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Original Article

Phytochemical analysis and *in vivo* anti-malarial activities of aqueous extracts of *Tithonia diversifolia* and *Parquetina nigrescens* leaves in mice

Mikhail O. Nafiu*, Musbau A. Akanji, Zaynab A. Raji, Taoheed A. Abdulsalam.

Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Nigeria.

*Corresponding author: Mikail Nafiu. Email: olumikail@gmail.com; Tel.; +2348054208511.

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ABSTRACT: This study was carried out to assess the acclaimed anti-malarial potentials of aqueous extracts of leaf of *Tithonia diversifolia* (TD) and *Parquetina nigrecsens* (PN) in mice. The phytochemical constituents and *in vivo* anti-malarial activities of individual and combined of aqueous leaf extracts of *Tithonia diversifolia* (TD) and *Parquetina nigrecsens* (PN) were investigated. Fifteen albino mice were infected by intraperitoneal injection of standard inocula (5×10^6) of chloroquine sensitive *Plasmodium berghei* (NK 65). The animals were randomly divided into 5 groups of 3 mice. Group I served as the control while group II received 5mg/kg body weight per oral of chloroquine diphosphate. Groups III – V were orally treated with 150mg/kg body weight extracts of TD, TD+PN and PN respectively. Phytochemical analysis revealed the presence of saponins, alkaloids and tannins in the aqueous extracts of TD and PN. There were 100, 90, 86 and 77 percent parasite inhibition in groups treated with Chloroquine, combination of *Tithonia diversifolia* and *Parquetina nigrescens* (TD+PN), *Parquetina nigrescens* (PN*)* and *Tithonia diversifolia* (TN*)* respectively on day 5. The mean survival time (MST) for the control animals was 7 days and chloroquine 25 days, while the TD+PN, PN and TD aqueous extracts recorded 19, 18 and 11 days respectively. The results indicated that the combined aqueous (TD+PN) extracts of *Tithonia diversifolia* and *Parquetina nigrescens* produced the best antimalarial activity, which provides a justification for their use in folklore medicine and may be promising alternative anti-malarial drug.

KEYWORDS: *Tithonia diversifolia; Parquetina nigrescens*, *Plasmodium berghei,* Anti-malarial, Phytochemical

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INTRODUCTION

Malaria is today not only a disease of poverty and underdeveloped countries, but remains an important health problem globally. It is one of the six killer diseases in the world today and it has been estimated that 40% of the world's population is at risk and 500 million people suffer from the disease annually (MIM, 2004). It has also been estimated that there are as many as 300 million acute cases of malaria worldwide each year, resulting in one million deaths. Ninety percent of these deaths occur in Sub-Saharan Africa and most victims are children aged less than five years (WHO, 2004).

In Nigeria, malaria is a major public health problem where it accounts for more cases and deaths than any other country in the world. Malaria is a risk for 97% of Nigeria's population. The remaining 3% of the population live in the malaria free highlands. There are an estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria. This compares with 215,000 deaths per year in Nigeria from HIV/AIDS. Malaria also contributes to an estimated 11% of maternal mortality (Nigeria malaria fact sheet, 2011).

Despite advances in modern medicine, malaria remains a disease which is difficult to be eradicated and is therefore a major health problem, for one main reason: emergence of

multidrug-resistance strains of *Plasmodium falciparum* and rapid spread of vector mosquito resistance to insecticides (Coker *et al*., 2000, Masaba, 2000). Hence, there is an urgent need to find alternative therapies that are effective against resistance in malaria disease.

Plant sources as anti-malarial agents has gain a lot of interests since the discovery of artemisinin, a compound found to be very active against drug resistant malaria parasites, from herb plant *Artemisia annua* (Klaymann, 1985). Nigeria has a huge biodiversity and some plants have been identified to possess medicinal values (WHO, 1987). Screening of plants for anti-malarial properties has not been fully explored and therefore creates the need for more investigations.

