Evaluation of Antimalarial Properties of *Ficus Platyphylla Del* Leaf Extract in Mice

ABSTRACT

Aim

This study was aim at investigating the effect of crude petroleum ether leaf extract of *Ficus platyphylla* Del on *Plasmodium berghei* infected mice.

Place and Duration of study

This research was carried out at the department of biochemistry, Federal university of technology minna, Niger state Nigeria in 2014.

Methodology

The crude plant extract of *F. platyphylla* was administered 72 hours at different doses post and pre infection for both the curative and prophylactic study respectively against residual infection. Mice were divided into 5 groups of 5 mice each, 3 of the groups where administered crude plants extract of *F. platyphaylla* at different doses (200, 400 and 600mg/kg body weight) while the other two serve as negative and positive control group and were administered 0.5ml and 50mg/kg body weight respectively.

Result

The extract at all doses produced significant ($P<0.05$) dose dependent chemo-suppressive activity with % inhibition of 38%, 61%, 74% and 81.8% for curative studies and 36.0%, 38.5%, 49.5% and 63.4% for prophylactic studies against the parasites at doses of 200mg/kgbw, 400mg/kgbw, 600mg/kgbw of the extract and 50mg/kgbw of Artesunate. All doses of the extract increased the survival time of the infected mice compared to the negative control group that was administered 0.5ml normal saline. The variation in the values of Packed Cell Volume (PCV) for treated group before and after extract administration was not significant at ($P<0.05$). The phytochemical screening of the plant extract showed the presence of tannin, saponin, flavonoids, terpenoids, steroids, anthroquinone and phenol.

Conclusion

The result of this study shows that *F. Platyphylla* leaf extract exhibited some antiplasmodial activity that could be exploited for safe, effective and affordable antimalaria regimen.

Keywords: Malaria, Ficus platyphylla, Plasmodium berghei, Anti- malarial, Suppressive.

INTRODUCTION

Despite efforts put in by government and non-governmental organization all over the world towards the eradication of malaria; the causative pathogen has thrived over the years, spreading far beyond their evolutionary origins in Africa and Southern Asia. Malaria still

22 remains a leading cause of death in most developing countries, especially in Africa. There
23 are estimated to be 300-500 million clinical cases of malaria annually [1] and it is estimated
24 to cause more than one million deaths annually, majority of which are children [2]. The
25 parasite exhibits its activity by cleaving the erythrocytes of its host immediately after the
26 release of the merozoites into the system. It achieves this with the aid of the protease
27 enzyme whose major function is to catalyze the breakdown of other proteins by hydrolyzing
28 their peptide bonds. Hence, clinical symptoms and signs of malaria occur shortly before or at
29 the time of red blood cells lysis. The associated fever is caused by the release of
30 merozoites, malaria pigment, parasites proteins and cellular debris. Chills or rigor followed
31 by high fever are observed normally in a cyclical pattern [3].

32 Medicinal plants material remains an important source to combats serious disease in the
33 world, being a store house to thousands of therapeutic phytochemicals. It has been
34 instrumental in traditional medicines to treat different disease from time immemorial in
35 various part of the world. In this regard, the first antimalarial was discovered by accident
36 from the barks extracts of *Cinchona* (Rubiaceae) species.

37 *Ficus platyphylla* Del Holl (Moraceae) is a deciduous plant locally known as 'Gamji'' among
38 the Hausa tribes in Nigeria, West Africa. It is widely distributed throughout the savannah
39 region of West African Coast. The leaves and stem bark of the plant are used traditionally to
40 treat malaria and anaemia [4]. Methanolic extracts of *F. platyphylla* barks have been
41 previously shown to possess significant anti-inflammatory effects [5]. A study has also
42 shown that the extract contains physoactive metabolites with potentials as an anti-epileptic
43 agent [6].

44 It is in view of these, that this study is aimed at evaluating the antimalaria properties of crude
45 leaf extract of *F. platyphylla* Del in mice.

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48 **MATERIALS AND METHOD**

49 **PLANT MATERIALS**

50 Fresh leaves of the plants were collected from Lokoja, North Central Nigeria and were
51 identified at the Department of Biological Science Ahmadu Bello University Zaria, Nigeria
52 where a voucher specimen was deposited at the departmental herbarium.

