Antiplasmodial Activity of Partially Purified Crude Pet Ether Leaf Extract of *Ficus Platyphylla* Del In Mice

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Abstract: **- Malaria is a leading cause of death in most developing countries. It is responsible for about 300 million clinical cases and 1-2 million deaths annually. The plant *Ficus platyphylla* is a deciduous plant that is used mostly in the northern part of Nigeria for the treatment of malaria and other illnesses. This study was aimed at investigating the antiplasmodial activity of partially purified leaf extract of *F. platyphylla Del* in mice. Safe dose determination was carried out to determine the LD_{50} of the plant extract. For the curative study, twenty one mice were divided into seven (7) groups with five mice in each group. Groups 1, 2, 3, and 4 were administered 600mg/kg body weight of each of the four fractions obtained from the partially purified leaf extract while groups 5 and 6 were administered 50mg/kgbw of artesunate and 0.2ml Normal saline respectively. Group 7 was left untreated. Treatment continued for five days and the level of parasitemia in the blood was determined and the body weight, Packed Cell Volume (PCV) and survival time were also evaluated.**

Acute oral toxicity of the crude plant extract indicates safety of the plant extract at all doses tested. It was also observed from the study that all fractions show remarkable inhibitory activities against malaria parasite compared to the control groups. There was a significant difference ($p<0.05$) in the PCV of the treated groups when compared to the control. Only few of the animals in all the treated groups survived beyond day 30 of the experiment. It can be deduced that the partially purified leaf extracts of *F. platyphylla* possessed antiplasmodial activity thus, indicating its potential as an antimalarial.

Keywords: **Malaria; Ficus platyphylla; Plasmodium bergei bergei; Packed Cell Volume; Parasiteamia.**

I. INTRODUCTION

Malaria is a major cause of morbidity and mortality around the world. The disease is present in 102 countries and is responsible for about 300 millions clinical cases and 1-2 million deaths each year [1]. Majority of those who die from malaria are infants and children living in sub-Sahara Africa. In Nigeria malaria is endemic throughout the country, the World Health Organization (WHO) estimated malaria mortality rate for children under five in Nigeria at 729 per 100,000. The Nigerian ministry of health reported that malaria is responsible for one out of ten deaths in pregnant women and the government spends over one billion naira annually in treating malaria [2].

Resistance of the vector mosquitoes to the current insecticides and the emergency of resistance by *plasmodium* species to widely use anti malaria drugs, such as chloroquine, have made malaria control and treatments more difficult [3]. In view of the problem associated with anti malaria drug resistance, new drugs have to be sourced to replace those compromised by parasites resistance, these drugs should exhibits a novel mode of actions or chemically different from currently used anti-malaria drugs [4]. Plants have always been considered as a possible alternative and rich source of new drugs. Most of the anti malaria drugs in used today such as quinine and artemisinin were either obtained directly from plants or developed using template of chemical structures of plant derived compound [5]. The plant *Ficus platyphylla* Del Holl (Moraceae) is a deciduous plant locally known as Ogbayikolo In Igala and Gamji in Hausa and it is widely distributed throughout the savannah region of West African coast. The leaves and stem bark of the plant are used traditionally to treat Malaria and anaemia in Africa [6]. The cold water extract of the roots or bark is used in various conditions as Dysphoria, Pain, and Insomnia. Also the plant has been reported to inhibit gastrointestinal motility [7], possesses analgesic [8] and anti-inflammatory and anticonceptive activities [9].

It is in view of these, that this research was aimed at evaluating the antiplasmodial activity of partially purified leaf extract of *F. platyphylla* Del Holl (Moraceae) in mice, in order to scientifically ascertain the traditional use of the plant in the management of malaria.

II. MATERIALS AND METHOD

A. Collection of Plant Materials

Matured fresh leaf samples of the *F. platyphylla* were collected from Lokoja, North-Central Nigeria and were identified at the Department of Biological Science, Ahmadu Bello University Zaria, Nigeria.

