## ට OPEN ACCESS Saudi Journal of Biomedical Research

Abbreviated Key Title: Saudi J Biomed Res ISSN 2518-3214 (Print) |ISSN 2518-3222 (Online) Scholars Middle East Publishers, Dubai, United Arab Emirates Journal homepage: <u>https://saudijournals.com</u>

**Original Research Article** 

## Exploring the Potential of Crude Methanolic Leaf Extract of Gossypium Hirsutum in Malaria Treatment, Pain Relief, and Inflammation Reduction

Umar Sheriff Itopa<sup>1,2\*</sup>, Olatunji Olalekan<sup>1</sup>, Yekeen Olaide Maruf<sup>1,2</sup>

<sup>1</sup>Department of Science Laboratory Technology, Federal Polytechnic Ile-Oluji, Ondo State, Nigeria <sup>2</sup>School of Computer, Mathematics and Natural Sciences, Morgan State University, Baltimore Maryland, U.S.A

DOI: 10.36348/sjbr.2023.v08i07.006

| Received: 23.06.2023 | Accepted: 19.07.2023 | Published: 24.07.2023

\*Corresponding author: Umar Sheriff Itopa

School of Computer, Mathematics and Natural Sciences, Morgan State University, Baltimore Maryland, U.S.A

## Abstract

The increasing resistance of Plasmodium falciparum to standard anti-malarial drugs has led to a search for new compounds with antimalarial properties. Throughout history, various plants have been used for the treatment of malaria. Gossypium hirsutum, a plant commonly used in Ondo state for malaria treatment, was the focus of this study. The crude extracts of Gossypium hirsutum were evaluated for their anti-plasmodial, analgesic, and anti-inflammatory properties. To assess the anti-plasmodial effect, *Plasmodium berghei* was inoculated into 12 mice divided into four groups, with three mice in each group. Groups 1 and 2 were treated with doses of 200mg/kg.bw and 400mg/kg.bw of the crude plant extract, respectively. Group 3 received 5mg/kg.bw of chloroquine phosphate, while group 4 received normal saline. The analgesic effects were evaluated using the Eddy's hot plate method and the Tail flick method in mice. The antiinflammatory effect was assessed using egg albumin-induced paw edema in rats. The crude methanolic extracts of Gossypium hirsutum demonstrated activity against Plasmodium parasites and a significant anti-inflammatory effect, suppressing paw edema by 49.7%. Animals administered with the extracts showed increased response time to thermal pain induction in both the hot plate and tail flick methods, similar to the effect observed with the standard drug. The crude plant extract reduced parasitemia in mice by 79.2%, accompanied by a slight but statistically insignificant decrease in packed cell volume (PCV) and the weight of treated animals. Overall, the crude extract of Gossypium hirsutum exhibited effectiveness in managing malaria, along with a moderate analgesic and anti-inflammatory potential. These findings suggest that the plant contains active compounds with promising antimalarial effects, which could be further isolated and studied.

Keywords: Gossypium hirsutum, Anti-plasmodium, Analgesia, Anti-inflammatory.

Copyright © 2023 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

## **INTRODUCTION**

Malaria, a parasitic disease transmitted through mosquito bites, is a significant global health issue that particularly affects tropical and subtropical regions [1]. It is classified as a re-emerging disease, with pregnant women and children under 5 years old being the most vulnerable population [2]. The World Malarial Report by WHO in 2022 revealed that malaria has a higher morbidity and mortality rate compared to other infectious diseases worldwide. Approximately 3.2 billion people, almost half of the world's population, are at risk of malaria. In 2021, there were 247 million malaria cases reported across 84 countries, resulting in 619,000 deaths. The majority of malaria-related deaths (96%) occurred in 29 countries, with Nigeria, the Democratic Republic of the Congo, the Niger, and the United Republic of Tanzania accounting for over half of the global malaria deaths. Shockingly, 76% of malaria deaths occur in children under 5 years old [3].

The reasons behind this situation are multifaceted, including the emergence of resistance to commonly used malaria treatments, insecticide resistance in mosquito vectors, and deteriorating living conditions and infrastructure in endemic areas due to population growth [4]. Malaria both affects and is affected by poverty, as the most affected populations have limited access to modern healthcare and often rely on traditional medicinal plants for treatment [5]. However, there is a lack of scientific studies evaluating the safety and efficacy of commonly used medicinal plants for malaria treatment. Therefore, this study aims to establish a scientific basis for the use of *Gossypium*  *hirsutum* in treating malaria, as well as managing associated symptoms such as pain and inflammation.

