Journal of Medical Science, Biology, and Chemistry (JMSBC)

ISSN: 3079-2576 (Online)

Volume 2 Issue 1, (2025)

https://doi.org/10.69739/jmsbc.v2i1.230

https://journals.stecab.com/jmsbc

Published by
Stecab Publishing

Research Article

In Vitro Antiplasmodial Activities and Safety of *Ocimum gratissimum* L and *Rhoicissus tridentate* L.f Extracts

*[1]Joan C. Koech, [1]M. Ngeiywa, [1]J. Makwali, [2]F. Kimani, [2]J. Gathirwa, [2]E. Kigondu

About Article

Article History

Submission: January 02, 2025
Acceptance : February 04, 2025
Publication : May 27, 2025

Keywords

Antimalarial Activity, Cytotoxicity, Ocimum Gratissimum, Phytochemical Analysis, Rhoicissus Tridentate

About Author

[1] Department of Biological Sciences, University of Eldoret, Kenya

[2] Kenya Medical Research Institute (KEMRI), Kenya

Contact @ Joan C. Koech
jkoech96@gmail.com

ABSTRACT

This study evaluated *Ocimum gratissimum* and *Rhoicissus tridentate* for their *In vitro* antimalarial activity and safety. The plant samples collected from Keiyo South in Kenya were processed and their compounds were extracted using inorganic and organic solvents. Phytochemical screening, antiplasmodial bioassays, cytotoxicity tests, and statistical analyses were conducted to validate and compare the efficacies of the two plants used traditionally in treatment of malaria in Kenya. *O. gratissimum* extracts showed strong inhibition and low toxicity, with methanol as the most effective solvent. Methanol and water solvents extracts demonstrated significant *In vitro* antiplasmodial activity with inhibition rates of 70.7% and 67.6%, respectively compared to the negative control, whereas *R. tridentate* extracts, especially those of hexane, had a lower inhibition rate of 36.1%. The Minimum Inhibitory Concentration (MIC) of *O. gratissimum* extracts was slightly higher than that of the standard antimalarial drug, Artemether-Lumefantrine indicating good antimalarial potency at the slightly higher dose. Cytotoxicity tests revealed that the methanol and water extracts of the two plants extracts were less toxic compared to hexane and dichloromethane solvent extracts. Phytochemical analysis found higher flavonoid content in *O. gratissimum* and higher carotenoid levels in *R. tridentate*. Methanol proved to be the most effective solvent, extracting more bioactive compounds than hexane and dichloromethane. The study highlights *O. gratissimum* as a promising antimalarial candidate and suggests that further optimization is needed to enhance its In vivo efficacy. The findings advocate for continued research into solvent selection, plant part efficacy, and phytochemical analysis to refine and potentially develop these plant extracts into effective therapeutic agents against malaria.

Citation Style:

Koech, J. C., Ngeiywa, M., Makwali, J., Gathirwa, J., Kimani, F., & Kigondu, E. (2025). *In Vitro* Antiplasmodial Activities and Safety of *Ocimum gratissimum* L and *Rhoicissus tridentate* L.f Extracts. *Journal of Medical Science, Biology, and Chemistry, 2*(1), 104-114. https://doi.org/10.69739/jmsbc.v2i1.230

1. INTRODUCTION

Malaria remains a significant global health challenge (Cibulskis *et al.*, 2016), in tropical and subtropical regions, caused by protozoan parasites of the *Plasmodium* species and transmitted through bite of infected female *Anopheles* mosquitoes (Fikadu & Ashenafi, 2023; WHO, 2023). Among the five *Plasmodium* species which can infect humans, *P. falciparum* and *P. vivax* are most serious. *P. falciparum* is notorious for causing severe malaria, which can be fatal if not treated promptly (Sato, 2021) while *P. vivax* is the most frequent and widely distributed cause of recurring malaria (Anstey *et al.*, 2024).

Efforts to control malaria face challenges due to increased resistance to traditional antimalarial drugs like Chloroquine, Sulfadoxine-Pyrimethamine, and Artemisinin (Alghamdi *et al.*, 2024; Roux *et al.*, 2021). Artemisinin resistance, particularly in Southeast Asia, threatens malaria control programs (Woodrow & White, 2017). In light of these challenges, there is a growing interest in medicinal plants as potential sources of new antimalarial agents. Historically, plant-derived compounds such as Quinine from *Cinchona* and Artemisinin from *Artemisia annua* have been crucial in malaria treatment (Kingston & Cassera, 2022). Resistance to these treatments demonstrates the need for alternative therapies (Singha & Soni, 2024). Recent research focuses on evaluating plant extracts' antimalarial potential using *In vitro* assays that measure their ability to inhibit parasite growth, with IC50 values indicating the concentration required for 50% inhibition (Bekono *et al.*, 2020). Cytotoxicity assays are also performed to ensure safety by assessing the impact of the antimalarials on human cells.

