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# *In vivo* Antiplasmodial Activity and GC-MS Analysis of *Vernonia colorata* (*Willd*) *Drake* Leaf

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors MHI, AYK and AM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MBB managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** To analyse the phytochemical components using GC-MS technique and evaluate the antiplasmodial activity of crude extract and partitioned fractions of *Vernonia colorata* leaf.
**Study Design:** Qualitative, gravimetric and Chromatographic methods of analysis were adopted for phytochemical analysis, while experimental design (*In vivo*) was used for antiplasmodial study.
**Place and Duration of Study:** Department of chemistry and Biochemistry, Federal University of Technology Minna Niger State, Nigeria, between April 2014 to April 2015.
**Methodology:** The crude extract and partitioned fractions were evaluated for their antiplasmodial activity *in vivo* against *Plasmodium bergei* at 200, 400 and 600 mg/kgbw using standard methods. The combined fractions obtained after column chromatography were analysed using GC-MS analysis. The antiplasmodial activity of *V. colorata* in mice infected with *P. berghei* showed effects

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of graded doses of the extract with dose dependent inhibition of parasitaemia with maximum effect at 600 mg/kg. The oral median lethal dose ($LD_{50}$) in mice was also evaluated and estimated to be greater than 5000 mg/kg. GC-MS analysis was carried out on combined fractions ($VC_{20}$) of *V. colorata* leaf.
**Results:** The analysis showed major chemical constituents such as 9-octadecenoic acid (12.86%), 3, 11-tetradecadien-1-ol (19.90%), 13-docosenoic acid (13.86%) and 4-methyl-1-decene (24.07%). The antiplasmodial evaluation carried out in this research shows a considerable antiplasmodial activity of *V. colorata.*

*Keywords: Vernonia colorata; antiplasmodial activity; Plasmodium bergei; GC-MS.*

## 1. INTRODUCTION

Malaria is an enormous public health problem nationwide killing over one to two million people every year, mostly children in Africa [1], having a great morbidity and mortality than any other infectious diseases [2,3,4].

*Vernonia* species are also being recognized for their medicinal value. *V. amygdalina* is well known as a medicinal plant with several uses attributed to it, such as diabetes, fever reduction, and recently a non-pharmaceutical solution to persistent fever, headache, and joint pain associated with AIDS, with infusion of the plant taken as needed. In North America, the 17 species of *Vernonia* (e.g. *V. altissima, V. fasciculata, V. flaccidifolia*) all have the same effective properties as a blood purifier and uterus toner, containing sesquiterpene lactone, which helps also to prevent atherosclerosis [5,6]. The species of the genus vernonia are characteristics of southern and tropical Africa where they are used from the native for their antimalarial, anti-ascorbic, anti- norexic, anti- helmintic and anti-diabetic activities [7].

*Vernonia colorata* has been worked on for various purposes especially in the treatment of ailments all over the world. *V. colorata* has been reported to have anti-bacterial, body weight dietary and anti-inflammatory activity [8,9,10]. The aqueous extract of *V. colorata* leaf is also used by African traditional medicine practitioners as a remedy for the treatment of diabetes [11].

Although traditional medicine is widely used to treat malaria and is often more available and affordable than orthodox medicine, there are few clinical data on their safety and efficacy. Therefore the identification of bioactive compound in plants, their isolation, phytochemical investigation, biological activity evaluation by various analytical methods is important. Numerous indigenous medicinal plants in Nigeria used in combating malaria are yet to be investigated, despite the rich Nigerian flora diversity. One of such plants is *Vernonia colorata*. Therefore, the present study has been designed to analyse phytochemical components and to evaluate in vivo the antiplasmodial activity of *Vernonia colorata* leaf used for malaria therapy in Nupeland Niger State, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant Material

*Vernonia colorata* was collected in July 2013 at Jipan village, near Kutigi, Lavun Local Government, Niger State, Nigeria.

### 2.2 Preparation of the Crude Extract

The crude extract was obtained from extraction of *Vernonia colorata* leaf using the maceration method at 70% methanol as described by (Mann, 2014).

### 2.3 Phytochemical Screening

The phytochemical analysis were carried out on the crude methanol extract of *Vernonia colorata* leaf to identify the metabolites qualitatively and quantitatively, namely: alkaloids, flavonoids, saponins, steroids, tannins, terpenoids, anthraqunoids, resins and phenols using standard methods [12,13,14].

