The Effect of the Aqueous Extract of Bush Cane (*Costus Afer)* Stem on *Plasmodium Berghei Berghei* Infected Albino Mice

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***Abstract*: The anti-plasmodial activity of bush cane (*Costus afer)* stem on albino mice infected with *Plasmodium berghei berghei* was evaluated. Bush cane collected from the wild were defoliated, washed and the stem macerated. The aqueous extract were test for various phytochemicals. Twenty-four albino mice were used for this experiment, and were divided into six groups of four mice per group. Group 1, 2 and 3 were normal, negative and positive controls respectively, while Group 4, 5 and 6 were treated with 100, 200 and 500mg/kg of bush cane extract based on body weights. Results showed that the groups treated with 100 and 200mg/kg had the same chemo suppressive effect of 89.1% while the group treated with 500mg/kg had a chemo suppressive effect of 96.4%. Also, the acute toxicity (LD_{50}) of the extract showed no mortality at the experimental groups. The hematological evaluation of the experimental mice showed that the extract had positive influence on the Erythrocytes (RBC), Leukocytes (WBC), Packed Cell Volume (PCV), Hemoglobin Concentration (HB), and Neutrophils (NEU) and Lymphocytes (LMYP). Data analysis showed that the chemo suppressive effect of *Costus afer* when compared with the chloroquine, at 500mg/kg was not statistically significant but was significant at 100 and 200mg/kg levels. This showed that bush cane has antiplasmodial property and should be recommended for treatment of malaria.**

***Keywords*: *Costus afar, Plasmodium berghei berghei,* Malaria, Albino mice, Bush cane stem.**

I. INTRODUCTION

Malaria is caused by plasmodium parasites. The parasites are spread to people through the bites of infected female Anopheles mosquitoes, called "malaria vectors" [1]. Malaria is a disease of global importance that results in 300 – 600 million cases annually and an estimated 2.2 billion people are at risk of infection [2]. In Nigeria, it is estimated that 300,000 deaths occur each year and 60% of outpatient visit hospitals and 30% hospitalizations are attributed to malaria [3]. Resistance to the commonly used anti-malaria are very high and toxicity to alternative. Indoor house spraying to prevent and control the vector has not totally solved the problem and the female anopheles mosquitoes have developed resistance. And several malaria programs have been hampered by financial and operational problems. Natural products, mainly plants are fundamental source of numerous drugs and therapeutics, and the most important drugs used in malaria treatment have been derived from the plants [4]. This has led many researchers to source for new antimalarial drugs from different sources, including higher plants. And *Costus afer* has been reported to have some antimalarial properties. The plant kingdom has proven to be the most successful in the treatment of nearly all ailments and they also provide an important source of raw materials for the world's pharmaceutical [5]. Bush cane (Costus afer) is a herb that belongs to the family Costaceae. It is a tall, perennial, herbaceous and un-branched tropical plant with a creeping rhizome and it is commonly called bush cane or monkey sugar cane. It was also reported that in Africa C. afer is found in the forest belt from Senegal, South Africa, Guinea, Niger and Nigeria [6]. In Nigeria bush cane is known by various local names such as Ukhuere-oha in Benin, Okpete or Okpoto among the Ikwerre and Igboid speaking tribes of the South-south and the South-eastern region of Nigeria. Parts of this plants (leaves, stem and rhizome) harvested from the wild plant are commonly used as medical herbs in the treatment of various ailments [6-7].

Aim/Objectives of the Study

The aim of this study is to investigate the anti-plasmodial effect of *Costus afer* on albino mice that is infected with *Plasmodium berghei berghei*. The objectives of this study include:

1. To determine the phytochemical constituents of the aqueous stem extract of *Costus afer.*
2. To investigate the anti-malarial effect of aqueous stem extract of *Costus afer* on *Plasmodium berghei berghei* infected albino mice.
3. To determine the efficacy of the extract at various dosages.
4. To confirm the acute toxicity of the aqueous extract of *Costus afer.*
5. To determine the comparative degree of *Costus afer* efficiency/potency to the parasite as compared to chloroquine.
6. To determine the effect of the aqueous extract on the hematological parameters of the Albino mice.

