IN VIVO ANTIMALARIAL ACTIVITIES OF ETHANOLIC CRUDE EXTRACTS AND FRACTIONS OF LEAF AND ROOT OF CARPOLOBIA LUTEA

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ABSTRACT

Carpolobia lutea (leaves and root) is used traditionally as malarial remedy by the Ibibios of Niger Delta of Nigeria and Benin. . This study was aimed to investigate the antiplasmodial potentials of the crude leaf and root extracts of this plant as well as their fractions in vivo in *Plasmodium berghei berghei* -infected mice to give scientific proof to the ethnobotanical claims and correlate with the reported *in vivo* activity. The ethanolic extracts of *Carpolobia lutea* leaf (245-735mg/kg/day) and root (7-21mg/kg/day) were screened for blood plasmocidal activity against chloroquine-sensitive *Plasmodium berghei* in mice. The antimalarial activity in 4-day and curative tests was evaluated. *Carpolobia lutea* leaf extract (245-735mg/kg/day) and fractions exhibited significant ($p<0.05$-0.01) antiplasmodial activity both in 4-day early infection test and in established infection with a considerable mean survival time which was incomparable to that the standard drug, chloroquine (5mg/kg/day). The root extract (7 - 21mg/kg/day) and fractions also demonstrated a promising blood schizontocidal activity in early and established infections. These plant extracts and fractions possess considerable antiplasmodial activities which justify their use in ethnomedicine and can be exploited in the control of malaria.

Keywords: Antimalarials, Antiplasmodial, *P. berghei berghei*, *Carpolobia lutea.*

INTRODUCTION

Herbal preparations have continued to enjoy the patronage of most people in rural and urban areas of Nigeria in malaria therapy despite the availability of conventional antimalarial drugs. This trend is independent of religion, region, tribe and class; perhaps due to cultural backgrounds and economic reasons. *Carpolobia lutea* G. Don (Polygalaceae) is a shrub or small tree up to 5m high. It is widely found in tropical Africa. *C. lutea* is called ikpafum (Ibibio) and cattle stick (English). Ethnobotanically, decoction of the root is used by the Ibibios of Akwa Ibom state of Nigeria as aphrodisiac (Ajibesin *et al.,* 2008; Nwafor and Bassey, 2007) and malarial remedy. Moreso, the leaves are used for the treatment of ulcer and diarrhoea (Nwafor and Bassey, 2007) as well as malarial remedy in some part of Nigeria. The leaves are also used as febrifuge and malarial remedy in Benin (Bero *et al*.,2009). Triterpene saponins have been reported in the leaves of *Carpolobia lutea* (Mitaine-offer *et al*., 2002). The root can also be used as anti-inflammatory and anti-arthritic agents (Irvine,1961; Iwu and Anyanwu, 1982), vermifuge, facilitate childbirth and to treat sterility and headache (Burkill,1985; Mitaine-offer *et al*., 2002). The leaves have been scientifically reported to possess in vitro antiplasmodial (Bero *et al*., 2009), antiulcer and antidiarrhoeal activities (Nwafor and Bassey, 2007; Nwidu and Nwafor, 2009), while antimicrobial activity have been reported on the leaves (Ettebong and Nwafor, 2009). However, information on the in vivo antiplasmodial activities of the ethanolic leaf and root extracts of *C. lutea* in *Plasmodium berghei* infection in mice is scarce. This work was aimed to investigate the in vivo antiplasmodial potentials of *C. lutea* leaf and root extracts as well as their fractions and correlate with previous report of its *in vitro* activity.

MATERIALS AND METHODS

Plant material

The leaves and roots of the plant were collected in May, 2009 from Nung Oku in Uruan Local Government Area of Akwa Ibom State and authenticated by Dr. Margaret Bassey, a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. A voucher specimen of the plant (UUH 126) was deposited previously at Department of Botany and Ecological studies of University of Uyo, Uyo. The leaves and roots were washed and dried on laboratory table for 2 weeks and then powdered.

