ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 2, pp: (173-178), Month: April - June 2019, Available at: www.researchpublish.com

An evaluation of *Eucalyptus camaldulensis* methanolic leaf extract as an alternative therapy for Malaria

Longdet Y. Ishaya[1], Adoyi L. Ewaoche[2]

[12]Department of Biochemistry, University of Jos, Jos, Nigeria.

[12]University of Jos, Jos, Nigeria.

Abstract: **The emergence of malarial drug resistance drives the search for new agents against the disease. *Eucalyptus camaldulensis* leaf extract was tested for antiplasmodial activity. Swiss albino mice were intraperitoneally infected with *Plasmodium berghei* NK65. The level of parasitaemia was monitored daily and treatment began 72 hours post-infection. Doses administered orally to the different groups were 100mg/kg, 200mg/kg and 300mg/kg extract, 25mg/kg Chloroquine (CQ) to the positive control. The negative control were infected and untreated, while the Normal control were uninfected untreated. The animals were treated daily for ten days after sighting parasites in the blood film and then observed. Pack cell volume (%PCV), bodyweight change and survival time were monitored. The extract exerted a dose dependent effect on parasitaemia, with the 300mg/kg group expressing the best effect. Phytochemicals present were flavonoids, tannins, anthraquinones, steroids, saponins, cardiac glycoside and carbohydrates. For PCV, 12% and 4% decrease was observed in the negative and positive control groups; while 8%, 7%, 7% decrease in the 100, 200, and 300mg/kg groups respectively. The normal control expressed a 1% increase in PCV. The mean body weight was compared before and after infection and the result showed a 2.5g, 0.9g, 1.3g, 0.17g, 0.63g decrease in the 100, 200, 300mg/kg, 25mg/kg CQ and negative control groups respectively. A 4.63g weight increased in the normal control. All animals in test groups survived beyond the experimental period. It is obvious that the methanol leaf extract of *Eucalyptus camadulensis* has antiplasmodial properties that could be exploited as alternative therapy for malaria.**

***Keywords: Eucalyptus camaldulensis, Plasmodium berghei* NK65, Malaria, Phytochemicals, Packed Cell Volume.**

I. INTRODUCTION

Malaria is a common and devastating disease caused by protozoan parasites of the genus Plasmodium. There are four known *Plasmodium species* (*P. falciparum, P. vivax, P. malariae and P. ovale*) that infect humans, but *P. falciparum* is known to be responsible for majority of the severity and loss of life associated with malaria [1]. Half of the world's population which is about 3.3 billion people is affected by malaria [2].

Malaria is endemic throughout most of the tropics, about ninety-five countries and territories have ongoing transmission [3]. It is the third principal reason for death of children under five years across the globe. The World malaria report 2018 estimates that there were 219 million cases of malaria in 2017.The 10 highest burden African countries saw an estimated 3.5 million more malaria cases in 2017 compared with the previous year [4].

There are growing evidences that Plasmodium parasite has developed resistance to most insecticides (pyrethroids) and drugs thereby making anti-malarial drugs less effective [5]. This creates a severe menace to treatment of malaria in Nigeria and the world at large. Furthermore, most anti-malarial drugs are not affordable by people and this necessitates the shift to self-medication using medicinal plants [6]. Therefore, there is high reliance on traditional medicine in the treatment of malaria, most of which are prepared from plants and available at affordable prices [7]. *Eucalyptus camaldulensis* leaf is used by Igala folks of Kogi state, Nigeria to treat malaria and fever. Hence, this study was designed to investigate and ascertain the preference and utilization of *Eucalyptus camaldulensis* in the treatment of malaria.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 2, pp: (173-178), Month: April - June 2019, Available at: www.researchpublish.com

II. MATERIALS AND METHODS

A. Materials

Preparation of plant material: The plant material (*Eucalyptus camaldulensis* leaves) was collected from University of Jos, Senior Staff Quarters, Bauchi Road, Jos, Nigeria on 10th May, 2018. The plant was identified at the Nigerian Forestry Research Institute (NFRI), National College of Forestry, Jos, Plateau state, Nigeria. The leaves collected were washed and dried at room temperature after which they was pulverized and stored in a plastic container pending extraction.