The current study evaluated the *In vitro* antiplasmodial activity of solvents extracts of *O. gratissimum* and R. tridentata, plants that are traditionally used in Kenya to treat malaria and other ailments. *O. gratissimum*, known as "Holy Basil," is recognized for its antimicrobial and anti-inflammatory properties (Monga *et al.*, 2017), while *R. tridentate* is noted for its potential health benefits (Brookes & Katsoulis, 2006). The research assessed these plants' efficacy and safety. Their potential in contributing to the development of new antimalarial treatments was also evaluated.

2. LITERATURE REVIEW

Malaria remains a global health challenge, particularly in tropical and subtropical regions where it is caused by protozoan parasites of the *Plasmodium* species, transmitted by the bite of infected female *Anopheles* mosquitoes. *Plasmodium falciparum* is the deadliest species, leading to severe complications, while *Plasmodium vivax* is the most widespread, causing recurring infections. Despite ongoing efforts to combat malaria, the emergence of resistance to conventional antimalarial drugs such as chloroquine, sulfadoxine-pyrimethamine, and Artemisinin has significantly hindered progress in malaria control programs (Cibulskis *et al.*, 2016; WHO, 2023). The spread of Artemisinin resistance, particularly in Southeast Asia, threatens the effectiveness of current treatments, underscoring the urgency to explore alternative therapies (Woodrow & White, 2017). This has led to increased interest in medicinal plants as potential sources of new antimalarial agents. Historically, plant-derived compounds like quinine from *Cinchona* and Artemisinin from *Artemisia annua* have played pivotal roles in the treatment of malaria (Kingston & Cassera, 2022). However, resistance to these compounds highlights the necessity for continuous research into novel plant-based therapies (Singha & Soni, 2024). This has prompted the use of *in vitro* assays to evaluate plant extracts' antimalarial potential and cytotoxicity, with the goal of identifying promising new treatments (Espíndola *et al.*, 2022). Medicinal plants are rich in secondary metabolites, many of which possess antimalarial activity. Flavonoids, alkaloids, and glycosides have been reported as key classes of compounds responsible for the inhibition of *Plasmodium* growth (Mamede *et al.*, 2020). Studies have shown that flavonoids, in particular, can interfere with the metabolism or cellular processes of *Plasmodium* parasites, inhibiting their growth (Aje, 2023). *Ocimum gratissimum* (Holy Basil) and *Rhoicissus tridentate* have been traditionally used in Kenya for the treatment of malaria and other ailments, with various reports indicating that both plants possess antimicrobial, anti-inflammatory, and antioxidant properties (Khan *et al.*, 2024). Phytochemical analysis of *O. gratissimum* has revealed the presence of flavonoids, alkaloids, and glycosides, all of which are linked to antimalarial effects (Mamede *et al.*, 2020). On the other hand, *R. tridentate* is known for its carotenoid content, which, while beneficial for general health, has yet to be proven effective in malaria treatment (Kudamba *et al.*, 2023). Understanding the phytochemical composition of these plants is vital in assessing their antimalarial potential, as specific compounds are often responsible for their therapeutic effects.

Solvent extraction plays a crucial role in isolating bioactive compounds from plant materials, with the polarity of the solvent influencing the types of compounds extracted. Studies have shown that polar solvents such as methanol and water tend to extract more bioactive compounds, including alkaloids, flavonoids, and glycosides, which have demonstrated antimalarial activity (Stéphane *et al.*, 2021). Conversely, non-polar solvents like hexane and dichloromethane may be less effective in extracting these bioactive compounds, often yielding lower antimalarial activity (Lefebvre *et al.*, 2021). In this study, methanol was found to be the most effective solvent for extracting bioactive compounds from both *O. gratissimum* and *R. tridentate*, with the highest yields obtained in methanol extracts for both plants. The higher efficacy of methanol as a solvent is further supported by previous studies, which advocate for its use in the extraction of bioactive phytochemicals (Bhadange *et al.*, 2024). This highlights the importance of solvent choice in optimizing the extraction process for the development of therapeutic plant-based antimalarial agents.