It is claimed that *Tithonia diversifolia* and *Parquetinas nigrescens* are used in the management of malaria in folk medicine in South-western Nigeria. *T. diversifolia* has been reported to possess anti-plasmodial activity (Ajaiyeoba *et al*., 2006; Goffin *et al*., 2002); anti-inflammatory and analgesic activities (Owoyele *et al*., 2004); bile, kidney, urinary and venereal diseases, testicular inflammation, frigidity, sterility, heavy menstruation, rheumatism and arthritis, upper respiratory tract infections, ranging from cough to tuberculosis, intestinal worms and schistosomiasis, cancer chemo-preventive activity (Jian-Qiao *et al*., 2002); cytotoxic properties (Wu *et al*., 2001) and antimicrobial activity (Ogundare, 2007; Singleton, 1999). *P. nigrescens* has been shown to possess haematological properties (Agbor and Odetola, 2001, 2005), cardiotonic and sympathomimetic effects (Datte *et al*., 1999; Datte and Ziegler, 2001) and uterotonic effects (Datte *et al*., 1996).

Although quite a number of scientific investigations have been undertaken to validate the local uses of these plants, there seems to be no scientific report on the analgesic, antipyretic and anti-malarial activities of *P. nigrescens* leaves. We therefore put forward the need to further evaluate the acclaimed efficacy of *P. nigrescens* in folk medicine against malaria. In this study, we compared the phytochemical constituents, individual and synergistic anti-malarial potential of the aqueous extracts of *Tithonia diversifolia* and *Parquetina nigrescens* leaves against *P. berghei* established malarial infection *in vivo* using Swiss albino mice.

MATERIALS AND METHODS

Drug and Reagents: Giemsa stain (stock solution) and Immersion oil were products of Ranjo Medix laboratories, and Zytaco Nigeria Limited respectively. Haemocytometer and Chloroquine phosphate were products of Super Main field, Germany and May and Baker respectively.

Plant Materials: Leaves of *Tithonia diversifolia* and *Parquetina nigrescens* plants were collected in November 2013 from Oke-odo, Tanke area and the premises of the University of Ilorin, Ilorin. The plants were authenticated at the Plant Biology Department, University of Ilorin, Ilorin, Nigeria, and Voucher specimens (UIH/594 and UIH/876) were prepared and deposited in the University herbarium.

Preparation of Plant Extracts: The leaves of *Tithonia diversifolia* and *Parquetina nigrescens* were rinsed with distilled water and air-dried in the laboratory at room temperature. The dried materials were pulverized using electric blender (Model HR 1727, Holland). 50g each of the powdered plant samples were extracted in 750 ml of distilled water for 24 hours. The resultant suspension of each was percolated through a musilin cloth and then filtered with Whatman No.1 (70 mm) filter paper. The filtrates were then concentrated under reduced pressure at 40^{o}C using a vacuum dryer under sterile conditions. The dried extracts were stored in a dessicator until used.

Experimental Animals: Fifteen Swiss albino mice with a mean weight of 20.27 g were obtained from the central animal house of the University of Ibadan, Ibadan, Nigeria. The mice were housed in clean, ventilated plastic cages, fed with commercial rat growers pellet (Bendel Feed Ltd, Ewu, Nigeria) and allowed free access to tap water. They were acclimatized to the animal house condition for two weeks before the commencement of the experiment. All animal treatments were in accordance with international ethical guidelines and the National Institute of Health (NIH publication 85-23, 1985) for laboratory animal care and use.

Malaria Parasite: *Plasmodium berghei* (chloroquine sensitive NK 65) was obtained from the Institute for Advanced Medical Research and Training (IMRAT), College of Medicine, University of Ibadan, Ibadan, Nigeria.

Phytochemical Screening: The aqueous extracts of *Tithonia diversifolia* and *Parquetina nigrescens* leaves were subjected each to phytochemical analysis, using standard procedures as described by Sofowora (1993), Harborne (1973) and Trease and Evans (1989).