53 **RODENT PARASITE (*PLASMODIUM BERGHEI BERGHEI*)**

54 The rodent parasite *Plasmodium berghei berghei* NK 65 used for the study was obtained
55 from National Institute for Pharmaceutical Research and Development (NIPRD), Abuja,
56 Nigeria and kept alive by continuous intra peritoneal passage in mice every four days at the
57 Department of Biochemistry Federal University of Technology Minna, Nigeria [7].

58 **ANIMALS**

59 Healthy Swiss albino mice of both sex of about 6 weeks old weighing 20-25g each was used
60 for the experiments. The animals were fed *ad libitum* with standard feed and had free access
61 to water. They were also maintained under standard conditions of humidity, temperature and
62 12 hours light/darkness cycle. Experiment were conducted in strict compliance with
63 internationally accepted principle for laboratory animal use and care as contained in the
64 Canadian council on animal care guidelines and protocol review [8].

65 **METHODS**

66 **PARASITE INOCULATION**

67 The method described by Kabiru *et al.,* [9] was used for the inoculation of parasite into
68 experimental animals. The inoculums consisted of 5×10^7 of *P. berghei berghei* parasitized
69 erythrocytes per ml. This was carried out by determining both the percentage parasitaemia
70 and erythrocytes count of the donor mouse and diluting the blood with phosphate buffer
71 saline in proportions indicated by both determinations.

72 **PREPARATION OF CRUDE EXTRACTS**

73 Fifty (50) grams of powdered leaves of *F. platyphylla* was weighed and macerated in 250ml
74 of 100% petroleum ether, the extraction lasted for 2 hours, thereafter it was filtered hot using

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75 muslin cloth and solvents removed under reduced pressure using a water bath. Extract
76 obtained was transferred into a sterile universal bottle and kept in the refrigerator at 4°C until
77 required for use [10].

78 **PHYTOCHEMICAL SCREENING**

79 To elucidate the secondary metabolites present, the crude leave extract was subjected to
80 qualitative phytochemical screening using the methods of Odebiyi and Soforowa, [11].

81 **RANE (CURATIVE TEST)**

82 Evaluation of curative potentials of *F. platyphylla* leaf extract (FLE) was carried out as
83 described by Ryley and Peters, [12]. Twenty five mice were selected and intraperitoneally
84 injected with 1 x 10^7 *P.berghei* infected erythrocytes. Seventy two hours after, the animals
85 were divided into 5 groups of 5 animals each. Group 1 was administered normal saline
86 (0.5ml/kg body weight), groups 2, 3 and 4 received 200, 400 and 600mg/kg body weight of
87 FLE respectively while group 5 received 50 mg/kg body weight of artesunate. All
88 administration of extracts, standard drug and normal saline were carried out daily through
89 oral routes. Treatment continued until the seventh day and a thin film was prepared with
90 blood collected from the tail of each mouse. The films were fixed with methanol, stained with
91 Giemsa stain and parasitemia was ascertained by microscopic examination in five different
92 fields.

93 **EVALUATION OF PROPHYLACTIC ACTIVITY**

94 The prophylactic activity of the extract was tested using the residual infection procedure
95 described by Saidu *et al.,* [13]. Adult mice of both sexes were weighed and randomized into
96 5 groups of 5 mice each. Mice in group 1 were administered 0.5ml normal saline/kg body
97 weight. Groups II, III and IV were administered 200, 400 and 600 mg FLE/kg body weight
98 orally while group V received 50mg/kg body weight of artesunate orally daily for 5 days. On
99 the fifth day, all the mice were inoculated with standard inoculum of 0.1 x 10^7 *P. berghei*
100 *berghei* NK 65 infected erythrocytes. Thin film of blood smears were made from each mouse
101 72 h after inoculation [14] and examined microscopically for the level of parasitaemia.

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DETERMINATION OF PACKED CELL VOLUME (PCV)

The packed cell volume was evaluated using the method of Daice and Lewis [15]. Blood sample was collected into a heparinized capillary tube from the tip of the tail of each mouse and sealed with a plastacin. The tube was then centrifuge using a micro hematocrite centrifuge at 11,000rpm, for 5minutes. PCV was read using the micro haematocrite reader.

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.

STATISTICAL ANALYSIS

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 .

RESULT

PHYTOCHEMICAL SCREENING

The preliminary phytochemical test carried out on warm sample of petroleum ether leaf extracts of *F. platyphylla* is presented in Table 1. The analysis reviewed the presence of tannins, saponins, anthroquinones, flavonoids, and terpenoids while steriods, phenols, alkaloids and cardiac glycosides were not detected.