Cotton, a significant natural fiber source utilized in the global textile industry, belongs to the Malvaceae plant family [6]. Among the cultivated cotton species, Gossypium hirsutum is one of the most commonly grown varieties. The distribution of compounds within the Gossypium genus varies across different parts of the cotton plant, and this variance corresponds to their distinct properties and functions within the plant [7]. The cotton plant contains a diverse range of compound classes, including terpenes, monoterpenes, sesquiterpenes, triterpenes, phenols, flavonoids, phenolic acids, tannins, phlorotannins, coumarins, alkaloids, fatty acids, carbohydrates, and proteins [8]. Gossypium hirsutum, specifically, shows potential for use in preventing and treating conditions such as oral candidiasis [9], respiratory ailments, reproductive health issues, and genitourinary conditions. Traditional uses of its leaves and roots involve decocting them to address digestive disorders like diarrhea and dysentery. Additionally, the decoction and infusion of cotton leaves are employed for treating injuries and diarrhea.

## **MATERIALS AND METHOD**

## **Chemicals and Reagents**

All chemicals and reagents used were of analytical grade. Some of the chemicals used for this study include 99% methanol (BDH), Chloroquine Sulphate Standard (Sigma Aldrich).

#### Animals

Adult Swiss albino mice and Rats were obtained from the Animal house of Federal Polytechnic Ile-Oluji, Ondo state. The animals were fed *adlibitum* with standard mice feed and maintained under standard conditions of humidity, temperature and 12hours light /darkness cycles. The animals were allowed to acclimatize for three weeks in the laboratory before the commencement of the study.

#### Parasites

The parasite, Chloroquine sensitive *Plasmodium berghei* (NK-65) was obtained from the National Institute of Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria and Kept alive at the laboratory of Department of Science Laboratory Technology, Federal Polytechnic Ile-Oluji, Ondo State by parasite serial passage in mice every four days.

## **Collection and Identification of Plant Material**

The fresh leaves of *Gossypium hirsutum* collected in May, 2021, from Ibule-soro, Ondo state within Ifedore local government in Ondo state, Nigeria. The herb was identified by a taxonomist by its local name, common name, scientific name and morphological descriptions.

# Preparation of Methanolic Leave Extract of Gossypium Hirsutum

The fresh leaves of *Gossypium hirsutum* were air dried and ground into fine powder. A 200g weight of powdered leave sample was soaked in 800ml methanol with regular agitation at an interval of 1 hour to uniformly mix the sample within the first 20 minutes and left to stand for 72 hours. Filtration was performed using What man filter paper. The filtrate was concentrated using heating mantle at a temperature of 60°C to escape all the methanol under reduced pressure. The extraction concentrate was kept in sealed containers at low temperature (4°C) until use in bioscreening experiments.

#### **Screening for Secondary Metabolites**

Qualitative phytochemical analysis of the plant samples was carried out using standard methods for phytochemical properties according to the methods of Harborne [10] and Sofowora [11], for the detection of Phyto-constituents present in the plant.

#### **Inoculation of Parasite**

Mice were infected by a blood sample collected from a donor mouse with a rising Parasitemia of about 20–30%. After the determination of the percentage Parasitemia in the donor mouse, it was sacrificed and blood was collected via incisions of the jugular vein into a test tube containing 3.8% trisodium citrate added as an anticoagulant. The collected blood was diluted by 0.9% physiological isotonic saline based on the Parasitemia of the donor mice and the RBC count of normal mice  $(4.5 \times 10^9 \text{ RBC/ml})$  in such a way that 1 ml blood contains  $5 \times 10^7 P$ . *Berghei*-infected erythrocytes. Each mouse to be used in the experiment was injected intraperitoneally with 0.2 ml of infected blood containing about  $1 \times 10^7 P$ . *Berghei*-parasitized erythrocytes [12].

#### **Determination of Parasitemia**

This was carried out using Rane's test as described by Umar et al., [13]. On the first day (D<sub>0</sub>), 12 mice were inoculated with 0.2ml normal saline, which contains 1.0x107 P. berghei parasitized erythrocytes. The 12 mice were then divided into 4 groups of 3 animals each. Drugs and extracts were administered orally. 2 groups (Group 1 and 2) received 200mg/kgbw and 400mg/kgbw of test extract and group 3 was administered with 5mg/kgbw of chloroquine phosphate as positive control while 20ml/kgbw of physiological saline as negative control and Group 4. The treatment started 72 hours after parasitemia challenge. The extracts were given once daily for 4 days. Thin blood smears were prepared from the tail snip of each mouse from day 3 after infection established to day 7 for the curative test on microscopic slides. The slides were dried, fixed with absolute methanol, and stained with 10% Giemsa at pH 7.2 for 10 minutes, and then, they were washed gently using distilled water and air-dried at room temperature. Finally, the slides were examined under a microscope with an oil immersion objective  $(\times 100 \text{ magnification power})$  by taking an average of six fields.