The assessment of *in vitro* antimalarial activity is essential in evaluating a plant's potential as an antimalarial agent. The *in vitro* assay measures the ability of plant extracts to inhibit the growth of *Plasmodium* parasites, with the IC_{50} value indicating the concentration needed for 50% inhibition of parasite growth (Kigondu *et al.*, 2009). In this study, *O. gratissimum* demonstrated strong antiplasmodial activity, with methanol and water extracts achieving inhibition rates of 70.7% and 67.6%, respectively, compared to the negative control. These findings are consistent with previous research that supports the antimalarial potential of *O. gratissimum* extracts (Kaou *et al.*, 2008). In contrast, *R.*

tridentate extracts exhibited significantly lower activity, with a maximum inhibition rate of only 36.1%. This difference in efficacy may be attributed to the differing phytochemical profiles of the two plants, as noted in other studies that have observed varying levels of activity in different plant species (Akhtar *et al.*, 2018). The Minimum Inhibitory Concentration (MIC) values for *O. gratissimum* extracts, though higher than the standard antimalarial drug Artemether-Lumefantrine, still suggest considerable activity. These findings are in line with the general understanding that plant-derived compounds may require higher doses than synthetic drugs to achieve similar efficacy (Habibi *et al.*, 2022). Furthermore, cytotoxicity tests using the MTT assay revealed that methanol and water extracts of both plants exhibited low toxicity, which is a crucial factor in their potential therapeutic application (Waiganjo *et al.*, 2020).

Plant-based therapies offer significant promise in the fight against malaria, sustainability remains a key issue. Overharvesting of medicinal plants can lead to depletion of natural resources, necessitating the development of sustainable cultivation practices or biotechnological methods such as tissue culture and genetic engineering to ensure a continuous supply of bioactive compounds (Alum, 2025). Research into the large-scale production of these compounds through biotechnological means will be essential for the commercialization of plant-based antimalarial agents. Moreover, continued research is needed to explore the potential of different plant parts, as certain parts may contain higher concentrations of bioactive compounds (Jha & Sit, 2022).

3. METHODOLOGY

3.1. Study area, plants collection, and identification

In February 2021, plant samples were collected from Tingwa Forest in Elgeyo Marakwet County, Kenya (latitude 0.24°N, longitude 35.65°E, altitude 1990 m). Roots and leaves of *Ocimum gratissimum* and *Rhoicissus tridentate* were collected, ground, and their constituent compounds extracted using inorganic and organic solvents at the University of Eldoret. The crude extracts were transported to KEMRI for screening and bioassays. The plants were identified by Dr. B. Wanjohi and voucher specimens deposited at the University of Eldoret. As UOE/0CGR/JCK/073/17 and UOE/RHTR/JCK/032/17 for *Ocimum gratissimum* and *Rhoicissus tridentate* respectively

3.2. Processing plant materials for solvent extraction

The collected plant materials were washed, chopped into small pieces, air-dried at room temperature for a period of three weeks in a well-aerated room. The grinding of plant materials into a coarse-fine powder was done using electric grinder in the school of agriculture at the university of Eldoret farm facility. The respective powders were packaged in well-labelled 1kg bags each and transported to KEMRI where extractions and bioassays experiments were conducted.

3.3. Aqueous solvent extraction

Distilled water was used for inorganic solvent extraction. 100 grams of the respective powdered plant materials was transferred into clean labelled 1000 ml capacity beakers and then 600 ml of distilled water with polarity of 1.00 was added. The respective concoctions in beakers were covered with aluminum foil papers and placed in a water bath (80°C) for 1.5 hours to facilitate extraction. The mixtures were then filtered through Whatman's number 1 filter papers before subjecting them to freeze drying for 48 hrs. The dry and lyophilized samples were transferred into clean, dry, pre-weighed universal bottles, and their respective percentage yields were determined and stored at -20°C in a freezer until use for screening and bioassays (Alamgir & Alamgir, 2018).

3.4. Organic solvent extraction

Sequential extraction using Hexane, Ethyl acetate, Dichloromethane and Methanol with polarity of 0.009, 0.228, 0.309, and 0.762 respectably was adopted. Briefly, 250 grams of the respective plant materials were soaked in 600 ml in 1000 ml capacity beakers at room temperature (25°C) for 48 hours. The mixtures were then filtered through double-layer Whatman's number one filter papers after which the filtrates were reduced *in vacuo* at respective solvent temperature set using a rotary evaporator. The respective extracts were soaked daily for three days in 600 ml volumes of Methanol in 1000 ml capacity beakers in the same way as for dichloromethane and other solvent extraction standards. The extracts were obtained by filtration and concentrated *in vacuo* using a rotary evaporator set at respective temperatures. The resultant extracts were transferred into clean pre-weighed glass bottles and their yields were determined and stored at -20°C in a freezer until use for screening and bioassays (Jha & Sit, 2022).