Table 1: Phytochemical Constituents of Petroleum Ether Leaf Extract of *F. platyphylla*

Bioactive Agent	Petroleum Ether Leaf Extracts
Tannins	+++
Saponin	++

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Flavonoids	+++
Terpenoids	++
Steriods	+
Anthroquinones	+++
Phenol	_
Alkaloids	_
Cardiac glycosides	_

126 Trace(+); Moderate (++); High (+++); Absent (-)

127

128 **RANE (CURATIVE TEST)**

129 FLE produced significant dose-dependent decrease in parasite counts at ($p<0.05$). The
130 mean percentage inhibition of parasitemia of the extract treated groups on day 7 were 38.4,
131 61.6 and 74.8% for groups administered with 200, 400, and 600mg/kg body weight of the
132 extract respectively while that of the artesunate treated group was 81.8% (Table 2.0)

133 **Table 2.0 Curative effect of FLE on parasitaemia in mice**
134

Treatment	Dose (mg/kgbw per day)	Day 2	Day 3	Day 4	Day 5	% Inhibition
Normal saline	0.5ml	9.6 ± 0.0^{c}	5.0 ± 0.20^{a}	9.33 ± 0.30^{bc}	10.6 ± 0.13^{d}	-
FLE	200	7.7 ± 0.10^{b}	7.9 ± 0.55^{a}	5.1 ± 0.30^{a}	5.6 ± 0.20^{c}	38.4
FLE	400	6.3 ± 0.30^{a}	6.0 ± 2.00^{a}	5.1 ± 0.50^{a}	3.7 ± 0.10^{b}	61.6
FLE	600	5.7 ± 0.10^{a}	5.5 ± 0.22^{a}	6.5 ± 0.10^{b}	2.3 ± 0.30^{a}	74.8
Artesunate	50	6.3 ± 0.10^{a}	5.8 ± 0.32^{a}	5.4 ± 0.0^{ab}	2.1 ± 0.10^{a}	81.8

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136 The packed cell volume of infected mice administered with FLE before and after extract
137 administration is represented in Table 3.0, from the result it can be deduced that there were
138 increase in the value obtained for PCV of mice in all treated groups but not in the negative
139 control group which shows a reduction in the value of PCV throughout the study period.

140 **Table 3.0 Effect of FLE on packed cell volume (PCV) of mice infected with**
141 ***Plasmodium berghei***
142

Treatment	Dose (mg/kgbw per day)	Mean PCV (Before Treatment)	Mean PCV (After Treatment)
Normal Saline	0.5ml	54.0 ± 2.06[a]	51.0 ± 4.73[a]
FLE	200	55.7 ± 7.62[a]	54.33 ± 7.31[a]
FLE	400	55.7 ± 5.3[a]	56.33 ± 4.17[a]
FLE	600	57.0 ± 3.05[a]	58.67 ± 3.18[a]
Artesunate	50	54.0 ± 4.93[a]	56.0 ± 4.61[a]

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144

145 The mean survival period in days were calculated to be 20.33± 0.67, 22.33±1.45,
146 27.00±2.08, 27.33±2.67 and 10.00±1.15 for 200, 400, 600mg/kg body weight of the plant
147 extract and the control group (0.5ml normal Saline) respectively (Table 4.0).

148 **Table 4.0 Effect of FLE on mean survival time of mice infected with *Plasmodium***
149 ***berghei***
150

Treatment	Dose (mg/kgbw per day)	Mean survival time

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Normal Saline	0.5ml	10.00 ± 1.15^{a}
FLE	200	20.33 ± 0.67^{a}
FLE	400	22.33 ± 1.45^{bc}
FLE	600	27.00 ± 2.08^{c}
Artesunate	50	27.33 ± 2.67^{c}

151

152 **PROPHYLACTIC TEST**

153 FLE exhibited significant ($p<0.05$) dose dependent reduction in the level of parasiteamia;

154 36.7, 38.5, 49.5 and 63.4% at 200mg/kgbw, 400mg/kgbw, 600mg/kgbw and 50mg/kg body

155 weight and artesunate treated groups respectively (Table 5.0)

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156 **Table 5.0: Prophylactic effect of FLE on parasitaemia in mice**

