*EJPMR, 2026, 13(2), 314-337* **Research Article** **Impact Factor: 8.158**

**EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH**

www.ejpmr.com

**ISSN (O): 2394-3211**
**ISSN (P): 3051-2573**
**Coden USA: EJPMAG**

# IN VITRO AND IN VIVO ASSESSMENT OF POTENTIAL ANTIMALARIAL ACTIVITY OF EXTRACTS AND SOLVENT FRACTIONS, ACUTE AND SUBACUTE TOXICITY OF AQUEOUS EXTRACT FROM TRICLISIA GILETTII (DE WILD) STANER ROOT BARKS (MENISPERMACEAE) ON SWISS ALBINO MICE

**Mangombo K.H.[1], Mampuya M.C.[2], Kikweta M.C.[2], Tshodi E.M.[2], Lusakibanza M.[1], Mesia K.G.[2], Tona L.G.[1], Cimanga K.R.[3]***

[1]Department of Pharmacology and Therapeutic, Faculty of Pharmaceutical Sciences, University of Kinshasa, B.P. 212, Kinshasa XI, Democratic Republic of Congo.
[2]Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Kinshasa, B.P. 212, Kinshasa XI, Democratic Republic of Congo.
*[3]Department of Pharmaceutical, Biomedical and Veterinary Sciences, Natural Products & Food-Research and Analysis (NatuRAPT), University of Antwerp, Universiteitsplein 1, B-2610, Antwerp, Belgium.

***Corresponding Author: Cimanga K.R.**

Department of Pharmaceutical, Biomedical and Veterinary Sciences, Natural Products & Food-Research and Analysis (NatuRAPT), University of Antwerp, Universiteitsplein 1, B-2610, Antwerp, Belgium.

**Email Id:** rkcimanga@gmail.com, **DOI:** https://doi.org/10.5281/zenodo.18479962

**How to cite this Article:** Mangombo K.H.[1], Mampuya M.C.[2], Kikweta M.C.[2], Tshodi E.M.[2], Lusakibanza M.[1], Mesia K.G.[2], Tona L.G.[1], Cimanga K.R.[3]*. (2026). In Vitro And In Vivo Assessment Of Potential Antimalarial Activity Of Extracts And Solvent Fractions, Acute And Subacute Toxicity Of Aqueous Extract From Triclisia Gilettii (De Wild) Staner Root Barks (Menispermaceae) On Swiss Albino Mice. European Journal of Pharmaceutical and Medical Research, 13(2), 314–337.


**Article Received on 05/01/2026** **Article Revised on 25/01/2026** **Article Published on 04/02/2026**

**ABSTRACT**

The present study was initiated to evaluate the antimalarial or antiplasmodial activity of aqueous, methanol 80% and total alkaloids extracts as well as solvent fractions from the partition of aqueous and methanol 80% extracts of Triclisia gilettii root barks against Plasmodium falciparum NF54 sensitive to chloroquine and Plasmodium falciparum KI resistant to chloroquine and pyrimethamine in vitro test, and towards Plasmodium yoeli 67 and Plasmodium berghei berghei ANKA in vivo test. Results revealed that in vitro test, aqueous, methanol 80% and total alkaloids extracts inhibited the growth of both selected Plasmodium strains with concentrations 50 (IC50) < 10 µg/mL as pronounced and produced selective index (SI) of 2.55±0.05, 2.57±0.03 and 2.16±0.04 against P. Plasmodium NF54 and 2.14±0.03, 1.71±0.06 and 1.63±0.03 towards P. falciparum K1 showing thus, their selective antimalarial effect. Choloroform, ethylacetate, n-butanol and residual aqueous solvent fractions from the partition of aqueous extract exhibited antiplasmodial activity against P. falciparum NF54 with selective index (SI) between 1.01±0.07 and 1.36±0.05 by inhibiting its growth with IC50 values ranging between 5.86±0.09 and 8.54±0.11 µg/ and exerted the same effect against P. falciparum K1 with IC50 values between 6.04±0.08 and 10.32±0.13 µg/mL with SI ranging between 0.83±0.05 and 1.07±0.05. The same solvent fractions from the partition of methanol 80% extract displayed antiplasmodial activity against P. falciparum NF54 with IC50 values between 4.14±0.05 and 8.34±0.06 µg/ml with SI values between 0.88±0.06 and 1.52±0.10 and IC50 values between 6.12±0.08 and 10.11±0.07 µ/mL towards P. falciparum K1 with SI values between 0.72±0.08 and 1.11±0.13 expressing their selective or non-selective antimalarial effect according the case. Artesunate and Quinine 2HCl displayed high antimalarial effect with IC50 values of 1.54±0.03 and 2.15±0.04 µg/ml and 2.23±0.04 and 4.13±0.02 µg/mL towards P. falciparum NF45 and K1 strains respectively with good SI against Plasmodiums NF54 and K1 of 6.77±0.06 and 7.00±0.04 and 4.67±0.04 and 3.64±0.04 respectively and showed high activity compared to extracts and solvent fractions. In vivo test, results revealed that aqueous, methanol 80% and total alkaloids extracts administered at dose oral of 400 mg/kg bw produced percentage chemosuppressions of 77.48±0.08, 85.24±0.13 and 85.70±0.04% against P. yolei and 75.13±0.11, 82.25±0.08 and 84.96±010% against P. b. berghei respectively. Artesunate and Quinine 2HCl used as reference antimalarial drugs engendered chemosuppression percentages of 90.96±0.08 and 89.36±0.05% against P. yolei 67 and 90.44±0.03 and 89.15±0.07% towards P. b. berghei respectively and showed high activity compared to the three extracts while total alkaloids ME-2 extract displayed high activity confronted to aqueous AE-1 and methanol 80% ME-1 extracts and this last extract exhibited high activity compared to aqueous EA-1 extract. All extracts from T. gilettii root barks and reference drugs showed good and interesting in vitro and in vivo antimalarial effects mainly exploitable in traditional medicine for the use particularly of aqueous extract for the treatment of uncomplicated malaria in humans in traditional medicine.

**KEYWORDS:** Triglisia gilleti, Menispermaceae, root barks, extracts, solvent fractions, Plasmodium strains, malaria.

## 1. INTRODUCTION

The disease malaria considered as a life-threatening infectious disease caused by Plasmodium parasites, may be caused by at least five different species like P. falciparum (most severe), P. vivax (relapsing), P. ovale, P. malariae, and P. knowlesi (from monkeys) (Patel et al., 2013, Yang et al;, 2020, Chani et al., 2022). But, there are approximately 156 named species of Plasmodium which infect various species of vertebrates. Four species are considered true parasites of humans, as they utilize humans almost exclusively as a natural intermediate host: P. falciparum, P. vivax, P. ovale and P. malariae. However, there are periodic reports of simian malaria parasites being found in humans and most reports implicating P. knowlesi. For un long time, it has not been determined if P. knowlesi is being naturally transmitted from human to human via the mosquito, without the natural intermediate host (macaque monkeys, genus Macaca). Therefore, P. knowlesi is still considered a zoonotic malaria. P. falciparum was the most dangerous parasite often causes malaria relapse reaching sometimes to death (Anonymous, 2025).

This disease is the major public health concern worldwide, particularly in tropical and subtropical regions especially in Sub-Saharan Africa where it is considered as one of the most serious health problems. Globally, there was estimated 229 million cases and 409.000 deaths from malaria in 2019 (WHO, 2020). This situation is due to the great increase in the resistance of malaria parasites to used antiplasmodial drugs and the limited number if effective drugs are the great challenge in the treatment of the disease. Most malaria cases and deaths occur in the WHO African countries and mainly affect children under 5 years and pregnant women (OMS, 2017, Wen-Hu et al., 2018).

Malaria generally occurs in areas where environmental conditions allow a in the vector. Malaria today is usually restricted to tropical and subtropical areas and altitudes below 1.500 m, although in the past, malaria was endemic in much of North America, Europe and even parts of northern Asia, and today is still present in the Korean peninsula. However, this present distribution could be affected by climatic changes and population movements. Plasmodium falciparum is the predominant species in the world. P. vivax and P. ovale are traditionally though to occupy complementary niches with P. ovale predominating in Sub-Saharan Africa and P. vivax in the others areas, but their geographical ranges do overlap. These two species are not always distinguishable on the basis of their morphologic characteristics alone, and the use of molecular tools will help clarify their diagnosis and exact distribution. P. malariae has wide global distribution being found in South America, Asia and Africa, but, it is less frequent than P. falciparum in terms of association with cases of infection. P. knowlesi is found in Southeast Asia (Anonym A , 2025a).

gMalaria is one of the major threats to human health, affecting an estimated number of 216 million peoples in 2016 all over the world, leading to 445,000 deaths, mainly in the African continent. The human malaria is caused by six different species of the genus Plasmodium, which are P. falciparum, P. vivax, P. ovale, P. malariae, Plasmodium knowlesi and P. simium, where the last one is exclusive to the Brazilian Atlantic forest (Anonym A, 2025).

Plasmodium was first described in 1880 by Laveran, who observed it in human erythrocytes. In human Plasmodium genus, the life cycle is very similar between species, being characterized by a sexual phase in vector Anopheles and an asexual phase in the human host that can be divided into liver phase and intraerythrocytic phase (Camargo, 2003).

Among the five Plasmodium species (P. falciparum, P. vivax, P. ovale, P. malariae, and P. knowlesi) known to infect humans, P. falciparum is considered the most virulent and and mortal. It is responsible for most severe malaria cases and deaths globally, particularly in sub-Saharan Africa (Kolade et al., 2023). In addition, P. falciparum is often associated with many fatal systems and organ complications, including severe anemia, acute renal failure, shock, blackwater fever, hepatic impairment, hypoglycemia, defective acid-base balance, neurological sequelae and cerebral malaria (Jensen et al. 2020, Manhas et al., 2022).

The symptoms of uncomplicated malaria can be rather non-specific and the diagnosis can be missed if health providers are not alert to the possibility of the disease. Since untreated malaria can progress to severe forms that may be rapidly (< 24 hours) fatal, malaria should always be considered in patients who have a history of exposure (mostly: past travel or residence in disease-endemic areas). The most frequent symptoms include fever and chills, which can be accompanied by headache, myalgias, arthralgias, weakness, vomiting, and diarrhea. Other clinical features include splenomegaly, anemia, thrombocytopenia, hypoglycemia, pulmonary or renal dysfunction and neurologic changes. The clinical presentation can vary substantially depending on the infecting species, the level of parasitemia and the immune status of the patient. Infections caused by P. falciparum are the most likely to progress to severe, potentially fatal forms with central nervous system involvement (cerebral malaria), acute renal failure, severe anemia, or acute respiratory distress syndrome. Others species can also have severe manifestations. Complications of P. vivax malaria include splenomegaly (with, rarely, splenic rupture), and those of P. malariae include nephrotic syndrome (Anonymous, 2025b).

Malaria is a severe illness, potentially fatal disease that requires immediate medical attention. The treatment involves antimalarial drugs medications prescription aimed at killing the parasite, with the specific drugs and

duration depending on the parasite species, the severity of the illness, the patient's age, and where the infection was acquired. Malaria is a severe and potentially fatal disease that requires prompt treatment with antimalarial drugs prescription to kill the parasite and prevent life-threatening complications. The specific treatment regime depends on several factors, including the infecting parasite species, the severity of the illness, the patient's age and health status (e.g., pregnancy), and the geographic area where the infection was acquired due to varying drug resistance patterns.

**a. The principal treatment of malaria includes**

Prompt diagnosis and treatment, species identification of specific Plasmodium species (e.g., P. falciparum, P. vivax) is crucial as different species respond to different medications and some can cause more severe complications or relapses. Severity Assessment categorizing patients having either uncomplicated or severe malaria. Severe malaria is a medical emergency requiring hospitalization and aggressive intravenous therapy. Drug Resistance considering the potential for drug resistance based on the geographic area where the infection was acquired, Combination Therapy: particularly for P. falciparum malaria, to increase effectiveness and prevent drugs resistance.

**b. Medications consists mainly in commonly used antimalarial drugs including:**

**b1. Medications for uncomplicated malaria are**

- Artemisinin-Based Combination Therapies (ACTs) for uncomplicated P. falciparum malaria in most parts of the world due to widespread resistance to older drugs like chloroquine. Examples include Artemether-Lumefantrine (Coartem) and Artesunate-Amodiaquine.
- Chloroquine phosphate preferred treatment only for chloroquine-sensitive parasites, which are now limited to specific geographic areas (e.g., some parts of Central America and the Caribbean) where it remains again effective.
- Atovaquone-Proguanil (Malarone): An effective option for chloroquine-resistant malaria.
- Quinine sulfate with Doxycycline or Clindamycin: Used for uncomplicated malaria, particularly if ACTs (Artemisinin-based combination therapies) are unavailable, or as an alternative in specific situations and recommended by the World Health Organization (WHO) as the first-line treatment.
- Parenteral Artesunate: recommended first-line treatment for all severe malaria cases, administered intravenously or intramuscularly.
- Primaquine or Tafenoquine: were necessary to clear dormant liver forms (hypnozoites) of P. vivax and P. ovale to prevent relapse, but require testing for G6PD deficiency before use due to the risk of severe anemia.

**b2. Special considerations are the followings**

- Artemisinin-Based Combination Therapies (ACTs): Examples include artemether-Lumefantrine (Coartem), Artesunate-Amodiaquine, and Atovaquone-Proguanil (Malarone).
- Chloroquine phosphate may be used only for Plasmodium species that are sensitive to the drug, such as in certain areas of Central America, Haiti, and the Dominican Republic. In most parts of the world, the parasites are resistant to chloroquine.
- Pregnancy: Malaria during pregnancy poses significant risks. Many antimalarial drugs are safe for use, and the benefits of prompt treatment generally outweigh the risks.
- Children: Doses are adjusted by weight for pediatric patients. Certain drugs like doxycycline are generally avoided in children under eight years of age.
- Self-treatment: In remote areas without immediate access to medical care, a reliable supply of emergency stand-by treatment may be carried, but medical evaluation must be sought as soon as possible.

**b3. Treatment for severe malaria**

Severe malaria is a life-threatening condition. The first-line treatment is with parenteral (intravenous or intramuscular) Artesunate for at least 24 hours and until the patient can tolerate oral medication.

- Parenteral Artesunate: This is the recommended treatment for severe malaria in adults, children, and pregnant women in all trimesters.
- Follow-up: After at least 24 hours of parenteral therapy, treatment is completed with a full course of an oral ACT.
- Alternatives: If Artesunate is unavailable (Anonym B, 2025)

**c. Preventing Relapse or recidism**

- For Plasmodium vivax and P. ovale infections, a second course of medication is necessary to kill the dormant liver-stage parasites (hypnozoites) that can cause a relapse.
- Primaquine phosphate or Tafenoquine: These drugs are used for anti-relapse or anti-recidivism treatment (radical cure).
- G6PD Deficiency Screening: A test for glucose-6-phosphate dehydrogenase (G6PD)
- deficiency is required before using Primaquine or Tafenoquine, as these drugs can cause severe anemia in deficient individuals. These medications are contraindicated during pregnancy.

Always consult a healthcare provider for proper diagnosis and a suitable treatment plan, as using the wrong medication or an incomplete course, can be dangerous and contribute to drug resistance (Anonym C, 2025).

Taking accounts of the wide-spread resistance of the parasite Plasmodium falciparum against the current synthetic antimalarial drugs not easly accessible to people in developing countries, they turn to traditional medicine using mainly aqueous extracts and other like decoctions and infusion based medicinal plant species claimed by traditional healers to be effective against malaria in their daily practices and find some reliefs after a long period of treatment (Wilcos and Bodeker, 2004, Kikweta et al., 2023).