The parasite count was done by an experiment blinded technician. Percentage parasitemia was

calculated by counting infected RBC and total RBC from Giemsa-stained thin blood films, and the percentage inhibition of parasitemia was calculated for each dose level by comparing the parasitemia in infected controls with that of treated mice with the following formulas.

% parasitaemia = 
$$\frac{Number of Parasitized RBC}{Total number of RBC counted} X 100$$

% inhibition =  $\frac{\bar{x} - ve \ control - \bar{x} \ of \ treated \ group}{\bar{x} \ of - ve \ control} X \ 100$ 

## **Determination of Body Weight**

The body weight of each mouse in Rane's test was measured on day 3 after the infection was established and on day seven, the last day of the treatment, using a sensitive digital weighing balance, to observe whether the test extract of the leave prevents body weight loss that commonly reduced with increasing *Parasitemia* in infected mice [14].

#### **Determination of Packed Cell Volume**

Blood was collected from the tail of each mouse in heparinized microhematocrit capillary tubes.

The capillary tubes were filled to 3/4<sup>th</sup> of their height with blood and sealed at one end with sealing clay. The tubes were then placed in a microhematocrit centrifuge, with the sealed end being outwards and centrifuged for 5 minutes at 11,000 rpm. The tubes were taken out of the centrifuge, and PCV was determined using a standard Micro-*Hematocrit* Reader [14]. PCV is a measure of the proportion of RBCs to plasma in the whole blood and is determined using the relation shown as follows

$$PCV(\%) = \frac{Volume \ of \ Erythrocyte \ in \ a \ given \ volume \ of \ blood}{Total \ Blood \ Volume} \ x \ 100$$

#### **Determination of Anti-Inflammatory Activity**

The anti-inflammatory activity of the extract was tested using egg albumin induced paw oedema in mice [15] as described by Jigam *et al.*, [2]. Adult rats were divided into 4 groups 5 rats each and used for the analysis. Inflammation was induced by the injection of 0.01ml egg albumin into the sub-planter surface on the right hind paw 30 min after administering the extracts.

The increase in volume(cm<sup>3</sup>) of the hind paw was measured with adigital Vernier caliper before and at 20 min interval after the injection of egg albumin for a period of 2 h. Control mice received an equivalent amount of normal saline while ASA (150 mg/kg.bw) served as reference. The percentage inhibition of oedema was calculated for each dose.

$$\% Inhibition = \frac{mean \ paw \ oedema \ of \ -VE \ control \ -mean \ paw \ oedema \ of \ treated \ group}{mean \ parasitemia \ of \ -VE \ control} X \ 100$$

#### Determination of Analgesic Activity Hot Plate Method

Albino mice of both sexes were randomly grouped into four groups of three mice each, fasted for 12 hours with adequate clean water provided ad libitum. Each of the mice was placed on a hot plate maintained at the temperature of 55  $\pm$  10 C and the pain reaction time (PRT) or latency period was determined as the time taken for the mice to react to the thermal pain by licking their paws or jumping. The reaction time was recorded before (0min) and at 15, 30, 45, and 60 min after the administration of the treatments. The maximum reaction time was fixed at 45 sec to prevent any injury to the tissues of the paws. This served as control pain reaction time. The mice were then treated as follows: Groups A and B received 200 and 400mg/kg of Hirsutum extract, group C received 100mg/kgof body weight Aspirin (positive control) while group D received normal saline (negative control) respectively.

Thirty minutes after drug and extract administration, the pain reaction time for each mouse was again determined and recorded [16].

#### **Tail Flick**

Twelve Mice divided into four groups of 3 animals each, were held in position in a suitable restrainer with the tail extending out. 3-4 cm area of the tail was marked and immersed in the water bath thermo-statistically maintained at 50°C. The reaction time (in seconds) was the time taken by the mice to flick its tail due to pain. The first reading was omitted and reaction time was taken as the average of the next two readings. The reaction time was recorded before (0min) and at 15, 30, 45, and 60minafter the administration of the treatments. The maximum reaction time was fixed at 15 sec to prevent any tail tissue injury. The mice were then treated as follows: Groups A and B received 200 and 400mg/kg of G.

*hirsutum* extract, group C received 100mg/kg of body weight Aspirin (positive control) while group D received 20mg/kg of body weight Normal saline (negative control) respectively. Thirty minutes after drug and extract administration, the pain reaction time for each mouse was again determined and recorded [17].

#### **Ethical Approval**

The principles governing the use of laboratory animals as laid out by the Federal Polytechnic Ile-Oluji, Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review were duly observed

#### Statistical Analysis

The data were analyzed using SPSS version 25. The results were expressed as mean  $\pm$  SEM (standard error of the mean). One-way ANOVA and Tukey's post hoc test for comparisons were used to compare differences in the groups. Results were considered significant at 95% confidence level at P value < 0.05.