II. MATERIALS AND METHODS

2.1Collection and Identification of Plant Materials

The stems of bush cane (*Costu safer)*were collected from a field at Aluu at latitude of 04^0 53' 14"N and longitude of 06^0 55' 08"E, in Ikwerre Local Government Area of Rivers State and the plant material was identified and authenticated in the herbarium unit of the department of Plant Science and Biotechnology, University of Port Harcourt.

2.2 Preparation of Crude Extracts

The stems of *C. afer* were washed and peeled to remove the axil thereafter; they were cut into bits and macerated using a porceline. The set up was allowed to extract in the aqueous medium for 11hours and the resultant filtrate was evaporated to dryness using a rotary evaporator. The extract was packaged in a sterile airtight plastic container and stored at room temperature of 25^0C until when required for use.

2.3 Acute Toxicity Study

The LD_{50} of the plant extract was tested to determine the safety of the agent using [8]. The study was carried out in two phases. In the first phase, nine mice were randomized into three groups of three per cage and where administered with crude extract of *Costus afer* orally with varied doses of 10mg/kg, 100mg/kg and 1000 mg/kg of the leaf orally. The mice were deserved for signs of toxicity which include paw licking, salivation, stretching of the entire body, weakness, sleep, respiratory distress, coma and death in the first four hours and subsequently for 4 days. In the second phase, another set of nine mice were also randomized into the three groups of three mice per cage and were administered 1600mg/kg,2900mg/kg and 5000mg/kg of the extract orally, based on the result of the first phase. The animals were observed for signs of toxicity and mortality for the four hours and thereafter for 4 days. The oral LD_{50} was calculated as the geometric mean of the highest non-lethal dose and the lowest lethal dose.

2.4 Phytochemical analysis of the plant extract

Phytochemical analysis of the aqueous stem extract of *Costus afer* was carried out using the procedure described by [9]. The phytochemical analysis examined the presence of the following chemical parameters in the plant extract: tannins, saponins, flavonoids, phenols, and alkaloids. About 0.5 -2g of aqueous extract of *Costus afer* stem was boiled and mixed with several reagents depending on the chemical parameter to be investigated using methods described by [10].

2.5 Experimental Animals

Swiss albino mice (18-22g) of both sexes obtained from the physiology laboratory of University of Port Harcourt, Rivers State. The animals were housed in plastic cages, covered with aluminum gauze at room temperature and moisture, under naturally illuminated environment of 12:12h dark/light cycle. They were fed on standard diet and given water at liberty. The mice were used in accordance with NIH guide for the care and use of laboratory animals.

2.6 Inoculation of Parasites

The method of [11] was used for the inoculation of parasites into experimental animal. The inoculums consisted of *plasmodium berghei berghei* parasitized erythrocytes. Each mice was inoculated on day 0, mintraperitoneally with 0.2ml of infected blood containing approximately 1 x 10^7 *Plasmodium berghei berghei* parasitized red blood cells. In addition, the newly inoculated animals were monitored daily to determine expression of parasite in circulation.

2.7 Experimental Design

A total of twenty-four (24) mice were used for this experiment. The mice were divided into six (6) groups of four (4) mice each, group 1 is the Normal control where the mice was not inoculated; group 2 is the untreated group where the mice was induced with *Plasmodium berghei berghei* but not treated*;* Group3 (P.C) was inoculated with *Plasmodium berghei berghei* and treated with 10mg/kg of the chloroquine drug*;* Group 4 (D1) was also inoculated with *Plasmodium berghei berghi* and treated with 100mg/kg extract of *C. afer;* Group 5 (D3) was equally inoculated with *Plasmodium berghei berghei* and treated with 500mg/kg extract of *C. afer;* while Group 6 (D3) was also inoculated with *Plasmodium berghei berghei* and treated with 500mg/kg extract of *C. afer*.