### 2.4 Evaluation of the Antimalarial Properties of *Vernonia colorata* Leaf

#### 2.4.1 Source of parasites and animals

Parasites (*Plasmodium berghei*) and Swiss strain albino mice of either sex bred and maintained at Animal Facility Centre (AFC) of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and

Development (NIPRD), Abuja, Nigeria were used for the study.

## 2.5 Ethical Clearance

Ethical clearance was given by Federal University of Technology, Minna/Nigeria ethical review board (CUERB) in accordance with international standard on the care and use of experimental animals.

## 2.6 Administration of the Crude Extract

Fifteen albino mice (22-27 g) were divided into 5 groups of 3 animals each were used for antiplasmodial activity of the crude extract. The animals were all inoculated with 0.2 ml of *P. berghei* inoculum and the treatment began orally after 72 hours of inoculation. Group AC received the aqueous preparation of the extract (200 mg/kg body weight daily), Group BC (400 mg/kg body weight daily), Group CC (600 mg/kg body weight daily), Group DC, standard drug (5 mg/kg body weight daily of chloroquine) and Group EC received an appropriate volume of normal saline (where C is the crude extract).

## 2.7 Administration of the Partitioned Fractions

The crude extract was partitioned into fractions of n-hexane, dichloromethane, ethyl acetate and methanol in order to determine the fraction with the best activity can be determined.

About 18 albino mice (Swiss strain) with weight 22-27 kg were used for antiplasmodial activity of the fractionated extract which were all inoculated with *P. berghei* and then grouped into 6 groups; Group AF received the aqueous preparation of the extract (n-hexane of 200 mg/kg body weight daily), Group BP (methanol 200 mg/kg body weight daily), Group CP (dichloromethane 200 mg/kg body weight daily), Group DP (ethyl acetate 200 mg/kg body weight daily), Group EP received the aqueous solution of chloroquine (5 mg/kg body weight daily) while Group FP received an appropriate volume of normal saline (where P is partitioned fractions).

## 2.8 Acute Toxicity Determination

The acute toxicity of the extract was determined using Lorkes method [16] with modifications. Animals were inoculated orally with crude extract of Vernonia colorata and the test was carried out in two phases. The first group containing nine animals was divided into three, of three animals per group. These were administered graded doses (100, 500 and 1000 mg/kg respectively) of the extract. Another nine mice were divided into three groups of three mice per group, which received graded doses (1600, 2900 and 5000 mg/kg) of the extract respectively. The number of deaths in each group within 24 h was recorded and the final LD50 values were calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred).

## 2.9 *In-vivo* Antiplasmodial Studies

The *in-vivo* antiplasmodial evaluation was carried out for both the crude and the partitioned fractions of the plant sample as described by standard methods referred to as Rane test [15] with slight modifications using albino Swiss mice. In summary, thin and thick film smear was done from the blood obtained from the tail of parasitized mice, follow by fixing with absolute methanol, staining with dilute giemsa stain, washed with distil water, and view under microscope at magnification of 100 after dry.

## 2.10 Packed Cell Volume Measurement

Packed cell volume (PCV) was measured to predict the effectiveness of the test extract and fractions in preventing hemolysis resulting from increasing parasitemia associated with malaria. Heparinized capillary tubes were used for collection of blood from tail of each mouse. The capillary tubes were filled with blood up to ¾$^{th}$ of their volume and sealed at the dry end with sealing clay. The tubes were then placed in a micro-hematocrit centrifuge (Gelma Awhksley, England) with the sealed end outwards and centrifuged for 5 min at 11,000 rpm. The tubes were then taken out of the centrifuge and PCV was determined using a standard Micro-Hematocrit Reader.

## 2.11 Weight of the Animals

Weights of animals were also monitored to predict the effectiveness of the test extract and fractions. This was monitored for weight loss effect which may be due to increasing parasitamia associated with malaria. A digitalized weighing balance was used.

### 2.12 Column Chromatographic Separation of the n–Hexane Fraction

The most active fraction obtained from partitioning with n–hexane, ethylacetate, dichloromethane and methanol is the n-hexane fraction which was mounted on the column for further purification. The combined fraction (VC20) obtained after the Thin Layer Chromatographic analysis of the derived fractions from the column chromatography with 95:5 ethyl acetate: methanol, and developed using a ratio of Hex: EtoAc, 7:3 solvent system Combined fractions VC20 was then analysed with GC-MS.