Inoculation of experimental Mice: Albino mice were infected by intraperitoneal injection of standard inoculums (0.2ml of 5 × 10^{6} infected erythrocytes) from a single donor mouse previously infected with *Plasmodium berghei* (29 percent parasitemia)

Study of the course of infection and antimalarial Activity: The course of infection following intraperitoneal inoculation in mice was studied in each experimental mouse that received standard inoculums of 0.2ml of 5 × 10^{6} infected erythrocytes. Thin blood films were prepared from the tail vein of infected mice, fixed with methanol and stained with 10% Giemsa stain for 30 min and then rinsed with tap water. Parasitaemia was monitored daily using oil immersion objective (×100) of a light

microscope. *In vivo* anti-malarial activity against *P. berghei* infection in mice was determined using Rane's test as described by Elufioye and Agbedahunsi (2004). The Rane test relies on the ability of a standard inoculum of *P. berghei* to kill the recipient mouse within 12 days of inoculation. Extension of survival beyond 12 days is regarded as activity (Peter and Anantoli, 1998).

Experimental design: The animals were randomly divided into five groups of 3 mice each, after the establishment of parasitaemia, 72 hours post infection. Group I (control) was left untreated but received 0.3 ml of distilled water orally once daily for four consecutive days. Group II were administered orally 0.3 ml of 5mg/kg body weight chloroquine diphosphate while groups III–V were treated with the same volume of 150mg/kg b.w per oral of the extracts of *Tithonia diversifolia* (TD), *Tithonia diversifolia* plus *Parquetina nigrescens* (TDPN) and *Parquetina nigrescens* (PN) respectively for the same period. Parasitaemia level were monitored during and after the treatment.

Estimation of Percentage Parasitaemia:

Percentage parasitaemia was estimated at the end of the observational period of 28 days using the following formula (WHO, 2005).

$$\text{Percentage Parasitaemia(\%)} = \frac{\text{Parasitized RBC}}{\text{Parasitized RBC} + \text{non-Parasitized RBC}} \times 100$$

RBC denotes Red Blood Cell

Estimation of Percentage Suppression:

The percentage suppression of parasite multiplication per days was calculated using the formula (Dikasso *et al*., 2006).

$$\text{Percentage Suppression (\%)} = \frac{\text{Parasitaemia in Negative Control} - \text{Parasitaemia in Study Group}}{\text{Parasitaemia in Negative Control}} \times 100$$

Estimation of Mean Survival Time (MST):

The number of death daily was recorded for the animals in each group for the experimental period. The Mean Survival Time (MST), was calculated using the formula:

$$\text{MST} = \frac{\text{Sum of survival of all animals in each group (in days)}}{\text{Total number of animals in each group}}$$

RESULTS

The phytochemical screening of the aqueous extracts of both *Tithonia diversifolia* (TD) and *Parquetina nigrescens* (PN) revealed the presence of saponins, alkaloids and tannins. Flavonoids and phlobatannins were also detected in TD and cardiacglycoside in PN extracts respectively (Table 1).

Table 1: Phytochemical constituents of the aqueous leaves extracts of *T. diversifolia* and *P. Nigrescens*

Phytochemicals	*T. diversifolia*	*P. nigrescens*
Saponins	+	+
Alkaloids	+	+
Phenolics	-	-
Steroids	-	-
Anthraquinones	-	-
Tannins	+	-
Triterpenes	-	-
Flavonoids	+	-
Phlobatannins	+	-
Cardiac glycosides	-	+
Cardenolides/Dienolides	-	-

Key: **+** = Detected
- = Not detected

The chloroquine treated group had parasite clearance on day 5 with 100 percent parasite inhibition while the individual extracts of TD and PN treated groups reduced the parasitaemia level when compared with the untreated group with 77 and 86 percent parasite inhibition respectively (Figure 1 and Table 2). The combined extracts of TD and PN treated group also reduced the produced 90 percent parasite inhibitioin and significant reduction in parasitaemia when compared with the control.