Table 1: A table showing the experimental design

S/N	Group	Administration	Inoculation
1.	NC	Healthy mice/no treatment	No
2.	Untreated	Induced mice/no treatment	Yes
3.	PC	10mg/kg of chloroquine	Yes
4.	D1	100mg/kg of *C. afer* extract	Yes
5.	D2	200mg/kg of *C. afer* extract	Yes
6.	D3	500mg/kg of *C. afer* extract	Yes

2.8 Determination of Suppressive Test

A 5 – day suppressive test was performed using the methods of David *et al*., 2004. Twenty-four albino mice of both sexes weighting (18-22g) were inoculated by intraperitoneal infection with standard inoculations of *p. berghei* containing 1 x 10^7 infected erythrocytes. The mice were randomly divided in 6 groups of 4 per cage and treated for 5 consecutive days with 100mg/kg ,200mg/kg and 500mg/kg of the extract, chloroquine and 0.2ml normal saline, all administered orally. On the sixth day, blood was collected from the tail of each mouse and thin films were made on slides. The films were fixed with methanol, stained with Giemsa and parasitemia density examined by microscopically counting the parasitized red blood cells in at least 1000 red blood cells in 10 different fields.

Expressed as % suppressive

$$= \frac{\%\ \text{parasitemia of untreated} - \text{Groups}}{\%\ \text{of untreated}} \text{ x } 100$$

2.9 Determination of Curative Test

Evaluation of curative potential of *C. afer* stem extract, was done adopting the method described by [12] with slight modification. Twenty-four mice were selected and intraperitoneally infected with 1 x 10^7*P. berghei berghei* infected erythrocytes on the first day. Ninety six hours after, the mice were grouped into 6 groups of 4 per cage and treated with 100mg/kg ,200mg/kg and 500mg/kg of the extract, chloroquine 10mg/kg and 0.2ml normal saline, all administered orally. Treatment continued daily until the 5^{th} day when thin films were prepared with blood collected from the tail of each mouse. The films were fixed with methanol, stained with Giemsa and parasitized red blood cells on at least 1000 red blood cells in 10 different fields.

This is expressed as

$$\text{PP} = \frac{\text{total number of PRBC}}{\text{total number of RBC}} \text{ x } 100$$

Where;

PP = Percentage parasitemia

PRBC = Parasitemia red blood cells

2.10 Collection of Samples and Determination of Hematological Parameters

The hematological parameters viz, WBC, RBC, HB content, differential counts etc. were determined by the standard method. On the 5^{th} day the animals were anesthetized by the use of chloroform**,** blood was collected through cardiac puncture into an anti-coagulant tubes shaken and taken for hematological parameter assessment.

2.11 Statistical Analysis

Results were analyzed statistically using SPSS version 20. Group means from the experiment were compared using one-way ANOVA (analysis of variance) followed by Duncan's test as a single post-hoc test. All results areexpressed as mean + s.e.m. (Standard error of the means).P< 0.05 is taken as statistically significant.

III. RESULTS

3.1 Suppressive Activities of Aqueous Extract of Bush Cane (Costus afer)

The group administered with 500mg/kg dose of *C. afer* showed a percentage suppression of 92.7% which is closely related to the percentage suppression of chloroquine which is 96.4%. This means that the 500mg/kg dose of extract was able to suppress more than the dosage of 100mg/kg and 200mg/kg which gave the same value of suppressive inhibition of 89.9%, this equally means that an increase in the dose of the extract within 100mg/kg and 200mg/kg will not show any significant difference on the level of the parasitemia. This is shown on table 2.

Table 2. Percentage Suppression Activity of *C. afer*

S/N	Group	% Parasitemia	% Suppression
1.	Normal	0.00 ± 0.00	100.00%
2.	Untreated	27.75 ± 1.11	0.00%
3.	Chloroquine	1.00 ± 0.41	96.4%
4.	100mg/kg	3.00 ± 0.92	89.1%
5.	200mg/kg	3.00 ± 0.81	89.1%
6.	500mg/kg	2.00 ± 0.41	92.7%

Data are represented as meant ±SEM. Obtained from a one way ANOVA Test using SPSS version 21.