B. Rodent parasite (Plasmodium berghei berghei)

The rodent parasite *Plasmodium berghei berghei* NK 65 was obtained from National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria and kept at the Department of Biochemistry Federal University of

Technology Minna. The parasites were kept alive by continuous intra peritoneal passage in mice as described by Jigam *et al.,* [10].

C. Laboratory Animals

Swiss albino mice and Wister rats of about 6 weeks old weighing between 20-30g and 200-300g respectively were obtained from NIPRD, Abuja were used for the experiments. The animals were fed *ad libitum* with standard feed and water. They were also maintained under standard environmental conditions and 12 hours light/dark cycle. The animals were acclimatized for two weeks before the commencement of the study. A standard protocol was drawn up in accordance with the Good Laboratory Practice regulations (ENV/MC/CHEM (98) 17, 1998). The Principle of Laboratory Animal Care (NH) Publication No. 85-23, 1985) was followed in this study.

D. Parasite Inoculation

The method described by Kabiru *et al.,* [11] was used for the inoculation of parasite into experimental animals. The inoculums consisted of $5x10^7$ of *P. berghei berghei* parasitized erythrocytes per ml. Each mouse was inoculated on day 0 with 0.2 ml of *P. berghei berghei* inoculums.

E. Preparation of Crude Extracts

The extract was prepared as described by Ogbadoyi *et al.,* [12] with slight modifications. Powdered leaves sample weighing 100g was macerated in 500ml of petroleum ether using reflux method. The extraction lasted for two hours after which the extract was filtered hot using a muslin cloth and solvents removed under reduced pressure using a water bath. Extract obtained was transferred into a sterile universal bottle and kept in the refrigerator at 4^{0c} until require for use.

F. Preliminary Toxicological studies

1) Safe Dose Determination

Safe dose determination was carried out in two phase, in phase I: Fifteen Wistar rats were divided into three groups of five rats per group. The three groups were administered orally with graded doses (100, 500, and 1000 mg/kg respectively) of the extract. Phase II: Another Fifteen rats were divided into three groups of five rats per group, which received graded doses (200, 3000 and 5000mg/kg) of the extract respectively. The number of death and clinical signs in each group within 24 hours were recorded and the final LD_{50} value was calculated as the geometric mean of the highest non-lethal dose (with no death) and the lowest lethal dose (where death occurred).[13]

G. Partial Purification of Crude Extract

The crude petroleum ether leaf extract of *F. platyphylla* was reconstituted in absolute n-Hexane and spotted on analytical thin layer chromatography (TLC), different solvent system at different ratio was used as mobile phase to determine the eluent with optimum performance. After each separation, the TLC plate was exposed to a UV lamp and the solvent system that gave the best resolution was adopted for column fractionation using the method described by Ogbadoyi *et al* [14].

H. Experimental design

1) Curative Antiplasmodial Test Using Partially Purified Extract

A total of thirty five mice were used for this study. On the first day (D0), standard inoculums of *P. berghei berghei* infected red blood cell were injected intraperitoneally. 72 hours later, the mice were divided into seven groups of five mice each. Dose of 600mg/kgbw of crude Pet ether extract of *F. platyphylla* was administered to a group, four groups were administered 600mg/kg body weight each of the four fractions obtained from the partially purified crude pet ether leaf extract of *F. platyphylla.* Artesunate (50mg/kgbw) was given to the positive control group and 0.2ml of normal saline was administered to the last group which serve as the negative control. The extracts were given once daily for 5days. Thin blood smear were prepared from the tail of each mouse on D2 to D7 of the study period to monitor the level of parasitemia. Variation in weight and PCV were also monitored. The mean survival time for each group was determined arithmetically by finding the mean survival (days) of the mice (post inoculation) in each group over a period of 30days (D0-D29).

Thin blood smears of mice that survived beyond 28days were evaluated to confirm if parasites had been cleared completely.

2) Determination of Packed Cell Volume (PCV) in Infected and Treated mice

The method according to Kabiru *et al* [15] was used to determine the PCV in both the infected not treated and treated mice. The PCV was then read using the micro haematocrite reader.