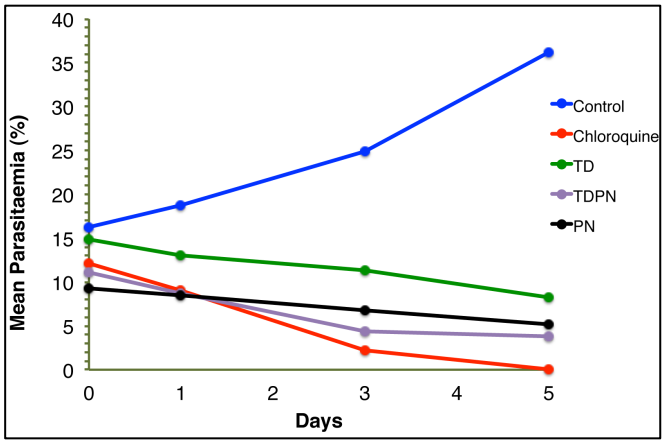

Figure 1: Parasitaemia Changes following Treatment with aqueous leaf extracts of TD, TDPN and PN.

TD = *Tithonia diversifolia*, PN = *Parquetina nigrescens*, TDPN = *Tithonia diversifolia* + *Parquetina nigrescens.*

Table 2: Effect of aqueous leaf extract of *T. diversifolia*, combined *T. Diversifolia* and *P. nigrescens* and *P. nigrescens* on established malarial infection in mice.

Treatment	% Parasitaemia and Suppression per day							
	Day 0		Day 1		Day 3		Day 5	
	% P	% S	% P	% S	% P	% S	% P	% S
Control	16.27 ± 0.27	0.0	18.73 ± 1.29	0.0	25.31 ± 1.34	0.0	36.16 ± 0.0	0.0
Chloroquine	12.10 ± 0.56	25.7	9.01 ± 0.43	51.9	2.21 ± 0.16	91.3	0.00 ± 0.0	100.0
150 mg/kg b.w TD	14.89 ± 0.56	8.4	13.01 ± 0.81	30.5	11.28 ± 0.26	55.4	8.21 ± 0.40	77.3
150 mg/kg b.w TDPN	11.09 ± 0.23	31.8	8.73 ± 0.35	53.4	4.39 ± 0.72	82.7	3.79 ± 0.27	89.5
150 mg/kg b.w PN	9.25 ± 0.59	43.2	8.47 ± 0.31	55.0	6.77 ± 0.60	73.3	5.18 ± 0.42	85.7

Data are mean of 3 replicates ± SEM % P = percentage parasitaemia, % S = percentage suppression, TD = *Tithonia diversifolia*, PN = *Parquetina nigrescens*, TDPN = *Tithonia diversifolia* + *Parquetina nigrescens*

Table 3 shows the mean survival time (MST), for the animals in each group. The least MST of 7 days was recorded for the control group that was left untreated, TD and PN extracts treated groups recorded MST of 11 and 18 days while the combined extracts and chloroquine treated groups had MST of 19 and 25 days respectively.

DISCUSSION

Plants have always been considered to be a possible alternative and rich source of new drugs. Majority of the anti-malarial drugs in use today such as, quinine and artemisinin were either obtained directly from plants or developed using chemical structures of plant-derived compounds as templates (Basco *et al.,* 1994). Results from this study revealed the presence of phytochemicals; saponins, alkaloids, tannins and flavoniods in trace amount, which are indicative of the anti-malarial properties of the plants. According to Stray (1998), pure isolated alkaloids and its synthetic derivatives have been used as basic medicinal agent because of their analgesic, anti-plasmodic and bacterial properties. Tannins have also been reported to exhibit potential antiviral, antibacterial and anti-parasitic effects (Kolodziej and Kiderlen, 2005; Lu *et al*., 2004; Akiyama *et al*., 2001). In addition to saponins, alkaloids and tannins, flavonoids and phlobatannin were both detected in *Tithonia diversifolia*, and cardiac glycosides in *Parquetina nigrescens* (Table 1).

