Assessing the influence of oils on the level of parasitemia following infection with *Plasmodium berghei* in a murine model

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Abstract

In some cultures, the consumption of oils is thought to predispose one to malaria attack. Therefore, this study sought to investigate this belief by examining the effects of some commonly consumed oils on mice infected with *Plasmodium berghei*. The oils were extracted from their respective food sources using the cold maceration method and their physicochemical properties were determined using instrumental methods. Next, a survey of the consumption patterns of oily foods among 230 adults was conducted using a questionnaire. Subsequently, forty (40) albino mice were randomly divided into 8 groups of 5 animals each, with each group receiving either the human equivalent doses (HEDs) of one oil or double that (DHED) for two weeks before infection. The control groups received distilled water. Afterwards, each experimental animal was inoculated with 1×10^7 of chloroquine-sensitive *P. berghei-infected* packed red blood cells through intraperitoneal injection. The treatment was continued once daily for one week. Thin smears stained with Giemsa were prepared from tail blood samples collected on the 4th-day post-inoculation to monitor parasitemia levels. There was a significant reduction ($p < 0.05$) in parasitaemia in animals treated with the HEDs of groundnut oil, and both the HED and DHED of coconut oil while Scumbia fish oil caused a significant reduction in parasitaemia at DHED. Coconut oil and Scumbia fish oil may possess antimalarial activities, contrary to the belief that consuming all oils may predispose one to malaria attack.

Keywords: malaria, parasitaemia, oils, susceptibility, severity

Running title: Food oils and malaria severity

Introduction

Plasmodium parasites are transmitted to humans through bites from infected female Anopheles mosquitoes, causing malaria. Of the species that can cause human malaria, *Plasmodium falciparum* and *Plasmodium vivax* are the most important (WHO, 2023). *P. falciparum* causes the most severe form of malaria, and is commonly found in Africa, while *P. vivax* is the parasite of interest in many areas beyond sub-Saharan Africa (WHO, 2023). In 2022, there were about 249 million malaria cases worldwide, with significant increases noted in Nigeria and several other countries (WHO, 2023). The WHO African Region accounted for about 94% of cases (233 million) in 2022, with children under five representing about 80% of fatalities in this region (WHO, 2023). Groups that are more vulnerable to severe malaria include young children and pregnant women as well as immunocompromised persons, such as those living with HIV/AIDS.

A higher incidence of malaria occurs in the rainy season because more favourable conditions for mosquito breeding occur (Nabatanzi *et al*., 2022). Severe malaria can lead to complications, such as anemia and nephrotic syndrome ((Buck *et al*., 2023).

Various beliefs exist about the causes of malaria and the likelihood of contracting it, as influenced by factors such as activity levels, diet, and environmental conditions (Zerdo *et al*., 2020). Inadequate nutrition can weaken the immune system, raising the risk of infections and mortality, especially in children (Gebreegziabher *et al*., 2023). However, nutrition deals more with the quality of food taken, than quantity, and excessive intake of unhealthy foods can increase susceptibility to infection (Morales *et al*., 2024). Malnourished children are at higher risk of developing complications associated with malaria (Gebreegziabher *et al*., 2023). Adequate diet, especially one that is rich in vitamins and trace elements can increase the body's ability to fight off infections like malaria (Onukogu *et al*., 2018).

In sub-Saharan Africa, coconut and palm oils are widely consumed, though they are often considered unhealthy due to their high saturated fat content (Boateng *et al*., 2016). So, it is recommended that saturated fats be consumed in limited quantities (Sacks *et al*., 2017). Groundnut (peanut) oil contains linoleic acid, whose antimalarial activity has been demonstrated in mice (Carballeira, 2007; Melariri *et al*., 2012).

Even though artemisinin-based combination therapies (ACTs) have shown a high level of effectiveness in treating malaria, therapeutic failures represent an ever-present concern. Therefore, a better understanding of the anti-malarial activities of some oily foods can offer a dietary approach for combating the malaria burden, alongside the use of ACTs.