3.2 Curative Activity of Bush Cane (Costus afer)

The result of the effect of the treatment with different concentrations of plant extracts on parasitemia density in mice is presented in Table 4.2. The parasitemia density for the untreated group progressively increased for the five days period, showing the mean number of the percentage parasitized cells as 14.25 ± 1.97 on the first day of post inoculation and 27.75 ± 1.11 by the fifth day. Treatment with the doses of plant extract (100mg/kg and 200mg/kg) showed a significant parasitemia reduction ($P < 0.05$) when compared to the untreated group. The highest parasitemia reduction was seen in chloroquine followed by 500mg/kg aqueous extract of *C. afer* stem on the fifth day. The 10mg/kg dose of chloroquine showed a significant reduction of parasitemia from 6.75 ± 0.85 on the first day to 1.00 ± 0.41 on the fifth day. The treated groups given 100mg/kg and 200mg/kg of *C. afer* extract of decreased progressively showing a similar mean number of parasitized red cells as 8.00± 1.58 and 8.50 ± 0.50 on the first day, and 3.00± 0.92 and 3.00 ± 0.81 on the fifth day. And 27.85 ± 1.55 on the first day post inoculation and 2.00 ± 0.41 on the fifth day for the 500mg/kg extract of *C. afer* stem. This simply means that the extract doses and chloroquine do not possess any curative effect but possess only suppressive effect.

Table 3. Curative Activity of Aqueous Extract of Bush Cane (*Costus afer)*Stem on *Plasmodium berghei berghei*

S/N	Group	Day 1	Day 3	Day 5
1.	Normal	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
2.	Untreated	14.25 ± 1.97	21.78 ± 0.75	27.75 ± 1.11
3.	Chloroquine	6.75 ± 0.85	2.50 ± 0.50	1.00 ± 0.41
4.	100mg/kg	8.00 ± 1.58	3.50 ± 0.65	3.00 ± 0.92
5.	200mg/kg	8.50 ± 0.50	4.25 ± 1.03	3.00 ± 0.81
6.	500mg/kg	27.75 ± 1.55	7.25 ± 1.31	2.00 ± 0.41

Data are represented as meant ±SEM. Obtained from a one way ANOVA test using SPSS version 21.

3.3 Phytochemical composition of aqueous extract of Bush Cane(Costus afer)

Results of the preliminary phytochemical test carried out on the aqueous extract of *Costus afer* showed the presence of

Alkaloids, Flavonoids, Saponins, Tannins and Phenols (Table 4). From the analysis, the aqueous extract of *C. afer* showed high content of flavonoids and Alkaloids, it showed the quantity of flavonoids and Alkaloids as 29 ± 0.18 and 4.6 ± 0.13. While Saponins, Tannins and Phenols had values of 2.6 ± 0.18, 2.54 ± 0.18 and 0.18 ± 0.03 respectively.

Table 4: Phytochemical composition of aqueous extract of Bush Cane (*Costus afer)*

Phytochemicals	Aqueous extract
Alkaloids	4.6 ± 0.13
Flavonoids	29 ± 0.18
Saponins	2.6 ± 0.18
Tannins	2.54 ± 0.18
Phenols	0.18 ± 0.03

Data are represented as meant ± SEM. Obtained from a one way ANOVA test using SPSS version 21.

3.4 Effect of Aqueous Extracts of Bush Cane (Costus afer) Stem on Some Hematological Parameters

From the table below, it can be seen that the extract had a significant effect at 0.05 significant level on the RBC, WBC, PCV, HB, NEU and LYMP.

RBC (Red Blood Cell): The normal RBC count is 5.13 ± 0.15 which is similar to the group administered with chloroquine having the mean value of 5.53 ± 0.15. In the untreated group, the RBC count reduced to 3.36 ± 0.18, this is due to the presence of parasites. When administered with 100mg/kg and 200mg/kg extract of *C. afer* stem RBC increased to 4.47 ± 0.09 and 4.93 ± 0.12 respectively while the group administered with 500mg/kg extract increased to 5.90 ± 0.11 which is similar to the Normal RBC count of 5.13 ± 0.14. This simply means that the plant extract is effective in reducing the parasite thereby helping to increase RBC.

WBC (White Blood Cell): The normal WBC is 6.72 ± 0.20, the untreated group was higher with a mean value of 11.23 ± 0.58, and this is due to the presence of parasite infection. When administered with 100mg/kg and 200mg/kg extract of *C. afer* stem, WBC was reduced progressively with mean values of 8.93 ± 0.15 and 5.47 ± 0.09 respectively. When administered with chloroquine and 500mg/kg extract the WBC count increased slightly to 6.10 ± 0.12 and 6.47 ± 0.12 respectively. This shows that the extract dose of 200mg/kg had more effect on the level of parasitemia than the other doses.