## RESULTS

S/N	Name of Constituent	Present	Absent
1	Alkaloid	•	
2	Tannin	•	
3	Flavonoids	•	
4	Glycosides		•
5	Steroids	•	
6	Terpenoids	•	
7	Phenol	•	
8	Saponin	•	

**Table 1: Results of Phytochemicals Screening** 

The phytochemicals screening shows that alkaloids, Tannis, Flavonoids, Steroids, Terpenoids,

Phenol, and Saponin was present and *glycosides* was absent.

Treatment	Dose mg/kgbw	Parasitemia $\overline{X} \pm \text{SEM}$	Inhibition (%)
Gossypium hirsutum	200	$12.33 \pm 2.40$	79.21
Gossypium hirsutum	400	$14.33 \pm 1.76$	75.85
Chloroquine	25	$5.33 \pm 1.20$	91.01
N. S	20	$59.33 \pm 2.40$	-

## Table 2: Percentage Inhibition of Parasitemia



Figure 1: Effect of Treatment on Weight of Albino MIC

Treatment with crude aqueous extracts (200 and 400mg/kg) of the leaf of *G. hirsutum* showed no

significance difference in the weight of the animals in the treated groups.



Figure 2: Effect of Treatment of on PCV of Albino MICE

No significant change in PCV was observed in treated groups while the untreated groups were significantly affected by the parasite.

Treatment	Dose (mg/kgbw)	Before egg albumin (mm)	120mins after egg albumin	Net	%inhibition	
G. hirsutum	200	$1.23 \pm 0.02^{a}$	$1.41 \pm 0.04^{b}$	0.18	48.342	
G. hirsutum	400	$1.09 \pm 0.01^{a}$	$1.26 \pm 0.06^{b}$	0.17	49.712	
ASA	150	$1.04 \pm 0.03^{a}$	$1.06 \pm 0.01^{a}$	0.02	70.260	
Normal Saline	0.01ml	$1.11 \pm 0.10^{a}$	$1.84 \pm 0.03^{\circ}$	0.73		

Table 3: Egg	Albumin	induced	ANTI-INFL	AMMATORY	<b>ACTIVITY</b>
I ubic of Ligg	1 III uIIIII	maacca	THIS IS A REAL OF A REAL ADDRESS OF A REAL ADDRESS		

Values are mean  $\pm$  SEM of 3 determinations. Values are mean  $\pm$  SEM of 3 determinations. Values along the same column with different superscripts are significantly different. (p< 0.05)

#### Table 4: Result of Analgesic Activity by Hot Plate Method

	<b>Pre-Drug Reaction Time</b>	Post-Drug Reaction Time			
Extract Dose		15min	30min	45min	60min
200mg/kg	$5.24 \pm 0.45$	$6.14 \pm 0.10^{a}$	$766 \pm 2.08^{ab}$	$7.00\pm0.22^{\rm a}$	$7.86\pm0.54^{\rm a}$
400mg/kg	$5.40 \pm 0.32$	$6.58\pm0.22^{\rm a}$	$7.46 \pm 1.53$ <sup>ab</sup>	$8.00 \pm 0.45^{b}$	$8.96 \pm 0.24^{b}$
A S A	$5.38 \pm 0.00$	$6.00 \pm 0.41^{a}$	$9.25 \pm 1.53$ <sup>a</sup>	$10.00\pm1.02^{\rm a}$	$11.33\pm0.68^a$
Normal Saline	$5.71 \pm 0.02$	$6.12 \pm 0.22^{a}$	$5.33 \pm 2.52^{\text{ b}}$	$5.02 \pm 0.00^{\circ}$	$5.02 \pm 0.41^{ab}$

Values are mean  $\pm$  SEM of 3 determinations. Values along the same column with different superscripts are significantly different at (p< 0.05).

	<b>Pre-Drug Reaction Time</b>	Post-Drug Reaction Time			
Extract Dose		15min	30min	45min	60min
200mg/kg	$3.48\pm0.45$	$4.02\pm0.10^{\rm a}$	$4.22 \pm 2.08^{ab}$	$5.61\pm0.22^{\rm a}$	$5.20\pm0.54^{b}$
400mg/kg	$3.26\pm0.32$	$4.56\pm0.22^{\rm a}$	$3.52 \pm 1.53^{ab}$	$4.21 \pm 0.45^{b}$	$5.35 \pm 0.24^{b}$
A S A	$3.22\pm0.00$	$4.12\pm0.41^{a}$	$5.31 \pm 1.53^{a}$	$7.64 \pm 12.00^{a}$	$7.52\pm0.68^{a}$
Normal Saline	$3.30\pm0.02$	$3.21\pm0.22^{\rm a}$	$3.54 \pm 2.52^{b}$	$2.94\pm0.00^{c}$	$3.01\pm0.41^{c}$

## Table 5: Result of Analgesic Activity BYON Tail Flick Method

Values are Mean  $\pm$  SEM for 3 determinations. Values along the same column with different superscripts are significantly different at (p<0.05)

## DISCUSSION

Phytochemicals are natural compounds found in medicinal plants that play a crucial role in their various biological activities. When examining G. *hirsutum*, a type of cotton plant, it was discovered through phytochemical screening that it contains several important compounds such as alkaloids, flavonoids, phenols, tannins, terpenes, steroids, and saponins. This finding aligns with previous studies on other *Gossypium* species, which also identified the presence of alkaloids, flavonoids, tannins, phenols, and similar compounds [18].