This study aims to evaluate the potential effects of some commonly consumed food oils on parasitaemia, a common indicator of disease severity. It is hoped that dietary control approaches will become more popular as the roles of diet are better understood.

Materials and methods

Extraction of oils

Fish oil, groundnut oil, and coconut oil were extracted using the cold maceration method. For coconut oil extraction, sun-dried coconut (700 g) was ground and mixed with petroleum ether in a stoppered container. This mixture was left at room temperature for three days, with regular shaking, until the soluble components dissolved. Afterward, the mixture was strained, the wet solid (marc) was pressed, and the liquid filtered and clarified.

For fish oil, one kilogram of fresh Scumbia fish was dried in a hot air oven at 50 degrees Celsius for 48 hours. The dried fish was then placed in a stoppered container with petroleum ether and allowed to stand at room temperature for three days, with frequent agitation until the soluble matter dissolved. The mixture was strained, the damp solid (marc) pressed, and the combined liquids were filtered and clarified.

In the extraction of groundnut oil, 750 grams of sun-dried groundnuts was blended into a powder. This powder was mixed with petroleum ether in a stoppered container and left at room temperature for three days, with regular shaking, until the soluble components dissolved. The mixture was then strained, the wet solid (marc) pressed, and the liquids were filtered and clarified.

Determination of moisture content

Moisture content was assessed as outlined by Obeagu *et al*. (2018) by drying the sample in an oven at 105℃. First, the weight of an aluminum dish was recorded, and the differences between the sample weights were noted. The dish containing the sample was placed in a controlled oven and dried at 105℃ until it reached a constant weight. The moisture content was calculated using the following formula:

$$Moisture\ content\ (\%) = \frac{(W2 - W3)}{(W2 - W1)} \times 100\ \%$$ Eq. 1

where W1 represents the initial weight of the empty crucible, W2 is the weight of the

crucible and sample before drying, and W3 is the final weight of the crucible and sample after drying.

Determination of the specific gravity of the oils

A 3ml aliquot of each oil was weighed and its density was calculated using the relationship:

$$\rho = \frac{mass\ of\ oil\ (g)}{volume\ of\ oil\ (ml)} \quad \text{Eq. 2}$$

The specific gravity (s.g) of the oil was calculated using the formula

$$\text{s.g} = \frac{density\ of\ oil}{density\ of\ water} \quad \text{Eq. 3}$$

Determination of percentage free fatty acid

Each oil's free fatty acid percentage was measured using the method outlined by the Association of Official Agricultural Chemists (AOAC, 1998). Two grams of oil were placed in a conical flask, to which 10 ml of 95 % ethanol was added. The mixture was then titrated with 0.1 M sodium hydroxide, with phenolphthalein as the indicator. The flask was shaken continuously until a stable pink color appeared for 30 seconds. The percentage of free fatty acid was calculated using the formula:

$$\%\ \text{free fatty acid} = \frac{\text{V} \times \text{M} \times 2.82}{Sample\ weight\ (g)} \quad \text{Eq. 4}$$

where V is the volume of 0.1 M sodium hydroxide used in ml, and M is the molarity of sodium hydroxide.

Determination of saponification value of the Oil

The saponification value of the oil was measured following the AOAC (1998) method. A sample of oil (2.054 g) was combined with 25 ml of 0.5N ethanolic potassium hydroxide in a flask equipped with a reflux condenser. The mixture was heated until the ethanol began to boil, and the flask was occasionally shaken using a magnetic stirrer until the oil fully dissolved. The solution was then boiled for 30 minutes. After the oil was completely dissolved, five drops of phenolphthalein indicator were added, and the resulting hot soap solution was slowly titrated with 0.5N hydrochloric acid, recording the volume used. A blank titration was also performed with the same amount of potassium hydroxide solution under identical conditions, and that volume was also recorded. The final saponification value (s. v.) was calculated using the formula:

$$\text{s. v.} = \frac{(\text{S}-\text{B}) \cdot \text{N} \cdot 56.1}{W} \quad \text{Eq. 5}$$

where W is the weight of the oil in grams, S is the sample titre volume in ml, B is the

blank titre volume in ml, and N is the normality of hydrochloric acid.