Trigisia gilettii, synoyms Triclisia flava Excell, Tiliacora gilettii De Wil, Tiliacora gilettii De Wild. in Bull. Jard. Bot. Etat Bruxelles 3: 255 (1911), Triclisia dictyophylla Diels, Tiliacora trichantha Diels in HGA.Engler (ed.), Pflanzenr., IV, 94: 63 (1910), Triclisia flava Exell in J. Bot. 64 (Suppl.): 12 (1926) Inst. Roy. Congo Belge 4: 430 (1933), Triclisia semnophylla Diels ex Engl. in H.G.A. Engler & O. Drude, Veg. Erde 9(III 1): 178 (1915), common names Gilettii Triclisa, Triclisia Gilettii and Gilettii Tree (selinawamuiu.com/plants/menispermaceae/Triclisia-giletii, 2025).

T. gilletii is a volubile liane, stem can reach 10 cm diameter, young twigs slightly pubescent, coming glabres, to greyish bark, striated longitudinaly. Leaves to robuste petioles, inflated at the base, geniculated and thickened to head, reaching 20 cm long, slightly tomentose, becoming glabrescent, limbs widely rounded, subcutaned to base, acuminated to head, reaching 32 cm long and 27 cm wide, coriace, glabre on 2 faces, nervures secondary nerves 3-5 pairs, whose have 1-2 basilars, the majority jointed between them to coast proximity, creating with tertiar nerves very strong network or mesh on the inferior face of the limbus. Inflorescences' ♂ in panicules rarely more than 3 cm long, bulding on adult twigs or to the basis of young twigs sheeted, rachis tomentose, hary or shaggy pediciled of ± 2 mm long, bracts 2, subcorded-ovales, of ± 0,5 mm long, hairy or shaggy-tomentose.

Flowers ♂ with 9 sepals, hairy or shaggy-tomentose : 3 exterior subcorded-ovales, of ± 1 mm long, 3 medians ± similar to exteriors, of ± 1 mm long, 3 interiors elliptic-obovales, of 4-4,5 mm long and 2-2,5 mm wide, petals 3, rounded, very small, of ± 0,5 mm long, stamens 6, free, of 4-5 mm long, to cylindric filets, tenuous or slight, of ± 4 mm long, anther ovoids, of ± 1 mm long, box or chest to dehiscence longitudinal.

Inflorescences' ♀ similar to inflorescences ♂.' flowers' ♀ to bracts and sepals as in flowers ♂; petal absents; carpels 50-60; ovaries tomentose; styles ± cylindric of± 1,5 mm long and glabres. Drupe obovoids, comprimed, truncated to the basis, caudated-acuminated to head, to acumen curved of ± 2,5 cm long and± 1,7 cm wide exocarps ± verruquous, mesocarp subleshy of ± 2 mm thinness in sicco, endocarp very heavy or rough, dur. Seeds of ± 1-1,5 cm long and 1 cm de wide. Its habitat is Equatorial primitive marshy forest and galleries forestry.

For its uses, the liana Efiri has subjected to several studies on biochemical point. In fact it has believed that this liana contained a strong antimalaric substance more effective than quinine. Results from the therapeutic essays with total plant extract at dose 3 to 10 more strong than used with quinine, have permitted to conclude the complete inefficiency or inefficacity of Efiri. The indigenous use it as remedy against diarrhoea, pyorrhea and arrow poison. (https://tropical.theferns.info/viewtropical.php?id=Triclisia+dictyophylla, 2025).

The macerated root is taken as an abortifacient and menagogue. A decoction is drunk as a vermifuge, whereas the roots are taken raw to treat venereal diseases. The grated roots are taken to treat snakebites and expel roundworms. The root pulp or root sap is rubbed into scarifications to treat joint pains, epileptic attacks, oedema, venereal diseases and anaemia. A bark or root decoction is used as a wash to calm palpitations. A decoction of the twig bark is widely taken to treat fever and malaria, and also to treat diarrhoea, stomach problems and purulent catarrh. The powdered stem bark is used in the treatment of leprosy. A root bark decoction is taken as a treatment for stomach problems and dysentery, convulsive coughing, pains and rhumatisms, and feverish stiffness of the limbs.

The leaf juice is used to ease coughs. The leaves are suspended from the ceiling to help children with breathing problems. The juice of young leaves is diluted and administered as a painkiller to patients with mental health problems during attacks. The characteristic bioactive compounds in the plant are bisbenzyl-isoquinoline alkaloids and related dioxin alkaloids (https://tropical.theferns.info/viewtropical.php?id=Triclisia+dictyophylla, 2025).

From phytochemical studies, the root barks of Trichisia gilettii (T. gilettii is the correct taxonomic name, likely what was intended by the user's query Triclisia gilettii, contains several chemical compounds, primarily belonging to the class of limonoids such as deacetylkhivorin and 3,7-dideacetyl-6α hydroxykhivorin. The leaves contain alkaloids (Owusu et al., 1981). Alkaloids, steroids and terpenoids, aminated compounds, saponins, coumarins, reducing sugars and polyphenolic compounds such flavonoids and tannins (gallic, cathechic and proanthocyanidins) are identified in the aqueous extract.

Anthocyanins, anthraquinones and cardiotonic heterosides (glycosides) were not detected in in stem bark. The chemical composition of aqueous extract was qualitatively similar to that of methanol 80% extract (Cimanga et al;, 2018). A range of alkaloids have been found in the roots, stems and leaves. Chromatography of

an extract of the leaves afforded the new bisbenzylisoquinoline dibenzo-p-dioxin alkaloids gilletine and isogilletine-N-oxide as well as two other bisbenzylisoquinoline alkaloids obamegine and stebisimine. (https://www.researchgate.net/publication/231715200_Constituents_of_West_African_Medicinal_Plants_XXVIII_Additional_Alkaloids_of_Triclisia_, 2025). Two antiprotozoal bisbenzylisoquinoline alkaloids from Triclisia gilettii stem bark (Cimanga et al., 2018a).

Drypemolundein B, (+)-nonacosan-10-ol stigmasterol 3-O-β-D-glucopyranosyl sitosterol, 3-O-β-D glucopyranosyl- stigmasterol, oleanic acid, myricetin, quercetin and 3-methoxyquercetindrypemolundein, (+)-nonacosan-10-ol, stigmasterol, 3-O-β-D glucopyranosylsitosterol, 3-O-β-D glucopyranosylstigmasterol, oleanic acid, myricetin, quercetin and 3-methoxyquercetin were isolated from the aerial parts of T. gilettii) (Tiam et al., 2019),

Phenols, fatty acids, vitamins and steroids were detected by Thermo Scientific DSQII single quadrupole gas chromatography (GC) and a Thermo Scientific liquid chromatography (LCQ Fleet system) tandem mass spectroscopy. Likewise, for LC-MS analysis kaempferol and dihydrovomifoliol-O-glucoside were detected (Olayeriju et al., 2022). Some mineral elements like de water, ash, proteins, lipids, fibers, glucides without fibers, Na, de Mg, de P, Cl, 0 K, Ca, Mn, Fe, 5 Cu, Zn, 0 Se were analyzed in T. dictophylla leaves for its valorization and improved alimentary security of poor menages or households in rural aeras (Mfulu et al., 2023).

The root barks of Trichisia giletii (T. gilettii was the correct taxonomic name), likely what was intended by the user's query Triclisia gilettii, contains several chemical compounds, primarily belonging to the class of limonoids such as deacetylkhivorin and 3,7-dideacetyl-6α hydroxykhivorin. The leaves contain alkaloids (Owusu et al., 1981). Alkaloids, steroids and terpenes, aminated compounds, saponins, coumarins, reducing sugars and polyphenolic compounds such flavonoids and tannins (gallic, cathechic and proanthocyanidins) were identified in the aqueous extract. Anthocyanins, anthraquinones and cardiotonic heterosides (glycosides) were not detected in in stem bark (Kuikweta et al., 2013, Cimanga et al., 2018).

About biological activity conducted on Triglisia gilettii, the observed antiplasmodial activity of extracts and solvent fractions from T. gilettii root barks reported in the present study, could be attributed to the presence of secondary metabolites identified in this plant part because these different phytochemical isolated other medicinal plant species such as alkaloids (Cimanga et al;, 2019, Soto-Vasquez et al;, 2021,Garcia-Duaz, 2022, 2025a,b, Okom et al;, 2025), flavonoids (Chepkrui et al;, 2021, Ranaivoarison et al;, 2020, Tchangollee et al;, 2022), saponins (Soeiro et al;, 2021), steroids (Safar et 2022, Ngnoung al;, 2020, Mianda et al., 2020, Ranaivoarison et al;, 2020, Tchangoue et al;, 2022, Springer et al., 2025) and terpenoids (Gabriel et al;, 2017, Greve et al., 2020, Olayemi et al;, 2020, Ekasari et al;, 2023, Ghorbani and Zargaran, 2025, 2025b) reported previously to be endowed with this biological activity expressed with different magnitudes.

Assessment of acute and subacute toxicological profiles of the aqueous extract (decoction) from the leaves of Triclisia gilettii (De Wild.) Staner (Menispermaceae) in Wistar rats was evaluated showing that this extract was devoid with toxic effects and did not induce the death of treated animals at high oral dose of 5000 mg/kg bodyweight (Cimanga et al;, 2015, 2018b). The effect of moonseed vine (Triclisia gilettii Staner) on ethane-1,2-diol-induced urolithiasis and its toxicity in Wistar albino rats was investigated (Olayerij et al., 2020). The antilithiatic potentials against ethane-1,2-diol induced nephrolithiasis of aqueous methanolic extract leaf of T. gilettii was investigated (Olayeriju et al., 2021).

Cellular antioxidant and peroxidase inhibition activities of decoctions from different parts of Triclisia gilettii (De Wild) Staner (Menispermaceae) was studied (Mutwale et al., 2022). In vitro evaluation of free radical scavenging and antioxidant, and spasmolytic potentials of extracts, solvent fractions and subfractions from Triclisia gilettii (de wild) Staner (Menispermaceae) stem bark was evaluated (Kikweta et al;, 2025).

Aqueous, methanol 80% and total alkaloids extracts, and solvent fractions chloroform, ethylacetate, n-butanol and residual aqueous phase from the partition of T. gilettii root and stem barks collected in Kinshasa were reported to exhibit strong antiplasmodial activity in vitro against isolated Plasmodium falciparum sensible to chloroquine infected human blood and against P. falciparum K1 strain resistant to chloroquine and pyrimethamine with IC50 values < 10 µg/mL and all tested samples were devoid with cytotoxic effect towards MRC5 cell lines showing good selective index. Extracts showed aslo good antiplasmodial activity against P. berghei berghei ANKA in vivo (Kikweta et al.., 2013).

The present investigation was conducted to evaluate the antimalarial activity of aqueous, methanol 80% and total alkaloids extracts, and solvent fractions from Triglisia gilettii root barks against Plasmodium falciparum NF54 sensible to chloroquine and P. falciparum K1 strain resistant to chloroquine and pyrimethamine and in vivo evaluation towards Plasmodium yolei 67 and P. berghei berghei ANKA. The acute and subacute toxicity of aqueous extract was also examined in vivo.

## 2. MATERIALS AND METHODS

### 2.1. Vegetal material

Fresh roots of Triclisia gilettii were collected in Kinzaweta collectivity in Bas-Congo (Democratic

Republic of Congo). The plant was identified at Institut National d'Etudes et de Recherches en Agronomie (INERA), Faculty of Sciences and Technologies, Department of Biology, University of Kinshasa/Kinshasa where a voucher specimen No Tgrb12480 was deposited in the herbarium of this institute.

**Figure 1: Triglisia gilettii leaves and twigs.**

**2.2. Preparation and frationation of extracts**

Roots were scratched with a knife to have barks which were dried in a hot for 3 days. 50 g of dried Triclisia gilettii root barks were cuted in small pieces and reduced to powder using a traditional mortal resulting in a powder kept in a brun bottle before use to avoid contamination. 50 g of root barks powder were mixed with 300 mL distilled water and heated on hot plaque at 100°C for 15 minutes. After cooling and filtration on sterile cotton and paper filter Whatman N° 1, the resulting filtrate was evaporated in rotative evaporator giving dried extract of 22.13 g denoted as Tgrb-1.

On the other hand, 20 g of plant material was mixed with 200 mL methanol 80% and macerated for 24h. After filtration in the same conditions described above, a macerate was obtained. The mark was exhaustively percolated with the same solvent and filtered in the same conditions as described above. The macerate and percolate were combined and evaporated in vaccuo giving a dried extract denoted as Tgrs-2 (12.67 g).

Next, an amount of extract AE-1 1and ME-1 and 2 (10 g) was dissolved in 200 mL distilled water and treated as described above. The resulting filtrates were separately, exhaustively and successively extracted with solvents of different polarities like chloroform, ethylacetate and n-butanol. All fractions including residual aqueous solvent fraction was treated as described above giving corresponding dried extracts denoted as AE-1.1 (1.25 g), AE-1.2 (2.65 g), AE-1.3 (1.52 g) and AE-1.4 (3.25 g) and ME-1 (1.54g) ME-1.2 (2.71 g), ME-1.3 (1.72 g) and ME-1.4 (3.45 g) for chloroform, ethylacetate, n-butanol and residual aqueous fractions from aqueous extract Tgrb-1 and methanol 80% extract respectively (Tgrb: Triclisia gilettii root barks).

**2.3. Chemical screening**

The confirmatory qualitative phytochemical screening of plant extracts was performed to identify the main classes of compounds (alkaloids, tannins, saponins, flavonoids, coumarins, polyphenols, glycosides, polysaccharides, reductor sugars, steroids, and terpenoids) in the extracts following standard protocols.

**Test for Tannins**

About 2 mg of the plant extract was dissolved in 5 mL distilled water and 1 mL Stiasny reagent (formol + HCl conc.) was added to the mixture giving a brun precipitate for catechin tannins as positive test. The mixture was filtered on paper filter Whatman n° 1 and in the filtrate, 1 mL FeCl3 5% was added giving grey color for gallic tannins as positive test.

**Test for Alkaloids**

The plant extract was dissolved in 5 mL of water, filtered, and Then, 1 mL of the heated mixture was combined with 1 mL of Mayer-Wagner's reagent or Dragendorff's reagent giving white yellowish and orange precipitate respectively as positive tests.

**Test for Saponins**

About 10 milliliters of aqueous solution extract were vigorously agitated. Then, the formation of foam persisting during10 minutes confirmed the presence of saponins.

**Test for flavonoids**

200 mg of the plant extract was mixed with 10 mL of ethanol and filtrated. Two mL of the filtrate, concentrated HCl, and magnesium ribbon (Shinoda reagent) were mixed. The formation of a pink or red color indicates the presence glycosides or flavonoids. After, adding 1 mL of distilled water and NaOH to 1 mL solution of extract, the formation of a yellowish color indicated the presence of flavonoids.

**Test for Steroids**

About 1 mL of the crude extract was combined with 10 mL of chloroform and 10 mL of sulfuric acid conc. (Liebermann-Burchard reagent), and the formation of the bilayer (red top layer and greenish bottom layer) reveals the presence of steroids.

**Test for Terpenoids**

The presence of terpenoids was determined by the formation of a reddish-brown color in the test for terpenoids, which included mixing of 0.5 mL of crude extract with 2 mL of chloroform and 3 mL of sulfuric acid conc. (Liebermann-Burchard reagent) or Salkowski reagent (0.5 M ferric chloride ( ) in 35% perchloric acid ( ) water and H2S04 concentred giving pink or red color as positive test.

**Test for Phenols**

About 1 mL of the extract was combined with three drops of FeCl3 5%, and 1 mL of K2Fe (CN6 (Barton

regeant). The formation of brun precipitate and blue color respectively confirmed the presence of phenols.

**Test for polysaccharides**
To1 ml solution of extract, formol + H2SO4 conc. was added giving various colors as positive test.

**Reductor sugars**
To 1 mL solution of extrct, 1Ml Felhing reagent composed of two separate solutions, Fehling A (blue) and Fehling B (colorless), mixed just before use, Fehling A contains aqueous copper(II) sulfate, while Fehling B contains potassium sodium tartrate (Rochelle salt) and sodium hydroxide in water, forming a blue tartrate-complex of Cu(II) that acts as an oxidizing agent in an alkaline environment to detect reducing sugars, was added, mixed and boiled giving a red color as positif test.

