© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720169

# Proximate Composition, Antimalarial Efficacy, and Haematological Effects of Fermented and Unfermented Cyperus esculentus Tubers

Omolara J. Fagbemi[1], Oreofe E. Fagbemi[2], Silas A. Oke[3], Stephen A. Adesida[4], Oluwatosin F. Olawuni[5], Wahab O. Quadri[6], Rowland M.O. Kayode[7], Charles C. Benedict[8], Kolade O. Faloye[9]

[1,9]*Department of Industrial Chemistry, Faculty of Sciences, University of Ilesa, Ilesa, Nigeria*

[2]*Department of Human Nutrition and Dietetics, Faculty of Basic Medical Sciences, University of Ilesa, Ilesa, Nigeria*

[3]*Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria*

[4, 5]*Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria*

[6]*Department of Anatomy, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria*

[7]*Department of Home Economics and Food Science, University of Ilorin, Ilorin, Nigeria*

[8]*Department of Biochemistry, Faculty of Science, University of Ilorin, Ilorin, Nigeria*

***Abstract-*** ***Cyperus esculentus is an edible medicinal plant widely cultivated in northern Nigeria and traditionally used for the management of various ailments. This study evaluated the proximate composition, antimalarial activity, and haematological effects of fermented and unfermented C. esculentus chaff. The chaff was fermented for 24, 48, and 72 h, oven-dried, and analysed for proximate composition using standard AOAC methods. Seventy Swiss albino mice infected with Plasmodium berghei berghei were randomly assigned to fourteen groups (n = 5). Fermented and unfermented chaff were administered orally at doses of 25, 50, and 100 mg/kg using the four-day suppressive test, while chloroquine (10 mg/kg) served as the positive control. Haematological parameters were also evaluated following treatment. Data were analysed using one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls post hoc test, with statistical significance set at $P < 0.05$. Fermentation influenced the nutritional composition of the chaff, with the 48 h and 72 h fermented samples exhibiting the highest moisture (8.93%), carbohydrate (50.40%), and crude protein (6.89%) contents, whereas the unfermented sample contained the highest crude fat (24.15%), crude fibre (16.63%), and ash (2.01%). The 72 h fermented chaff at 100 mg/kg produced the highest chemosuppressive activity (94.98%) and the lowest $ED_{50}$ (20.99 mg/kg) and $ED_{90}$ (57.85 mg/kg), comparable to chloroquine. Treatment also produced variable changes in haematological indices. These findings demonstrate that fermentation enhances the antimalarial efficacy of C. esculentus chaff while improving its nutritional profile, supporting its potential as a promising source of affordable plant-based antimalarial agents.***

***Keywords: Cyperus Esculentus, Proximate Composition, Antimalarial, Haematology***

## I. INTRODUCTION

Infectious diseases are among the top-ranked diseases contributing to the decreased life expectancy of the world's inhabitants (White *et al*., 2014). Malaria is among the deadly infectious diseases that are closely associated with the increasing level of mortality and morbidity of men, pregnant women and young children (Kogan, 2020). According to the World Health Organization (2025), malaria occurrence is predominant in Asia, Latin America, and Africa, with more than 40% of the world's inhabitants at risk of the disease. The continuous dominance of the disease has been widely attributed to the development of resistance to available drugs developed to inhibit the action of *Plasmodium falciparum* (Siqueira-Neto *et al*., 2023).

The resistance of pharmaceuticals is well known in malaria therapy, and this fore-knowledge has led to the continuous investment of resources in the

development of new drug candidates and therapeutic plant agents that can effectively treat the disease (Siqueira-Neto *et al*., 2023). Though synthetic therapeutic agents have dominated antimalarial research for several years, considerable attention has been shifted towards the investigation of nature in identifying cheaper, safer and effective antimalarial candidates with a view to curing the disease (Habibi *et al*., 2022; Bantie *et al.*, 2020). Medicinal plants present people living in developing economies with affordable and accessible healthcare. The antimalarial potential of many medicinal plants has been attributed to the presence of bioactive phytochemicals such as flavonoids, alkaloids, phenolic compounds, tannins, saponins and terpenoids, which have been reported to inhibit parasite growth through antioxidant activity, interference with parasite metabolism, inhibition of haem detoxification, and modulation of host immune responses (Habibi *et al*., 2022; Bantie *et al*., 2020).

Fermentation is a process which involves the breakdown of complex organic substances as well as toxic substances (Liu *et al.,* 2023). It plays an important role in the human diet since the development of civilization and represents a special feature of some dietary systems (Robles Hernandez *et al.,* 2026). During fermentation, bacteria synthesize vitamins, minerals, and biologically active peptides with enzymes such as proteinase and peptidase, improve plants' organoleptic properties, enhance nutritional qualities of plants, and also remove toxins and non-nutrients (Şanlier *et al*., 2017; Olorunnisola *et al.,* 2023). Pharmacologically active compounds are produced by bacteria responsible for fermentation, and these therapeutic compounds contribute significantly to the health benefits of fermented food materials (Voidarou *et al.,* 2020). Furthermore, fermentation may enhance the biological activity of medicinal plants by increasing the bioavailability of phytochemicals, converting inactive phytochemical precursors into more active metabolites, reducing anti-nutritional factors, and improving the extraction and absorption of bioactive constituents, thereby potentially enhancing their therapeutic efficacy (Şanlier *et al*., 2017; Olorunnisola *et al*., 2023; Voidarou *et al.,* 2020).