Preparation of animals: The animals used for the study, experimental mice of both sexes, were obtained from the Animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria. All animals were handled humanely and were granted clearance by the Institutional Animal Care and Use (IACU) in collaboration with the Office of Laboratory Animal Welfare (OLAW), through the University of Jos Ethic Committee, with a Reference number: F17-00379.

Plasmodium Parasite: The *Plasmodium species*, *Plasmodium berghei* NK65, was obtained from Institute of Malaria Research, Ibadan, Nigeria. The parasite was subsequently maintained in mice.

B. Methods

Preparation of crude extract: The methanol extract was obtained by macerating 100g of the pulverised plant sample in 200 ml methanol for 24 hours. This was then filtered through Whatman filter paper using a Speedvac vacuum pump. The filtrate was then poured into dishes and exposed at room temperature to evaporate to dryness to obtain the crude extract and the yield was determined relative to the starting material.

Parasite inoculation: The method described by [8] was used for the inoculation of parasites into the experimental animals. Each mouse was inoculated on day zero intraperitoneally with 0.2ml of parasite infected blood containing approximately 1×10^7 *Plasmodium berghei* parasitized red blood cells. In addition, the newly inoculated animals were monitored daily to determine expression of parasites in circulation.

Preparation of stock solution of extract: The stock solution was prepared just before use by dissolving the methanol extract in 10% Dimethylsulfoxide (DMSO) as described by [9] 1g of the crude methanol extract was weighed into a sample container and 1ml of the concentrated stock DMSO was aspirated into it, the volume was then topped up to 10ml by adding 9ml distilled water to the mixture. The mixture was agitated for several minutes until complete dissolution was achieved.

Administration of the crude extract: *Plasmodium berghei* infected mice in Groups A, B and C were treated with the crude methanol extract of *Eucalyptus camaldulensis* leaf orally at concentrations of 100mg/kg, 200mg/kg and 300mg/kg body weight of mice respectively. The treatment was done daily starting 72hours post infection for 10 days.

Antiplasmodial Test: The method described by [10] was used to screen for antiplasmodial property of the extract. Fifteen of the eighteen mice of average weight 25 g consisting of both sexes were inoculated with 0.2ml of diluted parasitized blood obtained 72 hours prior to the test from highly parasitized mice. Groups A, B, and C were given a daily dose of 100, 200, and 300mg/kg per body weight respectively, of the plant extract orally while group D was treated with 25mg/kg bodyweight of the standard drug, Chloroquine phosphate, and group E was left untreated.

Monitoring of Parasitaemia: Parasitaemia was monitored as described by [8]. Thin smear of the blood film was prepared on a microscope slide. The film was allowed to dry for about 3–5 minutes, fixed with methanol, and stained with a diluted Giemsa at pH 7.2 for 30 minutes. The stained slide is washed under a running tap and allowed to dry before viewing under the microscope at ×100 magnification to access the level of the parasitaemia. The thin blood film was methanol-fixed and stained with diluted Giemsa stain using buffered water at pH 7.2 to emphasize the parasite inclusion in the red blood cells. The average parasitaemia could be evaluated by this for each of the doses. Any death that occurred during this period was noted and used to determine the mean survival time as described by [11].

Monitoring changes in percentage Packed Cell Volume (%PCV) and weight of experimental mice: Blood sample was collected from the tail of each mouse with a capillary tube. The tube was sealed with plasticine at one end. The sealed capillary tubes were then arranged on the hematocrit centrifuge and set to spin at 12,000 revolutions per minute for five

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 2, pp: (173-178), Month: April - June 2019, Available at: www.researchpublish.com

minutes. The PCV was read on the hematocrit reader and recorded. The bodyweight of the mice were also monitored during the experiment by weighing them at specific interval of days.

Phytochemical Screening of crude extract: The crude extract was subjected to phytochemical screening using the methods described by [12], [13] and [14].

III. RESULTS

Extract Yield: The percentage yield of the leaf extract was shown in Table 1. The yield of methanol leaves extract of *E. camaldulensis* was 9.17%.

TABLE I: THE PERCENTAGE (%) YIELD OF THE CRUDE EXTRACT

E. camaldulensis	**Weight (g)**
Leaf powder	100.00
Methanol extract	9.17
Extract yield (%)	9.17

Phytochemical compositions of crude extract of Eucalyptus camaldulensis: The crude extract was found to contain high level of Flavonoids and Carbohydrates. Saponins, Tannins, and Cardiac glycosides were moderately present; meanwhile low levels of Steroids and Anthraquinones were observed.