**Thin Layer Chromatography (TLC) Test**
Thin layer chromatography was performed on TLC plate (silica gel pre-coated with layer thickness of 0.2 mm, Merck, Germany) to confirm the presence some phytochemical using hexane/ethyl acetate 8:2 as mobile phase for steroids and terpenoids using Liebermand-Bouchard as reagent, BAW 4:1:5 (top layer) for flavonoids using ACl3 reagent, CHCl3/MeOH/NH3 10: 9:1:1for alkaloids using Dragendorff reagent and Coumarin under UV using NaOH. Spots were applied using capillary tube 1.5 cm from the bottom marked by a line ruled using a pin. The sample spotted on the plate was allowed to dry before the plate was placed into the chromatographic tank which was covered immediately. When the solvent reaches the top of the plate, the plate was removed, marked and dried. The number of the spots was detected under UV at 254 and 366 nm wavelengths and spraying with appropriate reagent (Harborne, 1998, Trease and Evans, 2000).

**3. Evaluation of antiplasmodial activity of extracts and solvent fractions**

**3.1. Antiplasmodial evaluation in vitro test**
The in vitro antiplasmodial activity of aqueous Trgb-1 and methanol 80% Trgb-2 extracts, and their respective solvent fractions Trgb-1.1 to 1.4 and Trgb-2.1 to 2.4 was evaluated against Plasmodium falciparum NF54 sensitive to chloroquine and P. falciparum K1 resistant to chloroquine and pyrimethamine from the laboratory of Prof. Cos, University of Antwerp, Belgium, using the lactacte dehydrogenase method previously described by Kuypers et al., (2006) and Mesia et al., (2008) in microplates using the test concentrations from 1 to 20 µg/mL of each sample.7

**3.2. Antiplasmodial evaluation in vivo test**
The in vivo antiplamodial activity of aqueous, methanol 80% and total alkaloids extracts of T. gilettii rook bark as well as reference drugs Artesunate and Quinine 2HCl, was evaluated in the 4-day suppressive test against Plamodium yolei 67 and P. b. berghei ANKA infected mice using the method previously described by Cimanga et al., (2004), with some modifications (Tona et al., 2004). These strains were obtained from the laboratory of Parasitology in University of Antwerp, Belgium. White Swiss (OF1) mice weighing 23.2±1.7 g were divided in following groups with 5 mice each:

- Group I received orally 5 ml saline solution (0.9% NaCl)/kg bodyweight (bw) as negative control,
- Group II received separately in the same way Artesunate and Quinine at oral dose of 10 mg/kg bw each as positive controls,
- Groups IIIa,b and IVa,b were separately orally administered 200 (a) and 400 (b) mg/kg bw of aqueous AE-1 and methanol 80% extracts respectively,

All selected mice were separately intraperitoneally injected with 1 x 107 of parasizited erythrocytes with Plasmodium yolei 67 and Plamsodium b. berghei ANKA respectively. The test mice were immediately treated daily from day 1 to 3 with respective doses of each extract. Each day from day-1 to 4, a film was prepared from tail-blood sample from each mice and stained with Giemsa so that the level of parasitaemia expressed in percentage could be recorded as half the number of schizonts with at least three nuclei each, counted in 200 erythrocytes. On day 4, the mean parasitaemia in each group was determined in order to calculate the chemosuppression percentage using formula:

$$\frac{A - B}{B} \times 100$$

Where A was the mean percentage parasiteamia in negative control and B the mean percentage parasitaemia in tested groups.

**3.3. Cytotoxicity evaluation**
The cytotoxicity of extracts and solvent fractions was evaluated on MRC-5 cells (human lung fibroblasts) cultured in MEM medium, supplemented with 20 mM L-glutamine, 16.5 mM NaHCO3, 5% foetal calf serum and 2% P/S (penicillin/streptomycin) solution according to the MTT procedure as previously described by Kuyper et al., (2006) and Mesia et al., (2008). The cytotoxic concentration 50% (CC50) for each sample was derived from the dose-response curves. A selectivity index (SI) corresponding to the ratio between cytotoxic and antiparasitic concentrations, was calculated as SI = CC50 / IC50 (Kupers et al., 2006, Mesia et al., 2008, Mabuza et al., 2025). If SI < 1: test sample was considered as cytotoxic and was not selective, while SI > 1, the test sample was considered as non cytotoxic and presents a selective effect (Camacho et al., 2003).

**3.4. Evaluation of acute and subacute toxicity of aqueous extract**
The acute toxicity test was performed according the experimental method Guideline 423 of the Organization of Economic Cooperation and Development (OECD) with an initial and only dose of 5000 mg/kg bw administered once while the subacute toxicity was

carried out by the daily oral administration separately oral doses of 500, 1000 and 5000 mg/kg bw to selected mice according OECD Guidelines No 412.

Mice were randomly and divided in 5 groups of 5 mice each.

- Group I received orally 5 mL distilled water as negative group,
- Group II with received once 5000 mg/kg bw of aqueous extract in acute toxicity,
- Groups III to V were orally and daily administered separately 500, 1000 and 5000 mg/kg bw of aqueous extract in subacute toxicity.

After administration, each mice was kept in an individual plastic cage and following parameters were observed during 28 days of the test: irritability, touch response, tail grip response, contortion, posterior train position, straigtening reflex, body tone ataxia, mobility, tremors, convulsion, death, etc. They were daily weighted.

## 3. RESULTS AND DISCUSSION

### 3.1. Chemical screening

The root barks of Triclisia gilettii (as Triclisia gilettii was the correct taxanomic name, likely what was intended by the user's query Triclisia gilettii) contained several chemical compounds primarily belonging to the class of limonoids such as deacetylkhinorin and 3,7-dideacetyl-6α-hydroxykhivorin. The major compounds were alkaloids. In the present study, major phytochemical identified in Triglisia gilettii root barks were presented in Table 1. Results revealed T. gilettii root barks contained alkaloids, flavonoids, coumarins, polyphenols, polysaccharides, reductor sugars, saponins, steroids, terpenoids, gallic and cathechic tannins, proanthcyanidins and amined compounds. Consequently, anthocyanins, anthraquinones and cardiotonic heterosides were not identified in T. gilettii root barks collected in Kinzaweta collectivity in Bas-Congo (Democratic Republic of Congo). This reported chemical composition was completely and qualitatively similar to that of the root and stem barks of the same medicinal plant collected in Kinshasa as reported by Kikweta et al., (2013), but, this was the first report on the chemical composition of T. gilettii root barks collected in Kinzaweta collectivity in Bas-Congo, DRCongo since to our knowledge, no other study was previously conducted on this point and found in the literature on the same plant from this collection in DRCongo.

**Table1: Major phytochemical identified.**

| Groups | Results | Groups | Results |
|---|---|---|---|
| Alkaloids | +++ | Polysaccharides | +++ |
| Anthraquinones | - | Proanthocyanidins | ++ |
| Anthocyanins | _ | Reductor sugars | +++ |
| Cardiotonic heterosides | - | Saponins | ++ |
| Coumarins | ++ | Steroids | ++ |
| Flavonoids | ++ | Terpenoids | ++ |
| Glycosides | ++ | Tannins (cathechic and gallic) | ++ |
| Polyphenols | +++ | Amined compo | ++ |

### 3.2. Antiplasmodial activity

Malaria was always treatable, yet the burden continued to increase mainly in Africa regions, especially in the sub-Saharan regions (Li et al., 2024). Despite the availability of various synthetic antimalarials currently used by people, the eradication of malaria continued to prove difficult to achieve its complete elimination . The widespread antimalarial drugs resistance further complicated the malaria burden and treatment (Nguyen et al., 2023). Thus, the need to constantly discover novel drugs, with few or no side effects and effective as antimalarial agents from medicinal plants, was imperative (Umumararungu et al., (2023).

Medicinal plants had long been considered as a source of nutrition and healing for humanity. Today, modern pharmaceuticals rely on these medicinal plant species to supply antimalarial bioactive ingredients (Habibi et al., 2022; Tajbakhsh et al., 2021) as mainly people in developing countries use different aqueous (macerates) and others as decoction and infusion preparations based medicinal parts to treat malaria and found some reliefs after long a period of treatment.

For good interpretation and understanding of the activity developed by test samples in the present study, following criteria were adopted: IC50 ≤ 10 µg/mL: pronounced activity, 10 < IC50 ≤ 20 µg/mL: good activity, 20 < IC50 ≤ 30 µg/mL: moderate activity, 30 < IC50 ≤ 50 µg/mL: weak activity, IC50 > 50µg/mL: inactive.

#### 3.2.1. Effects of extracts and solvent fractions in vitro test against selected Plasmodium strains

Results revealed that, tested against Plasmosium falciparum NF54 sensitive to chloroquine, the majority of tested samples from T. gilettii root barks inhibited the growth of this Plasmodium strain with inhibitory concentrations 50 (IC50) less than 10 µg/mL as pronounced activity. The most active sample was methanol 80% ME-1 extract exhibited this activity with IC50 of 2.07 µg/mL followed by aqueous AE-1 extract

showing IC50 value of 2.13±0.06 µg/mL and this difference of activity level must be due to the nature of extractive solvent (water versus methanol 80%) and methanol 80% ME-1 extract was considered as good solvent for the extraction of active antiplasmodial principles in greater amount. The less active sample towards P. falciparum NF54 was n-butanol AE-1.3 solvent fraction (IC50 = 8.54±0.11 µg/mL) compared to respective remaining solvent fractions EA-1.1, 1.2 and 1.4, and EM-1.1 to 1.4 (Table 1).

All solvent fractions from the partition of aqueous EA-1 extract EA-1.1 to 1.4 with IC50 values between 5.75±0.05 and 8.54±0.11 µg/ml exhibited pronounced against this Plasmodium strain, but low compared to ME-1.1 to 1.4 solvent fractions from the partition of methanol 80% ME-1 exerting also pronounced activity by producing high IC50 values ranging between 4.14±0.05 and 8.34±0.06 µg/mL. The less active sample was n-butanol AE-1.3 solvent fraction (IC50 = 8.54±0.11 µg/ml) compared to remaining samples (Table 1).

**Table 1: Antiplasmodial activity of extracts and solvent fractions against Plasmodium strains and cytotoxic activity against MRC5 cell lines in vitro**

| Sample codes | MRC-5 ($CC_{50}$, µg/ml | PFNF54, $IC_{50}$, µg/mL | PFK-1, $IC_{50}$, µg/ml | SI/PNF54 | SIPFK-1 |
|---|---|---|---|---|---|
| **AQUEOUS EXTRACT** | | | | | |
| AE-1 | 5.45±0.04 | 2.13±0.06 | 2.54±0.04 | 2.55±0.05 | 2.14±0.03 |
| AE-1.1 | 7.63±0.06 | 5.86±0.09 | 8.23.34±0.10 | 1.30±0.05 | 0.92±0.06 |
| AE-1.2 | 6.45±0.03 | 5.75±0.05 | 6.14±0.08 | 1.21±0.06 | 1.06±0.08 |
| AE-1.3 | 8.63±0.11 | 8.54±0.11 | 10.32±0.13 | 1.01±0.07 | 0.83±0.05 |
| AE-1.4 | 9.72±0.13 | 7.11±0.04 | 9.05±0.08 | 1.36±0.05 | 1.07±0.05 |
| **METHANOL 80% EXTRACT** | | | | | |
| ME-1 | 5.32±0.02 | 2.07±0.04 | 3.11±0.06 | 2.57±0.03 | 1.71±0.06 |
| ME-1.1 | 7.32±0.07 | 8.34±0.06 | 10.11±0.07 | 0.88±0.06 | 0.72±0.08 |
| ME-1.2 | 6.30±0.05 | 4.14±0.05 | 6.12±0.07 | 1.52±0.10 | 1.03±0.08 |
| ME-1.3 | 8.26±0.08 | 7.55±0.04 | 9.67±0.03 | 1.10±0.05 | 0.85±0.04 |
| ME-1.4 | 9.41±0.11 | 6.23±0.05 | 8.42±0.05 | 1.51±0.12 | 1.11±0.13 |
| ME-2 | 3.34±0.05 | 1.54±0.04 | 2.05±0.06 | 2.16±0.04 | 1.63±0.03 |
| Artesunate | 10.43±0.05 | 1.54±0.03 | 2.23±0.04 | 6.77±0.06 | 4.67±0.04 |
| Quinine 2HCl | 15.03±0.06 | 2.15±0.04 | 4.13±0.02 | 7.00±0.04 | 3.64±0.04 |

AE: aqueous extract, AE-1-1 to 1.4a and ME-1.1 to 1.4: chloroform, ethylacetate, n-butanol and residual aqueous solvent fractions from the partition of aqueous EA-1 extract and methanol 80% ME-1 extract and ME-2: total alkaloids extract respectively, PFNF54: Plasmodium falciparum NF54, PFK-1: Plasmodium falciparum K1, SI/PNF54: selective index on Plasmodium falciparum NF54, SIPFK-1: selective index on Plasmodium falciparum K1.

When tested against. P falciparum K1 strain resistant for chloroquine and pyrimethamine, the majority of test samples displayed inhibition growth of this Plasmodium strain with IC50 value < 10 µg/mL as pronounced activity. Methanol 80% ME-1 (IC50 = 3.11±0.06 µg/mL) showed high activity compared to aqueous AE-1 extract (IC50 = 2.54±0.04 µg/mL) with good selective index (Table 1). ME-1.1 to 1.4 solvent fractions from the partition of methanol 80% ME-1 extract producing IC50 ranging between 6.12±0.07 and 10.11±0.07 µg/ml displayed greater antimalarial effects when tested towards P. falciparum K1 with SI values from 072±0.08 to 1.11±0.13 confronted to AE-1.1 to 1.4 solvent fractions from the partition of aqueous AE-1 extract presenting low IC50 values from 6.04±0.08 to 10.32±0.13 µg/mL with SI values between 0.83±0.05 and 1.07±0.05, and significant difference was observed ($p < 0.05$). The less active samples were n-butanol AE-1.3 (IC50 = 8.54±0.11 µg/mL) with SI value of 10,32±0,13 compared to remaining samples (Table 1). Our results were in good agreement with Cimanga 1997, Kikweta et al., 2013, Abibakar et al., 2022 for the antiplasmodial activity of alkaloids from medicinal plant species.

Moreover, the majority of solvents AE-1.1 to 1.4 and ME-1.2 to 1.4 from both extracts AE-1 and ME-1 respectively, presented good selective index (SI) > 1 expressing thus their selective antimalarial effect against both Plamsodium parasites.

But someone such as chloroform AE-1.1 (SI = 0,92±0,08) and n-butanol AE-1.3 (SI = 0,83±0,03) from the partition of aqueous AE extract on P. falciparum K1, and chloroform ME-1.1 (SI = 0,88±0.06 and 0.72±0,08) and n-butanol ME-1.3 (SI = 0,85±0,04) on P. falciparum NF54 and K1, and K1 respectively, presented SI < 1 as a sign of their cytotoxic effect and had not selective antimalarial effect (Camacho et al., 2003).

Figure 2 showed the effects of aqueous AE-1, methanol 80% ME-1 extracts, Artesunate and Quinine 2HCl as reference antiplasmodial drugs on Plasmodium falciparum NF54 growth. Tested at low concentrations from 0.5 to 2 µg/mL, extracts produced low inhibition percentages tn 50ha% from 12.52 to 39.21% for aqueous

AE-1 extract and from 23.45 to 45.34% for methanol 80% extract. These inhibition percentages knew significant ($p < 0.05$) increase with.