*Cyperus esculentus* (Cyperaceae), also known as tiger nut, is a medicinal plant that produces rhizomes from its base and is widely consumed because of its sweetness (Edo *et al.,* 2023). *C. esculentus* is widely found in Spain, the Arabian Peninsula, and across West and North Africa (Edo *et al.,* 2023; Sánchez-Zapata *et al*., 2012). It is classified as nutritious and medicinal due to its wide therapeutic use in drinks and in the treatment of diseases (Edo *et al*., 2023). Ethnomedicinally, *C. esculentus* is used in the treatment of constipation, skin diseases, tooth disorders, circulatory disorders, boils, scars, acne, pimples, and diabetes (Adenowo & Kazeem, 2020; Edo *et al.,* 2023). Pharmacological reports on the plant include antidiabetic, antioxidant, antimicrobial, anti-inflammatory and antibacterial activities (Akanbi *et al*., 2022; Wang *et al*., 2023). These pharmacological properties suggest the presence of biologically active phytochemicals that may also possess antiplasmodial activity, although this potential has received limited scientific investigation.
Malaria infection is frequently accompanied by haematological alterations, including reductions in packed cell volume, haemoglobin concentration and erythrocyte count, as well as changes in leukocyte profiles resulting from parasite-induced haemolysis and immune responses. Consequently, the evaluation of haematological parameters provides important information on the physiological effects of infection and the ability of a test agent to ameliorate malaria-associated haematological abnormalities.

Although the nutritional and several pharmacological properties of *C. esculentus* have been documented, there is limited information on the effect of fermentation on its antimalarial efficacy and associated haematological responses. Furthermore, the relationship between fermentation-induced changes in the nutritional composition of *C. esculentus* and its potential antiplasmodial activity has not been adequately explored. Addressing these is important in assessing whether fermentation could enhance the therapeutic value of this widely consumed plant. Therefore, we evaluated the proximate composition of fermented and unfermented chaff of *C. esculentus*, investigated their antimalarial activity, and assessed the associated haematological changes in treated mice.

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720169

## II. METHODOLOGY

### Plant Collection and Fermentation

*Cyperus esculentus* tubers were purchased from Orisumbare Market in Osogbo, Osun State. The tuber was authenticated at the Faculty of Pharmacy Herbarium, Faculty of Pharmacy, Obafemi Awolowo University. The voucher number FPI2256 was given, and the voucher specimen was deposited at the herbarium. Three separate glass jars containing 1.2 kg of *C. esculentus* tubers were fermented with distilled water for 24, 48 and 72 h, respectively. Both fermented and unfermented chaff of *C. esculentus* were oven-dried at 50 °C and kept in an air-tight polythene bag for future use. The powdered chaff was administered directly in the antimalarial study to evaluate the biological activity of the whole fermented material, thereby preserving the combined effects of its nutritional constituents, dietary fibre and fermentation-derived bioactive compounds that may otherwise be altered or selectively lost during solvent extraction.

### Determination of Proximate Composition of Fermented Chaff and Unfermented *Cyperus esculentus* Tubers

Proximate composition of fermented tiger nut chaff was determined using standard procedures recommended by AOAC International. For each analysis, 2 g of the sample was used. Moisture content was determined by oven-drying the sample at 105 °C to constant weight. Crude fat was determined by Soxhlet extraction using an organic solvent for continuous reflux. Crude protein was analysed by the Kjeldahl method, involving acid digestion of the sample, steam distillation of the liberated ammonia and titration, and the nitrogen content obtained was converted to crude protein using a factor of 6.25. Crude fibre was determined on the defatted sample by sequential digestion with dilute sulphuric acid and sodium hydroxide, followed by drying and ashing of the residue. Total ash content was obtained by incinerating the sample in a muffle furnace at 550 °C to constant weight. Total carbohydrate content was finally calculated by difference from the measured values of moisture, crude fat, crude protein, crude fibre and ash. All proximate analyses were performed in triplicate (n = 3), and the reported values represent the mean of three independent determinations.

### Animals and Parasite

The Animal House, Faculty of Basic Medical Sciences, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria, provided the seven-week-old Swiss mice of either sex, weighing between 18 and 24 g (male and female, not pregnant). They were kept in aluminum cages with wood shavings as bedding and had unrestricted access to food (Growers' mash) and water within a 12-hour day/night cycle. Before being used, they were acclimated for a week. The Institute of Advanced Medical Research and Training (IMRAT), University College Hospital, Ibadan, provided the chloroquine-sensitive *Plasmodium berghei berghei* strain NK65. Blood from an infected mouse was serially transferred into an uninfected mouse in order to retain the parasite strain. The NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 83-123, revised 1985) was followed in the handling and care of the mice used in the study. The Animal Health Research Ethics Committee, Institute of Public Health, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria (HREC No. IPHOAU/12/1772) approved the experimental protocol.

### $LD_{50}$ Determination

Prior to conducting the chemosuppressive assay, the acute toxicity profile of the fermented and unfermented *C. esculentus* chaff was evaluated using Lorke's method. A total of twenty-four mice were utilized, and the procedure was carried out in two sequential phases. In Phase I, nine mice were assigned to each of the fermented and unfermented samples, making three groups of three mice per treatment. The animals received oral doses of 10, 100, and 1000 mg/kg of either the fermented chaff or the unfermented plant powder. Following administration, the mice were monitored for 24 hours to assess behavioural changes and record any mortality. If no deaths occurred during this phase, the study progressed to Phase II; otherwise, the median lethal dose ($LD_{50}$) would have been estimated based on Phase I outcomes. Phase II involved three mice per sample type (fermented chaff and unfermented *C. esculentus* tuber powder), with each mouse placed in

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720169

a separate group. Higher doses of 1600, 2900, and 5000 mg/kg were administered orally. The animals were observed for another 24-hour period to evaluate toxic manifestations and mortality. The absence of death at this stage indicated that both the fermented chaff and unfermented powder were non-toxic under the tested conditions Lorke, 1983). Subsequently, the $LD_{50}$ value was determined using the appropriate Lorke's formula.

