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Research Article

Effect of ethanolic extract of *Olea europaea* on *Plasmodium falciparum* infected mice

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Abstract

Malaria persists as a major health burden in Africa in spite of all efforts at prevention and control. In traditional medicine *Olea europaea* root, bark or leaf decoction is used to treat malaria and other fevers. This study aimed at analyzing *Olea europaea* to establish its scientific basis in treating malaria. 30 Swiss albino mice were divided into 6 groups of 5 rats each. Group A Control (no induction, no treatment); group B experimental control (0.2 ml of O^+ human parasitized blood of *Plasmodium falciparum* induced but no treatment administered). Group C, D, and E were induced with *Plasmodium falciparum* intraperitoneally and treated with 40, 80 and 120 mg/kg body weight (bwt) ethanolic extract of *Olea europaea* respectively. Malaria parasite was confirmed 72 hours after induction using a compound microscope. Group F was induced with the O^+ human parasitized (*Plasmodium falciparum*) blood and treated with a reference drug (Artesunate 50 mg/kg). Phytochemical screening of *Olea europaea* leaf plant extracts revealed high content of alkaloids, saponin, flavonoids, and cardiac glycosides. Proximate analysis of the *Olea europaea* leaf extract revealed 48.80% carbohydrate content, 6.60% moisture content, 12.92% crude fiber, 24.08% crude fat, 7.60% ash content. The 40 mg/kg, 80 mg/kg and 120 mg/kg bwt of the extract were able to inhibit 33.4%, 59.0% and 79.1% of parasitemia respectively. All infected animals had reduced packed cell volume following infection and increased with treatment. The dose 120 mg/kg bwt of *Olea europaea* extract was more potent compared to anti-malarial drug (artesunate 50 mg/kg)

Keywords: Parasitaemia, *Plasmodium falciparum,* blood, *Olea europaea,* artesunate.

1. Introduction

Malaria is an infectious disease caused by Plasmodium which infects red blood cells[1]. It is the most significant public health problem in Nigeria. According to World Health Organisation, there are four species of malaria parasites namely *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium falciparum and Plasmodium malariae*[2,3]. Around the world, malaria is becoming more resistant to a number of drugs, thus a need for remedies. Thus, there have been continuous search for novel and more effective antimalarial compounds especially from medicinal plants extracts which can combat malaria infection[4,5,6].

Olive tree (*Olea europaea*) commonly known as African wild olive, is one of the most important fruit trees in Mediterranean countries and account for almost 98% of the world crop. This demonstrates the great economic and social importance of this crop and the possible benefits to be derived from utilization of any of its byproducts[5,7,8]. *Olea europaea* is widely studied for its alimentary use (the fruits and the oil are important components in the daily diet of a large part of the world's population), whereas the leaves are important for their secondary metabolites such as the secoiridoid compounds oleacein and oleuropein.[9,10,11,12] In traditional medicine *Olea europaea* root, bark or leaf decoction is used to treat malaria and other fevers[9,13]. Bark decoction helps in the healing of skin rashes and other irritations, and is also used as a laxative and as an anthelmintic (i.e., to expel parasites), especially as a remedy for tapeworms[14]. Owing to increased resistance of malaria to orthodox antimalarial drugs and the renewed interest in plant drugs, this study is aimed to analyze *Olea europaea* to establish the scientific basis for their use in treating malaria. Also, antiplasmodial activity of extracts of the plant was tested in artesunate sensitive Plasmodium falciparum treated mice.

2.0 Material and Methods

2.1 Materials

2.1.1 Collection and preparation of *Olea europaea*

Fresh leaves of *Olea europaea* were collected from Hawan kibo Southern part of Jos, Nigeria, identify and authenticated by Mrs Tarfa D. Florence, The H.O.D, Medicinal Chemistry and Quality Control, National Institute of Pharmaceutical Research and Development, Abuja, Nigeria. Voucher specimen number of the plant NIPRD/02/014. These leaves were dried for fifteen days at ambient laboratory temperature (20-28°C). It was milled to a fine powder using commercial blender (Blendtec), and stored in the dark at laboratory temperature in closed containers until required.