3) Determination of Mean Survival Time of infected and treated mice

Mortality was monitored daily and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse in the treatment and control groups throughout the follow up period. The mean survival time (MST) for each group was calculated as:

$$MST = \frac{\text{Sum of survival time of all mice in a group (days)}}{\text{Total number of mice in that group}}$$

I. Ethical clearance

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

J. Statistical Analysis

All experiments were carried out in triplicate. A completely randomized design was used throughout this study and data

was subjected to one-way analysis of variance and mean comparison with Duncan's Multiple Range Test (significance level of $P < 0.05$)using Statistic Package for Social Sciences (SPSS 22.0 for Windows: SPSS Inc., Chicago, IL, USA .

III. RESULT

Safe dose determination of the crude *F. platyphylla* leaf extract indicates there were no mortality in animal at all doses of the extract up to 5000mg/kgbw(Table I). Ruffled fur, reduced motility, and increase drowsiness were the only behavioural signs of toxicity shown by the animal at 5000mg/kgbw these disappeared within 24hrs of the first administration of extract.

Table I: SAFE DOSE DETERMINATION OF *Ficus platyphylla* LEAF EXTRACT

Doses (mg/Kgbw)	Mortality	Change Observed
100	0/5	None
500	0/5	None
1000	0/5	None
200	0/5	None
3000	0/5	None
5000	0/5	Ruffled fur, Reduced Motility and increased drowsiness

About 80 fractions were obtained from the partial purification of the crude leaf extract of *F. platyphylla* using chromatography. These fractions were subjected to thin layer chromatography and fractions with similar Retardation factor (RF) values were pulled together. Four fractions were finally obtained and curative test was carried out using these fractions.

From the study it was observed that all the fractions showed inhibitory activity with Fraction 2 having the highest inhibitory activity (66.2%) compared to other fractions, the activity of fraction 2 compared favourably with that of the group treated with 50mg/kg body weight of artesunate (73.5%) (Figure III).

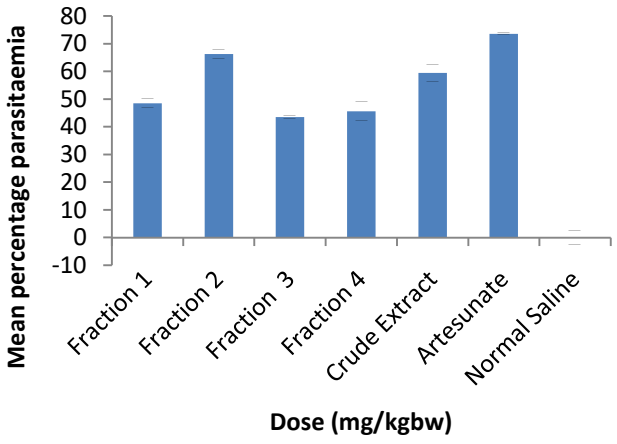

Figure I: Curative effect of *F. platyphylla* fractions against *P. bergei* infection in mice.

There was a significant increase in weight of mice after extract administration in all the treated groups however the negative control group had a decrease in weight after 5 days of the experiment (Figure II).

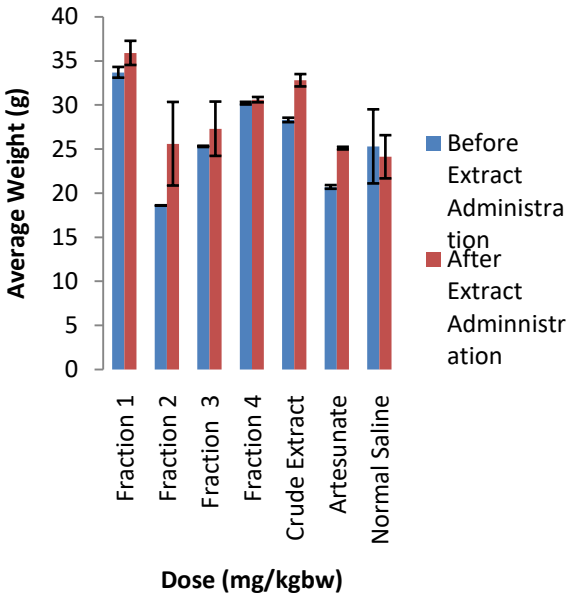

Figure II: Average weight of mice before and after 5days of treatments with fractions of *F. platyphylla*.