$LD_{50} = \sqrt{(D_0 \times D_{100})}$

where $\sqrt{(D0\ x\ D100)}$ $D_0$ = Highest dose that gave no mortality, $D_{100}$ = Lowest dose that produced mortality.

*In vivo* antimalarial assays

Parasite inoculation

The donor mouse was euthanised, and blood was collected via cardiac puncture into a heparinised container for preparation of the parasite inoculum. The collected blood was subsequently diluted with physiological saline to achieve a concentration such that 0.2 mL of the suspension contained approximately $1.0 \times 10^7$ parasitised erythrocytes. The required dilution factor was determined by calculating the total parasite load and the volume of blood to be administered per mouse. Each experimental mouse then received 0.2 mL of the prepared inoculum intraperitoneally prior to commencement of the antiplasmodial evaluation.

Four-Day Suppressive Test

The chemosuppressive effects of the fermented chaff, unfermented *C. esculentus* tuber powder, and the reference antimalarial drug chloroquine (CQ, 10 mg/kg) were assessed against early-stage infection (Peter *et al.,* 1995). A total of seventy Swiss albino mice were randomly allocated using a simple randomization procedure into fourteen groups, with five animals in each group, for the four-day suppressive test.

Group 1 → infected + not treated; negative control (NC)
Group 2, 3 and 4 → Infected + treated with 24 h fermented chaff (25, 50 and 100 mg/kg)
Group 5, 6 and 7 → Infected + treated with 48 h fermented chaff (25, 50 and 100 mg/kg)
Group 8, 9 and 10 → Infected + treated with 72 h fermented chaff (25, 50 and 100 mg/kg)
Group 11, 12 and 13→ Infected + treated with unfermented chaff (25, 50 and 100 mg/kg)
Group 14 → Infected + treated with 10 mg/kg chloroquine

The level of parasitaemia was determined for each mouse on Day 4 (D4) by withdrawing a thin blood smear from the tail, fixing it with methanol, staining with Giemsa, and examining it microscopically by counting parasitized erythrocytes in five microscopic fields. The average parasitaemia in each group of five mice was calculated in order to determine the percentage chemosuppressive activity using the formula:

$$\%\ Parasitaemia = \frac{(A - B)}{A}\ X\ 100$$

Where A and B; are the mean parasitaemia in the negative control and the test groups, respectively. The chemosuppressive antimalarial activity for each the extract and fractions was determined by the percentage reduction of parasitaemia in treated groups compared with the untreated infected group using the formula:

$$Percentage\ Chemosuppression = \frac{\%\ Parasitaemia\ in\ Negative\ Control - \%\ Parasitaemia\ in\ Treated\ Group}{\%\ Parasitaemia\ in\ negative\ Control}\ X\ 100$$

Statistical Analysis

All values were expressed as mean ± SEM and analyzed using Microsoft Excel 2010. One-way Analysis of Variance (ANOVA) was used to determine statistical significance, followed by the Student–Newman–Keuls post hoc test to identify the source of significant differences among treatment groups. Values of $P < 0.05$ were considered statistically significant. $ED_{50}$ and $ED_{90}$ values were estimated from the dose–response relationship using regression analysis of percentage chemosuppression against dose.

Haematological Analysis

Through microscopic analysis of thin blood smears created on microscope slides, the percentage of parasitized erythrocytes was ascertained. The microhaematocrit technique was used to determine the packed cell volume (PCV) (Cole, 1986).

Differential leukocyte counts were established in triplicate.

*Results and Discussion*

Proximate analysis

The results obtained for the proximate composition of fermented and unfermented *C. esculentus* are presented in Table 1.

Table 1: Proximate composition of fermented tiger nut chaff

| Sample | Moisture content (%) | Crude fat | Crude protein | Ash | Crude fibre | Total carbohydrate |
|---|---|---|---|---|---|---|
| UF | 7.83±1.37[a] | 24.15±0.33[b] | 4.70±1.10[a] | 2.01±0.01[a] | 16.63±0.21[a] | 44.68±0.12[a] |
| F24 | 8.53±0.60[a] | 23.96±1.32[b] | 5.26±1.24[a] | 0.50±0.01[c] | 15.80±0.13[a] | 45.95±0.23[a,b] |
| F48 | 8.93±0.59[a] | 20.68±1.17[a] | 6.13±0.62[a] | 0.51±0.01[c] | 13.35±0.24[b] | 50.40±0.15[a] |
| F72 | 7.81±0.51[a] | 22.45±1.20[a,b] | 6.89±0.16[a] | 1.50±0.01[b] | 13.24±0.31[b] | 48.11±0.23[a,b] |

Values are means of triplicate determinations (n = 3) ± SD. Means within the same column bearing different superscripts are significantly different ($p \leq 0.05$). Key: UF = unfermented *C. esculentus* chaff; F24 = *C. esculentus* chaff fermented for 24 h; F48 = *C. esculentus* chaff fermented for 48 h; F72 = *C. esculentus* chaff fermented for 72 h.