2.1.2 Extraction of *Olea europaea* leaves

The air-dried defatted powdered plant material (500g) was extracted with 500 ml ethanol (C_2H_5OH) using Soxhlet apparatus for 15 h. The residue was dried over night and then extracted with 250 ml water (H_2O) using shaking water bath at 70°C for 2 h. The extraction with water was repeated three times. The water filtrates were mixed together. The ethanol and water extracts were filtered and evaporated by using a rotary evaporator (Rotary evaporator RE-52A, SHANGAI YA RONG BIOCHEMISTRY INSTRUMENT FACTORY) and freeze dryer to give the crude-dried extract. The dried extracts were stored at −20°C until used.

2.1.3 Animals

Experimental mice were purchased from Benue State University. The animals were housed in steel cages and kept at room temperature. The animals had no history of drug consumption that is; they had not been used for any investigation. The animals were allowed to acclamatized for 14 days prior to the commencement of the experiment and were given water and pelleted standard laboratory feeds. All authors hereby declare that "Principles of laboratory animal care" (NIH Publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee of the Bingham University, Karu, Nigeria, Animal ethical committee approval number

BHU/02NRP. All authors hereby declare that all experiments have been examined and approved by appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

2.2. Methods

2.2.1 Experimental design

Thirty Swiss albino mice of body weight ranging from 20 – 30 g, were divided into different groups of six animals. Groups A, B, C, D, E and F. Group A served as normal control (no induction, no treatment). Group B served as experimental control (0.2 ml O^+ human parasitized blood induced but no treatment administered). Group C, D, and E were all induced with 0.2 ml O^+ human parasitized blood (*Plasmodium falciparum*) intraperitoneally, and treated with 40, 80 and 120 mg/kg body weight (bwt) ethanolic extract of the leaves of *Olea europaea*. Malaria parasite was confirmed 72 hours after induction. Treatment continued for 3days. Group F served as standard control, the animals in this group were induced with the 0.2ml O^+ human parasitized (*Plasmodium falciparum*) blood and treated with a reference drug (50 mg/kg Artesunate).

2.2.2 Proximate analysis

Moisture content, Crude fiber, crude fat, ash content and carbohydrate content were determined using (AOAC) 1990 method.[15]

2.2.3 Ash content determination

5g of the crude extracts were put into a weighed crucible and incinerated in an oven at 300^0C for 3 days. The weight after incineration was taken.

2.2.4 Moisture content

In determining the moisture content, exactly 10g of the extract was dried at 103^0C to a known weight in an oven.

2.2.5 Crude fat

Crude fat was determined by defatting a known weight of the seed sample in 25 ml petroleum ether for 30 minutes. The supernatant was decanted into weighted crucibles and oven dried for 45minutes at 103^0C.

2.3 Phytochemical screening

The ethanol extract of *Olea europea* leaves were screened for presence of phytochemical compounds as described by Evans, 1996.[16]

The ethanol extract of the plant material was subjected to preliminary phytochemical analysis using the following methods:

2.3.1 Test for alkaloids

0.1ml of the ethanol extract was added to few drops of dragendroff's reagent. An orange colour developed indicating the presence of alkaloid.

2.3.2 Test for flavonoids

0.1ml of the ethanol extract was heated with 10ml of ethyl acetate over a steam bath for 3min. The mixture was filtered and 4L of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration was observed

2.3.3 Test for Tannins (ferric chloride test)

1.0ml of the ethanol extract was diluted with 4.0ml distilled water (in ratio 1:4) and few drops of 10% ferric chloride solution were added. Blue or green precipitate or coloration formed indicates the presence of tannins.

2.3.4 Test for saponins

To 0.5 ml distilled water is added and shaken vigorously for 2 mins. Then few drops of olive oil were added. Formation of emulsion indicated the presence of saponins.

2.3.5 Test for steroids

2 ml of acetic anhydride was added to 0.5 ml of the ethanol extract with 2ml H_2SO_4. The colour changed from violet to blue indicating the presence of steroids.