In all the groups treated with fractions of the partially purified extract of *F. platyphylla*, there were significant differences in PCV after the fifth day of the experiment when compared to the negative control group (Figure III).

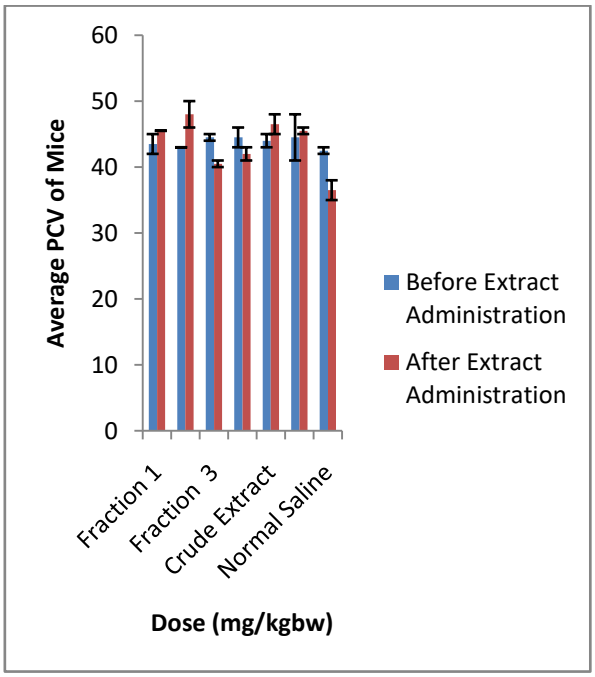

Figure III: Effect of *F. Platyphylla* Fractions on packed cell volume (PCV) in mice. (Curative test).

The mean survival periods in days were calculated to be 21.5 ± 1.50, 29 ± 1.0, 13.5 ± 1.50, 20 ± 2.0, 28.5± 0.5, 30.0 ± 0.0 and 12.0 ± 2.0 for Fractions 1,2,3, 4,crude pet ether leaf extract, artesunate (50mg/kgbw) and negative control groups respectively (Figure IV).

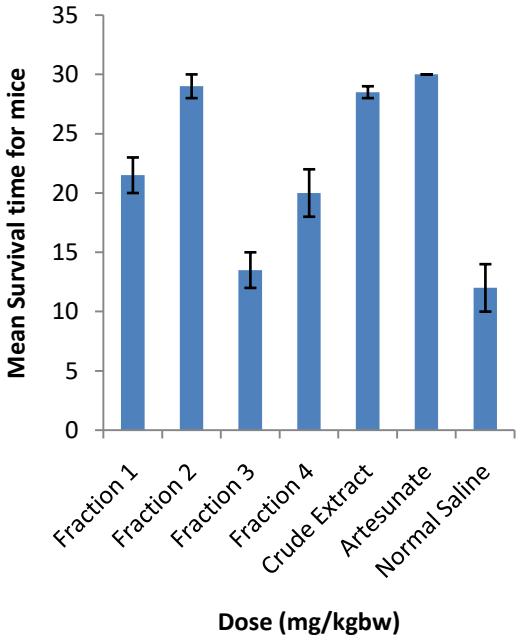

Figure IV: Mean days of survival of *Plasmodium bergei* infected mice treated with fractions *F. Platyphylla*

IV. DISCUSSION

The present study was carried out to evaluate the antiplasmodial activity of *Ficus platyphylla* a widely used traditional plant for the treatment of malaria in Kogi state, North-Central Nigeria. Traditional remedies are common in regions where patients cannot afford to use chemically synthesized drugs. Poverty, traditional beliefs and lack of available health centres have caused plants to be used as the major source for treatment of various ailments [16].