The results of the proximate analysis showed that F48 recorded the highest moisture content (8.93%), whereas F72 had the lowest moisture content (7.81%). The UF sample had the highest crude fat content (24.15%), while F48 recorded the lowest crude fat content (20.68%). F72 exhibited the highest crude protein content (6.89%), whereas the UF sample had the lowest crude protein content (4.70%). For ash content, the UF sample recorded the highest value (2.01%), while F24 had the lowest ash content (0.50%), which was comparable to F48 (0.51%). The highest crude fibre content was observed in the UF sample (16.63%), whereas F72 recorded the lowest crude fibre content (13.24%). F48 exhibited the highest carbohydrate content (50.40%), while the UF sample recorded the lowest carbohydrate content (44.68%).

This study performed a proximate analysis of the fermented chaff and unfermented *C. esculentus* tuber, which showed that fermentation generally increased crude protein and carbohydrate contents while reducing crude fat and crude fibre contents. Although moisture content increased up to 48 h of fermentation, a slight decline was observed at 72 h, with no significant difference among the fermented samples. F72 had the lowest moisture content and the highest crude protein content, whereas F48 had the highest carbohydrate content. The UF sample exhibited the lowest crude protein and carbohydrate contents. These findings are similar to those of Adejuyitan (2011), who reported increases in moisture, protein, and carbohydrate contents with increasing fermentation time. Likewise, crude fat and crude fibre generally decreased with fermentation, although crude fat increased slightly at 72 h without a significant difference.

Dose-response antiplasmodial study of the fermented chaff and unfermented powder of *Cyperus esculentus* tuber using the 4-day suppressive test

The average percentage parasitaemia, average percentage chemosuppression, and effective doses ($ED_{50}$ and $ED_{90}$) of the fermented chaff and unfermented powder are summarized in Tables 2, 3 and 4, respectively.

© JUL 2026 | IRE Journals | Volume 10 Issue 1 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV10I1-1720169

Table 2: Average percentage parasitaemia of the fermented chaff and unfermented powder of *C. esculentus* Tuber

| Drug | F24 | F48 | F72 | UF |
|---|---|---|---|---|
| NC | 22.90±1.20$^{e}$ | 22.90±1.20$^{c}$ | 22.90±1.20$^{b}$ | 22.90±1.20$^{c}$ |
| 25 mg/kg | 8.27±0.56$^{d}$ | 6.99±1,43$^{b}$ | 2.89±0.32$^{a}$ | 2.01±0.14$^{a}$ |
| 50 mg/kg | 6.21±0.32$^{c}$ | 2.02±0.47$^{a}$ | 1.46±0.07$^{a}$ | 3.51±0.34$^{b}$ |
| 100 mg/kg | 4.15±0.31$^{b}$ | 2.52±0.23$^{a}$ | 1.13±0.07$^{a}$ | 1.46±0.08$^{a}$ |

Keys: Data are presented as mean ± SEM (n = 5). NC (negative control) = Tween 80 in normal saline; CQ = chloroquine (10 mg/kg). Values with different superscripts within the same column are significantly different ($p < 0.05$, one-way ANOVA followed by the Student–Newman–Keuls post hoc test).

The results demonstrated that parasitaemia decreased with increasing dose in all treatment groups. F72 consistently produced the lowest parasitaemia values at all tested doses (2.89 ± 0.32, 1.46 ± 0.07 and 1.13 ± 0.07% at 25, 50 and 100 mg/kg, respectively), while F24 generally showed the highest residual parasitaemia among the fermented samples.

The antimalarial effect of the fermented *C. esculentus* chaff and unfermented tuber was investigated using the standard 4-day suppressive test in *Plasmodium berghei*-infected mice. The antiplasmodial activity of the fermented chaff and unfermented powder was both dose- and time-dependent, with the highest dose (100 mg/kg) producing the greatest reduction in parasitaemia. In the F72 sample, parasitaemia was reduced to a level that was statistically comparable to the chloroquine-treated group. This level of suppression suggests strong schizontocidal activity of the F72 chaff against the erythrocytic stages of the parasite. Lower doses also produced significant reductions in parasitaemia, although the effect was less pronounced, indicating a clear dose-response relationship. Similar dose-dependent antiplasmodial effects have been reported for other medicinal plant extracts evaluated using the 4-day suppressive test (Bantie et al., 2020; Akanbi et al., 2022).

Chemosuppressive activity

The results of the chemosuppression study showed that F72 exhibited the highest percentage chemosuppression, increasing from 87.46% at 25 mg/kg to 94.98% at 100 mg/kg. F24 showed the lowest chemosuppressive activity, increasing from 63.48% to 81.81% over the same dose range. F48 and UF also demonstrated high chemosuppressive activities, although their responses were less consistent across the tested doses. Overall, the chemosuppressive activity followed the order F72 > UF > F48 > F24 at the highest tested dose.

Table 3: Average Percentage Chemosuppression of the fermented chaff and unfermented powder of *C. esculentus* Tuber

| Drug | F24 | F48 | F72 | UF |
|---|---|---|---|---|
| NC | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 25 mg/kg | 63.48±3.36$^{b}$ | 68.78±7.21$^{b}$ | 87.46±0.88$^{b}$ | 91.15±0.66$^{c}$ |
| 50 mg/kg | 72.78±1.38$^{c}$ | 90.79±2.41$^{c}$ | 93.58±0.28$^{c}$ | 84.40±2.13$^{b}$ |
| 100 mg/kg | 81.81±1.24$^{d}$ | 88.85±1.30$^{c}$ | 94.98±0.43$^{d}$ | 93.51±0.64$^{c}$ |
| PC | 95.77±0.27$^{e}$ | 95.77±0.27$^{c}$ | 95.77±0.27$^{d}$ | 95.77±0.27$^{c}$ |

Keys: Data show the mean ± SEM, $n = 5$: NC (negative control): Tween 80 in normal saline; CQ = Chloroquine (10 mg/kg). Only values with different superscripts within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keul's post hoc test).