Antiplasmodial activity of crude extract: Figure 1 indicates that parasites were significantly suppressed in the 100, 200 and 300mg/kg body weight in a dose dependent pattern. Though the curative activity was observed in the standard drug (Chloroquine) treated group. After 10 days of treatment, it shows that the parasitaemia was best lowered in the grouped treated with 300mg/kg bodyweight of the extract when compared with the other dose groups.

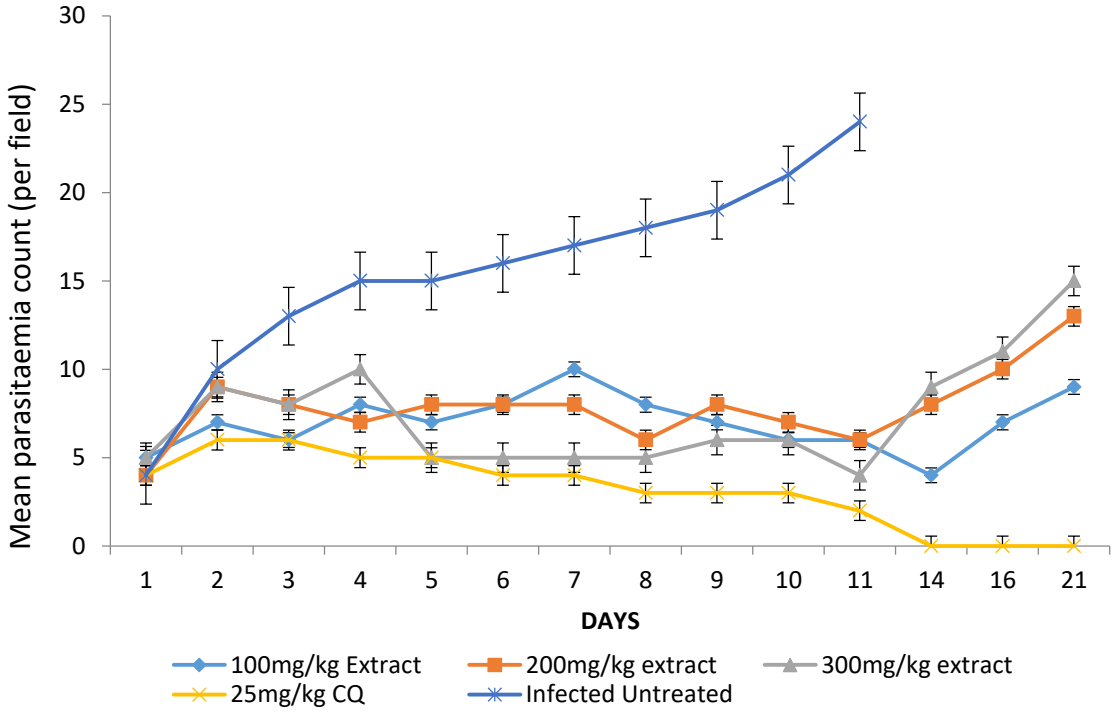

Fig. 1: Antiplasmodial activity of crude methanolic extract of *Eucalyptus camaldulensis* leaf in *Plasmodium berghei*-infected mice.

Effect of Crude Methanolic extract of E. camaldulensis on %PCV: Effect of the crude extract on PCV of Plasmodium berghei infected mice is as represented in Figure 2. As expected, one of the pathologic effects of malarial infection is the increased anaemia, but in this case, the crude extract expressed ability to manage this effect. The %PCV of the negative

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 2, pp: (173-178), Month: April - June 2019, Available at: www.researchpublish.com

control decreased significantly, conversely in the three treatment groups, the effect was managed; with the 300mg/kg group expressing a better effect. Nonetheless, the value increased for the normal control group, and the decrease was minimal in the CQ-treated group (Positive control).