3.5. Phytochemical screening methods

The *O. gratissimum* and *R. tridentate* extracts were subjected to qualitative and quantitative phytochemical screening for the detection and identification of the plants metabolites following standard methods with some modifications. Shinoda's test for the detections of Flavonoids, Dragendorff's test for Alkaloids, the Ferric Chloride test for Tannins, the Froth test for Saponins, and the NaOH paper test for coumarins. Phenols are detected with Ferric chloride, carotenoids via solvent extraction and spectrophotometry, flavonoids with aluminum chloride, phytosterols through saponification and thin-layer chromatography, glycosides using the Molisch test and quinones by color changes in alkaline solutions. Quantitative testing of phytochemicals employs various analytical techniques: - Phenols are quantified using the Folin-Ciocalteu method. Carotenoids and Quinones are typically measured using UV-spectrophotometry. Flavonoids can be quantified using the aluminum chloride colorimetric method, phytosteroids, High-Performance Liquid Chromatography (HPLC) method, Tannins are often analyzed using the vanillin-HCl method, and for glycosides, the phenol-sulfuric acid method was used (De Silva *et al.*, 2017).

3.6. Preparation of Stock solutions of plant extracts and standard drugs

Stock solutions of the extracts (200µg/ml) were prepared in sterile deionized water and filtered through 0.22µm membrane filters under aseptic conditions in a laminar flow hood. The water insoluble extracts were first dissolved in 0.02% dimethyl

sulphoxide (DMSO) before diluting them to the required concentrations using sterile deionized water. All the prepared stock solutions of plant extracts and the standard drug were stored at -20°C and retrieved only during their use (Ong, 2004).

3.7. Culture of *P. falciparum* parasites

Two *P. falciparum* strains; Chloroquine-sensitive (D6) and Chloroquine-resistant (W2)) were obtained from the Malaria Laboratories of Centre for traditional medicine research and development (CTMRD) in KEMRI Nairobi. The culture medium was a variation of what was previously described by Trager and Jensen (Kimingi *et al.*, 2022). Briefly, the medium consisted of RPMI 1640 supplemented with 10% human serum, 25 mM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES), 25 mM $NaHCO_3$, and 50 mg/ml gentamycin (0.5ml). Human type O+ erythrocytes (<28 days old) served as host cells, and the cultures were incubated at 37°C in an atmosphere of 3% CO_2, 5% O_2, and 92% N_2 as previously described (Jensen, 2002).

3.8. Cytotoxicity test (bioassay)

Cytotoxicity of *O. gratissimum* and *R. tridentate* extracts was evaluated using the Methyl Thiazolyl Blue (MTT) assay. This assay measures cell viability based on the reduction of tetrazolium salts by mitochondrial dehydrogenases in living cells, forming insoluble formazan crystals. Vero (E6) cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% Fetal Bovine Serum (FBS), seeded at 10,000 cells per well in 96-well plates, and incubated for 24 hours at 37°C and 5% CO_2. Following this, cells were exposed to extract concentrations ranging from 1.56 to 1000 µg/ml, and Coartem (100 µg/ml) was used as a positive control. After 48 hours, 10 µl of 10 mg/ml MTT reagent was added, and cells were incubated for 4 hours. The formazan crystals were dissolved in 100 µl DMSO and absorbance was measured at 560 nm. The viability of cells was calculated using the equation:

$$\% \text{ Viability} = (A_{sample} / A_{control}) \times 100\%$$

Where $A\text{-}_{sample}$ was the absorbance sample at 560 nm and $A\text{-}_{control}$ was the absorbance of DMEM medium. The percentage of cell viability was then plotted and regressed linearly to obtain the CC_{50} values. The selectivity index (SI) values were calculated based on the ratio between the CC_{50} value of cytotoxicity and anti-plasmodial activity against *P. falciparum* D6 and W2 strains for each extract IC_{50} (Espíndola *et al.*, 2022).