157

Treatment	Dose (mg/kg bw per day)	Day 2	Day 3	Day 4	Day 5	% inhibition
FLE	200	4.10 ± 0.1^{b}	3.9 ± 0.61^{a}	7.5 ± 1.10^{b}	8.10 ± 0.5^{a}	36.0
FLE	400	5.90 ± 0.1^{c}	5.5 ± 1.0^{ab}	5.0 ± 0.20^{a}	6.6 ± 0.58^{b}	38.5
FLE	600	2.90 ± 0.9^{c}	2.1 ± 3.20^{a}	6.8 ± 0.20^{b}	5.6 ± 0.40^{ab}	49.5
Artesunae	50	1.47 ± 0.29^{a}	1.43 ± 0.3^{a}	4.30 ± 1.3^{a}	4.73 ± 1.7^{a}	63.4
Normal saline	0.5ml	8.4 ± 0.40	8.20 ± 0.22^{b}	9.3 ± 0.33	10.2 ± 0.0^{c}	Normal saline

158
159
160 There was a slight increase in PCV value for all the treated groups after extract
161 administration except for that of the negative control group in which no change in PCV value
162 was observed. However PCV value for all the treated groups drops 72hours after infection
163 with *p. berghei* (Table 6.0).

164 **Table 6.0 Effect of FLE on packed cell volume (PCV) of mice infected with**
165 ***Plasmodium berghei* (Prophylactic test)**
166

Groups	Dose (mg/kgbw per day)	PCV before treatment	PCV after treatment	PCV 72 hours after inoculation
FLE	200	55.7 ± 1.94^{a}	56.33 ± 1.74^{a}	53.3 ± 1.31^{a}
FLE	400	62.67 ± 0.71^{a}	63.0 ± 0.5^{a}	61.33 ± 0.03^{a}
FLE	600	61.67 ± 0.03^{a}	63.67 ± 0.33^{a}	62.0 ± 0.08^{a}
Artesunate	50	60.0 ±1.15^{a}	60.67 ± 1.85^{a}	57.3 ± 1.45^{a}
Normal saline	0.5ml	61.0 ± 1.52^{a}	61.0 ±1.51^{a}	57.7 ± 1.45^{a}

167
168
169 Table 7.0 shows mean survival periods in days calculated to be 20.67±3.17, 21.0± 3.29,
170 25.33 ± 4.67, 26.67 ± 3.33 and 10.0 ± 1.0 for 200, 400, 600mg/kgbw of crude pet ether leaf
171 extract of *F. platyphylla*, 50mg/kg body weight of artesunate and the control group (0.5ml of
172 normal saline) respectively.
173

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176 **Table 7.0: Mean days of survival of Mice infected with *P.berghei* and treated**
177 **with FLE (Prophylactic test)**
178

Treatment	Dose (mg/kgbw per day)	Mean Survival time
FLE	200	20.67 ± 3.17[ab]
FLE	400	21.0 ± 3.79[ab]
FLE	600	25.33 ± 4.67[b]
Artesunate	50	26.67 ± 3.33[b]
Normal saline	0.5ml	10.0 ± 1.0[a]

179
180
181 **DISCUSSION**

182 The present study was carried out to evaluate the antimalaria properties of *F. platyphylla* Del
183 leave extract widely used in traditional treatment for malaria in some parts of Nigeria.
184 Traditional remedies are common in regions where patients cannot afford to use chemically
185 synthesized drugs. Poverty, traditional beliefs and moribund health centers have driven
186 patients to use plants as the major source for treatment of various ailments [16].

187 The analytical result of qualitative phytochemical analysis of *F. platyphylla* showed the
188 presence of tannins, saponins, flavonoids, terpenoids, steriods, anthroquinones and phenol,
189 these findings agrees with the previous studies on the phytochemical constituents of