Determination of peroxide value of the oils

The peroxide value of each oil was assessed using the AOAC (1998) method. To measure the peroxide value, 2.16 g of oil was dissolved in 30 ml of a glacial acetic acid and chloroform mixture (3:2, v/v). Next, 0.5 ml of 20% potassium iodide was added, and the solution was swirled in the dark for one minute. Afterward, 75 ml of distilled water was introduced. The mixture was titrated with 0.1 M sodium thiosulfate while shaking vigorously until the yellow iodine color faded. Then, 0.5 ml of starch indicator was added to create a blue color, and titration continued until the blue color completely disappeared.

The peroxide value was calculated using the formula:

$$\text{p.v.} = \frac{(\text{S} - \text{B}) \times \text{M} \times 1000}{W}$$

Eq. 6

where S is the sample titre volume in ml, B is the blank titre volume in ml, and M is the molarity of sodium thiosulfate.

Experimental design for determining the animal equivalent dose

To determine the human equivalent dose, a cross-sectional descriptive survey was conducted with adult participants from both urban and rural areas. The survey was carried out between February, 2022 and April, 2022 . The study instrument was distributed conveniently among 230 persons during Afor market days, in Agulu town of Anambra state, being a time when a large number of persons come from neighbouring towns come to the Afor market to make sales. Agulu is a large town in Anaocha Local Government in the central senatorial district of Anambra State, Nigeria.

A total of 230 questionnaires were distributed in the market day but only 195 were correctly filled (30 were incorrectly filled with 5 missing). The questionnaires were distributed only to those who granted oral consent, and those who could not read were assisted by the research assistant until the projected number was reached and the rest were offered writing materials to ensure that the questionnaires were filled promptly.

Research instrument and validation

A 22-item self-administered questionnaire was developed, validated and used for the study. The questionnaire was developed by consulting a pharmacist in public health and a food nutritionist to derive a concept on how to extrapolate feeding frequency of a particular food (the number of times per day

a feed is provided**)** to obtain a human equivalent dose (human equivalent concentration, HEC) in an animal model, which is the quantity of a chemical that, when administered to humans, produces an effect equal to that produced in test animals by a smaller dose (Minnesota Department of Health, 2011).

The instrument was structured into three different sections. The first section included socio- demographic characteristics. The second section consisted of 16 questions that assessed respondents' feeding frequencies of several oil-rich foods. Section three contained information used to evaluate the frequency of malaria attacks.

The survey instrument was independently face validated by an expert in the Department of Clinical Pharmacy and Biopharmaceutics, Enugu State University of Science and Technology, a hospital pharmacist specially trained on nutrition and a food and nutrition scientist (both of the University of Nigeria Teaching Hospital). The instrument was then pilot-tested to assess its feasibility in Agbani, Enugu state. Agbani is a semi-urban town located in Nkanu West Local Government area of Enugu State, Nigeria. The questionnaire was distributed to 20 persons in Agbani after getting each person's consent. Retrieved questionnaires were coded, and entered into SPSS 23, and the internal consistency of the questions was checked using Cronbach's alpha (CA) with an alpha value of ≥ 0.70 considered reliable. The corrected item-total correlation of each item was calculated and items with a corrected item-total correlation value of ≥ 0.3 were retained.

Retrieved copies of the questionnaire were collected and classified into sections and each section was coded and entered into Microsoft Excel and analyzed using SPSS version 23. Descriptive statistics was conducted for the demographics and percentage mean scores for each domain were computed and a correlation of the dependent variable against the independent variable was carried out.