PCV (Packed Cell Volume): Thenormal control for PCV is 44.67 ± 1.45, the untreated group went as low as 28.67 ± 2.33, this is because there was no under production of the cells due to the presence of parasite infection. When administered with 100mg/kg, 200mg/kg and 500mg/kg extract of *C. afer* stem the PVC count increased progressively with mean values of 42.33 ± 0.88, 45.33 ± 1.21 and 49.00 ± 1.53 respectively. That of chloroquine had the highest value of PVC count which is 50.00 ± 1.15. This shows that the extract was able to suppress the parasitemia load and increase the PCV count back to normal.

HB (Hemoglobin Concentration): The normal level of HB is 14.57 ± 0.30, in the untreated group it's reduced to 9.83 ± 0.37. The group treated with chloroquine had a normal mean value of 15.13 ± 0.12, while the group administered with 100mg/kg, 200mg/kg and 500mg/kg extract of *C. afer* stem increased progressively having values of 14.03 ± 0.09, 14.57 ± 0.09 and 15.13 ± 0.12 respectively. This shows that after the infection treatment with the extract boost the level of hemoglobin concentration.

NEU (Neutrophils): This is another special type of WBC, during infection their number reduces. The normal level of NEU is 73.67 ± 1.20, the untreated group reduced, having a mean value of 65.00 + 1.53, when treated with chloroquine their mean value increased to 74.00 ± 1.15. When administered with 100mg/kg, 200mg/kg and 500mg/kg extract of *C. afer* the level of NEU increased progressively from 69.67 ± 0.88, 71.67 ± 0.89 to 75.00 ± 1.15. This shows that the treatment was able to fight against the parasite as it brought the level of NEU to normal.

LYMP (Lymphocytes): The normal level of LYMP is 19.67 ± 0.33. In the untreated group it had a high value of 27.67 ± 1.20 due to the presence of parasite. When administered 100mg/kg and 200mg/kg the LYMP level increased to 21.33 ± 0.89 and 21.00 ± 1.15 respectively while that of chloroquine and 500mg/kg of reduced the level of LYMP to 18.67 ± 0.88 and 18.00 ± 1.15 respectively. This shows that there was significant progress in the treating of the parasite with the extract and chloroqiune.

Table 5: Effect of Aqueous Extracts of *Costus afer* stem on Some Hematological Parameters

S/N	Group	RBC	WBC	PCV	HB	NEU	LYMP
A	Normal	5.13 ± 0.15	6.27 ± 0.20	44.67 ± 1.45	14.57 ± 0.30	73.67 ± 1.20	19.67 ± 0.33
B	Untreated	3.36 ± 0.18	11.23±0.58	28.67 ±2.33	9.83 ± 0.37	65.00 ± 1.53	27.67 ± 1.20
C	Chloroquine	5.53 ± 0.15	6.10 ± 0.12	50.00 ± 1.15	15.13 ± 0.12	74.00 ± 1.15	18.67 ± 0.88
D	100mg/kg	4.47 ± 0.09	8.93 ± 0.15	42.33 ± 0.88	14.03 ± 0.09	69.67 ± 0.89	21.33 ± 0.89
E	200mg/kg	4.93 ± 0.12	5.47±0.09	45.33 ± 1.21	14.57 ± 0.09	71.67 ± 089	21.00 ± 1.15
F	500mg/kg	5.90 ± 0.11	6.47±0.12	49.00 ± 1.53	15.13 ± 0.12	75.00 ± 1.15	18.00 ± 1.15

Data are represented as meant ±SEM. Obtained from a one way ANOVA test using SPSS version 21

IV. DISCUSSION

Malarial, a life threatening disease is known to deplete the levels of red blood cell and eventually cause anemia and loss of life in most cases [13].

The development and spread of drug resistant strains of *Plasmodiumfalciparum* have limited the effectiveness of the currently used malaria drugs and this creates the need for new anti-malarial drugs, plant have always been considered to be alternative and rich sources of new drugs and most of the antimalarial drugs on use today such as quinine and artemisinin were either obtained directly from plants or developed using chemical structures of plants derived compounds as templates.

