight gave significant suppression ( $p < 0.05$ ) of parasitemia following five days administration in established infection [56]. White blood cell components fight against invading pathogens

and are usually mobilized in large number. In this study, the components of the WBC increased in correspondence to the increased parasite load of the Swiss mice after infection, which decreased after treatment with the chloroquine, artesunate, 400 and 600 mg/kg body weight of methanolic leaf extract of *C. papaya*. The reduction in the HCT and HGB in the untreated group could be due to the destruction of the RBCs by the parasite as it is known to cause anemia but the improvement in the HCT and HGB in the treated groups showed that the methanolic leaf of *C. papaya* has antianemic properties and could improve hemopoietic system. Momoh et al. [14] study shows that methanolic leaf extract of *Morinda lucida* significantly ( $P < 0.05$ ) increase HCT, HGB but WBC count decreased significantly ( $P < 0.05$ ) in Swiss mice induced with *Plasmodium berghei* NK65. Liver oxidative damage in mice infected with *P. berghei* was detected through significant ( $P < 0.05$ ) elevation of malondialdehyde (MDA), decrease in total protein (TP), superoxide dismutase (SOD) % inhibition, SOD unit, reduced glutathione (GSH) and catalase (CAT) in the negative control group mice (group B) when compared to other mice in other group. The extract and the standard drugs significantly ( $P < 0.05$ ) help in the restoration of hepatic TP, SOD % inhibition, SOD unit, GSH and CAT in the treated mice. The levels of hepatic MDA values was significantly ( $P < 0.05$ )

reduced in the treated group mice compared to the untreated group mice. Study has shown that MDA level was significantly ( $P < 0.05$ ) decreased and an increase in the total protein, catalase, SOD % inhibition, SOD unit and Vitamin A levels in the liver homogenate of animals treated with chloroquine, artesunate and extract of *M. lucida* were obtained when compared to the untreated mice [14]. The protective properties of the methanolic leaf extract of *C. papaya* on hepatic tissues through reinstating the normal levels of the oxidative stress markers and impeding the progression of hepatic fibrosis upon *P. berghei* induced hepatic damage indicate that the plant is good for consumption and reduces oxidative stress in the infected Swiss mice.

## 5. CONCLUSION

The study shows that methanolic leaf extract of *Carica papaya* possess antimalarial activity in Swiss mice infected with *Plasmodium berghei* NK 65.

## ETHICAL APPROVAL

The mice were used in accordance with NIH Guide for the care and use of laboratory animals; NIH Publication revised (1985) NIPRD Standard Operation Procedures (SOPs).

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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