© 2023 | Published by Scholars Middle East Publishers, Dubai, United Arab Emirates

The traditional use of G. hirsutum in treating malaria can be attributed to the presence of these phytochemicals, which act as the active components in the plant. Some of the secondary metabolites identified in this study have been linked to anti-plasmodium activities, indicating their potential in combating the malaria parasite. The anti-malarial properties of certain plants are often associated with the presence of alkaloids, flavonoids, and terpenes, all of which are present in G. hirsutum. Alkaloids, in particular, have been extensively utilized in malaria treatment, exemplified by the use of quinine, a cinchona alkaloid belonging to the aryl amino alcohol group of drugs. Due to its basic nature, quinine is typically found in the form of a salt and concentrates in the food vacuoles of P. falciparum, a parasite responsible for malaria. The proposed mechanism of action for quinine and similar anti-malarial drugs involves their toxicity against the malaria parasite, specifically by interfering with the breakdown and digestion of hemoglobin. Quinine demonstrates rapid schizonticidal action against malaria parasites within red blood cells and possesses analgesic properties as well [19]. The anti-oxidant flavonoid and phenolic compounds have also been shown to exert anti-plasmodium activity byelevating the red blood cell oxidation and inhibiting the parasites protein synthesis [20]. This activity counteracts the oxidative damage induced by the malaria parasite.

In the 4-day curative test, groups of mice treated with a crude extract of Gossypium hirsutum exhibited a significant decrease in parasitemia compared to the negative control group. It has been suggested that crude plant extracts are more effective in inhibiting parasite multiplication (plasmodistatic) rather than directly destroying the parasite (plasmodicidal). The presence of unpurified bioactive components in the extract requires conversion processes that allow the parasite to proliferate during a time lag [2]. Other studies have reported the antibacterial activity of Gossypium hirsutum [9]. It is worth noting that drugs with antibacterial properties like tetracycline and its derivatives have been associated with malaria treatment. This supports the idea that the plant may contain compounds responsible for its antimalarial activity. The administration of the crude extract of G. hirsutum to infected animals resulted in a gradual decrease in parasitemia over time. The two different doses used for treatment showed no significant difference in their antimalarial effects, suggesting that the minimum effective dose required to produce antimalarial activity was surpassed in this study. The significant antimalarial activity observed in the crude extract from of G. hirsutum may explain its widespread use in herbal medicine. While all doses of the extract reduced parasitemia after two administrations, the standard drug chloroquine exhibited its activity immediately after the first dose. This delay in activity could indicate the need for a loading dose or suggest that the extract has a delayed onset of action. It might also suggest the requirement for more frequent administrations throughout the day.

The antimalarial activity of G. hirsutumcould be attributed to the presence of any its constituent secondary metabolite which have previously been established to possess antimalarial activity. However, it is possible that a combination of its secondary metabolites which would include alkaloids, flavonoids, terpenoids, and phenolic compounds etc. are responsible for this activity. These metabolites have been reported in literature of different plant species as having different extents of antimalarial activity [21, 22]. In vivo anti-plasmodial activity can be classified as "moderate", "good", and "very good" if an extract displays a percentage parasitemia suppression  $\geq 50\%$  at a dose of 500 mg/kg, 250 mg/kg, and 100 mg/kg body weight per day, respectively [23]. Based on this classification, the crude extract of G. hirsutum has a good anti-plasmodial activity.

Packed cell volume (PCV) serves as an additional parameter for evaluating the antimalarial activity of a substance by determining its ability to prevent hemolysis caused by the invading Plasmodium parasite [24]. In this study, the PCV was measured to assess the effectiveness of the crude methanolic extract of G. hirsutum in preventing hemolysis resulting from an increase in parasitemia levels. Both doses of the crude extract of G. hirsutum led to a slight, albeit statistically insignificant, decrease in PCV. There was no significant difference in the initial and final PCV values among the groups treated with the alkaloidal extract. Anemia in malaria can arise from various mechanisms, including the clearance and/or destruction of infected red blood cells (RBCs), the clearance of uninfected RBCs, as well as erythropoietic suppression and dyserythropoiesis [25]. Each of these mechanisms has been associated with anemia caused by malaria in both humans and mice. The observed minor reduction in PCV could potentially be attributed to the presence of saponins in the extracts, as saponins are known to have hemolytic effects [26].