2.3.6 Test for Terpenoids (Salkowaski test)

0.5 ml of the ethanol extract was mixed in 2ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of Terpenoids.

2.3.7 Test for cardiac glycosides (keller-killani test)

1ml of extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the tin layer.

2.4 Induction of Malaria Parasite

0.2 ml of parasitized human blood of blood group O^+ obtained was injected into the mouse of about 30.75g in weight and was observed for 3 days (72 hours) for general symptoms of malaria.

2.5 Haematocrit determination

Packed cell volume (PCV) was determined with the aid of micro-hematocrit centrifuge. A small volume of blood was collected from the tail of the mice into heparinized capillary tube and then placed in the hematocrit centrifuge and spun for about 5minutes. The packed cell volume was determined by reading the value directly on a microhematocrit reader which gives the value in percentage.

2.5 Determination of parasitemia level

The parasitemia level in experimental animals was determined haematologically by preparing a thick or a thin film of the blood on a glass slide and viewed in oil immersion under a microscope at x100 objectives. The parasitemia level was determined once daily and was closely monitored for three days.

2.6 Statistical Analysis

The mean values were compared with one-way analysis of variance (ANOVA) using SPSS v16.0. Duncan test was used as the *post hoc* test. A $P<0.05$ was considered statistically significant.

3.0 Results

The results of the phytochemical screening of the plant samples are shown in Table 1 below. The preliminary phytochemical test reveals; the most active constituents in *Olea europaea* leaf are flavonoids and glycosides. Table 2 revealed the proximate analysis of the plant extract. Doses of ethanolic extract of *Olea europaea* leaf was varied to know the effect of the extract on the level of packed cell volume in mice infected with malaria parasite, this can be seen in Table 3. The results of parasitaemia level were presented in Tables 4, with variation in the doses of the extract administered to different groups.

Table 1: Phytochemical Screening of *Olea europaea* leaf extract

Phytochemicals	Leaf extract
Alkaloids	+++
Flavonoids	+++
Tannins	++
Saponins	+++
Terpenoids	++
Sterols and steroids	++
Glycosides	+++

Legend: +++ = High, ++ = Moderate; + = Low; ± = Inconclusive.

Table 2: Result of Proximate analysis of *Olea europaea* leaf extract

Parameters	Composition (%)
Moisture content	48.80
Carbohydrate content	6.60
Crude fat	24.08
Crude fiber	30.92
Ash content	7.60

Table 3: Effect of the varying doses of ethanolic extract of *Olea europaea* leaf on the Level of PCV in mice infected with malaria parasite in percentage (%)

Group/Treatment	Post Infection Days		
	Day 1 (% PCV)	Day 2 (% PCV)	Day 3 (% PCV)
A (Baseline)	56	55	54
B (Infected+normal saline)	47	32	18
C (Infected+40mg/kg extract)	37	40	43
D (Infected+80 mg/kg extract)	36	42	49
E (Infected+120 mg/kg extract)	42	49	52
F (Infected+50 mg/kg artesunate)	47	50	54

Table 4: Effect of varying doses of *Olea europaea* leaf extract on parasitemia level

Group/Treatment	Post Infection days		
	Day 1 (mg/kg)	Day 2 (mg/kg)	Day 3 (mg/kg)
A (Baseline)	-	-	-
B (Infected+normal saline)	24 ± 3.16^{a}	33 ± 3.16^{b}	39 ± 2.55^{c}
C (Infected+40mg/kg extract)	27 ± 3.08^{a}	21 ± 2.55^{b}	16 ± 2.24^{c}
D (Infected+80 mg/kg extract)	19 ± 4.58^{a}	17 ± 3.54^{a}	12 ± 3.00^{b}
E (Infected+120 mg/kg extract)	17 ± 5.20^{a}	13 ± 2.55^{b}	9 ± 2.35^{c}
F (Infected+50 mg/kg artesunate)	18 ± 1.22^{a}	15 ± 2.35^{b}	5 ± 2.55^{c}

Results of parasitemia level are presented as mean ± SD. Values carrying superscripts different from the control for each parameter across the column are significantly different ($p<0.05$).