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190 *F.platyphylla* [17, 18]. The observed antimalaria activity of FLE in this study may be
191 attributed to the presence of saponin. These compounds have been previously shown to be
192 responsible for the antimalaria activities in many plants. [19].
193 The determination of percentage inhibition of parasitemia has been noted to be a reliable
194 parameter for assessment of antimalaria effect of a test compound [20]. With respect to the
195 curative test, FLE exerted significantly suppressive effects in mice treated with 400mg/kgbw
196 and 600mg/kgbw (Table 2.0). This effect was however lower in group that received a lower
197 dose while we observed a daily increase in parasitemia in the negative control group on day
198 7. The observed significant suppressive effects of FLE against *P. berghei* in mice is a
199 confirmation of an earlier report in which stem bark ethanolic extracts of *F. platyphylla*
200 significantly inhibited *Plasmodium berghei*, *invitro,* in mice [21]. Only one of the mice in the
201 group administered 600mg/kgbw of petroleum ether leaf extract survived up to 28 days,
202 when compared with the experimental animal in the artesunate-treated group, where two (2)
203 of the animals survived beyond 28 days.
204 PCV is a widely known index of anemia [22], FLE was able to prevent a drastic reduction in
205 PCV in infected mice when compare to infected untreated experimental control, thus,
206 showing its efficacy in ameliorating malaria-induced anemia. This was consistent with the
207 marked decrease in parasite load observed in the cause of infection in the groups of mice
208 treated with the 400mg/kg body weight and 600mg/kg body weight doses of FLE. The
209 increase in PCV of extract-treated groups, as well as artesunate-treated groups, may be as
210 a result of clearance of the parasite from circulation thereby enabling the cells to gradually
211 divide and replenish the blood [23].
212 In the prophylactic study, FLE significantly at ($p<0.05$) exerted a dose dependent reduction
213 in the parasitemia level in the extract-treated groups while the standard drug artesunate has
214 the highest chemo-suppressive effect. Although the result from the antimalaria study of the
215 crude petroleum ether extract of *F. platyphylla* suggest that the extract has more curative
216 effect, than prophylactic effect as evident from the percentage prophylaxis (Table 5.0). This

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217 low activity may be due to rapid hepatic clearance of the active component of the plant
218 extract and so parasite clearance may not be completely cleared from the blood stream.
219 The level of packed cell volume of the mice in FLE-treated groups and that of the untreated
220 group drastically reduced 72 hours after inoculation of parasite, which may be as a result of
221 short duration of action of the extract occasioned by rapid metabolism. As a result,
222 *plasmodium* parasites which are usually localized in cell lyse the red blood cell and thus the
223 percentage packed cell volume is affected. The survival rate of mice infected with
224 *plasmodium berghei* and treated with crude pet ether leaf extract compares favourably with
225 group treated with standard artesunate.

226 **CONCLUSION**

227 The result shows that *F. platyphylla* leaf extracts exhibited some antimalaria properties as
228 claimed by local herbal traditionalist, thus justifying their long use in local malaria treatment.
229 Hence, the plant could be exploited for safe, effective and affordable antimalaria regimen.

230 **COMPETING INTEREST**

231 There are no competing interests among authors.

232

233 **CONSENT**

234 It is not applicable

235 **ETHICAL CLEARANCE**

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

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240 **REFERENCES**

241

242 1 World Health Organisation (WHO). Geneva, world malaria report, Global Malaria
243 programme. 2011.

244 2 World Health Organization, World Malaria Repor*t*. Geneva, Global Plan for
245 Insecticide Resistance Management in Malaria Vectors (GPIRM). 2011; 13.
246 3 Lamar, J., Martschinske, R., Tetreault, G., and Doud, C. Navy Medical Department
247 Pocket Guide to Malaria Prevention and Control. 2007; 1-112.
248 4 Nadembega, P., Boussim, J. I., Nikiema, J. B., Poli, F., Antognoni, F. "Medicinal
249 plants in baskoure, kourittenga province, Burkina Faso: an ethnobotanical study,"
250 *Journal of Ethnopharmacology*, 2011; vol. 133, no. 2, pp. 378–395.
251 5 Chindo, B., Joseph A., and Amos, A. Anticonvulsant properties of Saponins from
252 *Ficus platyphylla* Stem Bark. Brain Research Bulletin 2009; 78 Issues 6: 276-282.
253 6 Chindo, B. A., Amos, S., Odutola, A. A., Vongtau, H. O., Abbah, J., Wambebe, C., &
254 Gamaniel, K. S. Central nervous system activity of the methanol extract of *Ficus*
255 *platyphylla* stem bark. *Journal of Ethnopharmacological,* 2003; 85:131–137.
256 7 Jigam, A. A., Usman, T. A., Halima, A. M., Tijani, F. O. Efficacy of thonningia
257 sanguine Vahl root extract against plasmodium chabaudi, inflammation and
258 nociception in mice. *Journal of applied pharmaceutical science*, 2012; 02 (01); 47-
259 51.
260 8 Canadian Council of Animal Care (CCAC) Guidelines and Protocol Review. 1997
261 9 Kabiru, A. Y., Okolie, N. L., Muhammad, H. L., Ogbadoyi, E. O. Preliminary studies
262 on the antiplasmodial potential of aqueous and methanol extracts of *Eucalyptus*
263 *camaldulensis*. *Asian Pacific Journal of Tropical Disease*, 2012; S809-S814.
264 10 Ogbadoyi, E. O., Akinsunbo, O. A., Adama, T. Z., Okogun, J. I. In vivo trypanocidal
265 activity of *Annona senegalensis* Pers. leaf extract against *Trypanosoma brucei*
266 *brucei*. *Journal of Ethnopharmacology,* 2007; 112: 85-89.
267 11 Odebiyi, O. O., Sofowora, E. S. Antimicrobial alkaloids from a Nigerian chewing stick
268 (Fagaraz anthoxyloides). *Planta Medicals,* 1979; 36, 204–207.
269 12 Ryley, J. F., Peters, W. The antimalarial activity of some quinone esters. Annals of
270 *Tropical Medicine and Parasitology* 1970; 84, 209–222.