### 2.13 GC-MS Analysis of the Combined Fractions

Gas Chromatograhy-Mass Spectrometry analysis was carried out on a Shamadzu (Kyoto, Japan) GC-MS model QP 2010 at National Research Institute for Chemical Technology, Zaria, according to the EN 14103 standard method [17]. This was done on the combined fractions after column chromatography analysis.

### 2.14 Identification of Phytochemical Components from the Combined Fractions

Interpretation of mass spectrum GC-MS was conducted by comparing the database peaks of National Institute Standard and Technology (NIST) library with those reported in literature [18]. The spectrum of the unknown component was compared with the spectrum of the standards in the NIST library. Component relative percentages were calculated based on GC peak areas without using correction factors. The name, molecular weight and structure of the components of the test materials were ascertained.

## 3. RESULTS

### 3.1 Table 1: Qualitative Analysis of Crude Methanolic Extract

Table 1 shows result of the qualitative analysis of the crude methanolic extract of *Vernonia colorata* leaf. The result revealed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids, resins and phenols. While anthraquinone glycosides and steroids were moderately present.

### 3.2 Quantitative Analysis of Crude Methanol Extract of *Vernonia colorata* Leaf

Table 2 shows result of the quantitative analysis of the crude methanol extract of *V. colorata* leaf. The result revealed the presence of some medically active constituents. Quantitative estimation of the crude chemical constituents in mg/100 g of the plant leaf studied is summarized in Table 2.

**Table 1. Qualitative analysis of the crude methanolic extract**

| Chemical constituents | Test | Results |
|---|---|---|
| Alkaloids | Wagner's reagent | + |
| | Merqui's reagent | + |
| | Hager's reagent | + |
| | Mayer's reagent | + |
| Tannins | Potassium ferricyanide | + |
| Flavonoids | Ammonia test | + |
| Saponins | Frothing test | + |
| Terpenoids | Lieberman-Burchard's test | + |
| Steroids | Salkowskii's test | + |
| | Lieberman-Burchard's test | + |
| Anthraquinone glycosides | Borntrager's test | + |
| Resins | Solubility test: | |
| | In water | - |
| | In acetone | - |
| Phenols | Ferric chloride | + |

*Key: += Present, - =Absent*

**Table 2. Quantitative phytochemical analysis of crudes methanol extract of *V. colorata* leaf (mg/100 g of dry weight)**

| Phytoconstituents | Results |
|---|---|
| Alkaloids | 296±9.17 |
| Flavonoids | 6.09±0.66 |
| Saponins | 113±0.31 |
| Tannins | 1.07±0.39 |

*Values are in means of three replicates ± standard deviations*

### 3.3 Acute Oral Toxicity (Estimation of $LD_{50}$)

The $LD_{50}$ was calculated from the geometric mean of the consecutive doses for which 0-100% survival rates were the acute toxicity study. This indicated that, the crude extract tested did not cause mortality of mice within 24 h up to 5000 mg/kg body weight shows that the extract did not inflict any lethality or toxic symptoms on the Swiss albino mice. The absence of death at doses up to 5000 mg/kg body of the extract show that the $LD_{50}$ of the aqueous leaf extract of *V. colorata* is higher than 5000 mg/kg [16].

### 3.4 Antiplasmodial Activities of *Vernonia colorata* Leaf

#### 3.4.1 Antiplasmodial activity of crude extract

The antiplasmodial activity of the crude methanol extract of *V. colorata* is illustrated in Fig. 1. The result of all the extract doses showed a dependent reduction of parasitaemia at the different doses employed. The crude extract dose-dependently reduced parasitemia by 81.8, 80.9 and 83.2% for 200, 400 and 600 mg/kg body weights groups respectively, compared to the chloroquine control with 100% inhibition. The inhibition obtained from chloroquine was significantly higher than the doses as expected. However, reverse was the case of the control infected not treated group as the parasitamia increased throughout the experiment.

#### 3.4.2 Antiplasmodial activity of partitioned fractions

The antiplasmodial activity of the partitioned fractions of *V. colorata* is illustrated in Fig. 2. The result of all the partitioned doses showed reduction of parasitaemia. The n- hexane fraction showed the highest parasite inhibition of 85.8% followed by 84.6, 84.6, and 84% for dichloromethane, ethyl acetate and methanol respectively, all in the dosage of 200 mg/kg body weight, with chloroquine showing highest percentage parasite inhibition of 100%. However, reverse was the case of the control infected not treated group as the parasitaemia increased throughout the experiment.