The safe dose toxicity of the plant was investigated to determine any adverse effect that may arise as a result of a short time animal exposure to the extract within 24 hours period. Although the plant has been used previously by Ugwah-Oguejifor *et al* [17] with no mortality due to toxicity, this claim has been validated by the lack of death at oral treatment of up to 5000mg/kg body weight. Nevertheless, the observed behavioural sign were ruffled fur, reduced motility and increase drowsiness. The result thus suggests that the leaf extract of *F. platyphylla* is safe at 5000mg/kg body weight. The low toxicity obtained may have been responsible for its widespread use in different ethno therapeutic intervention.

Evaluation of the curative effect of the partially purified crude extracts of *F. platyphylla* on established infection shows that all fractions exhibits some level of antiplasmodial activity with fraction 2 having the highest average inhibitory activity (Figure III) relative to the fractions and the crude extract.

The high inhibitory activity of fraction 2 may be an indication that efficacy improved as the extracts got purer. Therefore it point out to the fact that the isolated pure compound when eventually obtained may be a drug able candidate. This agrees with the findings of Ogbadoyi *et al* [14] on their work on the therapeutic evaluation of *Acacia nilotica* stem bark extract in experimental Africa *trypanosomiasis* that crude extracts when purified exhibits a high inhibitory activity, whereas the minimal activity of the other fractions (1, 3, 4) when compared to fraction 2 might have resulted from the fractionation due to loss of synergy. Similar cases of loss of activity due to fractionation were reported by McKerrow *et al* [16], the partitioning of hexane and ethylacetate fractions of *Phyllantus amarus* (leaf) resulted in decreased antiplasmodial activity.

Haematological indices such as PCV were studied to assess the toxic effect of the parasite on blood component. Anaemia is one of the established major pathological features of most protozoa including the malaria parasite [19]. Therefore the presence and severity of anaemia is good indicator of disease status. The significant decrease level of PCV of the negative control group treated with 0.2 normal saline is an indication of anaemic condition cause by the parasite.

There was a significant difference ($P<0.05$) in weight and PCV of all group before and after treatment. This effect of the fractions and crude extracts of the plant on the PCV is normal to due to the effect of the extracts on the cells in the blood especially the Red Blood Cell (RBC) since the *plasmodium* parasites are usually localized in the cells and consequently affect the PCV. This condition is usually temporary because once the parasites are cleared from circulation, the cell begins to gradually divide and replenish in the blood.

There was a variation in the weight of the infected mice which was expected as malaria is characterised by loss in weight due to loss of appetite, however the animals started to gain weight after treatment with the extract which was as a result of the clearance of the parasite from the red blood cell.

V. CONCLUSION

The partially purified pet ether leaf extract fractions of *F. platyphylla* exhibits a good antiplasmodial activity, it therefore shows that the plants posses significant potential as a candidate in the development of antimalaria therapy. This study has also scientifically established the basis for its continuous use in traditional malaria treatment.

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REFERENCES

[1]. Oaks, S. C., Mitchell, VS., Pearson, GW. & Pearson, CC.J., eds.. (1991) *Malaria: Obstacles and Opportunities* (National Academy Press, Washington, DC).

[2]. Odugbemi, T. O., Akinsulire, O. R., Aibinu, IE., Fabeku, P. O. (2007). Medicinal plants useful for malaria therapy in Okeigbo, Ondo State, Southwest Nigeria. *Afr, J of Tradl Compl and AlterMed.* 4, 191–198.

[3]. O'Neill P. M., Bray P. G., Hawley S. R., Ward S. A and Park B. K., (1998). *Pharmacol Ther*. 77 (1), 29-58.

[4]. Jigam, A. A., and Akanya, H. O. (2007). Phytochemical, antimicrobial and antiplasmodial screening of Cochlospermum tinctorium root extracts. *J. Sci. Edu. Tech.* 1(1):4-8.

[5]. Basco, L. K., Mitaku, S., Skaltsounis, AL., Ravelomanaintsoa, N., Tillequin, F., Koch, M., Le Bras, J., (1994*). Antimicrobial Agents and Chemotherapy* 5, 1169–1171.