Costus afer has been shown by this study to contain phytochemical constituents which can act as secondary metabolites with certain degree of therapeutic effects, which has been shown by other previous researches to be responsible for the healing effects of most plants parts [14].

The results of this study showed that the stem extract produced significant suppressive effect against early infection at safe doses. The aqueous extract of *C. afer* exhibited a LD_{50} above 5000mg/kg having shown no mortality at all the doses tested. Based on Lorke's recommendation [8], the extract is assumed to be safe. Invariably, the experimental doses used were relatively safe. The aqueous extract of *C. afer* demonstrated significant antiplasmodial activity in mice infected with *Plasmodium berghei berghei* that was comparable to that of chloroquine. At the dose of 500mg/kg, aqueous extract of *C. afer* produced chemo suppression of 92.7% which was close to that of chloroquine (96.4%) at 10mg/kg and this shows that the plants antimalarial property can be deduced as being moderate.

This finding is in accordance with the finding of [15], which states that in *vivo* antimalarial activity of plant extracts can be categorized as moderate, good and very good of the extract showed 50% or more chemo suppression at 500mg/kg, 250mg/kg and 100mg/kg/day extract dose respectively.

Hematological indices were considered in this study because the most pronounced changes related to malaria involve the blood and blood forming system. Anemia is a fairly common problem encountered in malaria [16]. This is evidenced by the decrease in RBC, WBC, PCV, HBand NEU on all the infected animal groups. The marked decrease of the parameters (Table 5) on the untreated group was consistent with the results reported by Cyril *et al.*, [17]. The hemolysis which is the destruction of red blood cells of parasitized mice may be due to high parasitemia in the red blood cell that causes changes of red cell antigen structure brought about by the parasitic invasion which stimulates the mice production of antibodies against the red cell. This triggers immune mediated red cell lyses. In addition, the growing parasites consumes and degrades the intracellular proteins which are mainly hemoglobin [17]. This may account for further reduction of hemoglobin. This reductions were considerably reversed in the infected mice treated with 500mg/kg and 200mg/kg of extract but chloroquine treated group showed little increase. This suggests that the extract may enhance the production of red blood cells. This might have contributed to the increase in PCV and HB observed on extract treated groups (Table 5).

An increase in the WBC of the group treated with 100mg/kg and 500mg/kg extract suggests a boost in the immune system by the extract as seen in the findings of [18].

Packed Cell Volume (PCV) which is a measure of the relative mass of cells present in the blood was observed to increases in all groups that where treated groups which signify the possible presence of anemia state which was also stated by [19].

The neutrophils levels us infected mice were found to be lower than that of the normal control (Table 5) which is in line with the report of [20] and the groups treated with the extract showed a significant increase in the level of neutrophils. The alterations in counts of lymphocytes were also observed in this study just as reported by [21].

V. CONCLUSION

Based on the findings, the result of the present study have shown that the aqueous stem extract of *Costus afer* possesses anti-malaria activity as seen in the ability to suppress *Plasmodium berghei berghei* infection in mice. The oral administration of the plant extract indicates to a far reaching end that C. *afer* stem extract would be a promising natural anti-malaria product devoid of side effects upon use as it also influences hematopoietic stem cells to produce RBC, especially when administered within the dose range of 100-500mg/kg body weight investigated in this study.

5.1 Recommendations

Based on the findings gotten form this research, the following are recommended:

1. Further studies should also be carried out on the leaf and root of the plant to identify its possible anti-plasmodial effect.
2. The plants should be used together with other plants, which have anti-malarial properties to see how the combination can increase the anti-malaria property of the plants.
3. Further work should be carried out on the plant to check the usefulness of the plant in preventive malarial therapy.

REFERENCES

[1]. WHO (2018). World Health Organization, Malaria https://www.who.int/new. Retrieved 12th July, 2019.
[2]. Singh, B., Kim, S.L., Matusop, A., Radhakneoshan, A., Shamsul, S.S., Cox-Singh, J., Thomas, A. & Conay, D.J. (2004). A large focus of naturally acquired plasmodium knowlesi infections in human beings. Lancet, 363: 1017-1024.