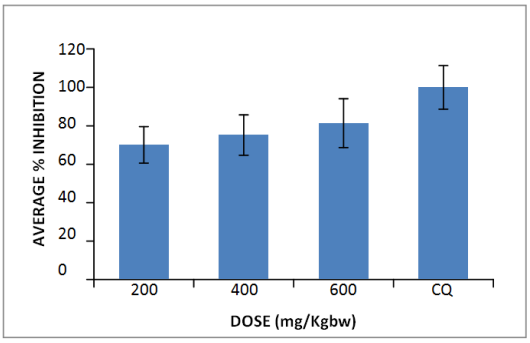


**Fig. 1. Average percentage inhibition of crude methanol extract of *V. colorata* leaf**

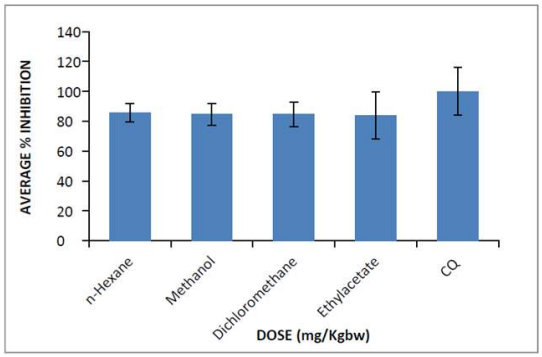


**Fig. 2. Average percentage inhibition of partitioned fractions of *V. colorata* leaf**

#### 3.4.3 Antiplasmodial effect of methanol crude extract of *V. colorata* leaf

Fig. 3 shows the antiplasmodial effect of crude methanol extract of *V. colorata* leaf during the five days treatment and parasitaemia counts on the 14$^{th}$ and 30$^{th}$ day post treatment. Treatment with the crude methanol leaf extract resulted in exponential decrease in parasite count, throughout the study period with chloroquine clearing up the entire parasite. However, the control not treated showed an increased parasite count of which most of the animals died after eight days of inoculation. This treatment showed dependence on dosage in mg/kg body weight with 600 mg/kg body weight which was the highest dose used for this study.

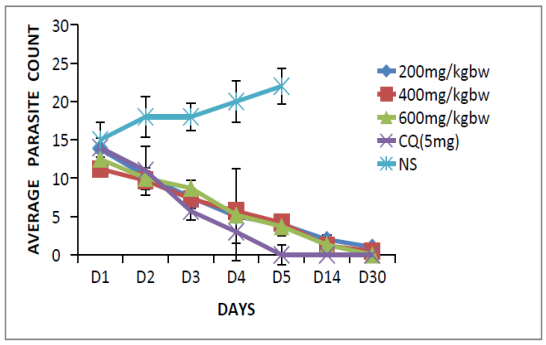


**Fig. 3. Antiplasmodial effect of crude methanol extract of *V. colorata* leaf**

### 3.4.4 Antiplasmodial effect of partitioned fractions of *V. colorata* leaf

Fig. 4 shows the antiplasmodial effect of partitioned fractions of *V. colorata* leaf during the five days treatment and parasitaemia counts on the 14th and 30th day post treatment. Treatment with the partitioned leaf extract resulted in exponential decrease in parasite count, throughout the study period with n-hexane showing the highest curative activity, followed by ethyl acetate, methanol and least was dichloromethane. Chloroquine standard drug gave the highest curative activity by clearing up the parasite completely by the 14th and 30th day after treatment. However, the control not treated showed an increased parasite count of which most of the animals died after eight days of inoculation.

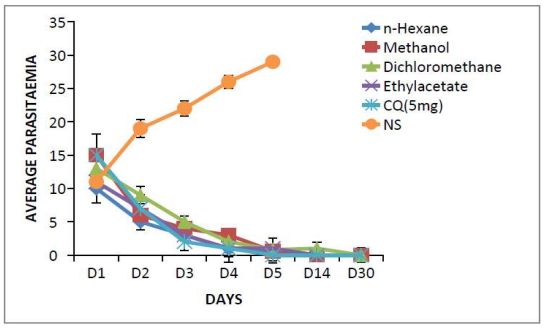


**Fig. 4. Antiplasmodial Effect of partitioned fractions of *V. colorata* leaf**

### 3.4.5 Antiplasmodial effect on packed cell volume (PCV) of animals

Figs. 5 and 6 shows the average percentage PCV of mice before infection, 5 days of treatment, 14 days and 30 days after treatment with both crude methanol and partitioned leaf extract. It can be deduced from the chart for each of the group treated with these extracts that there was a rapid drop in PCV which started to rise again by the 14th and 30th days. Except for the group treated with standard drug, which showed a slight drop which increased rapidly on the 14th and 30th days. However, the negative control which did not survive for long had a drastic fall in PCV in both cases.