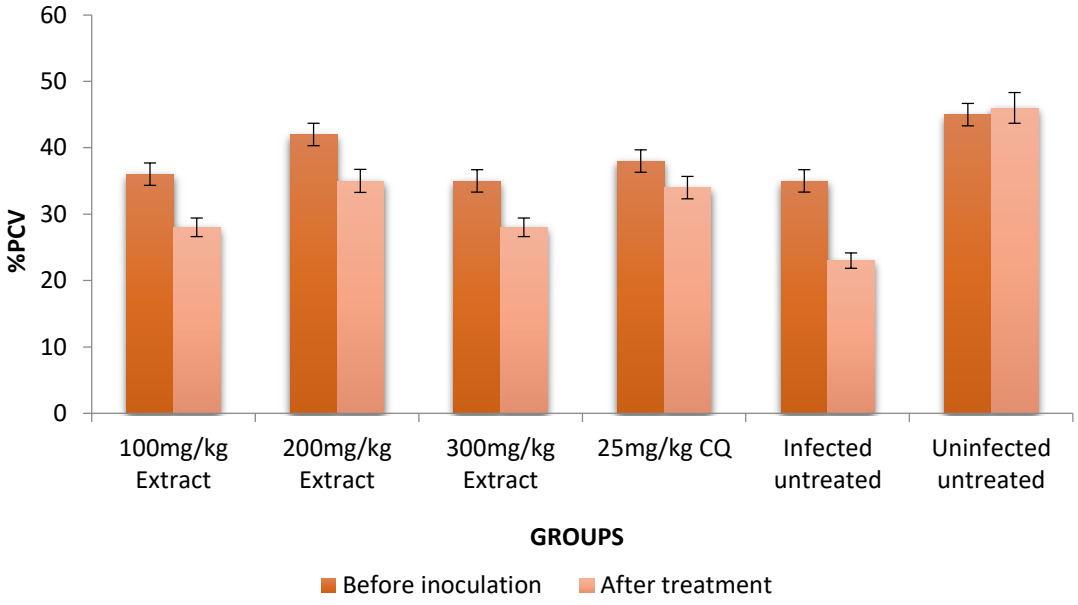

Fig. 2: Effect of crude methanol extract of *E. camaldulensis* leaf on the percentage pack cell volume (%PCV) of *P. berghei*-infected mice.

Effect of Crude Methanolic extract of E. camaldulensis on Body weight: The effect of the methanolic extract of E. camaldulensis on bodyweight of *Plasmodium berghei* infected mice before and after infection and treatment is as shown on Figure 3. From the result of the test carried out on the mice, there was no significant change in the body weight of the mice before and after inoculation in each of the respective doses.

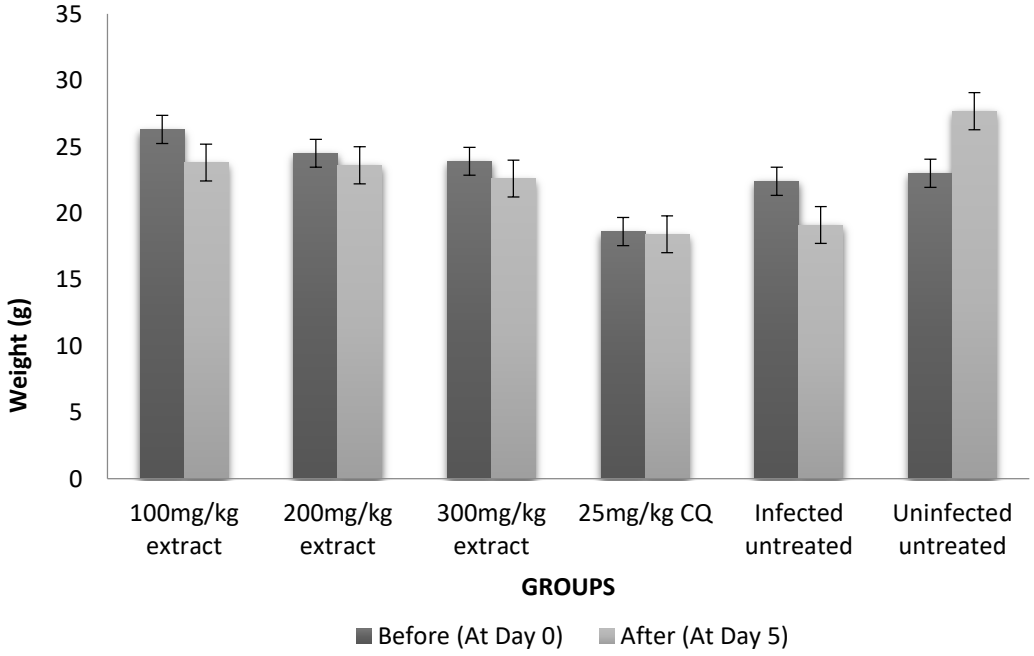

Fig. 3: Effect of crude methanol extract of *Eucalyptus camaldulensis* leaf on the mean body weight of *Plasmodium berghei*-infected mice.