The second section consisted of 16 questions which are items in which feeding frequencies were captured as ‘always’ ‘often’ sometimes’ ‘rarely’ ‘never’ or ‘no’ responses, were scored ‘5’, ‘4’ ‘3’ ‘2’ ‘1 and ‘0’ respectively. In some items, feeding frequency was denoted as ‘0’, ‘1-2’, ‘3-4’ ,‘5-6’ or more than ‘7’. These frequencies were transferred to a spreadsheet and the human equivalent dose (HED) was calculated in each case (as seen in the formula below):

$$\text{HED (mg/kg)} = \text{Animal dose (mg/kg)}.\frac{Animal\ K_m}{Human\ K_m}$$

Eq. 7

In this formula, HED represents the human equivalent dose, and the Km factor for each species is constant, which simplifies the calculations to:

$$\text{HED (mg / kg)} = \text{Animal dose (mg/kg)}.K_m \text{ ratio} \quad \text{Eq. 8}$$

The Km ratio is derived from a human equivalent dose calculation table published by the US FDA (2005). The above enabled the determination of the appropriate doses for the test animals.

Infection of animals

A total of 40 Swiss albino mice (weighing 15–25 g) were sourced from the Animal House of the Department of Pharmacology of the Faculty of Pharmaceutical Science, Enugu State University of Science and Technology, Enugu State, Nigeria. All animal experiments were conducted following the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (Pub. No. 85-23 Revised 1985), and approval was obtained from the University's institutional animal care and use committee (Approval No: ESUT/AEC/0371/AP294 of September 2, 2022). Chloroquine-sensitive strains of *P. berghei* (strain ANKA) were acquired from the Nigeria Institute of Medical Research (NIMR) in Yaba, Lagos, Nigeria, in the form of cryo-frozen stocks of parasitized red blood cells (PRBCs). The parasites were propagated through two cycles of passage in mice. Donors with a parasitaemia level of 40-50% were sacrificed, and blood was collected via cardiac puncture into heparinized tubes. The blood was then diluted with phosphate-buffered saline (PBS) to achieve a concentration of 5×10^7 parasites per 1 mL of blood, according to the parasitaemia level of each donor (Basir *et al*., 2012).

Testing of the effects of the oils

Forty albino mice were randomly assigned to eight groups, each containing five mice (groups 1 to 8). Groups 1, 3 and 5 received the HEDs of coconut oil, fish oil and groundnut oil while groups 2, 4 and 6 received DHEDs of the respective oils. Groups 7 and 8 served as control groups and both received water, with Group 8 receiving twice the dose offered to group 7. All treatments were administered orally for two weeks before infection. The mice were then infected with 1 × 10^7 chloroquine-sensitive PRBCs via intraperitoneal injection, and the oil treatments continued for another week. To monitor the level of parasitemia, Giemsa-stained thin smears were prepared from tail blood samples on the fourth-day post-inoculation, following WHO (2016)

methods. Each slide was examined microscopically at 100× magnification to determine the mean percentage of parasitemia, calculated using the formula: % Parasitaemia = (Total number of parasitized red blood cells) × 100 (WHO, 2016). Data were analyzed using descriptive and inferential statistics.

Data analysis

Microsoft Excel and Statistical Package for Social Sciences version 23 were used in the analysis. Where necessary, data in tables are presented as mean ± standard deviation. P values of less than 0.5 were considered as significant.

Results

Table 1 shows the results from selected physicochemical parameters which include free fatty acid (acid value), peroxide value, saponification value, refractive index, density, and moisture content. The tests were done in triplicate and the values are presented as mean ± standard deviation. Unfortunately, the yields from the extraction process are not available. Table 2 shows the frequency of consumption of the tested oils. From the responses, groundnut is the most frequently consumed food oil.