### 3.4.6 Antiplasmodial effect on weight of animals

The animals exhibited a loss of weight associated with infection at all dose levels. Body weight reduction caused by inoculation of the parasite was observed. However, the animals immediately started to gain the lost weight upon treatment except for the negative control group which had a consistent weight loss due to accumulated parasitemia. Figs. 7 and 8 shows the antiplasmodial effect on the weight of animals inoculated with the *Plasmodium berghei*.

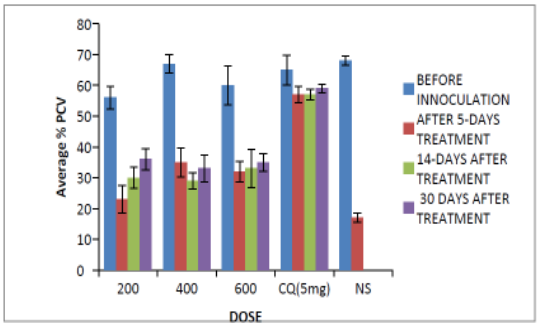


**Fig. 5. Average percentage PCV of animals treated with crude methanol extract of *V. colorata* leaf**

*Result presented in mean ± standard mean error*

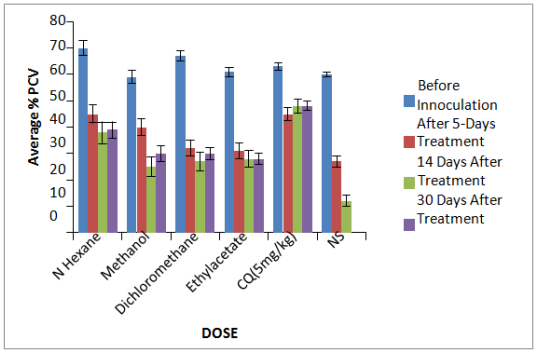


**Fig. 6. Average percentage PCV of animals treated with partitioned fractions of *V. colorata* leaf**

*Result presented in mean ± standard mean error*

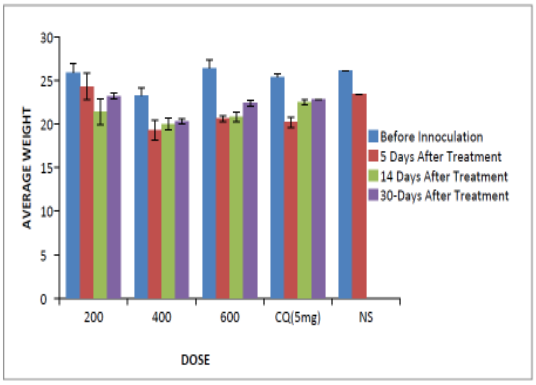


**Fig. 7. Mean weight of animals treated with crude methanol extract of *V. colorata* leaf**
*Result presented in mean ± standard mean error*

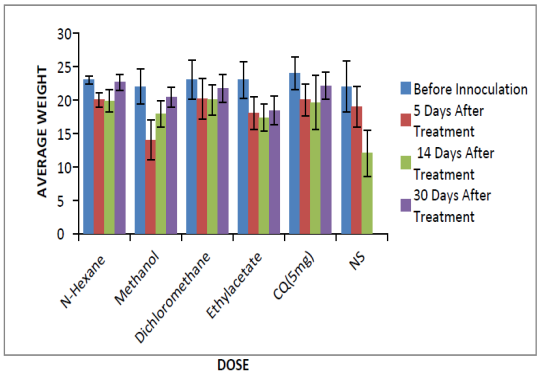


**Fig. 8. Mean weight of animals treated with partitioned fractions of *V. colorata* leaf**
*Result presented in mean ± standard mean error*

### 3.4.7 Antiplasmodial effect on packed cell volume (PCV) of animals

Figs. 9 and 10 shows the mean days of survival of P. berghei infected mice treated crude methanolic and partitioned fractions of *V. colorata* leaf. The animals were observed for 30 days after infection and the number of deaths recorded. These graphs hence show the survival of these animals during the investigation.