Although the unfermented powder also reduced parasitaemia, its efficacy was consistently lower than

that of the fermented chaff powders at corresponding doses and time points. The improved activity observed after fermentation may be associated with biochemical changes that occur during the fermentation process. Fermentation processes are known to improve the solubility and bioavailability of secondary metabolites while reducing fibrous or inert components that may hinder absorption (Olorunnisola *et al*., 2023). Therefore, the enhanced antiplasmodial activity of the fermented chaff observed in this study is discussed as a possible consequence of fermentation and requires confirmation by phytochemical analysis.

At 100 mg/kg, chemosuppression in F72 was comparable to that of the positive control (chloroquine) (Table 3). According to established criteria, plant extracts that achieve ≥50% chemosuppression are considered active, whereas those exceeding 90% are classified as highly active antiplasmodial agents (Willcox *et al*., 2021). Based on this classification, the fermented *C. esculentus* tubers qualify as highly active, particularly at higher doses. Although the unfermented powder also showed substantial chemosuppression, its values were consistently lower than those of the fermented chaff, further confirming the enhanced efficacy of the fermented preparation.

$ED_{50}$ and $ED_{90}$ estimation

Table 4: $ED_{50}$ and $ED_{90}$ of the fermented and unfermented *C. esculentus* Tuber

| Drug | $ED_{50}$ | $ED_{90}$ |
|---|---|---|
| F24 | $39.64\pm0.84^{b}$ | $65.74\pm3.35^{a}$ |
| F48 | $34.83\pm1.61^{a}$ | $76.76\pm1.61^{b}$ |
| F72 | $30.99\pm0.10^{a}$ | $57.85\pm0.20^{c}$ |
| UF | $32.20\pm0.37^{a}$ | $59.02\pm0.74^{c}$ |

Keys: $ED_{50}$ and $ED_{90}$ are doses of the extracts that gave 50 and 90% activity respectively. Only values with different superscripts within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keuls' post hoc test).

The results obtained for $ED_{50}$ and $ED_{90}$ showed that F72 had the lowest $ED_{50}$ (30.99 ± 0.10 mg/kg) and $ED_{90}$ (57.85 ± 0.20 mg/kg), indicating the greatest antiplasmodial potency among the tested samples. F24 recorded the highest $ED_{50}$ value (39.64 ± 0.84 mg/kg), whereas F48 had the highest $ED_{90}$ value (76.76 ± 1.61 mg/kg).

The progressive decrease in $ED_{50}$ values from 39.64 mg/kg at 24 h to 30.99 mg/kg at 72 h indicates increasing efficacy with prolonged fermentation. A similar trend was observed for $ED_{90}$ values, reflecting improved parasite clearance. Extracts with $ED_{50}$ values below 50 mg/kg are generally classified as highly active in vivo, placing the fermented *C. esculentus* chaff within this category (Fidock et al., 2019). The slightly higher $ED_{50}$ and $ED_{90}$ values observed for the unfermented powder further support its comparatively lower potency. The findings demonstrate that both preparations possess notable antiplasmodial activity; however, the fermented chaff consistently exhibited superior efficacy, marked dose dependence, and enhanced activity as fermentation progressed. Although the observed antiplasmodial activity may be related to bioactive phytochemicals previously reported in *C. esculentus*, including flavonoids, phenolics, alkaloids and saponins, these compounds were not quantified in the present study. Consequently, any proposed mechanisms, including inhibition of haem polymerisation, induction of oxidative stress, disruption of mitochondrial function or interference with parasite protein synthesis, should be regarded as possible explanations rather than confirmed mechanisms (Mojarrab *et al*., 2022; Lawal *et al*., 2024).

Effect of fermented and unfermented chaff of *C. esculentus* on haematological parameters

The packed cell volume, haemoglobin, red blood cells, white blood cells, lymphocytes, neutrophils, monocytes and eosinophils are presented in Table 5. The results showed no significant differences in packed cell volume, red blood cell count, lymphocyte count or neutrophil count among infected mice treated with fermented and unfermented *C. esculentus* chaff at the various doses. However, significant differences were observed in white blood cell counts following treatment with 25, 50 and 100 mg/kg of the fermented and unfermented preparations. Significant differences were also

observed in monocyte counts in mice treated with 25, 50 and 100 mg/kg of F24, 100 mg/kg of F48 and 50 mg/kg of F72 compared with the negative and positive controls.

The white blood cell component of blood plays a significant role in combating pathogen invasion and preventing the development of diseases, including malaria (Maina et al., 2010; Okpe et al., 2019). In this study, the white blood cell count of the infected mice increased significantly in the negative control group, indicating mobilization of immune cells in response to the increased parasitic burden compared with the treated groups. The reduction observed in the PCV of the negative control group may be due to the development of anaemia associated with malaria infection. However, the treated groups demonstrated increased PCV values, indicating that both fermented and unfermented *C. esculentus* tubers may possess anti-anaemic activity.