Table 1: Physicochemical analysis of extracted oil samples

S/N	Source of oil	Coconut	Fish	Groundnut
% Free fatty acid		5.58 ± 1.03	2.35 ± 0.68	2.83 ± 0.50
Peroxide value (mil/eq.)		3.27 ± 0.13	2.58 ± 0.07	0.79 ± 0.00
Saponification value (mgKOH/g)		260.44 ± 44.23	96.38± 34.56	145.31 ± 09
Refractive index (at 25ºC)		1.45 ± 0.06	1.47 ± 0.03	1.47 ± 0.01
Density (g/mL)		1.14 ± 1.44	0.81 ± 1.38	0.81 ± 0.08
Moisture content (%)		37.00 ± 1.53	44.60 ± 2.52	5.60 ± 0.51

Values are means ± SD of triplicate determinations, n = 3

Table 2: Frequency of consumption of oil foods/malarial attacks reported by respondents

Frequency of consumption	Scumbia fish	Groundnut	Coconut	Frequency of malaria
Valid	195	195	195	195
Missing	5	5	5	5
Mean	2.99	3.66	3.02	.96
Median	3.00	4.00	3.00	1.00
Mode	3	4	3	1
Std. Deviation	1.208	1.030	.974	.846
Skewness	-.274	-.513	.128	.998
Std. Error of Skewness	.174	.174	.174	.174

Key: frequency of consumption: 1 - Never; 2 - Rarely; 3 - Sometimes; 4 - Often; 5 - Always

Figure 1 shows the frequency of malaria attacks reported by respondents per three (3) month period. Most respondents reported experiencing only one malaria attack in three months, as depicted. Table 3 shows parasitaemia levels on the fourth-day post-inoculation. The values represent the mean of five replicates (± standard deviation).

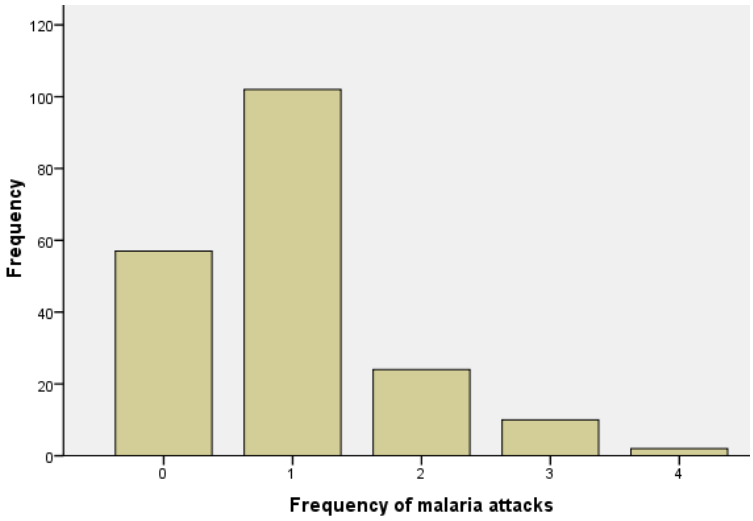


Figure 1: Frequency of malarial attacks in respondents in the space of three months
Key: 0 - Not sure; 1 - Once; 2 - Two times; 3 - Three times; 4 - Five times

Table 3: Influence of different oils on percentage parasitaemia four days after inoculation of mice with *P. berghei*

Treatment	% parasitaemia	
	HED (Human equivalent dose)	DHED (Double HED)
Groundnut oil	5.32±0.54*	6.4±2.77
Coconut oil	5.36±1.28*	5.24±2.42*
Scumbia fish oil	7.4±5.32	5.12±0.901*
Control	6.94±6.77	-

* parasitaemia significantly lower than the control. The oils were administered for two weeks prior to infection and continued for a week after.

Discussion

The physicochemical analysis of the oil showed that coconut oil had higher peroxide value compared to the fish oils. This is not supposed to be so because the peroxide value of an oil is linked to its content of polyunsaturated fatty acids, or its degree of unsaturation. Oils such as coconut which are made up of mainly saturated fats (90%) (Boateng *et al*., 2016) are expected to have a lower peroxide value than unsaturated oils such as fish and groundnut oils since the latter have oxidizable double bonds. The deviation in the peroxide values recorded with coconut oil in this case may represent the effect of storage on the 10% unsaturated fat component. Dietary lipids may affect parasite metabolism in different ways, especially in the case of parasites lacking the ability to synthesize their fatty acids, which then depend on lipids scavenged from host sources. Accumulation of lipids from the surroundings in this manner may expose them to lipotoxicity, especially in the case of the monounsaturated fatty acid - oleic acid and Apicomplexa parasites (Shunmugam *et al*., 2022).