[3]. FMOH (2009). Strategic plans 2009-2013. A road map for malaria control in Nigeria, Nigeria and national malaria control programme, Abuja, Nigeria.
[4]. Ukpabi, A.K., Eze, R.H. & Ugwu, E.C. (2012). Chemical component of Costus afer plant. International Journal of Pharmatech Research, 4(2): 7-10.
[5]. Ezejiofor, A.N., Igweze, Z., & Amadi, C.N. (2017). Evaluation of some biological properties of Ethianolve leave extract of Costus afer (Ker gawl). Journal of Pharmacy and Biological Science.
[6]. Omokhua, G.E. (2011). Medicinal and socio-cultural importance of Costus afer (Ker Gauwl) in Nigeria. International Multidisciplinary Journal, Ethiopia 5(5): 282-287.
[7]. Anyasor, G.N., Ogunwenmo, K.O., Olatunji, A.D., Blessing, E.A. (2010). phytochemical, proximate and mineral element composition of stem of Costus afer (bush cane). Asian Journal of Plant Science and Research 2(5): 607-612.
[8]. Lorke, D. (1983). A new approach for acute toxicity testing. Archives of Toxicology, 54: 275-289.
[9]. Gboeloh, L.B., Okon, O.E. & Udoh, S.E. (2014). Antiplasmodial effect of authocleista vogelii on Albino Mice experimentally infected with Plasmodium berghei berghei (NK, 65). Journal of Parasitology Corporation, Pp. 1-2.
[10]. Sofoworo, E.A. (1993). Medicinal plants and traditional medicine in Africa. Ibadan: Spectrum Books Ltd.
[11]. Kapp, S.H. (2001). Explaining the transcription of the malaria sporozrite stage. Proc. Nat CAcad, Sc. USA, 98, 9895-9900.
[12]. Ryley, J.F. and Peters, W. (1970). The antimalarial activity of some quinolone esters. American Tropical Medical Parasitology, 84:209-222.
[13]. WHO (2015). Fact Sheet on World Malaria Report. Geneva: World Health Organization. https://www.who.int/malaria/media/world-malaria-report-2015/en.
[14]. Friedman, M. (2014). "Chemistry and multibeneficial bio-activities of carvacrol (4-isophyl-2-methylphenol), a component of essential wills produced by aromatic plants and spices". *Journal of Agricultural andFood Chemistry*, 62(31): 7652-7670.
[15]. Deharo, E., Bourdy, G., Quenevo, C., Munoz, V., Ruiz, G. & Sauvain, M. (2001).A Search for Natural Bioactive Compounds in Bolivia through Multidrsciplinary Approach. Part V. Evaluation of the Antimalarial Activity of Plants used by the Tacana Indians,Journal of Ethnopharmacology, 77;918.
[16]. Boareto, A.C., Muller, J.C., Bufalo, A.C., Botelho, G.G.K., De Araujo, S.L., Foglio, M.A., Morais, R.N. & Delsenter, P.R. (2008). Toxicity of artemisinin (Artemisia annua, L) in two different periods of pregnancy in Wister rat. Reproductive Toxicology, 25: 239-246.
[17]. Wilcox, M.L. & Bodeker, G. (2004). Traditional herbal medicines for malaria. British Medicinal Journal, 329: 1156-1159).
[18]. Florens, L. Washburn, M.P., Raine, J.D., Anthony, R.M., Grainger, M., Haynes, J.D., Moch, J.K., Muster, N., Sacci, J.B., Tabb, D.L., Witney, A.A., Wotters, D. & Gardner, M.J. (2002). A proteomic view of the Plasmodium falciparum lifecycle. Nature, 419: 520-526.
[19]. Akbar S. (2011). Andrographis paniculata: A review of pharmacological activities and clinical effects. Alternative Medicine Reviews 16, 66-77.
[20]. Ekvall H (2003). Malaria & Anemia. Current Opinion Hematology, 10: 108–114.
[21]. Adebayo, J.O. & Krettli, A.U. (2010). Potential antimalarials from Nigerian plants: A review. Journal of Ethnopharmacology, 133:289-302