Effect of the Crude Extract on the Survival Time of P. berghei-Infected Mice: The survival time of the mice treated with the extract at different doses indicates that the antiplasmodial activity was dose-dependent, because the mice treated with the highest dose (300mg/kg body weight) survived longer than the 20mg/kg groups. However, mice treated with CQ survived all through the experimental period because it was cured, while mice in the negative control group died within 14 days after infection.

IV. DISCUSSION

The results showed that the extract was capable of reducing the level of parasites in circulation following the treatment.The crude methanol extract reduced the level of parasitaemia in a dose-dependent manner with the highest dose (300mg/kg) reducing the parasitaemia more efficiently after compared to the 100 and 200mg/kg treated groups respectively. This was different when compared to the Chloroquine treated group which reduced the parasitaemia best but worse in the case of the infected untreated. This observation is in conjunction with [15] findings were the reduction in the level of parasites was dose-dependent, with the highest dose (400mg/kg) giving the best result amongst the treatment groups.

The phytochemical screening of crude methanolic extract of *Eucalyptus camaldulensis* leaf revealed the presence of flavonoids, tannins, anthraquinones, steroids, saponins, cardiac glycoside and carbohydrates. This compares well with the work done by [16] on the same plant using same solvent, but steroids were absent. The potency of medicinal plants depends solely on their active phytochemical components which produces a definite physiological action on the human body and is responsible for their numerous bioactivities [17]. Phytochemical compounds such as terpenoids and flavonoids are thought to be responsible for antiprotozoal and antiplasmodial activity of most plants such as Artemisinin, the main active component of the traditional Chinese antimalarial plant (Qinghaosu) [18], [19], [20], [21] and [22].

The percentage packed cell volume (%PCV) generally decreased after infection because anaemia is a common problem that is associated with malaria [8]. However, the treated mice had improved PCV compared to the negative control group. [22] Observed an apparent increase in the haemoglobin concentrations among other parameters across the animal experimental groups owed to the presence of some constituents of iron which are of great importance in the production of blood.

The change in the weights of infected mice is expected because malaria is characterized by loss in weight due to loss of appetite. The mean body weights of the mice in the 100, 200 and 300mg/kg treated groups tend to have better PCV than those in the negative control group. This implied that the treatment helped managed the pathology of the disease. It also implies that other than direct parasiticidal or parasitistatic effects, the plant may possess other pharmacological benefits to the hosts.

The mean survival time for the mice treated with the extract at all doses used indicated that the antiplasmodial activity of the extract was dose-dependent, because the group treated with the highest dose survived all through the experimental period and yet still expressed the least parasitaemia trend over the period. There is an indication in this result that a higher dose of extract could produce a longer survival time just as stated by [15].

V. CONCLUSION

The results justify the use of this plant in traditional medicine for the management of malaria fever. It is convenient to conclude from the results obtained that the methanol leaf extract of *Eucalyptus camaldulensis* possesses phytochemicals with antiplasmodial properties and so can be explored as an alternative therapy for malaria.

REFERENCES

[1] Bhatt D, Weiss, E, Cameron, D, Bisanzio, B, Mappin, U, Dalrymple, K, et al. (2015) The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. Nature, 526 (757): 207–211. DOI: 10.1038/nature/15535.

[2] Obisesan A, and Owoseni, O. (2017) Preference of Medicinal Plants in the Treatment of Malaria Fever in Akure South Local Government Area, Ondo State Nigeria. Available: https://mpra.ub.uni-muenchen.de/81641/

[3] Longdet IY, and Adoga EA. (2017) Effect of Methanolic Leaf Extract of *Carica papaya* on *Plasmodium berghei* Infection in Albino Mice. European Journal of Medicinal Plant 20(1): 1-7. DOI: 10.9734/EJMP/2017/34698.

[4] World Health Organization. World Malaria Report 2018. Geneva: World Health Organization; 2018. Licence:CC BY-NC-SA 3.0 IGO.