Table 5: Effect of treatment of infected mice with C. esculentus fermented chaff and unfermented powder on haematological parameters

| Plant material | Groups | PCV | HB | RBC | WBC | LYM | NEUT | MONO | Eosin |
|---|---|---|---|---|---|---|---|---|---|
| 24 h fermented C. esculentus tuber | NC | 43.10±1.10$^{a}$ | 14.01±0.79$^{a}$ | 7.26±1.78$^{a}$ | 5100±2.19$^{e}$ | 72.01±2.00$^{e}$ | 25.93±0.58$^{c}$ | 2.93±3.11$^{a}$ | 1.22±0.56$^{a}$ |
| | F24 (25 mg/kg) | 46.21±0.99$^{a}$ | 15.30±1.00$^{a}$ | 7.52±1.32$^{a}$ | 4500±1.87$^{d}$ | 73.32±1.99$^{e}$ | 23.21±0.82$^{c}$ | 2.69±2.91$^{a}$ | 1.51±0.81$^{a}$ |
| | F24 (50 mg/kg) | 46.13±1.21$^{a}$ | 15.40±0.88$^{a}$ | 7.47±1.88$^{a}$ | 4150±1.88$^{c}$ | 76.09±2.21$^{c}$ | 22.56±0.77$^{c}$ | 1.03±2.21$^{a}$ | 1.24±1.03$^{a}$ |
| | F24 (100 mg/kg) | 46.02±1.03$^{a}$ | 16.40±0.91$^{a}$ | 7.49±1.59$^{a}$ | 3650±2.31$^{b}$ | 76.11±2.13$^{a}$ | 22.38±0.59$^{a}$ | 0.01±0.23$^{a}$ | 1.32±0.99$^{a}$ |
| | CQ (5 mg/kg) | 53.00±0.89$^{a}$ | 17.20±0.86$^{a}$ | 8.41±1.12$^{a}$ | 3500±1.88$^{a}$ | 78.08±2.37$^{a}$ | 19.57±1.02$^{a}$ | 2.31±1.53$^{a}$ | 1.44±0.69$^{a}$ |
| 48 h fermented C. esculentus tuber | NC | 43.10±1.10$^{b}$ | 14.01±0.79$^{a}$ | 7.26±1.78$^{a}$ | 5100±2.19$^{e}$ | 72.01±2.00$^{e}$ | 25.93±0.58$^{c}$ | 2.93±3.11$^{a}$ | 1.22±0.56$^{a}$ |
| | F48 (25 mg/kg) | 45.10±1.11$^{a}$ | 14.30±1.02$^{a}$ | 7.39±1.09$^{a}$ | 5000±2.52$^{d}$ | 74.05±1.86$^{e}$ | 22.88±0.79$^{c}$ | 1.55±1.88$^{a}$ | 3.12±1.11$^{b}$ |
| | F48 (50 mg/kg) | 47.11±0.89$^{a}$ | 15.20±0.95$^{a}$ | 7.43±1.68$^{a}$ | 3650±1.90$^{c}$ | 75.51±2.35$^{a}$ | 21.39±1.03$^{c}$ | 3.22±0.19$^{a}$ | 3.23±1.00$^{b}$ |
| | F48 (100 mg/kg) | 50.12±1.31$^{a}$ | 16.00±0.86$^{a}$ | 8.14±0.99$^{a}$ | 2950±2.33$^{b}$ | 75.06±2.99$^{a}$ | 19.07±1.11$^{a}$ | 1.08±0.89$^{b}$ | 3.34±1.24$^{b}$ |
| | CQ (5 mg/kg) | 53.00±0.89$^{a}$ | 17.20±0.86$^{a}$ | 8.41±1.12$^{a}$ | 3500±1.88$^{a}$ | 78.08±2.37$^{a}$ | 19.57±1.02$^{a}$ | 2.31±1.53$^{a}$ | 1.44±0.69$^{a}$ |
| 72 h fermented C. esculentus tuber | NC | 43.10±1.10$^{b}$ | 14.01±0.79$^{a}$ | 7.26±1.78$^{a}$ | 5100±2.19$^{e}$ | 72.01±2.00$^{e}$ | 25.93±0.58$^{c}$ | 2.93±3.11$^{a}$ | 1.22±0.56$^{a}$ |
| | F72 (25 mg/kg) | 46.31±0.97$^{a}$ | 15.30±1.01$^{a}$ | 7.20±1.66$^{a}$ | 4500±1.89$^{d}$ | 73.12±2.11$^{a}$ | 23.59±0.98$^{a}$ | 2.39±1.21$^{a}$ | 1.55±0.21$^{a}$ |
| | F72 (50 mg/kg) | 50.23±0.99$^{a}$ | 16.00±0.99$^{a}$ | 8.39±1.39$^{a}$ | 3000±1.92$^{c}$ | 77.09±2.62$^{d}$ | 21.76±0.67$^{d}$ | 1.07±0.59$^{b}$ | 1.21±0.55$^{a}$ |
| | F72 (100 mg/kg) | 52.05±1.05$^{a}$ | 16.00±0.93$^{a}$ | 8.42±1.33$^{a}$ | 3100±1.22$^{b}$ | 77.17±1.76$^{a}$ | 18.02±1.32$^{a}$ | 2.85±1.99$^{a}$ | 1.92±0.86$^{a}$ |
| | CQ (5 mg/kg) | 53.00±0.89$^{a}$ | 17.20±0.86$^{a}$ | 8.41±1.12$^{a}$ | 3500±1.88$^{a}$ | 78.08±2.37$^{a}$ | 19.57±1.02$^{a}$ | 2.31±1.53$^{a}$ | 1.44±0.69$^{a}$ |
| Unfermented C. | NC | 43.10±1.10$^{b}$ | 14.01±0.79$^{a}$ | 7.26±1.78$^{a}$ | 5100±2.19$^{e}$ | 72.01±2.00$^{e}$ | 25.93±0.58$^{c}$ | 2.93±3.11$^{a}$ | 1.22±0.56$^{a}$ |