3.9. Selectivity index

To estimate the potential of the extracts to inhibit the growth of *P. falciparum* parasites without toxicity, the selectivity index (SI) was calculated as:-

$$SI = CC_{50} \text{ Vero cells} / IC_{50} \textit{ P. falciparum}$$

3.10. *In vitro* antiplasmodial assays

The antiplasmodial activity of *O. gratissimum* and *R. tridentate* extracts was assessed against Chloroquine-sensitive (D6) and Chloroquine-resistant (W2) *P. falciparum* strains. Parasites were cultured in human O+ red blood cells using RPMI-1640 medium with Gentamycin, sodium bicarbonate, HEPES, and O+ serum. A 1% parasitemia suspension was prepared and placed in a 24-well plate with 5% hematocrit. After synchronization to the ring stage using 5% sorbitol, extracts, and Artemether-Lumefantrine "Coartem" (positive control) were dissolved in DMSO, diluted to 0.01-100 µg/mL, and added to the wells. Negative controls used DMSO only. The cultures were incubated for 48 hours at 37°C, 5% CO_2, and 95% humidity. Post-incubation, thin smears were prepared, fixed, and stained with Giemsa. Parasite counts were assessed microscopically to determine growth inhibition relative to negative controls (Nanyingi *et al.*, 2009).

The percentage of parasitemia was calculated using the formula:

$$\%\text{Parasitemia} = \sum\text{infected erythrocytes} / \sum\text{Total erythrocytes} \times 100\%$$

The percentage of inhibition was determined using the equation:

$$\%\text{Inhibition} = (\text{Tp}/\text{NC} \times 100\%)$$

The percentage of growth was calculated using the formula:

$$\%\text{Growth} = \%\text{Parasitemia} - \%\text{Parasitemia Do}\%$$

Growth= Parasitemia X – Parasitemia Do

Where:

Tp = Standard Treatment parasitemia

NC = Negative control parasitemia

X = % parasitemia in each extracts concentration

Do = % parasitemia at the start

Σ infected erythrocytes = the number of infected erythrocytes of total erythrocytes

The probit analysis was conducted to calculate the IC50 values.

3.11. Statistical analyses

Statistical analyses using Gen-Stat Software Version 14.1 involved descriptive statistics (mean ± SD) and one-way ANOVA for comparing treatment outcomes. Student's t-test assessed differences and compared extract activities and IC_{50}, MIC, and CC_{50} values against controls. Significance was set at $P \leq 0.05$.

3.12. Ethical clearance

Ethical clearance for the research was given by the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton (UEAB/ISERC/01/05/2022), and a research permit was obtained from NACOSTI (NACOSTI/23/P/23/23966), laboratory procedures were conducted following SERU and ACUC guidelines of KEMRI

4. RESULTS AND DISCUSSION

4.1. Parasites inhibition activity of *o. Gratissimum* and r. Tridentata extracts.

In the current study, the antiplasmodial activity of *Ocimum gratissimum* and Rhoicissus tridentata extracts was assessed against the sensitive (D6) and resistant (W2) *P. falciparum* strains. In the D6 strain experiment, the control treatment with DMSO only demonstrated minimal inhibition at 7.1%. The methanol extract of *O. gratissimum* exhibited the significant ($p = 0.001$) highest inhibition at 71.7%, outperforming *R. tridentate* methanol extract (58.2%). Other solvent extracts of *O. gratissimum* (hexane, Dichloromethane (DCM), ethyl acetate) also demonstrated higher inhibition than their *R. tridentate* counterparts. The aqueous extract of *O. gratissimum* had 67.6% inhibition, significantly ($P < 0.05$) higher than the 58.7% observed for *R. tridentate* aqueous extract. In the W2 strain experiment,

O. gratissimum methanol and water extracts demonstrated 70% and 65% inhibition, respectively, compared to 57% and 56.7% for *R. tridentate*. However, Artemether-Lumefantrine was the most effective, with 77.9% and 76.4% inhibition for D6 and W2 strains, respectively. *O. gratissimum* extracts demonstrated potent antimalarial activity, surpassing *R. tridentate* extracts and nearing the effectiveness of the standard Artemether-Lumefantrine drug.