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271 13 Saidu, K., Onah, J., Orisadipe, A., Olusola, A., Wambebe, C., Gamaniel, K.
272 Antiplasmodial, analgesic, and anti-inflammatory activities of the aqueous extract of
273 the stem bark of *Erythrina senegalensis*. *Journal of Ethnopharmacology* 2000; 71,
274 275–280.
275 14 White, N., Nosten, F., Bjorkman, A., Marsh, K., Snow, R. W. *The Lancet,* 2004; 363:
276 1160.
277 15 Daice, J. V., Lewis, S. M. Practical Haematology, 5th Edition, Churchill-living stone,
278 Edinburgh. 1975.
279 16 Tshibangu, J. N., Chifundera, K., Kaminsky, R., Wright, A. D., Konig, GM. Screening
280 of African medicinal plants for antimalarial inhibitory activity. *Journal of*
281 *Ethnopharmacol*ogy 2002; 80: 25-35.
282 17 Ugwah- Oguejiofor, C. J., Bello, S. O., Etuk, E. U., Igbokwe, V. U., Ugwah, O. M.,
283 Okolo, R. U. Preliminary toxicity and phytochemical studies of the aqueous extract of
284 *Ficus* platyphyllain female albino rats. *International research journal of pharmacy*
285 *and pharmacology,* 2011; Vol 1 (5) pp. 086- 092.
286 18 Olugbenga, A. M., Musa, A. E., Oluwatoyin, A. H. Toxicological activity of crude
287 saponin extract of *ficus platyphylla,Asian journal of pharmaceutical and clinical*
288 *research*, 2012; Vol 5(1) pp 30-33.
289 19 Okokon, J., Ofodum, K.C., Ajibesin, K. K., Danlandi, B., Gamaneil, K. S.
290 Pharmacological screening and evaluation of antiplasmodial activity of *Croton*
291 *zambesicus* against *P. berghei* infection in mice. *Indian Journal of Pharmacology,*
292 *2005;* 37:243-246.
293 20 Salawu, O. A., Tijani, A.Y., Babayi, H., Nwaeze, A. C., Anagbogu, R. A., and
294 Agbakwuru, V. A. Anti- malaria activity of ethanolic stem bark extract of
295 *FaidherbiaAlbida (Del)* a. Chev (Mimosoidae) in mice; *Archives of applied science*
296 *research*, 2010; 2 (5): 261- 268.

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297 21 Shittu, I., Emmanuel, A., Nok, A. J. Antimalaria effect of the ethanolic stem bark
298 extracts of *Ficus platyphylla* del. *Journal of parasitological research,* 2011; 10(5),
299 1155- 1160.
300 22 Esievo, K. A. N., Saror, D. L., Ilemobade, A. A., and Hallaway, A. M. Variation in
301 erythrocytes surface and free serum sialic acids concentration during experimental
302 trypanosome vivax infection in cattle, *Research in vet sci,* 1982; 32, (1) 1-5.
303 23 Kabiru, A. Y., Abdulkadir, A., Gbodi, A. T., Bello, U. M., Makun, H. A., Amah, D. J.,
304 Ogbadoyi, E. O. Evaluation of haematological changes in plasmodium- berghei-
305 infected mice administered with aqueous extract of phyllantus amarus, *Pakistan*
306 *journal of biological science*, 2013; Vol 16 (11): 510- 516.

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