Treatment with crude aqueous extracts of G. hirsutum leaf at doses of 200mg/kg and 400mg/kg did not result in a significant difference in the weight of the animals between day 0 and day 4 of treatment. However, the untreated group exhibited a notable decrease in weight, which could be attributed to the loss of appetite, a common symptom of malaria [27]. In the chloroquine group, there was no significant difference in the weight of the treated animals. This effect could be attributed to the presence of active anti-malarial agents, which are expected to prevent weight loss in mice infected with P. berghei [28]. These agents demonstrated a significant prevention of weight loss across all dose levels, indicating the potential localization of appetite-suppressing components,

nutrients, and other immunomodulatory substances even at lower doses of this fraction [23].

The crude extract of G. hirsutum has been shown to possess a significant anti-inflammatory effect [29]. The presence of various secondary metabolites such as terpenoids, saponins, flavonoids, alkaloids, and tannins indicate that the plant extract may have medicinal qualities, including anti-inflammatory, analgesic, anti-diabetic, and anti-cholesterolemia effects [30]. Multiple studies have provided evidence for the anti-inflammatory properties of flavonoids, saponins, anthraquinones terpenes. and using different inflammation models [31]. It is possible that one or a combination of these secondary metabolites is responsible for the significant anti-inflammatory effect exhibited by the plant extract. For example, flavonoids are antioxidants that also possess anti-inflammatory properties due to their ability to inhibit enzymes involved in the production of inflammatory mediators [32].

The measurement of egg albumin-induced paw edema is widely accepted as a representation of the acute stage of inflammation and is commonly used in the discovery of potential anti-inflammatory drugs [33]. This model is particularly useful for assessing the effects of cyclooxygenase inhibitors and has been employed to evaluate the impact of nonsteroidal antiinflammatory drugs (NSAIDs), which inhibit prostaglandin synthesis. The initial stage of inflammation is characterized by the release of bradykinin, serotonin, and histamine, followed by the release of prostaglandins after three hours, which persists for approximately six hours post egg albumin challenge. The final stage involves the maximal vascular reaction, marked by the migration of leukocytes to the inflamed area [34]. In this study, it was observed that the crude extract of G. hirsutum at dose levels of 200 mg/kg (48.34%) and 400 mg/kg (49.97%) reduced paw edema in rats. These findings suggest the anti-inflammatory potential of the crude extract by blocking the inflammatory mediators in the acute stage of inflammation, as well as potentially inhibiting the synthesis pathway of prostaglandins (PGs).

This study observed a significant increase in the pain threshold of animals treated with the crude methanolic extract of *G. hirsutum*, indicating the involvement of peripheral pain pathways. The analgesic effect of the extract may occur through central mechanisms, including the opiate, dopaminergic, descending noradrenergic, and serotonergic systems, or through peripheral mechanisms that inhibit the production of prostaglandins, leukotrienes, and other endogenous substances involved in inflammation and pain.

The crude leaf extract of G. *hirsutum* exhibited significant analgesic activity in both pain response time

models used in the study. The concentrations of 200mg/kg and 400mg/kg extended the response time of mice at 60 minutes after treatment, showing a significant difference compared to the untreated group but significantly lower than the response time of the animals in the standard drug group (11.33  $\pm$  0.68s). The findings of our study indicate a notable potential for pain relief in the hot plate and tail flick models, suggesting that the analgesic activity of G. hirsutum resembles that of non-narcotic analgesics. Previous research has established the presence of flavonoids, terpenoids, saponins, tannins, and alkaloids in G. hirsutum. These phytochemicals, including tannins, saponins, flavonoids, and alkaloids, are bioactive compounds known for their diverse pharmacological effects which can include their effectiveness in pain management [35].

## **CONCLUSION**

The findings of this study demonstrate the promising antimalarial activity of the crude leaf extract of G. hirsutum. Additionally, the alkaloidal extract of M. senegalensis has shown potential as an analgesic, although its anti- inflammatory effect is moderate, possibly due to other phytochemicals present in the plant. Moving forward, it is crucial to isolate, identify, and characterize the active component responsible for these effects. Understanding the mechanism of action of this active component can also provide valuable insights for future research and potential development of synthetic analogues. Furthermore, further analysis of other phytochemical components in the plant can shed light on their therapeutic activity and their role in the plant's diverse pharmacological effects

#### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## REFERENCES

- Greenwood., B. M., Fidock, D. A., Kyle, D. E., Kappe, S. I., Alonso, P. L., Collins, F. H., & Duffy, P. E. (2008). Malaria: progress, perils, and prospects for eradication. *Journal of Clinical Investigation*, 118, 1266–1276.
- Jigam, A. A., Akanya, H. O., Dauda, B. E. N., & Ogbadoyi, E. O. (2011). Anti-plasmodial, analgesic and anti-inflammatory effects of crude *Guierasenegalensis* Gmel (Combretaceae) leaf extracts in mice infected with *Plasmodium berghei*. *Journal of Pharmacognosy and Phytotherapy*, 3(10), 150-154.
- WHO (2022) World malaria report (2022). Geneva: World Health Organization; License: CC BY-NC-SA 3.0 IGO
- Hyde, J. E. (2014). Drug-resistant malaria an insight. Federation of European Biochemical Society Journals, 274, 4688-4698.
- 5. Bourdy, G., Willcox, M. L., Ginsburg, H., Rasoanaivo, P., Graz, B., & Deharo, E. (2008).

Ethnopharmacology and malaria: new hypothetical leads or old efficient antimalarials? *International Journal for Parasitology*, *38*, 33-41.

- Thompson, C. N., Hendon, B. R., Mishra, D., Rieff, J. M., & Lowery, C. C. (2019). Cotton (Gossypium hirsutum L.) mutants with reduced levels of palmitic acid (C16:0) in seed lipids. *Euphytica*, 215, 112.
- Egbuta, M. A., McIntosh, S., Waters, D. L., Vancov, T., Liu, L. (2017). Biological Importance of Cotton By-Products Relative to Chemical Constituents of the Cotton Plant. *Molecules*, 22(1), 93. doi: 10.3390/molecules22010093. PMID: 28067842; PMCID: PMC6155835.
- Chandrashekhar, R., Ram, B., & Bhavani, N. L. (2019). Quantitative analysis of phytochemical compounds in the cotton (Gossypium) Seed Extracts; an important commercial crop plant. *Bulletin of Pure & Applied Sciences- Botan*, *38b*(1), 56-62. DOI: 10.5958/2320– 3196.2019.00007.7
- Balliana, R. C. S., Jamal, C. M., & Maifrede, S. B. (2014). Evaluation of the anti-fungal activity of plant extracts and oral antiseptics against Candida albicans. *Brazilian. Dent. Sci.* 17, 98. https://doi.org/10.14295/bds.2014.v17i4.1044
- Harborne, J. B. (1973). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. London, Chapman and Hall Ltd., 278.
- Sofowora, A. (1993). Medical Plants and Traditional Medicine in Africa. (2<sup>nd</sup>ed.). New York, John Wiley and Sons Ltd., 97-145.
- Okokon, J. E., Ofodum, K. C., Ajibesin, K. K., Danladi, B., & Gamanil, K. S. (2005). Pharmacological screening and evaluation of antiplasmodial activity of *Croton Zambesicus* against *Plasmodium berghei* in mice. *Indian Journal Pharmacology*, 379, 243–246.
- Umar, S. I., Ndako, M., & Jigam, A. A. (2019). Anti-plasmodial, Anti-inflammatory, antinociceptive and safety profile of Maytenus senegalensis root bark extract on hepato-renal integrity in experimental animals. *Comp Clin Pathol*, 28, 1571–1579 https://doi.org/10.1007/s00580-019-02965-4
- Dikasso, D., Mekonnen, E., & Debella, A. (2006). Antimalarial activity of Withania somnifera L. dunal extracts in mice. *Ethiopian Medical Journal*, 44(3), 279–285.
- 15. Winter, C. A., Risley, E. A., & Nuss, G. V. (1962). Carrageenin induced oedema in hind-paw of rats as an assay for anti-inflammatory drugs. *Proceedings* of the Society for Experimental Biology and Medicine, 3, 544-547.
- Mothana, R. A., Al-Said, M. S., Al-Rehaily, A. J., Thabet, T. M., Awad, N. A., Lalk, M., & Lindequist, U. (2012). Anti-inflammatory, antinociceptive, antipyretic and antioxidant activities and phenolic constituents from Loranthus

regularis Steud. Ex Sprague. Food Chemistry, 130, 344–349.