Preparation of extracts

The dried and powdered leaves and roots of *C. lutea* (1kg each) were exhaustively macerated separately in 70% ethanol (5L each) for 72 hours. The liquid filtrates obtained were concentrated in vacuo at 40^{o}C. The yields were 3.85% and 2.23% leaf and root extracts respectively. The extracts (2g each) were partitioned with a 50:50 mixture of distilled water and chloroform. The aqueous fractions were evaporated to dryness in a water bath at

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60°C and the chloroform fractions air-dried. The ethanolic extracts (leaf and root), the acqueous and chloroform fractions were stored at -4°C until used in a refrigerator.

Animal

Swiss albino mice (21-25g) of both sexes used for the experiments were obtained from the University of Uyo animal house, Uyo, Nigeria. The animals were housed in standard cages and acclimatized for a period of 10 days. The mice were maintained on standard pelleted diet and water *ad libitum.* Approval for the study was obtained from the Animal Ethics Committee, University of Uyo.

Microorganism

A strain of *P. berghei berghei* (ANKA) that was chloroquine -sensitive was gotten from the National Institute of Medical Research (NIMER), Lagos and was maintained by subpassage in mice.

Parasite inoculation

Each mouse used in the experiment was infected intraperitoneally with 0.2ml of infected blood containing about 1 x 10^7 *P. berghei berghei*- parasitized erythrocytes. The inoculum consisted of 5x10^7 *P. berghei berghei* - parasitized erythrocytes per ml. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations (Odetola and Basir, 1980).

Drug administration

The standard drugs (chloroquine and pyrimethamine), and extract used in the antiplasmodial study were orally administered with the aid of a stainless metallic feeding cannula.

Evaluation of antiplasmodial activity of the extract/fraction

Evaluation of suppressive activity of the extract and fractions (4-day test).

This test was used to evaluate the schizontocidal activity of the extract, fractions and chloroquine against early *P. Berghei berghei* infection in mice. This was done as described by Knight and Peters (1980). On the first day (D_o), the seventy-two mice were infected with the parasite and randomly divided into various groups (n=6). These were administered with the extract/fraction and chloroquine. The mice in group 1 were administered with 245mg/kg, group 2, 490mg/kg and group 3, 735mg/kg of the crude leaf extract, groups 4 and 5 were administered with the 490mg/kg of the acqueous and chloroform fractions of the leaf respectively. While group 6 was administered with 7mg/kg, group 7, 14mg/kg and group 8, 21mg/kg of the root extract. Groups 9 and 10 recieved 14mg/kg of the acqueous and chloroform fractions of the root respectively. Chloroquine was given to the positive control group (group 11) and 10ml/kg of distilled water to negative control group (Group 12) for four consecutive days ($D_0 - D_3$) between 8am and 9am. On the fifth day (D_4), thin blood film was made from tail blood. The film was then stained with Leishman's stain to reveal parasitized erythocytes out of 200 in a random field of the microscope. The average percentage suppression of parasitaemia was calculated in comparison with the controls as follows:

$$\frac{\text{Average \% parasitaemia in negative control} - \text{Aaverage \% parasitaemia in positive groups}}{\text{Average \% parasitaemia in negative control}}$$

Evaluation of curative activity of the leaf and root extracts (Rane's test)

This was used to evaluate the schizontocidal activities of the extracts and chloroquine in established infection. This was done as described by Ryley and Peters (1970). *P. berghei* was injected intraperitoneally into groups of mice (n=6) on the first day (D_o). Seventy-two hours later (D_3), the mice was divided randomly into groups of six mice each. Different doses of the leaf extract, 245mg/kg, 490mg/kg and 735mg/kg were orally administered respectively to mice in groups 1-3, the root extract, 7mg/kg, 14mg/kg and 21mg/kg were administered to groups 4-6 respectively. 5mg/kg/day of chloroquine (positive control) and 10ml/kg of distilled water (negative control) were respectively given to mice in Groups 7 and 8. The extract and drugs were administered once daily for 5 days. Leishman's stained thin smears were prepared from tail blood samples collected on each day of treatment to monitor parasitaemia level. The mean survival time (MST) of the mice in each treatment group was determined over a period of 29 days ($D_0 - D_{28}$).

Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using ANOVA (One-way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means were considered significant at 1% and 5% level of significance i.e. $P \leq 0.01$and 0.05.

RESULT

Suppressive test

Evaluation of suppressive activity of leaf extract during 4-day test shows that the leaf extract demonstrated a dose-dependent chemosuppresive effect at the various doses used in this study (245, 490, 735mg/kg/day) with a percentage chemosuppressions of 28.20%, 31.32% and 42.14% respectively (table 1). Acqueous and chloroform fractions of the leaf had chemosuppression of 45.40 and 49.46% respectively. Similarly, the ethanolic root extract of *C. lutea* produced a dose-dependent chemosuppresive effect at the different doses employed (7, 14 and 21 mg/kg) with a chemosuppression of 70.01%, 73.95% and 76.71% respectively. Acqueous and chloroform fractions

of the root had chemosuppression of 75.12 and 81.35% respectively. The effects of these extracts/fractions were significant ($p<0.05$-0.001) when compared to the control. The standard drug, chloroquine (5 mg/kg/day) caused 81.37% suppression (table 1).

Effect of extracts on established infection

Treatment of the *P. berghei* infected mice with the plants' extracts resulted in a daily reduction in parasitaemia in the extracts- treated groups similar to that of chloroquine-treated group and these reductions were dose- dependent, while a daily increase in parasitaemia was observed in the control group. Day 7 percentage parasitemia in the groups treated with leaf extract of *C. lutea* 245, 490 and 735 mg/kg/day were 14.3%, 12.0% and 10.4% respectively, while percentage parasitaemia of 5.0 and 33.0% were recorded for chloroquine-treated and control groups respectively (fig. 1). Root extract-treated groups had percentage parasitaemia of 15.3, 12.9 and 11.2% respectively for 7, 14 and 21mg/kg doses of extract-treated groups (fig. 2).

Mean survival time (m.s.t) of 30.0±0.00 (mean ± SEM) days was observed for chloroquine-treated groups compared to 8.66±0.21, 13.0±0.33 and 14.64±1.63 days respectively observed for the groups treated with 245, 490 and 735mg/kg of ethanolic leaf extract of *C. lutea.* The mice in the control group survived for 7.51±0.12 days only. Animals treated with 7, 14 and 21mg/kg of *C. lutea* root extract had m.s.t. value of 10.33±0.55,

Table 1: Suppressive activities of leaf and root extracts and fractions of *C. lutea* during early *P. berghei berghei* infection in mice (4- day test)

Drug/Extract	Dose (mg/kg/day)	Average % Parasitaemia	Average % Suppression
C. lutea leaf extract	245	32.02± 0.58*	28.30
	490	30.54 ± 0.15*	31.32
	735	25.66± 0.53**	42.54
Acqeous	490	24.36 ±0.23**	45.40
chloroform	490	22.55 ±0.32**	49.46
Root extract	7	13.38±0.15**	70.01
	14	11.62 ± 0.47**	73.95
	21	10.39 ± 0.24**	76.71
Acqeous	14	11.10±0.33**	75.12
chloroform	14	8.32±0.21**	81.35
Chloroquine	5	5.66± 1.11**	87.32
Distilled water (control)	0.2ml	44.62 ± 3.10	-

Data are expressed as mean ± SEM for six animals per group.*$p<0.05$, **$p<0.001$ when compared to control

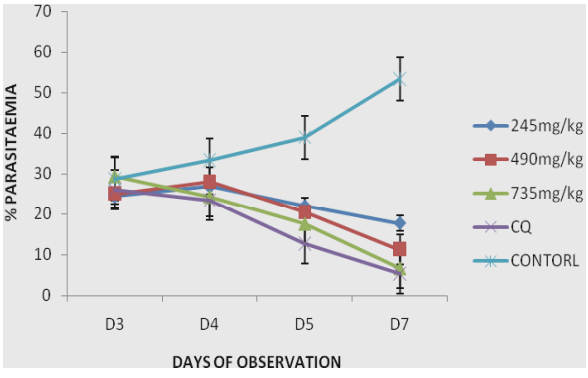

Fig. 1: Curative effect of leaf extract of *Carpolobia lutea* on established *Plasmodium berghei* infection in mice.