[5] Arese C. (2001) Malaria: Its Human Impact, Challenges and Control Strategies in Nigeria. Havard Health Policy Review, 2(2): 1-3.

[6] Muregi F, Chabra S, Njagi E, Langat-thoruwa C, Njue W, Orago A, Omar S, and Ndiege I. (2003) *In vitro* Anti-plasmodial Activity of Some Plants Used in Kisssii, Kenya against Malaria and their Chloroquine Potential Effect. Journal of Ethopharmacology. 84 (2): 235-239.

[7] Alves R, and Rosa I. (2007) Biodiversity, Traditional medicine and Public Health: Where do they meet? Journal of Ethnobiology and Ethnomedicine. 3(4):3-14.

[8] Kabiru YA, Ibikunle GF, Innalegwu DA, Bola, BM, and Madaki FM. *In Vivo* Antiplasmodial and Analgesic Effect of Crude Ethanol Extract of *Piper guineense* Leaf Extract in *Albino* Mice.*Scientifica*. 2016. Available: http://dx.doi.org/10.1155/2016/8687313.

[9] Kabiru YA, Ogbadoyi, EO, Okogun JI, Gbodi TA, and Makun HA. (2013) Anti-trypanosomal Potential of *Eucalyptus Camaldulensis*. British Journal of Pharmacology and Toxicology. 4(2): 25-32.

[10] Souleymane S, Adama G, and Lamoussa O. (2013) *In vitro* antiplasmodial and cytotoxic properties of some medicinal plants from western Burkina Faso. African Journal of Laboratory Medicine, 2(1): 1–7.

[11] Aarthi N, and Murugan K. (2011) Antimalarial activity and phytochemical screening of ethanolic leaf extract of *Phyllanthusniruri* and *Mimosa pudica*. International Journal of Pharmaceutical Research and Development, 3.

[12] Harbone JB. (1973) Phytochemical methods: A guide to modern techniques of plant analysis. Chapman A and Hall, London. pp 279.

[13] Trease EG, and Evans CW. (1978) Laboratory Methods in Antimicrobial Chemotherapy. Churchill Livingstone, Edinburgh. p 35-38.

[14] Sofowora A. (1993) Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. p 289.

[15] Kabiru YA, Okolie NL, Muhammad HL, and Ogbadoyi EO. (2012). Preliminary studies on the antiplasmodial potential of aqueous and methanol extracts of eucalyptus camadulensis leaf. Asian Pacific Journal of Tropical Disease. 2012. S809-S814.

[16] Mahama A, Saidou C, Tofel HK, Ali A, Adji MB, and Nukenine EN. (2018) Efficacy of *Eucalyptus camaldulensis* leaf extracts against the pea beetle *Callosobruchus maculatus* and their impact on biochemical and microbiological properties of the treated bambara groundnut grains. Journal of Entomology and Zoology Studies. 6(2): 869-877.

[17] Ekpo IA, Osuagwu AI, Agbor AB, Okpako EC, and Ekanem BE. (2013) Phytochemical composition of Afromomum melegueta and Piper guineense seeds. Journal of Current Research in Science, 1:24–27.

[18] Kim YC, Kim HS, and Wataya Y. (2004) Antimalarial activity of lavandulyl flavanones isolated from the roots of *Sophoraflavescens*. Biological and Pharmaceutical Bulletin. 27(5): 748–750.

[19] De Monbrison F, Maitrejean M, and Latour C. (2006) *In vitro* antimalarial activity of flavonoid derivatives dehydrosilybin and 8-(1;1)-DMA-kaempferide. Acta Tropica, 97 (1): 102–107.

[20] Tasdemir D, Kaiser M, and Brun R. (2006) Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: in vitro, in vivo, structure-activity relationship, and quantitative structure-activity relationship studies, Antimicrobial Agents and Chemotherapy, 50 (4): 1352–1364.

[21] Asase A, Akwetey GA, and Achel DG. (2010) Ethnopharmacological use of herbal remedies for the treatment of malaria in the Dangme West District of Ghana. Journal of Ethnopharmacology, 129 (3): 367–376.

[22] Dakheel MH, Mohammed WA, Murad, H.M, and Fadhil I.A. (2017) Effect of Ethanolic Extracts leaves of *Eucalyptus camaldulensis* on heamatological parameters of Males wistar rats. International Journal of ChemTech Research, 10 (4): 448-452.