| Plant material | Groups | PCV | HB | RBC | WBC | LYM | NEUT | MONO | Eosin |
|---|---|---|---|---|---|---|---|---|---|
| esculentus tuber | | | | | | | | | |
| | UF (25 mg/kg) | 47.03±1.01$^{a}$ | 15.20±1.01$^{a}$ | 7.58±1.08$^{a}$ | 5100±1.55$^{c}$ | 75.09±2.33$^{a}$ | 22.07±1.79$^{a}$ | 2.36±1.19$^{a}$ | 0.21±0.43$^{b}$ |
| | UF (50 mg/kg) | 50.12±1.32$^{a}$ | 16.00±1.01$^{a}$ | 8.26±0.97$^{a}$ | 3650±2.56$^{b}$ | 76.11±2.02$^{a}$ | 22.13±1.12$^{a}$ | 2.57±1.58$^{a}$ | 1.52±0.33$^{a}$ |
| | UF (100 mg/kg) | 51.33±1.03$^{a}$ | 16.10±0.89$^{a}$ | 8.35±1.55$^{a}$ | 3600±2.78$^{b}$ | 76.58±2.59$^{a}$ | 22.08±0.90$^{a}$ | 2.38±1.33$^{a}$ | 1.48±0.38$^{a}$ |
| | CQ (5 mg/kg) | 53.00±0.89$^{a}$ | 17.20±0.86$^{a}$ | 8.41±1.12$^{a}$ | 3500±1.88$^{a}$ | 78.08±2.37$^{a}$ | 19.57±1.02$^{a}$ | 2.31±1.53$^{a}$ | 1.44±0.69$^{a}$ |

PCV= Packed cell volume; HB- haemoglobin; RBC= red blood cells; WBC= white blood cells; LYM= lymphocytes; NEUT= neutrophils; MONO= monocytes; EOSIN= eosinophils

## III. CONCLUSION

The present study determined the proximate composition of fermented and unfermented *C. esculentus* tubers and evaluated there in vivo antiplasmodial activity and haematological effects in *Plasmodium berghei*-infected mice. Fermentation improved the nutritional profile of the tubers, with F72 exhibiting the highest crude protein content, while F48 recorded the highest carbohydrate content. The fermented chaff, particularly F72, demonstrated the greatest antiplasmodial activity, as evidenced by reduced parasitaemia, higher chemosuppression, and lower $ED_{50}$ and $ED_{90}$ values compared with the unfermented sample. Treatment with both fermented and unfermented preparations also improved selected haematological parameters, including increased packed cell volume and reduced white blood cell counts relative to the negative control, suggesting recovery from malaria-induced haematological alterations. Overall, these findings demonstrate that fermentation enhanced the in vivo antiplasmodial activity of *C. esculentus* in the *P. berghei* mouse model. However, the present findings are limited to this experimental model and do not establish clinical efficacy in humans. Furthermore, the mechanisms underlying the enhanced antiplasmodial activity remain uncertain because the phytochemical constituents responsible for the observed effects were not quantified in this study. Therefore, further studies involving phytochemical characterization, toxicity evaluation, mechanistic investigations, and clinical studies are recommended to better establish the therapeutic potential of fermented *C. esculentus*.

### Ethical Approval

Animal Health Research Ethic Committee, Institute of Public Health at Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria (HREC NO: IPHOAU/12/1772) approved the experimental protocol. No clinical trial was performed in the study.

## REFERENCES

[1] Adejuyitan, J. A. (2011). Tigernut processing: Its food uses and health benefits. *American Journal of Food Technology, 6*, 197–201. https://doi.org/10.3923/ajft.2011.197.201

[2] Adenowo, A. F., & Kazeem, M. I. (2020). Tiger nut as a functional food, pharmacological and industrial agent: A mini review. *Annals of Science and Technology, 5*, 31–38. https://doi.org/10.2478/ast-2020-0004

[3] Akanbi, O. M., Omonkhua, A. A., & Cyril-Olutayo, C. M. (2022). In vivo antiplasmodial activity of medicinal plant extracts in *Plasmodium berghei*-infected mice. *Journal of Parasitology Research, 2022*, 1–8.

[4] AOAC International. (2019). *Official methods of analysis of AOAC International* (21st ed.). AOAC International.

[5] Bantie, L., Assefa, S., & Teklehaimanot, T. (2020). In vivo antimalarial activity of medicinal plants used in traditional medicine. *Evidence-Based Complementary and Alternative Medicine, 2020*, 1–10.

[6] Behera, S. S., Ray, R. C., & Zdolec, N. (2019). Lactic acid fermentation of tubers and

vegetables: An overview. *Microorganisms, 7*(205).

[7] Burns, E. R., Goldberg, S. N., & Lawrence, C. (2019). Hematologic responses during infectious diseases. *Clinical Hematology International, 1*, 45–52.

[8] Ceravolo, I. P., Sanchez, B. A., & Sousa, T. N. (2021). Drug discovery for malaria: Challenges and new perspectives. *Frontiers in Pharmacology, 12*, 679647.

[9] Cole, E. H. (1986). *Veterinary clinical pathology* (4th ed.). WB Saunders.