[6]. Nadembega, P., Boussim, J. I., Nikiema, J. B., Poli, F. & Antognoni, F (2011). ("Medicinal plants in baskoure, kourittenga province, Burkina Faso: an ethnobotanical study," *Journal of Ethnopharmacology*, vol. 133, no. 2, pp. 378–395.

[7]. Amos, S., Binda, L., Chindo, B., Akah, P., Abdulrahman, M., Danmallam, H. U.,... Gamaniel, K. (2001). Evaluation of methanolic extract of *F. platyphylla* on gastrointestinal activity**.** *Indian Journal of Experimental Biology*. **39:**63-67.

[8]. Wakeel, O. K., Aziba, P. I., Ashorobi, R. B., Umukoro, S., Aderibigbe, A. O. & Awe, E. O. (2004) "Neuropharmacological activities of *Ficus platyphylla* stem bark in mice," *African Journal of Biomedical Research*, vol. 7, no. 2, pp. 75–78.

[9]. Amos, S., Chindo, B., Edmond, I., Akah, P., Wambebe, C., Gamaniel, K. (2002). Antinociceptive and anti-inflammatory properties of *F. platyphylla* stem bark**.** *Journal of Herbs Spices Medicinal Plants*. **9:**47-53.

[10]. Jigam, A. A., Usman, T. A., Halima, AM and Tijani, FO. (2012). Efficacy of thonningia sanguine Vahl root extract against plasmodium chabaudi, inflammation and nociception in mice. *J of Appl. Pharmacol, Sci.* 02 (01); 47-51.

[11]. Kabiru A. Y., Okolie N. L., Muhammad H. L., and Ogbadoyi E. O. (2012). Preliminary studies on the antiplasmodial potential of aqueous and methanol extracts of *Eucalyptus camaldulensis*. *Asian Pacific J of Trop Disease* December Supplement S809-S814.

[12]. Ogbadoyi, E. O., Akinsunbo O. A., Adama, T. Z., & Okogun, J. I. (2007). In vivo trypanocidal activity of *Annona senegalensis* Pers. leaf extract against *Trypanosoma brucei brucei*. *J of Ethnopharmacol* 112: 85-89.

[13]. Lorke, D. (1983). A new approach to practical acute toxicity test. Arch. Toxicology. 54: 275-286.

[14]. Ogbadoyi, E. O., Garba, Mirielle. T., Kabiru, A. Y., Mann, A., Okogun, J. M. (2011). Evaluation associated with restorative potentials associated with Acacia nilotila (Linn) originates will often bark extract inside trial and error African-american trypanosomiasis. Intercontinental Diary associated with Appl, Investigation inside Organic Goods 4(2): 11-18

[15]. Kabiru, A. Y., Abdulkadir, A., Gbodi, AT., Bello, U. M., Makun H. A., Amah, D J. & Ogbadoyi, E. O (2013). Evaluation of haematological changes in plasmodium- berghei- infected mice administered with aqueous extract of *phyllantus amarus*, *Pak. J. of Biol Sci*, Vol 16 (11): 510- 516.

[16]. McKerrow, J., Sun, A., Rosenthal, V. J., Bouvier, J (1993). The actual proteases along with pathogenicity associated with parasitic protozoa. *12-monthly Evaluation Microbiology forty seven*: 821-53.

[17]. Rosenthal, P. J., Meshnick, S. R (1996). Hemoglobin catabolism and iron utilization by malaria parasites. *Mol Biochem Parasito*l. 83:131-9.

[18]. Tshibangu, J. N., Chifundera, K., Kaminsky, R., Wright, A. D., & Konig, G. M. (2002). Screening of African medicinal plants for antimalarial inhibitory activity. *Journal of Ethnopharmacol*ogy 80: 25-35.

[19]. Ugwah- Oguejiofor, C. J., Bello, S.O., Etuk, E. U., Igbokwe, V. U., Ugwah, O. M. & Okolo R. U (2011). Preliminary toxicity and phytochemical studies of the aqueous extract of ficus platyphylla in female albino rats. *International research journal of pharmacy and pharmacology*, Vol 1 (5) pp. 086- 092.