From the survey responses, groundnut oil is the most frequently consumed. The feeding frequency refers to the number of times per day a feed is provided while the human equivalent dose (HED) or human equivalent concentration (HEC) refers to the dose of a chemical that produces the same magnitude of toxic effect in humans as the experimental animal species if the toxic responses elicited in targeted tissues are similar (Minnesota Department of Health, 2011). This determination is an important step in the clinical testing of drugs, where animal models are frequently used in predicting human toxicity.

In this study, only coconut oil elicited a reduction in parasitaemia at all the doses used for the study. The level of parasitaemia is an important indicator of the severity of malaria disease, and hyper parasitaemia has been recognized by the World Health

Organization (WHO) as a criterion for severe falciparum malaria for more than two decades (Kotepui *et al*., 2015). The antiparasitic activity of an oil is influenced by its phytochemical composition (dos Santos Sales *et al*., 2018). Some oils contain compounds that fight parasitic infections, and oils with strong anti-parasitic properties like coconut oil likely contain high concentrations of such components (El Naggar *et al*., 2023). The rise of drug-resistant parasites makes it necessary to recognize the potential anti-parasitic activities of some plant essential oils, such as coconut oil (Anthony *et al*., 2005). Coconut oil is also believed to help prevent various chronic health conditions, including heart disease, diabetes, cancer, and infections (Boateng *et al*., 2016). However, the composition of medicinal oils of this nature may be influenced by various factors, such as growth conditions, climate, development stage and agricultural practices, processing method, time of harvest and storage conditions (Moghaddam *et al*., 2017).

In the study, DHED of fish oil led to lower parasitaemia levels relative to the control. This supports previous reports on the anti-plasmodial activities of oils rich in unsaturated fatty acids, such as fish oil (Godfrey, 1957; MalariaWorld 2015). Fish oil and oils from other marine foods contain essential unsaturated fatty acids like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Grønn *et al*., 1992). Both omega-3 and omega-6 fatty acids can limit parasite growth and survival due to their anti-inflammatory properties, thereby conferring protection against protozoal diseases like malaria. Omega-3 polyunsaturated fatty acids (ω-PUFAs) can modulate immune response to infection by inhibiting arachidonic acid metabolism and producing anti-inflammatory mediators (Calder, 2017; Rahman *et al*., 2024). Polyunsaturated fish oils have been demonstrated to cause vitamin E deficiency in mice and suppress parasite growth. Vitamin E, being an antioxidant, reduces oxidative stress in the parasites if present, and can result in increased parasitaemia (Levander *et al*., 1993; Taylor *et al*., 1997). In other reports, mice fed a diet containing 20% menhaden fish oil for four weeks showed partial protection against the central nervous system complications caused by *Plasmodium berghei* infection (Levander *et al*., 1995). Blok *et al*. (1992) observed that fish oil supplementation influenced eicosanoid production, reducing interleukin-1 (IL-1) and tumor necrosis factor (TNF) levels, with cerebral malaria developing in only 23% of fish-oil-fed mice compared to 75% in the control group. The same influences may explain the level of reduction in parasitaemia seen with DHED of Scumbia fish oil.

Conclusion

Both Scumbia fish oil and coconut oil may possess significant anti-plasmodial activity, depending on the dose. Coconut oil resulted in a statistically significant reduction in parasitaemia at both the HED and DHED. Contrary to some of the cultural beliefs referred to, the results indicate that ingestion of some dietary oils may reduce the severity of malaria infection (as measured by parasitaemia). More studies are recommended to unravel the molecular basis for this influence.

Conflicts of Interest

The authors report no conflicts of interest

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