Treatment with chloroquine at 5 mg/kg body weight showed total parasite clearance indicating that the malaria model used *P. berghei*, is sensitive to chloroquine. This conforms to other study; hence, it was an appropriate parasite for the experiment (Muregi *et al.,* 2003). However, administration of the plant extracts at a dose of 150 mg/kg body weight once daily for 4 consecutive days against established malarial infection in this study has revealed that the two plant extracts exhibited significant level of anti-malarial activity.

The combination of the aqueous extracts of *T. diversifolia* and *P. nigrescens* exhibited the most potent anti-malarial activity and 90% parasite inhibition at the same dose (Table 2). The mean survival time (MST), recorded for this group was 19 days (Table 3). This could be due to the synergistic action of different phytochemicals present in both plants on the inhibition of parasite multiplication as described by Tyler (1999). In this respect, Kaufman et al. (1999) extensively documented how synergistic interactions underline the effectiveness of a number of phytomedicines. Okeola *et al*. (2011) reported that crude methanolic extract of *Nigella sativa* suppress *P. yoelli* infection *in vivo* by 94%. Similarly, extracts from the roots and aerial part of *Asparagus africanus* were observed to inhibit *P. berghei* parasitaemia in mice by 46.1% and 40.7% respectively (Dikasso *et al*., 2006). These results show that the combined effects of the extracts compared favourably with chloroquine, a reference anti-malarial drug.

T. diversifolia leaf extract at a dose of 150 mg/kg body weight exhibited less anti-malarial activity and parasite inhibition compared with what was observed in similar dose of *P. nigrescens* treated group (Table 2). This may be an indication of its weak potency as an anti-malarial agent. Goffin et al. (2002) reported that sequiterpene lactones taginin C isolated from *T. diversifolia* is an active component against *Plasmodium* .Our study thus supports the earlier investigations on the anti-malarial activity of *T. diversifolia* (Goffin *et al*., 2002; Bidla *et al*., 2004; Elufioye and Agbedahunsi, 2004).

Table 3: Mean survival time (MST) of mice in the different treatment groups.

Groups	Mean Survival Time (days)
Control	7
150 mg/kg b.w TD	11
150 mg/kg b.w PN	18
150mg/kg b.w TDPN	19
Chloroquine	25

TD = *Tithonia diversifolia*, PN = *Parquetina nigrescens*, TDPN = *Tithonia diversifolia* + *Parquetina nigrescens*

The *P. nigrescens* aqueous extract displayed a very good activity against the *P. berghei* malarial parasite *in vivo*. A significant reduction in the percentage parasitaemia with 86% chemo-suppression on day 5 and 18 days MST was recorded (Table 2 and 3). So far, there is no report on the *in vivo* anti-malarial activity of *P. nigrescens* aqueous leaf extract. *Eurycoma longifolia* and *Ardisia crispa* were both reported as potent anti-malarial agent with 80% parasitaemia inhibiton at 10mg/kg body weight (Bassir *et al*., 2012).

The mean survival time (MST) of mice above 12 days is an indication that the extracts possess anti-malarial activity when used separately and in combination. It has been reported that the survival of experimental animals beyond 12 days of malarial infection is regarded as activity (Ajaiyeoba *et al*., 2006; Abosi and Raseroka, 2003; Obih and Makinde, 1985; Peters, 1980).

We conclude that this study has provided scientific evidence for the anti-malarial activities of *Parquetina nigrescens and* its combination with *Tithonia diversifolia* as anti-malarial combination therapy. Further studies is however, suggested to elucidate the active agent(s) responsible for the anti-malarial activities and the possible mechanism of action of the two plant extracts.

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