### 3.4.8 Chemical compounds deduced from GC-MS spectrum

The GC-MS analysis of the combined fraction (VC20) revealed the presence of eleven (11) compounds; 14-methyl ester (4.70%), butyl octyl ester (1.66%), n- hexadecanoic acid (9.71%), 9-octadecenoic acid (12.86%), n- octadecenoic acid (2.04%), 13-docosenoic acid (13.86%), n-hexadecanoic acid (2.91%), 2, 3-dihydroxypropyl ester (5.57%), 3,11- tetradecadien-1-ol (19.90%), 9-methyl- Z-10-pentadecen-1-ol (2.73%), and 4-methyl-1- decene (24.07%). The chemical constituents with their retention times (RT), molecular formular, molar mass, area (%) and fragmentation peaks are presented on Table 3.

## 4. DISCUSSION OF RESULTS

The qualitative analysis of the crude methanolic extract of V. colorata (Table 1) can be said to be in conformity with result of the analysis [19] for the anti- bacterial activity and phytochemical composition of extracts of three medicinal asteraceae species from Burkina Faso, where V. colorata was reported to contain saponins, tannins, polyphenols, cardenolids, coumarins, sterols and triterpene.

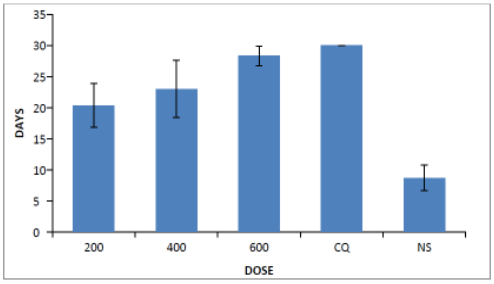


**Fig. 9. Mean days of survival of *P. berghei* infected mice treated with crude methanol extract of *V. colorata* leaf**

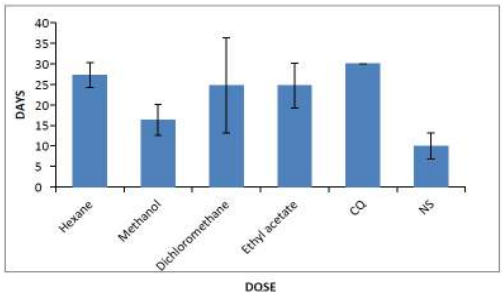


**Fig. 10. Mean days of survival of *P. berghei infected* mice treated with partitioned fractions of *V. colorata* leaf**

Many secondary metabolites of plants' origin have been shown to have antiplasmodial activity [20]. The presence of phytochemicals in *V. colorata* are said to be responsible for its therapeutic effects against these ailments it is used to cure in respective localities. Phytochemicals such as alkaloids (296±9.17 mg/100 g) and flavonoids (113± mg/ 100 g) with high concentrations in the extract maybe associated with its use for treatment of malaria.

**Table 3. Chemical compounds deduced from GC-MS spectrum ($VC_{20}$)**

| Line no. | IUPAC name | Molecular formula | Molar mass | R.T peaks | Area (%) | Fragmentation |
|---|---|---|---|---|---|---|
| 1 | 14-Methyl pentadecanoate | $C_{17}H_{34}O_2$ | 270 | 18.116 | 4.70 | 41,43,57,(74),87,270 |
| 2 | Butyl Octyl ester | $C_{20}H_{30}O_4$ | 334 | 19.345 | 1.66 | 41,57,104,(149),223 |
| 3 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 19.501 | 9.71 | 41,(43),60,73 |
| 4 | 9-Octadecenoic acid | $C_{19}H_{36}O_2$ | 296 | 21.169 | 12.86 | 41,43,(55),69,98,296 |
| 5 | n-Octadecenoic acid | $C_{19}H_{38}O_2$ | 298 | 21.491 | 2.04 | 41,43,57,(74),87,298 |
| 6 | 13-Docosenoic acid | $C_{22}H_{42}O_2$ | 338 | 22.200 | 13.68 | 41,(55),69,83,97,338 |
| 7 | Butyl octyl ester | $C_{16}H_{32}O_2$ | 256 | 22.438 | 2.91 | 41,(43),60,85,129,298 |
| 8 | 3, 11 –Tetra deca dien-1-ol | $C_{14}H_{26}O$ | 210 | 25.555 | 19.90 | 41,(55)67,81,95,210 |
| 9 | 9-Methyl-Z-10-pentadecen-1-ol | $C_{16}H_{32}O$ | 240 | 25.749 | 2.73 | 41,(43),57,83,240 |
| 10 | 4-Methyl-1-decene | $C_{11}H_{22}$ | 154 | 26.246 | 24.07 | 41,43,(57),71,154 |