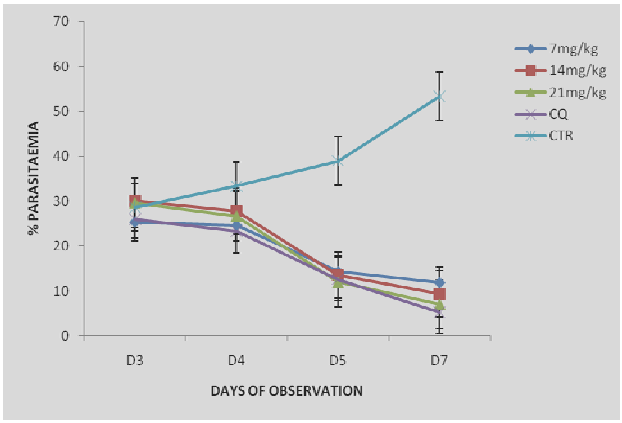

Fig. 2: Curative effect of root extract of *Carpolobia lutea* on established *Plasmodium berghei* infection in mice.

17.68±0.47 and 23.39±0.51 days respectively. The mice in the control group survived for 7.51±0.12 days only.

Table 2: Mean survival time of mice receiving the various doses of ethanolic extracts of leaf and root extracts of *C. lutea* during an established *P.berghei* infection in mice.

Drug/Extract	Dose (mg/kg/day)	Mean Survival Time (day)
C. lutea leaf extract	245	8.66± 0.21*
	490	13.0 ± 0.33*
	735	14.64 ± 1.63*
Root extract	7	10.33 ± 0.55*
	14	17.68 ± 0.47*
	21	23.39 ± 0.51*
Chloroquine	5	30.0 ± 0.00*
Distilled water (control)	0.2ml	7.51 ± 0.12

Data are expressed as mean ±SEM for six animals per group.*P<0.05 when compared to control

DISCUSSION

The ethanolic leaf and root extracts and fractions of *Carpolobia lutea* used as malarial remedy in Niger delta region of Nigeria and Benin were evaluated for antiplasmodial properties using standard models.

The results show that *C. lutea* leaf and root crude extracts and fractions possess considerable antiplasmodial activity as evident from the chemosuppressions obtained during the 4- day early infection test. The leaf and root extracts also exhibited significant curative effects during established infection incomparable to the standard drug, chloroquine (5mg/kg/day) as demonstrated in the mean survival time of the mice in the extract and chloroquine-treated groups. Although, the antimalarial activities demonstrated by the crude leaf and root extracts of *C. lutea* are low due to the crude nature of these extracts. Their activities were enhanced by further purification of these extracts as evident in the suppressive activities of leaf and root fractions. *C. lutea* leaf and root extracts have been reported above to contain some phytochemical compounds like alkaloids, terpenes (monoterpenes), triterpene saponins, tannins, anthraquinones and flavonoids(Mitaine-offer *et al.,* 2002; Nwafor and Bassey, 2007; Etebong and Nwafor, 2009). Some secondary metabolites of plants are said to have antiplasmodial activity. Among these metabolites are flavonoids and triterpenoids such as quassinoids (Philipson and Wright, 1991; Christensen and Kharazmi, 2001; Kirby *et al.,* 1989). Flavonoids are reported to chelate with nucleic acid base pairing of the parasite (Lui *et al.,* 1992) and triterpenes like quassinoids are potent protein inhibitors (Liao *et al.,* 1976). These compounds (flavonoids and triterpenoids) present in this plant extracts may in part have contributed to the plasmocidal activity of this extract and therefore explained the mechanism of antiplasmodial effect of the extract and its fractions. Moreso, the chloroform fractions of the leaf and root were found to possess enhanced activity in this study. These corroborate the results of Bero *et al*.,(2009), which the chloroform extract was found to be more active than other suggesting the possible localization of the active ingredients.

The results of the present study indicate that the extracts of the leaf and root of the *Carpolobia lutea* possess antimalarial activity. These confirm their uses in ethnomedicine in the treatment of malaria. Therefore it would be interesting if the active principles are isolated and characterized.

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