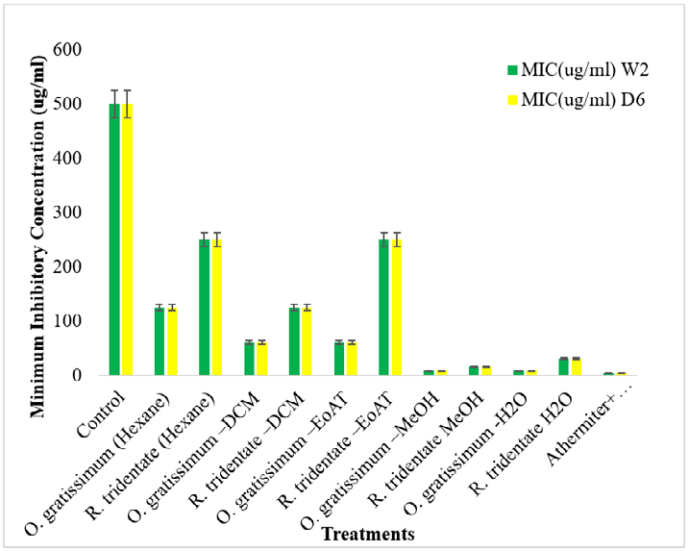
Table 1. *In vitro* antiplasmodial inhibition activity of *O. gratissimum* and *R. tridentate* extracts at 100µg/ml against 1x105 Sensitive (D6) and Resistant (W2) P.

Treatment	%Inhibition ± SE(D6)	%Inhibition ± E(W2)
Negative Control (DMSO)	0.0[a]	0.0[a]
O. gratissimum (Hexane)	41.5 ±0.02[b]	40 ±0.01[b]
R. tridentate (Hexane)	36.1±0.02[b]	35±0.01[b]
O. gratissimum –DCM	58.1 ±0.02[c]	56 ±0.01[c]
R. tridentate –DCM	45.2 ±0.02b[c]	45 ±0.01[bc]
O. gratissimum –EoAT	49.5 ±0.02b[c]	50 ±0.01[bc]
R. tridentate –EoAT	45.4±0.02b[c]	43±0.01[bc]
O. gratissimum –MeOH	71.7 ±0.02[cd]	70 ±0.01[cd]
R. tridentate MeOH	58.2±0.02[c]	57±0.01[c]
O. gratissimum -H_2O	67.6 ±0.01[cd]	65 ±0.01[cd]
R. tridentate H_2O	58.7±0.01[c]	56.7±0.01[c]
Artemether-Lumefantrine	77.9±0.01[d]	76.4±0.01[d]

**Legend: O. G = O. gratissimum; Hex = Hexene, RT = R. tridentate; DCM = Dichloromethene; EoAT= Ethyl acetate; MeOH = Methanol; H_2O = Water; Artem+Lum = Artemether + Lumefantrine "Coartem." Means that do not share a superscript letter are significantly different while those with same letters are not significantly different at P ≤ 0.05. Results are presented as Mean ± Standard Error; n =3*

4.2. Determination of Minimum Inhibitory Concentration (µg/ml)

The results in Figure 1 demonstrate that *Ocimum gratissimum* extracts exhibited superior antiplasmodial activity compared to Rhoicissus tridentatae with lower Minimum Inhibitory Concentration (MIC) values against both Chloroquine-sensitive (D6) and Chloroquine-resistant (W2) *Plasmodium falciparum* strains. *O. gratissimum* 's methanol extract showed the lowest MIC at 20 µg/ml for both strains, while its water extract also demonstrated notable activity with MICs of 22 µg/ml in both D6 and (W2) compared to 24 µg/ml in *R. tridentate* Methanol extracts. These results suggest that polar solvents like methanol and water are highly effective in extracting potent bioactive compounds from *O. gratissimum*. The hexane extract of *O. gratissimum* (122 µg/ml) showed weaker activity, indicating that non-polar compounds may have lesser antiplasmodial effects. In contrast, *R. tridentate* required higher concentrations for inhibition across all solvents, with MIC values of 250 µg/ml for hexane and ethyl-acetate extracts, and 120 µg/ml for DCM. RT's methanol and water extracts were more effective (26 µg/ml and 24 µg/ml, respectively), yet still less potent than *O. gratissimum*'s extracts. The comparison highlights the greater antimalarial potential of *O. gratissimum*, particularly when extracted with polar solvents, suggesting it contains more effective antiplasmodial compounds. Artemether-Lumefantrine (Coartem) was the most potent, with an MIC of 10 µg/ml.