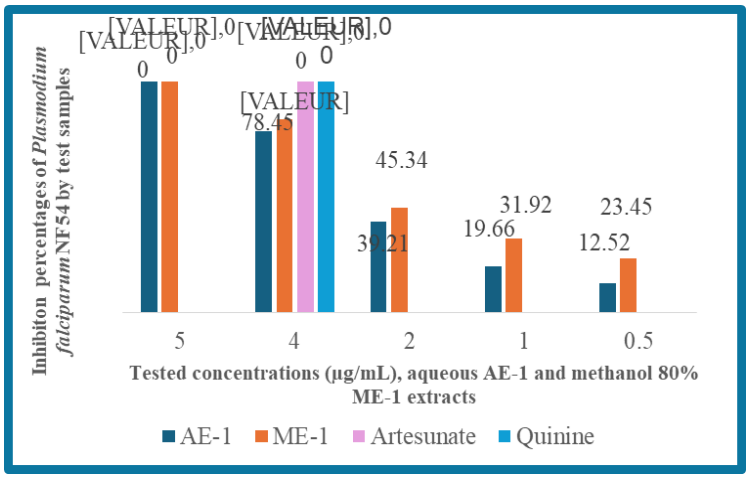


**Figure 2: Effects of aqueous AE-1, methanol 80% ME-1, Artesunate and Quinine 2HCl on Plasmodium falciparum NF54 growth.**

The use of high test concentration of 4 µg/ml at which aqueous AE-1 extract and methanol 80% ME-1 extracts showed 78.45 and 83.16% inhibition of this parasite growth. At this test concentration, Artesunate and Quinine 2HCl as reference drugs carried away 100% inhibition of the growth of P. falciparum NF54. The same effect was also obtained with both extracts tested at high concentration of 5 µg/mL (Fig. 2).

Figure 3 pointed out the effects of aqueous AE-1, methanol80% ME-1 and total alkaloids ME-2 extracts as well as Artesunate and Quinine 2HCl on Plasmodium K1 growth. Tested at low concentrations from 1 to 2 µg/ml, aqueous AE-1 and methanol 80% ME-1 extracts generated low inhibition percentages than 50% accredited from 15.45 to 27.83 for aqueous AE-1 extract, from 36.86 to 43.67% for methanol 80% ME-1 extract and tasted high inhibition percentages when tested at high concentration of 4 µg/mL furnishing 76.45 and 81.78% inhibition attributed to aqueous AE-1 and methanol 80% ME-1 extract respectively. Total alkaloids ME-2 (M-3) extract exerted this inhibitory effect by production high inhibition percentages than 50% of 66.23, 75.45 and 93.24% at tested concentrations of 1, 2 and 4 µg/mL respectively and caused 100% inhibition at high concentration of 5 µg/mL, at which also this effect was also observed with aqueousAE-1 and methanol 80%-ME-1 extract (Fig. 3).

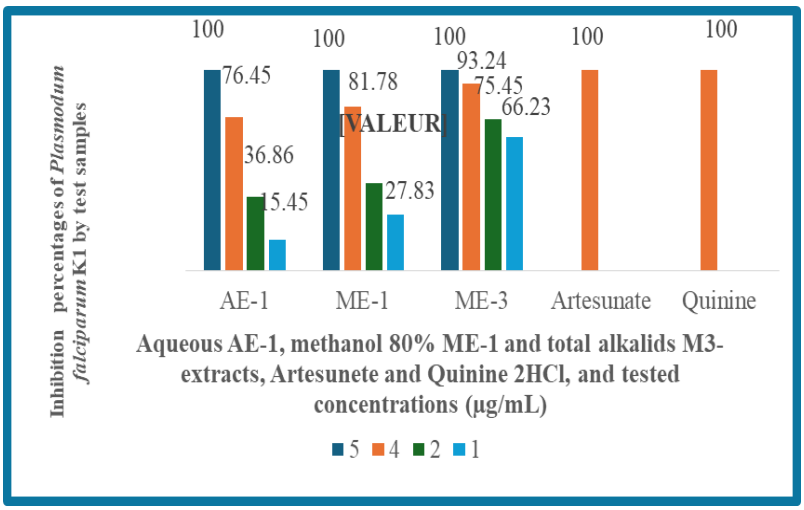


**Figure 3: Effects of aqueous AE-1, methanol 80% ME-1 and total alkaloids ME-3 extracts as well as Artesunate and Quinine 2HCl on Plasmodium falciparum NF54 growth.**

100% inhibition at high concentration of 5 µg/mL, at which also this effect was also observed with aqueousAE-1 and methanol 80%-ME-1 extract (Fig. 3).

Figure 4 reported the parasiteamia percentage inhibitions of parasiteamia induced by injection of Plasmodium yolei 67 in mice. The injection of this Plasmodium in mice (NCSs) carried away high production of parasitemia to level of 28.34%. Afterwards, the oral

administration of all extracts at doses of 200 and 400 mg/kg bw carried away significant ($p < 0.05$) reduction of this parasitemia in dose-dependent manner (Fig. 4). Administrated at the high oral dose of 400 mg/kg bw, they provoked significant reduction ($p < 00.05$) of this parasiteamia to 8.14, 6.04 and 4.11% attributed to aqueous AE-1, methanol 80% ME-1 and total alkaloids ME-2 extract respectively. Artesunate and Quinine 2HCl used as reference antiplasmodial drugs caused also remarkable decrease of this parasitemia to very low values of 2.54 and 3.05%. This lowering of parasiteamia in treated mice by these samples and reference drugs clearly demonstrated that they were endowed with interesting antiplasmodial or antimalarial activity in vivtro.

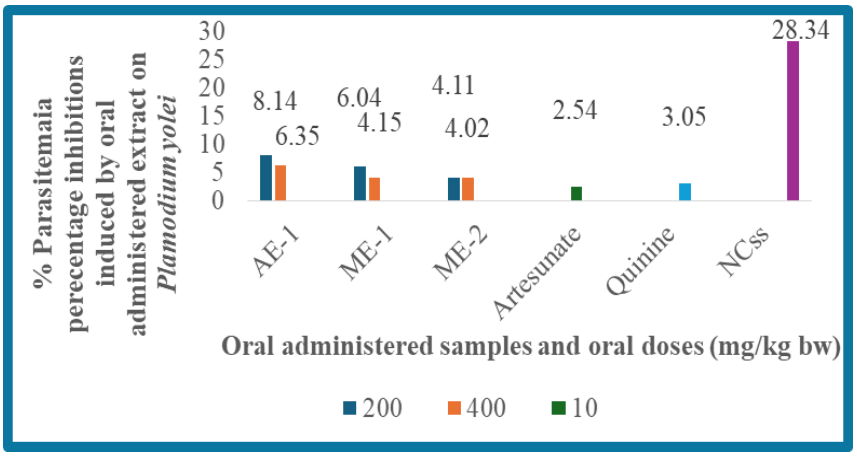


**Figure 4: Parasiteamia percentage inhibitions caused by oral administration of aqueous AE-1, methanol 80% ME-1and total alkaloids M-2 extracts, negative control (NCss) as well as Artesunate and Quinine 2HCl against parasiteamia induced by Plasmodium yolei 67 in mice.**

Figure 5: pointed out the parasiteamia percentage inhibitions of parasiteamia induced by injection of Plasmodium b. berghei ANKA in mice. The injection of this Plasmodium in mice (NC) carried away high production of parasitemia to level of 25.15%. The oral administration of all extracts at oral doses of 200 and 400 mg/kg bw brought about this parasitemia in significant ($p < 0.05$) reduction in dose-dependent manner (Fig. 5).

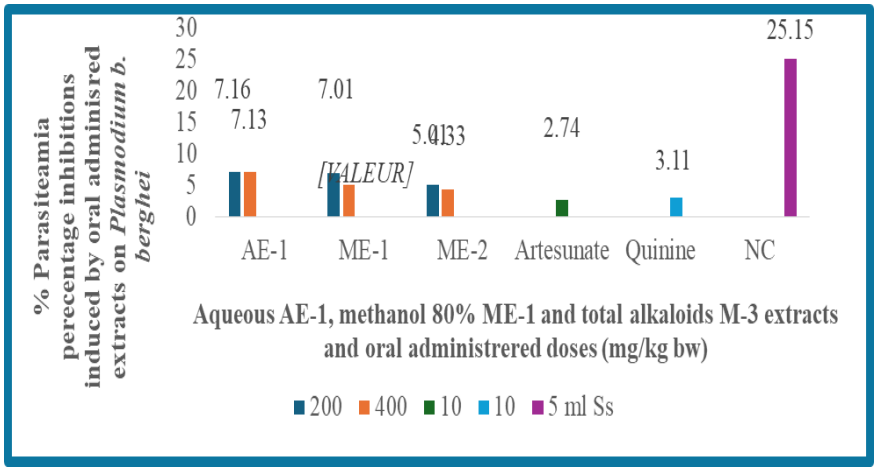


**Figure 5. Parasiteamia percentage inhibitions caused by oral administration of aqueous AE-1, methanol 80% ME-1and total alkaloids M-2 extracts as well as Artesunate and Quinine 2HCl against parasiteamia induced by Plasmodium b. berghei ANKA in mice (NB. ME-2=ME-3: is the same extract).**

Served orally at high oral dose of 400 mg/kg bw, these extracts caused significant ($p < 0.05$) decrease of this parasitemia to values of 7.13, 5.02 and 4.33% assigned to aqueous AE-1, methanol 80% ME-1 and total alkaloids ME-2 extracts respectively. In addition, the oral administration of Artesunate and Quinine at oral dose of 10 mg/kg bw, carried this parasiteamia to very low values of 2.74 and 3.11%. This parasiteamia reduction showed by these samples and reference drugs, clearly demonstrated they were endorsed with interesting antiplasmodial or antimalarialc properties in vivo.

Served orally at high oral dose of 400 mg/kg bw, these extracts caused significant ($p < 0.05$) decrease of this parasitemia to values of 7.13, 5.02 and 4.33% assigned to aqueous AE-1, methanol 80% ME-1 and total alkaloids ME-2 extracts respectively. In addition, the oral administration of Artesunate and Quinine at oral dose of

10 mg/kg bw, carried this parasiteamia to very low values of 2.74 and 3.11%. This high parasiteamia reduction showed by these samples and reference drugs, clearly demonstrated they were endorsed with interesting antiplasmodial or antimalaric properties in vitro.

The comparison of antiplasmodial activity in vitro of these samples revealed that methanol 80% extract exhibited high activity confronted to aqueous extract towards both selected Plasmodium falciparum strains, and this was due to nature of extractive solvent and the high capacity of methanol 80% ME-1 extract to fournishfurnish high amount of antiplasmodial principles as already denoted abvove while total alkaloids ME-2 extract exhibited high activity compared to aqueous AE-1 et methanol 80% ME-1 extract due particularly to its alkaloids content as active principles. Artesunate and Quinine 2HCl showed high activity compared to all extracts (Fig. 4 and 5)..

Moreover, all solvent fraction showed weak activity against both selected P. falciparum strains compared to their respective parent aqueous AE-1 and methanol 80 ME-1. This observation suggested that these different solvent fractions could act in synergistic manner to restore the high activity their respective parent extracts. Both ethylacetate AE-1.2 and ME-1.2 from AE-1 and ME-1 extracts respectively, displayed high activity compared the remaining solvent fractions from AE-1 and ME-1 extracts respectively, although ME-1.2 showed high effect confronted to AE-1.2 against P. falciparum NF54 and K1 strains. n-butanol AE-1.3 solvent fraction (IC50 = 8.54±0.11 and 10.32±0.13 µg/ showed weak activity against P. falciparum NF54 and K1 respectively, compared to all extracts and remaining solvent fractions form AE-1 and ME-1 extracts (Table 1).

In addition, our results compared to those reported previously by Kikweta et al. (2013) using P. falciparum sensitive to chloroquine isolated in infected blood of some patients in hospital and another P. falciparum K-1 strain for aqueous and methanol 80% ME-1 extract and their respective solvent fractions from of T. gilettii root barks collected in Kinshasa, revealed that these samples exhibited high antiplasmodial activity in vitro against both selected clinical isolated P. falciparum and K1 strains in vitro and against P. b. berghei ANKA in vivo compared to the same samples from T. gillettii root barks collected in Kinzaweta collectivity in Bas-Congo in the same country. Thus, this difference of activity may be due to the origin of the used Plasmodium strains in vitro and in vivo tests and the geographical collection of the plant material as well as to the variations in the plant's chemical composition (Machado et al., 2016, Jessica and Jazmin, 2020, Panţuroiu et al., 2025).

### 3.2.2. In vivo antiplasmodial activity of aqueous AE-1, methanol 80% % ME-1 and total alkaloids ME-2 extracts

Results recorded in antiplasmodial in vivo test were presented in Table 2. They revealed that the oral administration of all oral dose of all extracts, caused significant ($p < 0.05$) reduction of parasitemia in all treated infected mice in dose dependent manner (Table 2). Administered at high dose of 400 mg/kg bw, they produced considerably percentage chemosuppressions of parasitemia in infected mice with P. yolei 67 by 77.48±0.08% and with P. b. beghei ANKA by 75.13±0.11% for aqueous extract AE-1. Methanol 80% ME-1 extract reacted in the same manner

**Table 2: Effects of extracts on selected Plamodium in vivo test.**

| Sample codes | Treatment (mg/kg bw) | %P. in *P. yolei* 67 | %P. in *b. berghei* ANKA | % Inhibition of *P. yolei* 67 | % inhibition on *P. b. berghei* ANKA |
|---|---|---|---|---|---|
| AE-1 | 200 | 8.14±0.05 | 7.16±0.07 | 71.05±0.04 | 75.0.2±0.08 |
| | 400 | 6.33±0.10 | 7.13±0.04 | 77.48±0.08 | 75.13±0.11 |
| ME-1 | 200 | 6.04±0.12 | 7.01±0.08 | 78.52±0.06 | 75.54±0.12 |
| | 400 | 4.15±0.08 | 5.02±0.10 | 85.24±0.13 | 82.25±0.08 |
| ME-2 | 200 | 4.11±0.07 | 4.87±0.12 | 85.38±0.06 | 83.01±0.08 |
| | 400 | 4.02±0.10 | 4.31±0.08 | 85.70±0.04 | 84.96±0.10 |
| Artesunate | 10 | 2.54±0.06 | 2.74±0.05 | 90.96±0.08 | 90.44±0.03 |
| Quinine 2HCl | 10 | 3.05±0.04 | 3.11±0.07 | 89.36±0.05 | 89.15±0.07 |

AE-1: aqueous extract, ME-1:methanol 80% extract, ME-2: total alkaloids extract.

as aqueous extract by generating 85.70±0.04 and 84.96±0.10% chemosuppressions towards both Plasmodium yoeli 67 and P. b. berghei ANKA respectively. Total alkaloid ME-2 extract acted also the same manner as aqueous EA-1 and methanol ME-1 by decreasing parasitemia in treated mice infected with both Plasmodium strains in dose dependent manner (Table 2). Given orally at high dose of 400 mg/kg bw, this extract produced chemosuppresion percentages of parasitemia towards both Plasmodium strains to 85.70±0.04% towards P. yolei 67 and 84.96±0.10% against P. b. berghei ANKA respectively. Its antiplasmodial in vivo was found to be high confronted that of aqueous AE-1 and methanol 80% ME-1 extract while that of methanol extract ME-1 was high compared to aqueous AE-1 extract (Table 2). Our results were only qualitatively in good agreement with Kikweta et al; (2013) who reported previously the antiplamsodial activity in vivo only against P. b. berghei ANKA for all extracts from T. gilettii root barks collected in Kinshasa. Thus, for the

present study and to our knowledge, it was the first time to report the antiplasmodial activity in vitro against P. falciparum NF54 of extracts and solvent fractions and in vivo against P. yolei 67 and P. b. berghei ANKA of extracts from T. gilettii root barks collected in Kinzaweta collectivity in Bas-Congo (Democratic Republic of Congo).