- Ameyaw, E. O., Boampong, J. N., Kukuia, K. K. K. (2014). Effect of xylopic acid on paclitaxelinduced neuropathic pain in rats. *Journal* of Medical and Biomedical Sciences, 2(4), 612.
- Ademilua, O. E., & Okpoma, M. (2018). Gossypium hirsutum and Gossypium barbadense: Differences in phytochemical contents, antioxidant and antimicrobial properties. *Ife Journal of* Science, 20(1).
- Ross, L. S., & Fidock, D. A. (2019). Elucidating Mechanisms of Drug-Resistant Plasmodium Falciparum. *Cell Host Microbe* 26, 35–47. Doi: 10.1016/j.chom.2019.06.001
- Ahmad, A., Kaleem, M., Ahmed, Z., Shafiq, H. (2015). Therapeutic potential of flavonoids and their mechanism of action against microbial and viral infections—A review. *Food Res. Int*, 77, 221–235. doi: 10.1016/j.foodres.2015.06.021.
- Khalid, S. A., Friedrichsen, G. M., Christensen, S. B., El Tahir, A., & Satti, G. M. (2007). Isolation and characterization of pristimerin as the antiplasmodial and antileishmanial agent of *Maytenus* senegalensis (Lam.) Exell. Archive for Organic Chemistry, 9, 129–134.
- Malebo, H. M., Nguruwe, R., Lugimbana, L., Sambu, E., Malecela, E., Kamugisha, M. L., Senkoro, K., & Magesa, S. M. (2005). Efficacy of Ocimum suave volatile oil formulation against man-biting mosquitoes in Muheza, north-east Tanzania. *Tanzania Health Research Bulletin*, 7, 25-30.
- Fentahun, S., Makonnen, E., Awas, T., & Giday, M. (2017). In vivo antimalarial activity of crudeextracts and solvent fractions of leaves of Strychnosmitis in Plasmodium berghei infected mice. *BMC Complementary and Alternative Medicine*, 17(1), 13.
- 24. Alehegn, A. A., Yesuf, J. S., & Birru, E. M. (2020). Antimalarial activity of crude extract and solvent fractions of the leaves of bersama abyssinica fresen. (Melianthaceae) against plasmodium berghei infection in Swiss albino mice. *Evidencebased Complementary and Alternative Medicine*, 2020, 1–14.
- Lamikanra, A. A., Theron, M., Kooij, T. W. A., Roberts, D. J. (2009) Hemozoin (Malarial Pigment) Directly Promotes Apoptosis of Erythroid Precursors. *PLoS ONE*, 4(12), 844-849.
- Inalegwu, B., & Sodipo, O. (2013). Phytochemical screening and haemolytic activities of crude and purified saponins of aqueous and methanolic extracts of leaves of Tephrosia vogelii Hook F. Asian J Plant Sci Res, 3(5), 7–11.
- Ndyomugyenyi, R., Magnussen, P., & Clarke, S. (2007). Diagnosis and treatment of malaria in peripheral health facilities in Uganda: findings from an area of low transmission in south-western Uganda. *Malar J.*, 2(6), 39. doi: 10.1186/1475-

 $<sup>\</sup>ensuremath{\mathbb{O}}$  2023 | Published by Scholars Middle East Publishers, Dubai, United Arab Emirates

2875-6-39. PMID: 17407555; PMCID: PMC1851016.

- Hagazy, K., Sibhat, G. G., Karim, A., Tekulu, G. H., Periasamy, G., & Hiben, M. G. (2020). Antimalarial activity of Meriandra dianthera leaf extracts in Plasmodium berghei-infected mice. *Evidence-Based Complementary and Alternative Medicine*, 2020. Article ID 8980212, 8 pages, https://doi.org/10.1155/2020/8980212
- da Silva, G., Tanica, M., Gomes E. T., & Silva, O. (2011). *Maytenus* heterophylla and *Maytenussenegalensis* leaves – chemical composition and antioxidant properties. *Future Trends in Phytochemistry*, 21, 324-327.
- Aquino, R., Morelli, S., Lauro, M. R., Abdo, S., Saija, A., & Tomaino, A. (2001). Phenolic constituents and antioxidant activity of an extract of *Anthuriumversicolor* leaves. *Journal Natural Products*, 64, 1019-1023.
- Chandel, R. S., & Rastogi, R. P. (2012). Review: Triterpenoid Saponins and Sapogenins. *Phytochemistry*, 19, 1889–1908.

- Bani, S., Kaul, A., Jaggi, B. S., Suri, K. A., Suri, O. P., & Sharma, O. P. (2006). Anti-inflammatory activity of hydro-soluble fraction of *Euphorbiaroyleana* latex. *Fitoterapia*, 71, 655-662.
- Barung, E. N., Dumanauw, J. M., Duri, M. F., & Kalonio, D. E. (2021). Egg white-induced inflammation models: A study of edema profile and histological change of rat's paw. *J Adv Pharm Technol Res, 12*(2), 109-112. doi: 10.4103/japtr.JAPTR\_262\_20. Epu PMID: 34159139; PMCID: PMC8177150
- Tomazetti, J., Avila, D. S., Ferreira, A. P. O., Martins, J. S., Souza, F. R., Royer, C., Rubin, M. A., Oliveira, M. R., Bonacorso, H. G., Martins, M. A. P., & Zanatta, N. (2005). Baker yeast-induced fever in young rats: Characterization and validation of an animal model for antipyretics screening. J *Neurosci Methods*, 147, 29–35.
- 35. Kim, S. K., & Van Ta, Q. (2011). Potential beneficial effects of marine algal sterols on human health. *Advances in Food and Nutrition Research*, 64, 191–198.