Legend: DCM= Dichloromethane; EoAT= Ethyl acetate; MeOH= Methanol; H_2O= Water; MIC= Minimum inhibitory concentration; Antimalarial Sensitive (D6) and Resistant (W2) P. falciparum

Figure 1. *In Vitro* Antiplasmodial inhibitory Activity and Minimum Inhibition Concentration of *O. gratissimum* and *R. tridentate* Extracts against Sensitive (D6) and Resistant (W2) *P. falciparum.*

4.3.Antiplasmodial and cytotoxicity assays

Table 2 compares the antiplasmodial activity of *Ocimum gratissimum* (OG) and *Rhoicissus tridentate* (RT) extracts against *Plasmodium falciparum* strains (W2 - resistant, D6 - sensitive), alongside Artemether-Lumefantrine (Coartem). *O. gratissimum* extracts exhibited notable antiplasmodial activity, with the Methanol extract showing the highest efficacy, boasting IC50 values of 3.5 ± 0.6 µg/mL (W2) and 3.7 ± 0.3 µg/mL (D6) and excellent selectivity indices (SI) of 89.3 (W2) and 84.5 (D6). The Water extract also demonstrated high activity with IC_{50} values of 4.1 ± 0.2 µg/mL (W2) and 3.8 ± 0.5 µg/mL (D6), and outstanding SIs of 104.8 (W2) and 113.1 (D6). In comparison, *R. tridentate* extracts showed lower efficacy, with higher IC_{50} values and lower SI values across all tested solvents. Hexane and Dichloromethane extracts from *R. tridentate* had IC_{50} values of 17.7 ± 3.1 µg/mL (W2) and 31.1 ± 5.2 µg/mL (W2), respectively, and Ethyl acetate extracts were less effective compared to those of *O. gratissimum.* Among the tested extracts, *O. gratissimum* -Methanol exhibited the highest SI values of 89.3 for W2 and 84.5 for D6, indicating a favorable therapeutic window and suggesting it is a promising candidate for further investigation due to its potent antiplasmodial activity coupled with relatively low toxicity. Similarly, *O. gratissimum* -H_2O and RT-Methanol

also demonstrated high SI values of 104.8 and 60.5, respectively, reinforcing their potential as effective antimalarial agents. In contrast, the extracts with lower SI values, such as *O. gratissimum* -DCM and RT-DCM (10.7 and 9.6, respectively), reflect a narrower therapeutic margin, which could pose risks in clinical applications. Overall, *O. gratissimum* extracts demonstrated potent activity comparable to Coartem, whereas *R. tridentate* showed consistently lower efficacy, indicating a less effective antimalarial potential. Groups with the same letters are statistically similar (P< 0.05), while different letters indicate significant differences.

Table 2. Antiplasmodial Activity of *O. gratissimum* and *R. tridentate* Extracts against Sensitive (D6) and Resistant (W2) Strains of *P. falciparum* and Cytotoxicity Activity of the extracts against Vero Cells.

Test Samples	IC_{50} W2 (µg/mL)	IC_{50} D6(µg/mL)	Antiplasmodial activity	LC_{50}µg/mL)	SI (W2)	SI D6)
OG-Hex	15.1 ± 2.3^{bc}	16.2 ± 1.3^{d}	Moderate	333.4 ± 8.5^{b}	22.1^{b}	20.6^{b}
RT-Hex	17.7 ± 3.1^{bc}	18.1 ± 2.1^{d}	Moderate	321.6 ± 5.2^{b}	18.2^{b}	17.8^{b}
OG-DCM	28.9 ± 0.5^{d}	26.3 ± 1.3^{e}	Moderate	308.1 ± 6.5^{a}	10.7^{a}	10.7^{a}
RT-DCM	30.6 ± 4.7^{d}	31.1 ± 5.2^{e}	Moderate	298.3 ± 10.2^{a}	9.8^{a}	9.6^{a}
OG-EoAT	9.5 ± 0.9^{c}	8.9 ± 1.3^{c}	Good	308.4 ± 10.3^{a}	32.4^{c}	34^{c}
RT-EoAT	10.8 ± 0.7^{c}	9.7 ± 1.3^{c}	Good	300.5 ± 10.1^{a}	27.8^{c}	27^{c}
OG-MeOH	3.5 ± 0.6^{ab}	3.7 ± 0.3^{ab}	High	312.5 ± 1.4^{a}	89.3^{e}	84.5^{e}
RT-MeOH	6.0 ± 1.4^{b}	5.4 ± 0.1^{b}	High	326.8 ± 3.3^{b}	54.5^{d}	60.5^{d}
OG-H_2O	4.1 ± 0.2^{ab}	3.8 ± 0.5^{ab}	High	429.6 ± 3.7^{c}	104.8^{f}	113.1^{f}
RT-H_2O	6.4 ± 1.3^{b}	5.1 ± 0.7^{b}	High	411.4 ± 2.5^{c}	64^{d}	80.7^{e}
Artem-Lum	0.14 ± 0.1^{a}	0.09 ± 0.43^{a}	High	-	-	-