[10] Edo, G. I., Ugbune, U., Ezekiel, G. O., Onoharigho, F. O., & Agbo, J. J. (2023). *Cyperus esculentus* (Tiger nut): Its application in agriculture, food, health and nutrition: A review. *Vegetos, 37*, 757–766. https://doi.org/10.1007/s42535-023-00672-8

[11] Fidock, D. A., Rosenthal, P. J., Croft, S. L., Brun, R., & Nwaka, S. (2004). Antimalarial drug discovery: Efficacy models for compound screening. *Nature Reviews Drug Discovery, 3*(6), 509–520. https://doi.org/10.1038/nrd1416

[12] Habibi, P., Shi, Y., Fatima, M., Grossi-de-Sa, M., & Khan, I. (2022). Plants as sources of natural and recombinant antimalarial agents. *Molecular Biotechnology, 64*, 1177–1197. https://doi.org/10.1007/s12033-022-00499-9

[13] Kogan, F. (2020). 1981–2019 vegetation health trends assessing malaria conditions during intensive global warming. In *Remote sensing for malaria: Monitoring and predicting malaria from operational satellites* (pp. 219–263). Springer.

[14] Lawal, B., Shittu, O. K., & Oibiokpa, F. I. (2024). Phytochemical constituents of medicinal plants with antiplasmodial activity. *Journal of Ethnopharmacology, 315*, 116557.

[15] Liu, Y., Jiang, B., Wang, K., et al. (2023). A review of fermented bee products: Sources, nutritional values, and health benefits. *Food Research International, 174*, 113506. https://doi.org/10.1016/j.foodres.2023.113506

[16] Lopez-Cortes, A., Salazar-Rios, J., & Garcia-Mendez, M. (2013). Nutritional composition and fatty acid profile of tiger nut (*Cyperus esculentus*). *Journal of Food Composition and Analysis, 31*, 92–98.

[17] Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology, 54*, 275–287. https://doi.org/10.1007/BF01234480

[18] Maina, R. N., Walsh, D., Gaddy, C., Hongo, G., Waitumbi, J., Otieno, L., Jones, D., & Ogutu, B. (2010). Impact of *Plasmodium falciparum* infection on hematological parameters in children living in western Kenya. *Malaria Journal, 9*, 1–11. https://doi.org/10.1186/1475-2875-9-54

[19] Marchyshyn, S., Budniak, L., Slobodianiuk, L., & Ivasiuk, I. (2021). Determination of carbohydrates and fructans content in *Cyperus esculentus* L. *Pharmacia, 68*, 211–216. https://doi.org/10.3897/pharmacia.68.e54762

[20] Mojarrab, M., Shiravand, A., & Delazar, A. (2022). Antiplasmodial activity of plant secondary metabolites. *Phytochemistry Reviews, 21*, 203–225.

[21] National Research Council (US) Committee... (2011). *Guide for the care and use of laboratory animals* (8th ed.). National Academies Press.

[22] Okpe, O., Ibrahim, S., & Nwozo, S. O. (2019). Hematological changes associated with malaria infection. *Journal of Parasitic Diseases, 43*, 412–418.

[23] Olorunnisola, O. S., Adetutu, A., & Balogun, E. A. (2023). Fermentation enhances phytochemical bioavailability in medicinal plants. *Food Chemistry Advances, 2*, 100210.

[24] Peters, W., Portus, H., & Robinson, B. L. (1975). The four-day suppressive in vivo antimalarial test. *Annals of Tropical Medicine and Parasitology, 69*, 155–171. https://doi.org/10.1080/00034983.1975.11686997

[25] Robles Hernandez MG, Beauséjour M, Wolodko J, Gänzle MG. What is the role of fermented plant foods in sustainable, safe, secure and health-sustaining food systems? Adv Food Nutr Res. 2026;120:1-43. doi: 10.1016/bs.afnr.2026.04.002. Epub 2026 Apr 27. PMID: 42436033.

[26] Sánchez-Zapata, E., Fernández-López, J., & Pérez-Álvarez, J. A. (2012). Tiger nut (*Cyperus esculentus*) commercialization: Health aspects, composition, properties, and food applications. *Comprehensive Reviews in Food Science and Food Safety, 11*, 366–377. https://doi.org/10.1111/j.1541-4337.2012.00190.x

[27] Şanlier, N., Gökcen, B. B., & Sezgin, A. C. (2017). Health benefits of fermented foods. *Critical Reviews in Food Science and Nutrition, 59*, 506–527. https://doi.org/10.1080/10408398.2017.1383355

[28] Siqueira-Neto, J. L., Wicht, K. J., Chibale, K., Burrows, J. N., Fidock, D. A., & Winzeler, E. A. (2023). Antimalarial drug discovery: Progress and approaches. *Nature Reviews Drug Discovery, 22*, 807–826. https://doi.org/10.1038/s41573-023-00772-9

[29] Voidarou, C., Antoniadou, M., Rozos, G., Tzora, A., Skoufos, I., Varzakas, T., Lagiou, A., & Bezirtzoglou, E. (2020). Fermentative foods: Microbiology, biochemistry, potential human health benefits and public health issues. *Foods, 10*(69). https://doi.org/10.3390/foods10010069

[30] Wang, H., Yuan, M., Li, G., Tao, Y., Wang, X., Ke, S., Zhuang, M., Wang, A., & Zhou, Z. (2023). Chemical characterization, antioxidant and immunomodulatory activities of acetylated polysaccharides from *Cyperus esculentus*. *Food Chemistry, 427*, 136734. https://doi.org/10.1016/j.foodchem.2023.136734

[31] White, N. J., Pukrittayakamee, S., Hien, T. T., Faiz, M. A., Mokuolu, O. A., & Dondorp, A. M. (2014). Malaria. *The Lancet, 383*, 723–735. https://doi.org/10.1016/S0140-6736(13)60024-0

[32] Willcox, M. L., Graz, B., & Falquet, J. (2021). Strategies for evaluating antimalarial activity of medicinal plants. *Malaria Journal, 20*, 356.

[33] World Health Organisation. (2025). *Malaria*. https://www.who.int/news-room/fact-sheets/detail/malaria