**Artesunate Quinine**

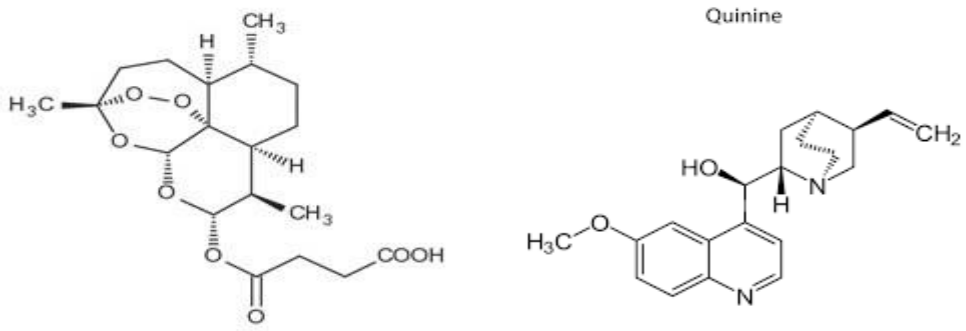


**Figure 6: Structures of Artesunate and Quinine as reference antiplasmodial drugs.**

Figure 7 displayed the chemossuprresion percetanges percentages of extracts and Artesunate and Quinine 2HCl on by parasiteamia caused by Plasmodium yolei 67 and P. b. berghei ANKA in infected mice. The intraperitoneal injection of these two parasites in mice carried away an enhancement of paraisteamia to high values of 28.34 and 25.15% respectively. After immediate oral treatment of infected mice administered orallly at oral dose of 200 and 400 mg/kg bw of extracts, it was observed that these extracts caused significant ($p < 0.05$) increase of chemosuppresion percentange reductions of parasiteamia in dose-dependent manner (Fig. 6). Served orallly at high dose of 400 mg/kg bw, they produced significant ($p < 0.05$) chemosuppression percentage reductions by 77.48 and 85.24%, 75.13 and 82,25% and 85,70 and 84,96% against P. yolei 67 et P. b. berghei ANKA respectively, imputed to aqueous AE-1, methanol 80% ME-1 and total alkaloids M-2 extracts respectively. Artesunate and Quinine 2HCl as reference antiplasmodial.

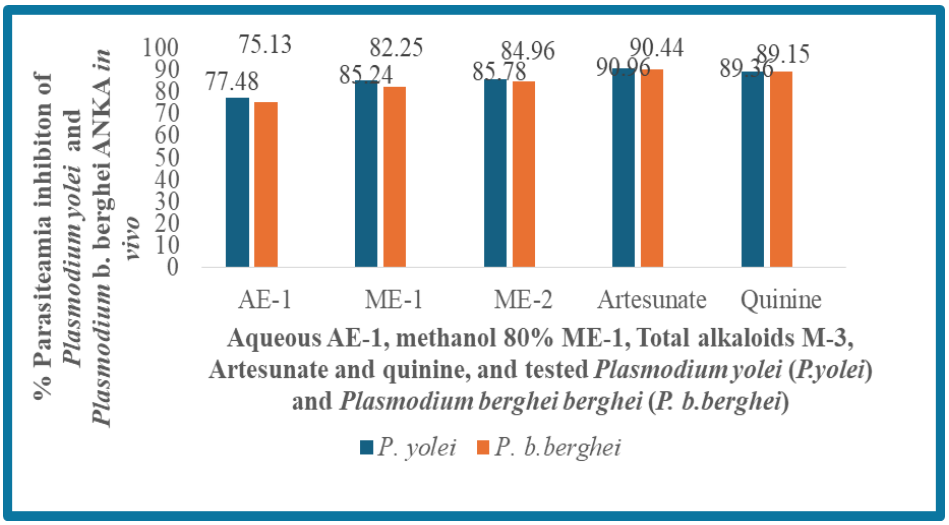


**Figure 7: Chemossuppression percentage reductions induced by aqueous AE-1, methanol 80% ME-1 and total alkaloids ME-2 extracts, Artesunate and Quinine 2HCl in infected treated mice.**

drugs, acted the same fashion by producing high chemosuppression percentage reductions in infected treated mice to values of 90.96 and 90,44% and 89.36 and 89,15% towards Plasmodium yolei 67 and P. b. berghei ANKA respectively.

This increase effect reduction of chemossuppression percentage reductions in infected treated mice clearly demonstrated that these samples were endowed with interesting and promising antiplasmodial activity or they exerted good antimalarial effect in vivo test. In both tests against P. yoelei 67 and b. berghei ANKA, total alkaloids ME-2 extract exerted high activity compared to aqueous AE-1 and methanol 80% ME-1 extract and this last showed similar effect compared to aqueous AE-1 extract for the same reasons already denoted above. Artesunate and Quinine 2HCl exerted this activity in elevated manner confronted to all extracts. However, all samples that had showed high chemosuppression percentage reductions than 80% can be considered as

candidates for future study in clinical trials leading to the formulation of ameliorated medicines for easy use by people.

Moreover, general mechanisms action of antiplasmodial activity of some secondary metabolites were previously:
Alkaloids acted as antiplasmodial by attacking Plasmodium parasite through multiple mechanisms, primarily by inhibiting hemozoin formation (preventing detoxification of heme), disrupting parasite membranes, interfering with DNA and RNA/protein synthestis, modulating parasite signaling pathways and targeting specific enzymes like PfATP4 (Plasmodium falciparum ATPase 4) which messed with ion balance leading to parasite death, often by affecting the digestive vacuole stage of the parasite as seen with the quinolinic alkaloid quinine interfering with heme polymerization and indolic alkaloid cryptolepine targeting multiple pathways offering hope against drug resistant and sensitive malaria by hitting distinct targets distinct from older drugs. They acted also as antimalarial agents by inhibiting the production of proteins and halting the conversion of toxic heme which resulted from the destruction of hemoglobin from being converted into hemozoin, an non-toxic pigment as previously reported by Herraiz et al., 2019 and Evbuomwan et al;, 2025.

Other particular mechanisms included:
- Hemozoin inhibition preventing the parasite from detoxifying heme into inerte hemozoin crystals leading to toxic buildup and parasite death.
- Ionic and osmotic homeostasis disruption that regulated sodium and osmotic balance causing parasite collapse.
- DNA/RNA protein interference could intercalate into or disrupt nucleic acid biosynthetisbiosynthesis and protein production, hindering parasite replication.
- Enzyme inhibition that could target vital parasite enzymes such as those in fatty acid.
- Synthetics pathway (FAS-II: Type II fatty acid synthase) as considered with Amaryllidaceae alkaloids for example.
- Synergistic effects that could enhance or increase the activity of existing drugs like chloroquine, offering new combination therapies.
- Targeting multiple pathways in which alkaloids possessed often multiple mode of action (multiple targeted) or slow-acting properties, making them effective towards drug-resistant where single-target drugs fail.

Alkaloids, including established drugs like quinine and newer research compounds, employed diverse mechanisms to act as antiplasmodial agents, primarily by targeting essential functions within the Plasmodium parasite.

**Their key mechanisms of action were**
- Heme detoxification interference: Many alkaloids, such as quinine and cryptolepine, were accumulated in the parasite's acidic digestive vacuole and inhibited the conversion of toxic heme (a byproduct of hemoglobin digestion) into non-toxic hemozoin. The resulting accumulation of free heme was toxic to the parasite, leading to its death. This was a major mechanism, especially for indole alkaloids effective against chloroquine-resistant strains.
- DNA Iintercalation and tTopoisomerase iInhibition: Some planar alkaloids, including cryptolepine and berberine, intercalated into the parasite's DNA, disrupting its structure and inhibiting essential processes like replication and transcription. They could also inhibit vital enzymes for DNA management, such as topoisomerase I and II.
- Metabolic pPathway dDisruption: Alkaloids could interfere with key metabolic pathways essential for parasite survival and virulence.

**Examples included**
- Inhibition of enzymes like Plasmodium falciparum lactate dehydrogenase (PfLDH).
- Disruption of glycolysis and the isoprenoid metabolic pathways.
- Interference with mitochondrial function, reducing the parasite's energy supply.

- Ion hHomeostasis Iinterference: Certain alkaloids could disrupt the parasite's ability to regulate ion levels and pH. Cipargamin, a spiroindolone derivative, worked by inhibiting PfATP4, a crucial plasma membrane Na+-ATPase that regulated sodium and osmotic balance which was a novel and highly effective mechanism against resistant strains.
- Membrane dDisruption and oOxidative sStress: Some alkaloids could compromise the integrity of the parasite's cytoplasmic or mitochondrial membranes. They might also induce oxidative stress by generating free radicals, further damaging parasite components.
- Protein sSynthesis and mMicrotubule aAssembly iInhibition: Alkaloids like Cipargamin had been shown to block protein synthesis in P. falciparum. Others might bind to tubulin and inhibited microtubule assembly, which wais crucial for cell division. The diverse range of mechanisms employed by alkaloids, often with some compounds exhibiting multiple modes of action onkes them as promising candidates for developing new anti-malarial drugs, especially to combat the problem of drug resistance (Frederich et al., 2008, Tajuddeen and Van Heerden, 2019, Graham , 2020, Uzor, 2020, Parvatar et al., 2024, García-Díaz, 2025).

Flavonoids acted as antiplasmodial agents by disrupting the malaria parasite’s vital functions, primarily by inhibiting fatty acid biosynthesis (FAS-II) pathway enzymes like FABg type II fatty acid synthase (FAS II)

system enzymes where FABG was the gene/protein name for β-ketoacyl-acyl carrier protein reductase (also known as 3-oxoacyl-[acyl-carrier-protein] reductase).

FABz was an essential bacterial enzyme that played a crucial role in the type II fatty acid biosynthesis (FAS II) pathway) that played a crucial role in the type II fatty acid biosynthesis (FAS II) pathway, FABi (an acronym that referred to several different entities, including an AI-powered data analysis platform, a Belgian federation of engineers, a research institute in South Africa, and a functional assessment-based intervention model) crucial for parasite survival, but absent in humans, and by interfering with heme detoxification and targeting multiple Plasmodium pathways such as inhibition parasite growth by interfering with crucial enzymes like falcipain-2, plasmepsin.

PfDXR and PfDXR were stands for Plasmodium falciparum 1-deoxy-D-xylulose 5-phosphate reductoisomerase as a crucial enzyme and a highly promising target for the development of novel antimalarial drugs, PFLDH appeared to be a typo or an unusual query syntax for PfLDH, which standed for Plasmodium falciparum lactate dehydrogenase and was an essential enzyme for the energy generation of the malaria parasite Plasmodium falciparum, and it was a key target for antimalarial drug development and diagnostic tests, disrupted heme detoxification (β-hematin formation), interfered with the parasite's metabolism/lifecycle, potentially blocked red blood cell invasion and couldan synergize with existing drugs like Artemisinin, making them promising for new antimalarial therapies, especially towards drug-resistant types.

They had been reported to inhibit the transportation of myoinositol and L-glutamine into Plasmodium-myoinositol and L-glutamine played significant ($p < 0.05$) roles of the cycle of the parasite as also reported previously by Hamilton et al., (2017) and Evbuomwan et al., (2025). Methoxylated flavonoids bounded to heme to prevent the parasite from neutralizing toxic heme products and also by affecting parasite nutrient uptake with some showing synergistic effects with existing drugs like Artemisinin.

**Particular mechanisms included**

- Enzyme inhibition by which flavonoids bounded to and inhibited essential parasite enzymes including proteases, metabolic enzymes PfDXR (deoxy-D-xylose-5 phosphate reductioisomerase) and pfLDH (lactose dehydrogenase),
- Heme detoxification inhibition where these compounds blockeding the formation of hemozoin (β-hematin), as a toxic byproduct of heme digestion leading to parasite death,
- Disruption of parasite lifecycle in which these secondary metabolites interfered with the parasite's metabolic processes and development at various stages,
- Red blood cell (RBC) invasion where some flavonoid compounds could prevent the parasite from entering new red blood cells,
- Synergy with Artemisinin by which flavonoids particularly quercetin increased the efficacy of Artemisinin suggesting potential for new combination therapies,
- Modulation of host pathways where flavonoids could influence host immune responses and cell signaling pathways involved in inflammation, further combating the disease malaria.

Flavonoids, as antiplasmodial agents, exhibited diverse mechanisms of action against the malaria parasite Plasmodium falciparum, often targeting multiple pathways.

**Their special mechanisms of action included**

- Inhibition of heme Detoxification (β-hematin formation): Flavonoids like quercetin could inhibit the formation of β-hematin (hemozoin), a critical detoxification process the parasite uses to manage toxic heme produced from digested host hemoglobin.

- **Enzyme Iinhibition**

- Proteases: flavonoids bounded to and inhibited Plasmodium proteases, such as falcipain- 2 (PfFP2), plasmepsin II (PfPlm II), and PfSUB2, which were essential for the parasite's growth, development, and invasion of red blood cells.
- DNA gyrase and other essential enzymes: Certain flavonoids inhibited key enzymes involved in parasite nucleic acid synthesis and replication, such as DNA gyrase, DNA PriA helicase, and specific P. falciparum enzymes like RIO-2, which was involved in cell cycle progression.
- Fatty Acid Biosynthesis: It was theorized that flavonoids might inhibit fatty acid biosynthesis (specifically the FAS-II pathway), a critical process for the synthesis of the parasite's cell membrane.

Dihydrofolate reductase-thymidylate synthase (DHFR-TS): Some flavonoids, like rutin and hesperidin, showed affinity for and inhibit enzymes such as Pf-DHFR-TS, disrupting the parasite's metabolism.

- Disruption of membrane function: Flavonoids could penetrate the parasite's cellular membranes, disrupting their integrity and function, which affected energy metabolism and nutrient transport. The lipophilicity of flavonoid aglycones (non-glycosylated forms) often allowed for better cell permeability and thus higher activity compared to their glycosylated counterparts.
- Induction of oxidative stress: In some cases, flavonoids might induce oxidative stress within the parasite, leading to cellular death.

- Modulation of Host Immune Response: Flavonoids could modulate the host's immune responses by inhibiting pro-inflammatory cytokines, thereby mitigating malaria-induced inflammation that could worsen clinical symptoms. Plasmepsins II and Falcipain-2 were proteases involved in hemoglobin breakdown.

Due to the complex, multi-target nature of their action, flavonoids often exhibited additive or synergistic effects when combined with other antimalarial drugs like Artemisinin, offering potential for new combination therapies to combat drug resistance (Tasdemir et al., 2006, Preething et al., 2017, Chepkirui et al., 2021, Tchangolle, 2022, Mamede, 2023, Foka et al, 2025, Olakunde et al., 2025).

Saponins were multi-faceted and primarily involved the disruption of the parasite's host cell invasion process and the inhibition of key parasite proteins. Their antiplasmodial effect was attributed to their amphipathic properties having both hydrophobic and hydrophilic parts which allowed them to interact with biological membranes and other cellular components. They had the ability to inhibit the growth of the malaria parasite Plasmodium falciparum with research indicating the work through multiple mechanisms including disrupting the parasite's membrane and targeting key proteins.

**Their special or key mechanims included**

- Membrane disruption and cholesterol binding in which these compounds with cholesterol in cell membranes, including those of the parasite and the host red blood cells invaded. This interaction perturbed and disorganized the cholesterol-rich lipid rafts, which were vital for the parasite's entry into cells and metabolism, thereby limiting host cell invasion,
- Protein invasion where saponin compounds could bind to specific parasite proteins. For example glycyrrhizin (GLR), a triterpenoid saponins from licorice (Glycyrrhizia glabra) bound to Plasmodium falciparum high-mobility group Box 1 (PfHMGB1) protein potentially inhibiting the parasite's transcriptional activity and growth,
- Enzyme inhibition in which some saponin metabolites like glycyrrhetic acid (GA) could inhibit the parasite's glyxalase-1 (GLO-1) enzyme, a detoxification system essential for parasite survival. Saponins exerted antiplasmodial effects through multiple mechanisms, primarily by disrupting the parasite's membrane function and cholesterol metabolism, and by interfering with parasitic and host protein activity.

**Membrane-Related Mechanisms**

Saponins were amphipathic compounds having both hydrophilic and hydrophobic parts that interacted with cell membranes.