Fig 1: Effects of varying doses of ethanolic leaf extract of *Olea europaea* (mg/kg body weight) on parasitemia count

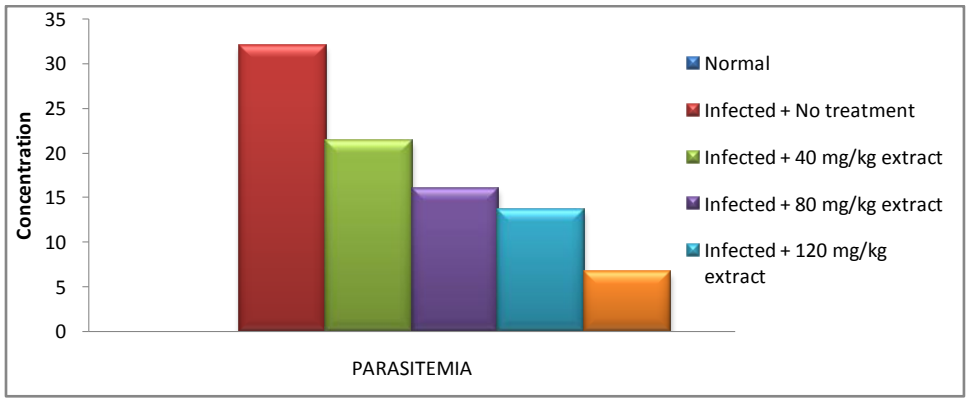


Fig 2: Effects of varying doses of ethanolic leaf extract of *Olea europaea* (mg/kg body weight) on % inhibition

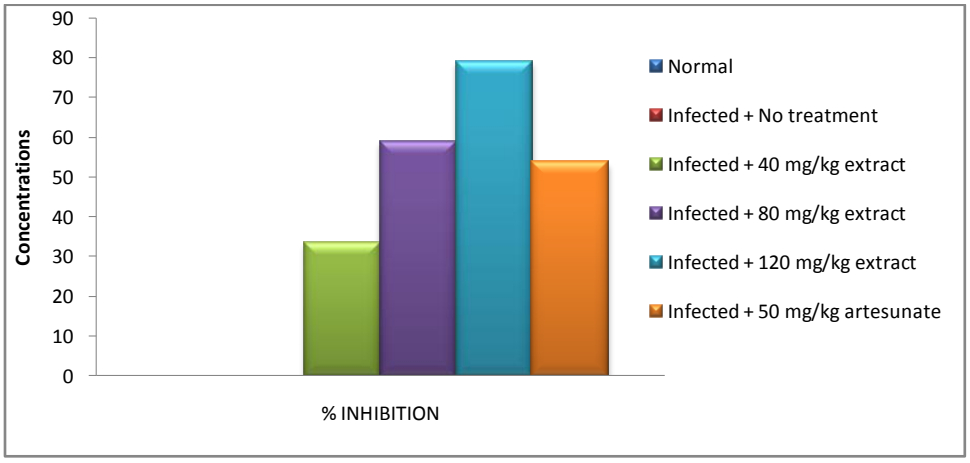
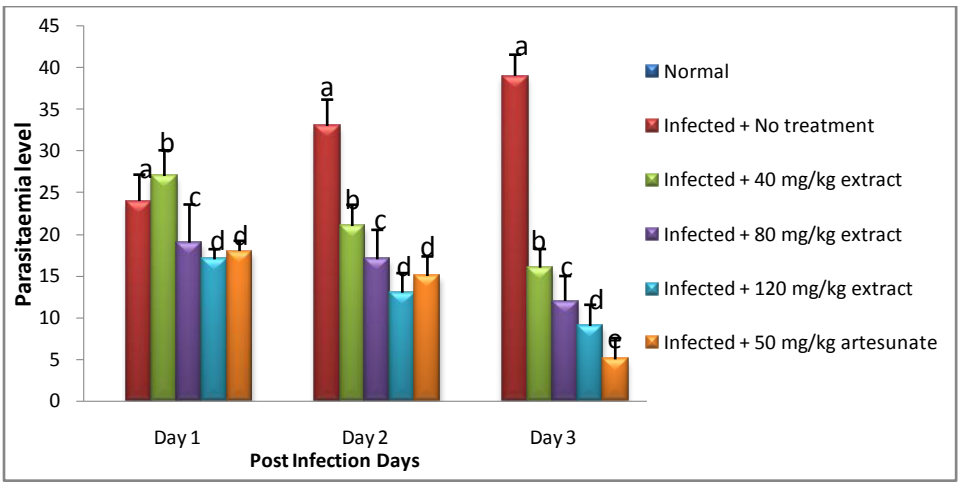