*Key: () is the base key*

The quantitative analysis of crude methanolic extract of *V. colorata* leaf reveals significant concentrations of flavonoids, alkaloids, tannins, saponins and phenols in *V. colorata*. The present study show a very high concentration of alkaloid (296±9.17 mg/100 g) with which this plant can reasonably be reported to be a source of malaria remedy, this is because alkaloidal compounds are noted for their antimalarial activity.

The acute toxicity study indicated that the extract did not cause mortality of mice within 24 h up to 5000 mg/kg body weight. There was also no visible sign of acute toxicity like hair erection, lacrimation and reduction in their motor and feeding activities. This result thus suggests that the *V. colorata* possess low toxicity as no mortality of the experimental animals were observed at high dose [21].

The antiplasmodial effects observed in this study maybe due to the presence of phytoconstituents such as 9- octadecenoic acid, 13-docosenoic acid, 3, 11-tetradecadien-1- ol, 4-methyl-1-decene, 2-ethylhexyl ester and also dioctyl ester reported in this study maybe associated with the antimalarial property. Though this plant has been used as malarial remedy but its efficacy is yet to be scientifically proved. Therefore, these compounds deduced maybe purified further in the search for novel source of drug from this plant.

The *in vivo* antiplasmodial study of both crude methanolic and partitioned extract showed appreciable curative effect. The antiplasmodial effect observed was ≥ 80% which was close to that of the standard drug (chloroquine, 100%). In standard screening studies, a mean parasitemia level ≤ 90% to that of treated control animals usually indicates that the test compound is active [22]. This may be due to active compounds present.

The high antiplasmodial effect of the n-hexane, ethyl acetate and dichloromethane fractions may be attributed to pure phytocompounds contained in these fractions. Therefore, n- hexane fraction was then considered for further purification using column chromatography and GC-MS analysis.

In this investigation, it was observed that the infected animals did last long after treatment. Animalstreated with n-hexane lasted longer followed by those treated with dichloromethane and ethylacetate, for an average of (27.3) days for n-hexane extract and (24.7) days for both dichloromethane and ethyl acetate.

Though a number of metabolites from plants such as alkaloids, terpenenes, flavonoids, glycosides, steroids etc., are reported to be responsible for the antiplasmodial activity of many medicinal plants [23]. It is therefore probable that some metabolites present in *V. colorata* are responsible for its antiplasmodial activity possibly by elevating red blood cell oxidation and inhibiting the parasite's protein synthesis as earlier reported by [24,25].

In the present research work, a total of 11 chemical constituents have been identified from the hexane fraction of V. colorata leaf by Gas Chromatography Mass Spectrometry (GC- MS) analysis. The results of this study offers a base for which further work on can be pursued in search of *V. colorata* is used as an alternative herb for synthesis of anti plasmodial agent [26].

## 5. CONCLUSION

The antiplasmodial activities of the methanolic extract of *V. colorata* conform to its use as traditional medicine in Northern Nigeria for the treatment of malaria. This could be related to the presence of certain bioactive agents with antiplasmodial potential. The results obtained in this study showed the effect of *V. colorata* on swiss albino mice in crude and partitioned portions of which the extract was nontoxic to the animals even on a high dose level. The drug also succeeded in suppressing the parasitaemia level ≥ 80% in the infected animals after it was administered compared to standard chloroquine at 100%. The animals also survived for some good number of days. *V. colorata* can be said to be dosage dependent as its efficacy increased with increase in dose level administered.

The GC-MS analysis of the purified fractions successfully identified the respective compounds suspected to be present. The presence of various bioactive compounds hence justifies the use of *V. colorata* as anti- malarial drug by the Nupe people of Niger State.

## 6. RECOMMENDATION

Large numbers of animals per group with appropriate statistical analysis are recommended for further research on this work. Furthermore, human malarial parasite such as *P. falciparum* should be used to reaffirm the efficacy of the antimalarial properties of the plant extract.

## CONSENT

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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