- Membrane permeabilization and lysis: A general characteristic of many saponins was their ability to bind to sterols, particularly cholesterol, in cell membranes. This interaction could lead to pore formation, increasing the permeability of the parasite's membrane and potentially causing cell lysis, although some specific saponins like glycyrrhizin (GLR) had low lytic profiles.
- Cholesterol sequestration and lipid raft disruption: Plasmodium falciparum, the parasite responsible for the most severe form of malaria, was unable to synthesize cholesterol and might acquire it from the host. Saponins, such as GLR and its metabolite glycyrrhetinic acid (GA), sequestered host cholesterol and disrupted cholesterol-rich membrane microdomains called lipid rafts. Since these rafts were crucial for the parasite's entry into red blood cells and for membrane trafficking during infection, their disruption limits the parasite's ability could invade new host cells and managed its metabolism.

**Protein interaction Mechanisms**

In addition to membrane effects, saponins could target specific proteins critical to the parasite's survival and the host's inflammatory response by:

- Binding to Plasmodium HMGB1 (PfHMGB1): The parasite produced its own high-mobility group box 1 (HMGB1) protein, which was involved in transcriptional regulation necessary for parasite growth and invasion. Saponins could bind to PfHMGB1, inhibiting its activity and thus limiting parasite growth.
- Sequestration of host HMGB1 (HsHMGB1): During severe malaria infection, infected red blood cells induce the release of human HMGB1 (HsHMGB1), a molecule that orchestrated the inflammatory response and was associated with severe disease outcomes. Saponins could sequester HsHMGB1, reducing inflammation and associated tissue damage in the host.
- Potential enzyme and uninfected and infected erythrocytes inhibition: It was also suggested that saponins might inhibit specific parasite enzymes, such as glyoxalase 1 (PfGLO-1) or lactate dehydrogenase (PfLDH), which were vital for the parasite's detoxification processes and energy production via glycolysis. (Barbosa, 2014, Marciani, 2018, Tajuddeen and Van Heerde, 2019, Nyandwaro et al., 2020, Soeiro et al., 2021, Anonymous, 2025f).

Steroids acted as antiplasmodial agents by various mechanisms depending on the specific compound, but primarily involved disrupting of the Plasmodium parasite-infected red blood cell (pRBC) membrane function, particularly the new permeability pathways (NPPs) the parasite created for nutrient uptake. Other mechanism proposed included interference with hemoglobin digestion and induction of apoptotic-like cell death. They possessed significant ($p < 0.05$) antiplasmodial (anti-malarial) activity with various

natural and synthetic steroid derivatives showing effectiveness against P. falciparum, the parasite causing malaria by disrupting its growth, affected its metabolic pathways like hemozoin formation or ion transport, and even induced parasite cell death through apoptosis-like mechanisms, leading to promising new drug candidates, particularly those with peroxide or specific side-chain modifications.

**Their key mechanism of action included**

- Inhibition of new permeability pathways (NPPS) demonstrated by some steroids such as those isolated from Solanum nudum (like SN2 and SN4) primarily acted on the infected red blood cell membrane by blocking the NPPS (National Procurement Policy Statement) that the parasite created to acquire nutrients and disposed of waste. By inhibiting these pathways, the steroids interfered with the parasite's development and survival leading to its death,
- Interference with hemoglobin digestion where some steroids like Arymathylamino steroids, e.g compound 1o, were through to interfere with the parasite's hemoglobin (Hb) digestion pathway, a crucial process for the parasite's survival and growth. The steroid moiety might facilitate rapid internalization into the parasite's food vacuole, where the active part of the molecule was biovactivated possibly involving metal or metal chelation to exert its toxic effect. This prevented the accumulation of toxic heme and preserved the food vacuoles's integrity.
- Induction of apoaptotic-like cell death caused by certain steroids as those from S. nudum which could induce characteristics of programmed cell death by apoptosis and autophagy and the parasite, like as a decrease in mitochondrial membrane potential, DNA fragmentation, and cytoplasmic acidification,
- Disruption of membrane function and merozoite invasion due to their lipophilic nature: Many steroidal compounds could incorporate into cell membranes, altering membrane properties like permeability and rigidity. This could inhibit the ability of new merozoites(the invasive stage) to invade uninfected red blood cells,
- Targeting parasite_specific proteins provoked by some hybrid steroid compounds like novel steroid-tetreoxane hybrids were being investigated for their ability to target specific parasite proteins such as P. falciparum Sarco/endoplasmic reticulum calcium-dependent ATPase (PfATP6 was crucial calcium-transporting enzyme (a SERCA pump) in the malaria parasite Plasmodium falciparum, essential for its survival by regulating calcium levels in the endoplasmic reticulum, it was a significant ($p < 0.05$) drug target, particularly for Artemisinin-based antimalarials, with mutations in PfATP6 linked to Artemisinin resistance, making it a focus for new drug development, crucial calcium-transporting enzyme (a SERCA pump) in the malaria parasite Plasmodium falciparum, essential for its survival by regulating calcium levels in the endoplasmic reticulum, it was a significant ($p < 0.05$) drug target, or cyclophilin, a protein liked to drug resistance.

In summary, steroid compounds acted as antiplasmodial agents through multifaceted mecahnisms, primarily by disrupting essential parasite processes, modifying host cell membranes and in some cases including cell death pathways as the specific mechanism was highly dependent on the compound's structure. The antiplasmodial action of certain steroids involved novel mechanisms distinct from their typical anti-inflammatory pathways. Specifically, these compounds appeared to target parasitic processes related to heme detoxification and protein synthesis.

**Mechanisms of action included:**

- Heme bioactivation and Quinone Methide formation: The most prominent proposed mechanism for novel antiparasitic steroids, such as Arylmethylamino steroids, involved bioactivation by metal ions, particularly iron (heme). Parasites like Plasmodium falciparum digest hemoglobin, producing toxic heme as a byproduct. The parasite normally converted this heme into a non-toxic crystal called hemozoin. The steroid compounds were though to interfere with this process:
- The lipophilic steroid moiety helped the compound penetrate the parasite's membrane and accumulate in the digestive vacuole.
- The attached hydroxyarylmethylamino group was essential and facilitates a chelate-based quinone methide mechanism, involving interaction with free iron or heme.
- This interaction leaded to the formation of a reactive species that likely covalently modified parasite proteins or disrupts the heme detoxification pathway, ultimately killing the parasite.
- Inhibition of parasite poteins: Other antiplasmodial steroids exhibited activity through different mechanisms such as:
- PfATP6 Inhibition: Some steroids, notably ergosterol-5,8-endoperoxide isolated from certain plants, demonstrated antiplasmodial activity by binding to and inhibiting the P. falciparum Sarco/endoplasmic reticulum calcium-dependent ATPase (PfATP6).
- Inhibition of elongation factor G (EF-G): Fusidic acid, a steroid-type antibiotic with antiplasmodial properties, inhibited bacterial protein synthesis by locking elongation factor G (EF-G) on the ribosome. Studies suggested it targeted the PfEF-Gs located in the parasite's apicoplast and mitochondria.

These mechanisms highlight that the antiplasmodial effected were specific to the parasite's unique biology and distinct from the classic glucocorticoid receptor-mediated anti-inflammatory actions of conventional steroids (Ng et al., 1997, Prasad and Gramer, 1999, Lopez et al., 2009, Krieg et al., 2017, Varo et al., 2018, Minton, 2021, Safar et al., 2022).

Terpenoids acted as antiplasmodial compounds trough multiple often interconnected mechanisms, primarily by disrupting essential metabolic and cellular functions in the Plasmodium parasite. They possessed significant antiplasmodial or anti-malarial activity acting the Plasmodium parasite by disrupting nutrient intake, inhibiting heme detoxification and targeting various stages of its lifecycle, with diverse structures such as diterpenoids and triterpenoids showing promise, exemplified by Artemisinin, highlighting their potential as new leads especially against resistant strains.

**Their key mechanisms of action included:**

- Heme-mediated activation as Artemisinin was a widely used sesquiterpene lactone antiplasmodial or antimalarial drugs, and its derivates were activated by heme (iron) within the Plasmodium parasite's digestive vacuole. This effect generated carbon-centered reactive oxidants that bounded to and inactivated crucial parasite proteins ultimately killing the parasite,
- Inhibition of phospholipid synthestis caused by some terpenoids such as those found in Garderma lucidum plant extracts and appeared to inhibit the synthestis of phospholipids in infected erythrocytes. This was a crucial process for the rapidly growing parasite which required high amount of membrane material to survive and multiply,
- Disruption of cell membrane integrity was due to their lipophilic (fat-loving) nature. Many terpenoids could penetrate the parasite's cell membrane and disrupted its structure and integrity. This leaded to metabolic instability, the loss of essential intracellular components like proteins, ions and event cell death,
- -Interference with protein prenylation induced by certain terpenoid compounds like farnesol and nerolidol having been shown to inhibit the post-translational modification (isoprenylation) of cell-growth-regulating proteins like Ras and Rap in Plasmoduim parasites. This disruption hindered the parasite's development, particularly in the schizont switched the parasite'stage,
- Inhibition of key metabolic enzymes as terpenoids could interfere with the parasite's isoprenoid biosynthetisbiosynthesis pathway by inhibiting enzymes like isopentenyl diphosphate synthases or DXR (1-deoxy-D-xylose-5-phosphate reductoisomerase). Thus, equilibrium leading to the accumulation of reactive oxygene species that the parasite's endogenous systems, could not manage causing oxidative damages an cell death.
- Interaction with and inbibitioninhibition of parasite proteins: computational studies of some experimental evidence suggested that certain terpenoids like cucurbitacin B and β-O-acetyl oleanolic acid could bind to and inhibited essential P. falciparum proteins like plasmepsin II, histo-aspartic protein and falcipain-2. Terpenoids acted as antiplasmodial agents through multiple, often interconnected, mechanisms that target key survival processes in the Plasmodium parasite. The specific action dependeds on the particular terpenoid compounds, with major mechanisms including membrane disruption, metabolic interference, and induction of oxidative stress.

**Key mechanisms of action included**

Disruption of the Parasite Cell Membrane: The lipophilic (fat-loving) nature of many terpenoids allows them to penetrate and disrupt the integrity of the parasite's cell membrane, leading to a loss of essential cellular components and ultimately cell death-.

- Interference with mMitochondrial rRespiration: Some terpenoids, such as farnesol and nerolidol, disrupted mitochondrial function and the electron transport chain by inhibiting the biosynthesis of crucial isoprenoid-containing molecules like coenzyme Q10 (ubiquinone). This process was detrimental to the parasite's energy production.
- - Inhibition of kKey pParasite Eenzymes and biosynthetic pathways were:
- Heme detoxification: The widely used antimalarial drug artemisinin (a sesquiterpene lactone) wais activated by heme within the parasite, generating carbon-centered free radicals that alkylated and inactivate multiple parasite proteins, leading to the parasite's demise.
- Isoprenoid sSynthesis: Terpenoids could inhibit enzymes in the parasite's non-mevalonate (MEP) pathway, such as DXR (1-deoxy-D-xylulose-5-phosphate reductoisomerase), which was vital for the synthesis of essential isoprenoids.

Calcium Homeostasis: Certain terpenoids could modulate calcium metabolism in Plasmodium by inhibiting P-type calcium ATPases (like PfATP6), causing an elevation in cytosolic calcium that was toxic to the parasite.

- Induction of oOxidative sStress: Many terpenoids caused a perturbation of the parasite's redox equilibrium, generating reactive oxygen species (ROS) faster than the parasite's defense systems (e.g., the trypanothione system) could neutralize them, leading to oxidative damage and cell death.
- Cell cCycle aArrest: Specific terpenoids, such as paclitaxel (though primarily studied for cancer), hadve demonstrated the ability to block the cell cycle progression of protozoan parasites like Leishmania, preventing them from multiplying.

Due to the structural diversity of terpenoids, their effects weare highly varied and often involved a combination of these mechanisms, making it difficult for parasites to develop resistance to them simultaneously ( Heloisa et al., 2018, Saito et al., 2016, Oluyemi et al., 2020, Dwivedi et al., 2021, Ishola et al., 2023, Dembitsk et al., 2025)

In general, the observed antiplasmodial activity of extracts and solvent fractions from T. gilettii root barks could be attributed to the presence ofd secondary metabolites identified in this palantplant part because these phytochemicals isolated from other medicinal species such as alkaloids (Cimanga et al., 2019, Soto-Vasquez et al., 2021, Garcia-Duaz, 2025a,b, Okom et al., 2025), flavonoids (Karama et al., 2020, Ranaivorarison et al., 2020, Chepkrui et al., 2021, Tchangolle et al., 2022), saponins (Soecero et al., 2021, Hakin et al., 2021), steroids (Safar et al., 2022, Ngnoung et al., 2020, Mianda et al., 2020, Ranaivoarison et al., 2020, Springer et al., 2025), and terpenoids (Heloisa et al., 2017, Greve et al., 2020, Olayemi et al., 2020, Dwivedi el al., 2021, Ekasari et al., 2023) were previously reported to be endowed with this biological activity expressed at different magnitudes.

Particularly, alkaloids could be considered as the one active principles antiplasmodial of T. gilettii root barks since the crude total alkaloids extract showed strong antiplasmodial effect in previous studies (Kikwueta et al., 2013) as confirmed in the present investigation.

Moreover, the phytochemical analysis of aqueous AE-1 et methanol 80% ME-1 extracts of T. gilettii root barks in the present study, revealed the presence of different phytochemical groups with flavonoids and alkaloids as major predominant in both extracts. Alkaloids acted as antiplsamodial agents by various mechanisms such as by inhibiting the production of proteins and halting the conversion of toxic heme resulting from the destruction of hemoglobin from being converted into hemozoin, a non-toxic pigment as previously reported by Herraiz et al., (2019) and Evbuomwan et al., (2025). Flavonoids had been reported to inhibit the transportation of myoinositol and L-glutamine playing significant role of the cycle of the parasite as also previously reported by Hamilton et al., (2017) and Evbuomwan et al., (2025). Based on this observation, it was suspected that the total alkaloids extract from T. gilettii root barks studied could also act in the same way to exhibit its antiplasmodial activity in vitro test.The aqueous extract of T. dictyophylla root presented hematic and antiplatelet proprieties, it presented likewise anticoagulant, antimicrobial and antifungal activities (Tiam, 2020).

Collectively, the antimalarial of the studied plant part in the present investigationng mightay be attributed to alkaloids and other phytoconstituents such as flavonoids, saponins, steroids and terpenoids, etc. acting probably in sinergistic manner to exert strong antiplasmodial activity in vitro and particularly to alkaloids pointed now as the one of the active antiplasmodial principles of the studied plant part.