Fig 3: Effect of varying doses of *Olea europaea* leaf extract on parasitemia level



4.0 Discussion

The results of the phytochemical screening of the plant samples are shown in Table 1. In our present study, the plant extracts may function as antioxidant due to their bioactive principles. Phytochemically, the plant samples are quite rich, containing alkaloids, saponins, flavonoids, and cardiac glycosides. Seventy-five percent of the test plants contained alkaloidsThe concentration of tannins was moderate in *Olea europaea*, and it has been reported that tannins may have antiplasmodial activity[19]. The cardiac glycosides are used for treating heart problems that may result from severe malaria attack[21,22]. The plant extracts may contain constituents that can inhibit Plasmodium growth by blocking the parasite choline intracellular transport necessary for the biosynthesis of the phosphatidylcholines which are essential molecules for the Plasmodium[23]. Therefore, phytochemical principles present in the plant extracts may have a proportional link with antiplasmodial activity[24,6].

Table 2 shows the proximate composition of the plant sample. Ethanolic extract of *Olea europaea* leaf contains substantial quantity of Crude fiber 30.92% and ash and it is recommended as a source of protein and energy supplement. Ash (mineral) content in *Olea europaea* implies that it is very nourishing and suitable for consumption[25]. It was reported that ethanolic extract of *Olea europea* leaf has crude fiber 12.92% [26] its high quantity of crude fiber in this study, may suggest apparent digestibility of nutrients and suitable supplement in food in addition to antimalarial activity. The proximate analysis also revealed the moisture content of 6.60%, which is within the acceptable limits of about 6 to 15% for most vegetable drugs. In addition to the broad range of biological activities previously reported for *Olea europaea* leaf extract, this extract displays also a significant inhibitory activity on the erythrocytic cycle of *P. falciparum*. Parasitemia was observed to steadily decrease as treatment progressed as shown in Table 4, Fig 1 and Fig 3. The effect was dose dependent as 120mg/kg was found to be more potent than the standard drug (artesunate 50mg/kg).120 mg/kg of extract decreased parasitemia by a maximum of 79.1%. The 40mg/kg and 80mg/kg treated groups, showed some level of decrease but not as efficient as the 120mg/kg treated group. 40mg/kg and 80mg/kg of the extract were able to inhibit 33.4% and 59.0% of the parasitemia respectively from the first day of treatment to the third day of the experiment as shown in Fig 2. 80mg/kg body weight of extract 59.0% has insignificantly higher value than the standard control drug 53.7% ($P<0.05$). In the negative control group, this was the infected untreated group, parasitemia rose till the end of the experiment characterized by a fall in the level of PCV value which dropped below the normal range. In this experiment, infected animals had reductions in their PCV following infection but increased when treatment was administered (Table 3). The increase was dose dependent and may have resulted from the effect of treatment as there was a steady reduction in parasitemia as shown by the ethanolic extract. The antiplasmodial property of the plant extracts may be attributed to presence of some phytochemicals which might have conferred some protective/antioxidative effect against oxidative stress induced in the host parasitized red blood cells (RBCs) by the malaria parasite [27,28,29,30]. This finding suggests that the ethanolic extract of *Olea europaea* leaves has anti-plasmodial activity in mice experimentally infected with malaria parasite *(Plasmodium falciparum)* than the standard drug.

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