### 3.4. Observations in acute and sub acute toxicity

After 28 days of observation in acute and subacute toxicity evaluation, no sign of toxic effects such as significant alteration in body weight, physical signs, symptoms, hematological, biochemical parameters, and body organ weights, hyperurination, abdominal muscle twitches, convulsions, salivation, aggression, rising furs, writhing, and general behavior, etc. were observed compared to the negative group. The liver, kidney, heart, lunchs and stomach histology did not show any drug-induced lesions confronted to negative control showing normal architecture as also reported previously by Alelign et al., (2020), Jawhari et al. (2021), Ur Rehman et al., (2022). But, mice in treated groups with aqueous extract in acute at subacute toxicity gained body weight compared to negative control as illustrated in figures 7 and 8 below:

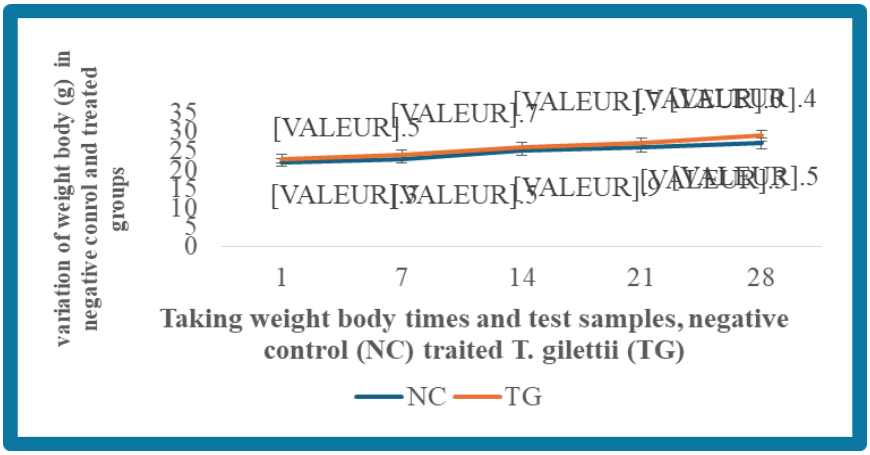


**Figure 7: Variation of bodyweights of mice in negatif control and treated T. gilettii (TG) in acute toxicity.**

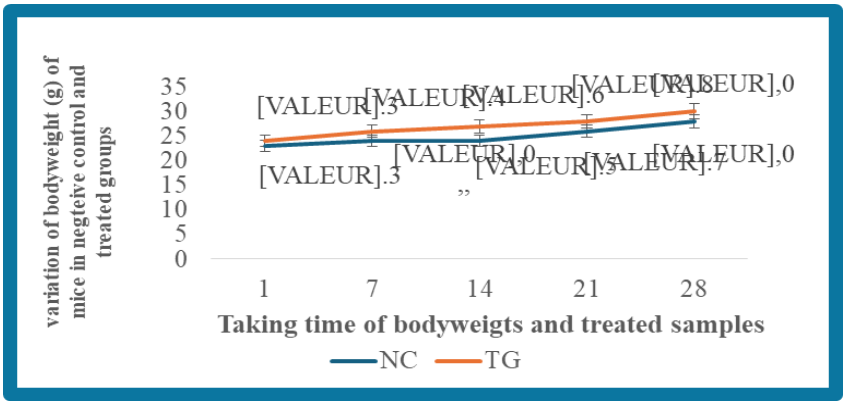


**Figure 8: Variation of bodyweights of mice in negatif control and treated group in subacute toxicity, negative control (NC) and trated T. gilettii (TG).**

It was also observed that mice in subacute toxixity administred daily oral aqueous at oral doses of 500, 1000 and 5000 mg/kg bw in subacute toxicity, had showed high bodyweight than mice treated once with 5000 mg/kg bw in acute toxicity as seen in figure 1 compared to figure 2. This difference could be due to fact the aqueous AE-1 extract administered at these multiple doses each day in subcute toxicity, has a positive impact on the stimulation of appetite of food and water intake of treated mice at these doses inducing high accumulation of appetizing or savoury substances like tannins (Anonym D, 2025). No mortality of treated mice in both tests toxicity was observed at high adminsiterd oral dose of 5000 mg/kg and the lethal dose of the administered aqueous extract was estimated to be greater than 5000 mg/kg bw. According to Suwandi et al., (2021) and Jegnie et al. (2023), this aqoeus AE-1 extract from T. gilettii root barks, could be considered or regarded as pratically non-toxic by this administrered way.

According to toxicity classification, substances with lethal dose 50 (LD50) values within the range of 1 to 5 g/kg bodyweight were generally considered to have low toxicity, while those having higher values than 5000 mg/kg bodyweight were generally reagarded pratically to be non-toxicby by the administered route (Piyachaturavat et al., 2002). Thus, as the high oral dose of 5000 mg/kg of aqueous extract AE-1 from T. gilettii did not induced mortality of treated mice after 28 days of observation and its LD50 consequently estimated to be greater than 5000 mg/kg bodyweight, this aqueous extrat AE-1 from Triclisia giletti root barks could be considered pratically non-toxic per os route as already noted above and was emerged safe to be used in the medicinal field for its pharmacological activity for a long period of treatment without disquietude or worry. Normally, this statement indicated that in toxicity tests involving mice, administration or management a high dosage of 5000 mg/kg bodyweight of a specific plant extract did not automatically result in any death among the treated animals. This suggested a low level or absence of acute and subacute toxicity for that particular medicinal plant extracts in treated animals at this dosage.

## 4. CONCLUSION

This study reported fort the first time the antiplamsodial activity of aqueous AE-1, methanol 80% ME-1 and total alkaloids ME-2 extracts and solvent fractions both extracts from Triglisia gilettii root barks collected in Kinzaweta collectivity in Bas-Congo (DRCongo) in vitro and in vivo tets. In both test, all test samples had showed good and intereisting evaluated biological activity. in vitro and in vivo test. In vitro test, it is important to note the the aniplasmodial activity of these samples was reported for the first time against Plasmodium falciparum NF54 sensible to chloroquine in vitro and against Plamodium yolei in vivo test. A great attention was made on extracts showing more than 80% chemosuppression percentages in vivo constituting valid candidates for clinical studies in humans leading to the formulation of ameliorated medicines based plant extracts for their easy use by people. These reported results can support and justify the traditional use mainly of aqueous extract of the plant part for the treatment of uncomplicated human malaria in tradtional medicine in DRCongo and other African countries where it knew the same medical use. Furture investigation were envisaged for the extraction of active principles from T. gilettii root barks based mainly on alkaloids since crude alkaloids showed high antiplamodial activity in the present study without forgoten other phytochemical groups.

## REFERENCES

1. Abibakar A, Ahmad NS, Akanya HO, Abdukadir A, Ambulkra AN. Antiplasmodial activity of total alkaloids and flavonoids of stem bark extracts of Enantia chlorantha in mice. Comparative Clin Prasitol, 2022; 29/ 873-881.
2. Alelign T, Chalchisa D, Fekadu N, Solomon D, Sisay T, Debella A, Petros B. Evaluation of acute and sub-acute toxicity of selected traditional antiurolithiatic medicinal plant extracts in Wistar albino rats. Toxicol Rep., 2020; 7: 1356-1365. https://doi.org/10.1016/j.toxrep.2020.10.001
3. Anonym A. Malaria-Diagnosis & Treatment, 2025.
4. Anonym B. Treatment of malaria, 2025e.
5. Anonym C. Preventing Relapse or recidism, 2025.
6. Anonym D. Tannins and appetite, 2025.

7. Barbosa AP. An overview of the biological and pharmacological activity of d. Int J Pharm Pharm Sci, 2014; 6(8): 47-50.
8. Camacho Md, Philipson JD, Croft SI, Solis PN, Marschall SJ, Chazanfar SA. Screening of plant extracts for antiprotozoal and cytotoxic activities. J Ethnopharmacol., 2003; 89(2): 185-191.
9. Camargo EP. Malária, maleita, paludismo. Ciência e Cultura., 2003; 55: 26-29.
10. Chepkirui C, Ochieng RT, Sarkar B, Hussain A et al.,. Antiplasmodial and antileishmanial flavonoids from Mundululea sericea. Fitoterapia, 2021; 149: 104796. https://doi.org/10.1016/j.fitote.2020.104796.
11. Cimanga K, De Bruyne T, Pieters L, Turger, CA, A.J. Vlietinck. In vitro and in vivo antiplasmodial activity of cryptolepine and related alkaloids from Cryptolepis sanguinolenta. J Nat Prod., 1997; 60: 688-691.
12. Cimanga K. R., Tona, L,. Luyindula, N., Mesia, K., Lusakibanza, M., Musuamba, C.T., De Bruyne T., Apers, S., Van Miert, S., Hermans, N., Totté, J., Pieters, L, and Vlietinck, A.J. Antiplasmodial activity of extracts and fractions from callus culture of fresh apical stems of Phyllanthus niruri (Euphorbiaceae). Part 2. J Ethnopharmacol., 2004; 95: 399-404.
13. Cimanga KR, Kikweta MC, Mbamu MB, Kambu KO, Apers S, Vlietinck AJ, Pieters, L. Assessment of acute and subacute toxicological profiles of the aqueous extract (decoction) from the leaves of Triclisia gilettii (De Wild.) Staner (Menispermaceae) in Wstar rats. Sch. Acad. J. Pharm., 2015; 4(7): 351-357.
14. Cimanga KR, Kikweta MC, Nsaka LS, Tshodi EM., Makila BMF, Kambu KO , B. Mbamu MB, Cos P, Maes L, Vlietinck AJ, Tuenter E, Foubert K, Pieters L. Isolation and structure elucidation of two antiprotozoal bisbenzylisoquinoline alkaloids from Triclisia gilettii stem bark. Phytochem Let., 2018; 28: 19-23. https://doi.org/10.1016/j.phytol.2018.09.008.
15. Cimanga KR, Kikweta MC, Mbamu MB, Nsaka LS, Tshodi M, Bakana PD, Opota OD, Kambu KO, Vlietinck AJ, Pieters L. Assessment of in vitro antioxidant activity, acute and sub-acute toxicity of aqueous extract (decoction) of Triclisia gilettii (De Wild.) Staner (Menispermaceae) stem bark in Wistar rats. Eur J Biomed Pharm Sci., 2018; 5(1): 130-140.
16. Cimanga KR, Nsaka LS, Tshodi EM, Mbamu MB, Kikwueta MC, Vlietinck AJ, Pieters L. In vitro and in vivo antiplasmodial activity of extracts and isolated compounds of Alstonia congensis root bark. J Ethnopharmacol, 2019; 242: 111736. https://doi.org/10.1016/j.jep.2019.02.019.
17. Dembitsky VM, Alexander O. Terent'ey. Endoperoxides: Highly Oxygenated Terpenoids with Anticancer and Antiprotozoal Activities. Creative Commons CC BY, 2025; 40. doi: 10.20944/preprints202512.1819. doi: 10.20944/preprints202512.1819.
18. Dwivedi MK, Mishra S, Sonter S, Singh PK. Diterpenoids as potential anti-malarial compounds from Andrographis paniculata. J Basic Appl Sci., 2021; 10: 7. https://doi.org/10.1186/s43088-021-00098-8.
19. Ekasari W, Makrdiani A, Putri NT. Terpnoids from Euphorbiaceae as source of antimalarial medicines. A literature review. Pharm Educ., 2023; 23(4): 294-299.
20. Evbuomwan IO? Adeyemi OS, Oluba OM. Aqueous extract of Enantia chlorrantha Oliv. dDemonstrates antimalarial activity and improves redox imbalance and biochemical alterations in mice. BMC Complement Altern Med and Ther., 2025; 25(73): 1-67.
21. Foka PVT, Mboh CA, Naoti KL, Tauris NI et al., From rural pratice to scientific evidence : synergistic antiplasmodial and active properties of combined Ageratum congroides and Bidens pilosa extracts. Explor Drug Sci., 2025; 3: 1008122. https://doi.org/10.37349/eds.205.1008122.
22. Frederic kM, Tits M, Angenot L. Potential antimalarial activity of indole alkaloids. Trans Royal Society Trop Med Hygiene, 2008; 102(1): 11-19. doi:10.1016/j.trstmh.2007.10.002
23. Gabriel HB, Rodrigo AC, Sussmann, Emila A, Kimura, Rodrigue AAM,. Terpenes as Potential Antimalarial Drugs. Edited by Shagufta Perveen and Areej Al-Taweel, 2017. DOI: 10.5772/intechopen.75108.
24. Garcia-Diaz I, Tuenter E, Arranz JLE, Maury GL, Cos P, Pieters L. Antiplasmodoal activity of alkaloids from Croton linenearis leaves. Exp Parasitol., 2022: 236-237. https://doi.org/10.1016.
25. Garcia-Diaz I. Antiplasmodial activity of alkaloids. Heidelbery. https://doi.org/10.1007/978-3-652-3608-6, 2025a.
26. García-Díaz, I. Antiplasmodial Activity of Alkaloids. In: Ramawat, K.G., Mérillon, JM. (eds) Natural Products. Springer, Berlin, Heidelberg, 2025b. https://doi.org/10.1007/978-3-642-36202-6-277-1
27. Ghorbani R, Zargaran A. Artemisinin: An Effective Natural Drug for the Treatment of Malaria and Prospects for Sustainable Production. Biol Molec Chem., 2025b: 94-105. https://doi.org/10.22034/bmc.2025.534193.1071.
28. Graham LP. Antimalarials Agents. Design and Mechanism of Action, 2020. Gen Chem., 622 pages. DOI: 10.106/22815-0-06338-9.
29. Habibi P, Shi Y, Fatima M, Grossi-de-Sa FM, I. Khan I. Plants as sources of natural and recombinant antimalarial agents. Mol. Biotechnol., 2022; 64: 1177., doi: 10.1007/s12033-022-00499-9.
30. Hakin MB, Adewuyi AI, Abdelsalam TA, Ashafa AOT. Antimalarial ctivityactivity and biochemistry effects of saponins-rich extract of Dianthus banutius Burst Davy in Plasmodium bergehei infected mice.

Adv Trad Med., 2021; 571: 1-11. https://doi.org/10.1007/s/13596-021-00571-w.
31. Hamilton WL, Claessens A, Otto TD, Kekre M, Fairhurst RM, Rayner JC,? Kiiatkowski D. Extreme mutation bias and high AT content in Plasmodium falciparum. Nucleic ACID Res., 2017; 45(4): 1889-18901.
32. Harborne JB. Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. Chapman & Hall Ltd. London UK, 1998.
33. Herraiz T. Guillén H, González-Peña D, Arán VJ. Antimalarial quinoline drugs inhibits-β-hematin and increase free hemin catalyzing peroxidative reactions and inhibition of cysteine proteases. Sci Rep., 2019; 9(1): 15398. https://doi.org/1? Éà&è1038/s41598-019-51604-.
34. Indrian I, Amoinab NS, Puspunigsih NN. Antiplasmodial activity of sStigmastane steroids from Dryobatanops oblongifolia stem bark. Open Chem, 2022, 18:259-296. https://doi;org/10.1515/chm.2020-0027.
35. Ishola AA, Adewole KE , Adebayo JO, Balogun EA. Potentials of Terpenoids as Inhibitors of Multiple Plasmodium falciparum Protein Drug Targets. Acta Parasitol., 2023; 4: 793-806. https://doi.org/10.1007/s11686-023-00711-z.
36. Jegnie M, Abula T, Woldekidan S, Chalchisa D, Asmare Z, Afework M. Acute and Sub-Acute Toxicity Evaluation of the Crude Methanolic Extract of Justicia schimperiana Leaf in Wistar Albino Rats. J Exp Pharmacol., 2023; 15: 467–483. doi: 10.2147/JEP.S441273.
37. Jessica GP, Jazmin MPR. Advances in Chemical Composition and Biological Activity of Mexican Propolis. Lupine Online. J Pharmacol Clin Res., 2020; 2(3): 194-200.
38. Karama I, Comaporé M, Djandi A, Traoré O, Caguika , Kiendrebengo M. Antiplasmodial activities of fkavonoidsflavonoids from leaves of Securidata longependuculata Fresen (Polygonaceae); Am J BioSc., 2020; 8(1): 1-5.
39. Kikweta MC, Tshiodi EM, Tona LG, Vlietinck AJ, Pieters L, Cimanga KR. In vitro evaluation of free radical scavenging and antioxidant, and spasmolytic potentials of extracts, solvent fractions and subfractions from Triclisia gilletti (De Wild) Staner (Menispermaceae) stem bark. World J Pharm Pharm Sci., 2025; (03): 26-58.
40. Kolade OF, Manish KT, Emmanuel GF, Awodayo OA, Stephen A, Adesida IO et al. Plasmepsin II inhibitory potential of phytochemicals isolated from African antimalarial plants: a computational approach. J Biomol Struct Dyn., 2023: 505-520. https://doi.org/10.1080/07391102.2023.2283146..
41. Krieg R, Jortzik E, Goetz AB, Blaudins S et al.,. Atylmethylamino steroids as antiparasitic agents. Nat Comm., 2017; 8(14478).
42. Kuypers K, Cos P, Ortega- Baria, Eduardo, Vanden Berghe D, Maes L. Bioassays from some parasitic protozoa: screening concepts and standard in vitro and in vivo laboratory models. In Gupta MP, Manda SS, Vanish K (Eds.): Biological Screening of Plant Constituents. International Centre for Since and High Technology, 2006; 1-18.
43. Li J, Docile HJ, Fisher D, Pronyuk K, Zhao L. Current status of malaria control and elimination in Africa: epidemiology, diagnosis, treatment, progress and challenges. J. Epidemiol Glob. Health, 2024; 14: 561-579. doi: 10.1007/S44197-024-00228-2.
44. Lopez Ml, Blair S Saiz J, Segura C. Effect of Solanum nudum steroids on unifected and Plasmodium falciparum-infected erythrocytes. Mem Inst Oswaldo Cruz, 2009; 105(5): 688-688.
45. Mabuza MJ, Kaizer M, Tshikalange JE, Yusuf AA, Bapela MJ. In vitro antiplasmodial activity and cytotoxicity of three Ziziphus (Rhamnaceae) species from Africa. J Ethnopharmacol., 2025; 349: 119918. https://doi.org/10.1016/j.ep.2O25.119918.
46. Machado BAS, Silva RPD, de Abreu Barreto G ,Samantha Serra Costa SS et al.,. Chemical composition and Biological Activity of Extracts Obtained by Supercritical Extraction and Ethanolic Extraction of Brown, Green and Red Propolis Derived from Different Geographic Regions in Brazil. PLoS ONE, 2016; 11(1): e0145954. https://doi.org/10.1371/journal.pone.0145954
47. Mamede L. Metabotolomicc investigation of antiplasmodial agents; Doctorat ThessisThesis In Biomedical and Pharmaceutical Sciences, Université de Liège, Liège, Belgique, 2023/2024.
48. Manhas A, Kumar S, Jha PC. Identification of the natural compound inhibitors against Plasmodium falciparum plasmepsin-II via common feature based screening and molecular dynamic simulations. J Biomol Struct Dyn., 2022; 40: 3-43.
49. Marciani DJ. Elucidating the Mechanisms of Action of Saponin-Derived Adjuvants. Trends Pharmacol Sci., 2018; 39(6): 573-585.
50. Mesia GK, Tona, GL, Nanga TH, Cimanga KR, Apers S, Cos P, Maes L, Pieters L, Vlietinck AJ. Antiprotozoal and cytotoxic screening of 45 plant extracts from Democratic Republic of Congo. J Ethnopharmacol., 2008; 115: 409-415.
51. Minton K. Steroid response to malaria. Nat Revs Immunol., 2021; 21: 472–473.
52. Mfulu EAL, Vweba JD , Lungu TK , Muanda GM. Contribution à l'analyse physico-chimique et biochimique des feuilles de Triclisia gilettii (De Wild.) Staner utilisées en alimentation au Kongo central en République Démocratique du Congo. Revue Afr Environ Agric, 2023; 6(2): 83-91.
53. Mianda SM, Moyo P, Maboane S, Pirkhaltz LM, Makaria VJ. Phyconstituents fromTurraea obtusifolia and their antiplasmodial activity. Nat Prod Res., 2023: 1-3. https://doi.org/10.080/14786419.2023.2255.
54. Moovendhan M. Polysaccharides and from seaveeds: A natural arsenal against drug-resistant malaria. J Fonct Foods, 2025; 134: 107050. https://doi.org/10.1012/j.jff.2025.107050.

55. Mutwale KP, Ondze JTrésor, Njakarinala R, Ange Mouithys-Mickalad A et al.,. Cellular Antioxidant and Peroxidase Inhibition Activities of Decoctions from Different Parts of Triclisia gilettii (De Wild) Staner (Menispermaceae). Pharmacogn. Commn., 2022; 12(3): 103-108.
56. Ng CKM, Mak AYK, Au TS, Lai ST, Lai JY. Plasmodium infection unmasked by corticosteroid therapy. Hong Kong Medi J., 1997: 3(3): 328-330.
57. Ngnoung GAA, Sdjni L, Leutiku P, Ditchou Y-YN et al.,. Antileishmanial and antiplasmodial activities of secondary metabolites from root of Antrpcaryon klaineanum Pierre (Anacardiaceae). Molecules, 2023; 28(6): 2730; https://doi.org/10.3390/molecules28062730.kal-04447765.
58. Nguyen TD, Gao B, Amaratunga C, Dhorda M, Tran TNA, White NJ, Dondorp AM, Boni MF, Aguas R. Preventing antimalarial drug resist, ance with triple artemisinin-based combination therapies. Nat. Commun., 2023; 14; 1-10. https://doi.org/10.1038/s41467-023-39914-3.
59. Nyandwaro K, Oyweri J, Kimani F, Mbugua A. Evaluating Antiplasmodial and Antimalarial Activities of Soybean (Glycine max) Seed Extracts on P. falciparum Parasite Cultures and P. berghei-Infected Mice. J Phatogens, 2020; 1-8. ttps://doi.org/10.1155/2020/7605730.
60. Ntie-Kang F, Arguéné PA, Lifongo L, Ndom JC, Sippl W, Meva'a Mbase L. The potential antimalarial compounds derived from African medicinal plants, part II: a pharmacological evaluation of non-alkaloids and non-terpenoids. Malaria J., 2014; 13 (81)/120.
61. Okom SU, Okpoghono J, Omyesam I. In vivo antiplasmodial activity of alkaloids, flavonoids and ethanol extracts from Stem bark and leaves of Phyllanthus amarus. Discov Apll Sci., 2025; 7: 280. https://doi.org/10.1007/542452-025-06769-w.
62. Olayeriju1 OS, Crown OO, Elekofehinti OO, Akinmoladun AC, Olaleye MT, Akindahunsi AA. Effect of moonseed vine (Triclisia gilettii Staner) on ethane-1,2-diol-induced urolithiasisandurolithiasis and its renotoxicity in Wistar albino rats. Afr J Urol, 2020; 26: 4. https://doi.org/10.1186/s12301-020-0018.
63. Olayerijua OS, Elekofehinti OO, Olaleyec MT , Akindahunsi AA. Activation of NRF2/HO-1 Pathway by aqueous methanolic leaf extract of Triclisia gilettii and selected identified compounds in Triclisia gilettii, modulates crystal binding genes (CD44/OPN) in Ethane-1,2-diol-induced nephrolithic rats. Phytomedicine Plus, 2021; 1(4): 1006. https://doi.org/10.1016/j.phyplu.2021.100066.
64. Olatunde A, Ssuhaiman O, Muhammad OB, Tyani H et al.,. Deciphering the inhibitory activity of flavonoids against Plasmodium falciparum proteases through molecular docking, drug-likeness and pharmacokinetic studies; Discover Chem., 2025; 2: 294. https://doi.org/10.1007/544371-025-00339-0.
65. Preethind I, Sussen SR. Antimalarial potential of isolated flavonoids-A review. Res J Pharm Technol., 2017; 10(11): 4057-4062.
66. Panţuroiu M, Gălăţanu ML, Manea CE, Popescu M, Sandulovici RC, Emilia Pănuş E.
67. Comparison of Matricaria chamomilla and Tripleurospermum inodorum. Compounds, 2025; 5(4): 50. https://doi.org/10.3390/compounds5040050.
68. Olayeriju OS, Papetti A, Colombo R, Mannucci B, Olaleye MT, Akindahunsi AA. Phytochemical profiling of aqeousaqueous methanolic leaf extract of Triclisia gilettii by gas chromatography (GC/MS) and liquid chromatography (HPLC-PDA–ESI/MS) tandem mass spectroscopy (MS): a pointer to its nephroprotection. Nat Prod., 2022; 36(8): 2171-2176.
69. Olatunde A, Sukaiman O, Muhammad OB, Tijani H et al.,. Deciphering the inhibitory activity of flavonoids against Plasmodium falciparum proteases through molecular docking, drug-likeness and pharmacokinetic studies; Discover Chem., 2025; 2: 294. https://doi.org/10.1007/544371-025-00339-0.
70. Oluyemi WM, Babatunde BS, Kaehley H, Zehl M et al.. Antiplasmdodial of triterpenoids isolated from the methanol leaf extract of Combretum racemosum P. Beauv. J Ethnopharmacol., 2020; 247: 1122O3. https://doi.org/10.1016/j;ep.2019.112203.
71. OMS (Organisation Mondiale de la Santé). Rapport sur le paludisme dans le monde. Aide mémoire N°, 2017; 94.
72. Organization for Economic Cooperation and Development. OECD Guideline for the testing of chemicals. Test Guideline No. 425. Acute Oral Toxicity: Up-and-Down Procedure. Paris, OECD Publishing, 2022.
73. Organization for Economic Cooperation and Development. OECD Guidelines on the testing of chemicals 412. 28-day (subacute) inhalation toxicity study. Paris, 2018.
74. Owusu PD, Slatkin DJ, Knapp JE, P. L. Schiff Jr PL. Constituents of West African Medicinal Plants. XXVIII. Additional Alkaloids of Triclisia gillettii. J Nat Prod, 1981, 44 (1): 61-66.
75. Prasad K, Garner P. Steroids for treating cerebral malaria. Cochrane Database Syst Rev., 1999 Jul 26; 1999(3): CD000972. doi: 10.1002/14651858.CD000972.
76. Mamede L. Metabolomic investigation of natural antiplasmodial agents. Doctorat ThsesisThesis in Biomedical and Pharmaceutical Sciences. Université de Liège, Belgique, 2023/2024.
77. Nyandwaro K, Oyweri J, Kimani F, Mbungua A. Evaluating antiplasmodial and antimalarial activities of Soybean (Glycine max) seed extracts on Plasmodium falciparum parasite cultures and Plasmodium berghei-infected mice. J Pthaog, 2020; 2020(1): 7605730. DOI:10.1155/2020/7605730.
78. Parvatkar PT, Daigne K, Zhao Y, Manetsch R. Indoquilonie alkaloids as antiplasmodials:

Advances,Challenges and Opportunities. Chem Med Chem., 2024 Sep 16; 19(18): e202400254. doi: 10.1002/cmdc.202400254.
79. Ranaivoarisoa RH, Ralambanirina STR, Randriamialinoro F, Randrianasola RR, Ratsimbason M, Rananivelo LF. Antiplasmodial activity of the extracts and flavonoids isolated from Helichrysum gymnocephalum Humbert (Asteraceae) from Madagascar. Africa Nat Plant Prod, 2020, III. DOI: 10.1021/bk.2022-1361.ch008.
80. Saito AY, Rodriguez AAM, Vega DSM, RAC, Kimura EA, Katzin AM. Antimalarial activity of the terpene nerolidol. Int J Antimicrob Agents, 2016; 48: 641–646. doi: 10.1016/j.ijantimicag.2016.08.017.
81. Safar HF, Ali AH, Zukana HH, Kama N et al.,. Steroids from Diplazium esculantum: Antiplasmodial activity and molecular docking studies to investigate their binding modes. Trop Biomed, 2022; 39 (4):/ 552-558.
82. Soeiro MNC, Vergoten G, Bailly C. Mechanism of action of glycyrrhizin against P. falciparum. Mem Inst Oswaldo Cruz, 2021; 116: e210084. doi: 10.1590/0074-02760210084.
83. Soto-Vaquez MR, Horna-Piwento MV, Tullin LR, Bastida J. Chemical composition and in vitro antiplasmodial activity of the total alkaloids of the bulbs of two Amaryllidaceae species from Northern Peru. Pharmacogn, 2021; 13(4): 1046-1052.
84. Springer E, Heimsch KL, Sidiropoulos N, Locker JR, Przyborski JM. Arylmethylamino steroid compound 10 interfere with flavonoids with Plasmodium falciparum's hemoglobin metabolism. Antimicrob Agents Chemther, 2025; 69(8): 1-20.
85. 83. Sudji IR. Bioactivity of Steroid,TriterpenoidSteroid, Triterpenoid Saponins: Influence on Membrane Permeability and Drug Absorption. Doctorat Thesis in Natural Sciences, Heidelberg, Germany DOI: 10.11588/heidok.0019012, 2015.
86. Suwandi W, Rostinawati T, Muchtaridi M, Subarnas A. Safety assessment of the Polypodium feei root extract: Acute and subchronic studies. Toxicolo Rep., 2021; 8: 696-704. https://doi.org/10.1016/j.toxrep.2021.03.013.
87. Tajuddeen andVan Heerden. Antiplasmodial natural products: an update, 2019; 18: 404. https://doi.org/10.1186/s12936-019-3026-1
88. Tasdemir D, Lact G, Bran R, Rüed P, Scapoza L, Perozzo R. Inhibition of Plasmodium falciparum Fatty Acid Biosynthesis: Evaluation of FabG, FabZ, and FabI as Drug Targets for Flavonoids; J Med Chem., 2006; 49(13): 3345-3353.
89. Tajbakhsh E, Kwenti TE, Kheyri P, Nezaratizade S, Lindsay DS, F. Khamesipour F. Antiplasmodial, antimalarial activities and toxicity of African medicinal plants: a systematic review of the literature. Malar. J., 20(2021): 1-50, doi:10.1186/s12936-021-03866-0.
90. Tiam ER, Bikoboa, DSN, Zintchema AAA, Nyemeck NM, Nde EDF et al.,.Secondary metabolites from Triclisia gilettii (De Wild) Staner (Menispermaceae) with antimycobacterial activity against Mycobacterium tuberculosis. Nat Prod Res., 2019; 33(5): 642–650, 2019.
91. Tiam ER. Structures et activités biologiques des constituants chimiques de Triclisia gilettii (De Wwild) Staner (Menispermaceae) et de Cordia batesii Wernham (Boraginaceae). These de Doctorat, Université de Yaoundé, Faculté des Sciences Département de Chimie Organique. Cameroun, 2020.
92. Tona, L, Cimanga, K. R., Mesia, K., Musuamba, C.T., De Bruyne T., Apers, S., Van Miert, S., Hermans, N., Totté, J., Pieters, L, and Vlietinck, A.J. (2004) Antiplasmodial activity of extracts and fractions from seven medicinal plants used in Democratic Republic of Congo. J Ethnopharmacol., 2004; 93: 27-32. DOI: 10.1016/j.jep.2004.02.022.
93. Umumararungu T, Nkuranga JB, Habarurema G, Nyandwi JB, Mukazayire MJ et al.,. Recent developments in antimalarial drug discovery. Bioorg. Med. Chem., 2023; 6: 88-89, doe: 10.1016/j.bmc.2023.117339
94. Tchangolle J, Eyade AN, Kamdem BP, Ngadjai AT et al.,. In vivo antiplasmodial properties of fractions and flavonoids of Pseudorthria hooheri Wrifgt & Arn (Fabaceae)n Nat Res Human Health, 2022; é (3): 313-321.
95. Trease GE, Evans WC. Textbook of Pharmacognosy, 13th Edition, Bailliere Tindail, London, 2000.
96. Ur Rehman MH, Saleem U, Ahmad B, Rashid M. Phytochemical and toxicological evaluation of Zephyranthes citrina. Front. Pharmacol., 2022; 13. | https://doi.org/10.3389/fphar.2022.1007310.
97. Varo R, Crowley VM, Sitoe1A, Madrid L, Lena Serghides4 L, Kevin C. Kain KC. Adjunctive therapy for severe malaria: a review and critical appraisal. Malaria J., 2018; 17(47). https://doi.org/10.1186/s12936-018-2195-7.
98. Wen -Hu P, Xin-Ya X, Ni S, Siu W, Tsangand HJZ. Antimalarial activity of plant metabolites. Int J Mol Sci., 2018; 19: 14-18. doi: 10.339/ijms19051382.0
99. WHO. World Malaria Report, world Health Organization, Geneva, Switzerland, 2020. https://www.whow;intq/news-room/fact-sheets/detail/malaria.
100. Yang W, Chen X , Li Y , Guo S, Wang Z , Yu X. Advances in Pharmacological Activities of Terpenoids. Nat Prod Comm., 2020; 15(3): 1-13.