Legend: O. G = O. gratissimum; Hex = Hexene, RT = R. tridentate; DCM = Dichloromethene; EoAT= Ethyl acetate; MeOH = Methanol; H_2O = Water; Artem+Lum = Artemether + Lumefantrine "Coartem."; IC_{50} = Half-Maximal Inhibitory Concentration; LC5 = Lethal Concentration 50; SI = Selectivity Index; Groups sharing different superscript letters are significantly different at P ≤ 0.05. Results are presented as Mean ± Standard Error; n =3

4.4. Quantitative screening of phytochemical

The methanol (MeOH) extracts of *O. gratissimum* and *R. tridentate* exhibit similar and distinct compositions of phytochemicals across different solvents, highlighting their potential pharmacological and nutritional benefits. *O. gratissimum* Methanol extracts contain Alkaloids, Flavonoids, Phytosterols, Coumarins, Quinones, Tannins and Phenols as shown in table 3. *R. tridentate* MeOH extracts contain Alkaloids, Flavonoids, Phytosterols, Coumarins, Quinones, Tannins Phenols and additional Carotenoids

Tannins are found in *O. gratissimum* and *R. tridentate* Methanol extracts, Phenols are extracted by all tested solvents in both plants, and carotenoids are present in all solvents extracts of *R. tridentate* and are absent in all solvents extracts of *O. gratissimum.* Water extracts Alkaloids, Flavanoids, Saponins, Coumarines, Quinones, and Phenols in both *O. gratissimum* and *R. tridentate.* The choice of solvent influences the phytochemicals that are extracted from a plant, with *O. gratissimum* showing a broader range of compounds extracted by organic solvents. These findings underscore the potential of both plants for pharmaceutical and nutritional applications, tailored to exploit their diverse bioactive properties based on solvent-specific extraction methods.

Table 3. Qualitative phytochemicals screening for Alkaloids, Flavonoids, Phytosterols, Saponins, Coumarins, Quinones, Tannins, Glycosides, Phenols and Carotenoids in *O. Gratissium* and *R. Tridentate* extracts.

	O. Gratissium **Extracts**					*R. tridentate* **Extracts**				
Phytochemicals	**Hex**	**DCM**	**EA**	**MeoH**	**H_2O**	**Hex**	**DCM**	**EA**	**MeoH**	**H_2O**
Alkaloids	+	+	-	+	+	+	+	+	+	+
Flavonoids	-	+	+	+	+	+	+	+	+	+
Phytosterols	-	+	-	+	-	-	+	-	+	-
Saponins	-	-	-	-	+	-	-	-	-	+
Coumarines	-	-	-	+	+	-		+	+	+
Quinones	-	+	-	+	+	-	-	-	+	+

Tannins	-	-	-	+	-	-	-	-	+	-
Glycosides	-	-	-	-	-	-	-	-	-	-
Phenols	+	+	+	+	+	+	+	+	+	+
Carotenoids	-	-	-	-	-	+	+	+	+	+

Hex= Hexane; DCM= Dichloromethane; EA= Ethyl acetate; MeoH= Methanol; H_2O= Water; Mean ± Standard Error; n =3

4.5. Quantitative screening of phytochemical

Quantitative screening of methanol extracts of *Ocimum gratissimum* and *Rhoicissus tridentate* shown in table 4 revealed notable differences in phytochemical concentrations in different parts of the plants. In *O. gratissimum*, alkaloids were highly concentrated in roots (6.2 ± 0.1) compared to leaves (5.7 ± 0.0). Conversely, *R. tridentate* has higher alkaloid levels in leaves (3.9 ± 0.1) than in the roots (1.3 ± 0.0). Carotenoid levels are significantly ($P < 0.05$) higher in *R. tridentate* roots (6.8 ± 0.2) compared to *O. gratissimum* roots (0.7 ± 0.1). Flavonoid levels are at similar levels in *O. gratissimum* leaves (1.3 ± 0.2) and roots (1.8 ± 0.2), while in *R. tridentate* flavonoids levels are lower in leaves (1.3 ± 0.0) compared to roots (0.8 ± 0.1). Glycosides, phenols and saponins distribution varied insignificantly in *O. gratissimum* and *R. tridentate* leaves and roots, indicating differential distribution and potential of the different plant parts for medicinal and nutritional uses.

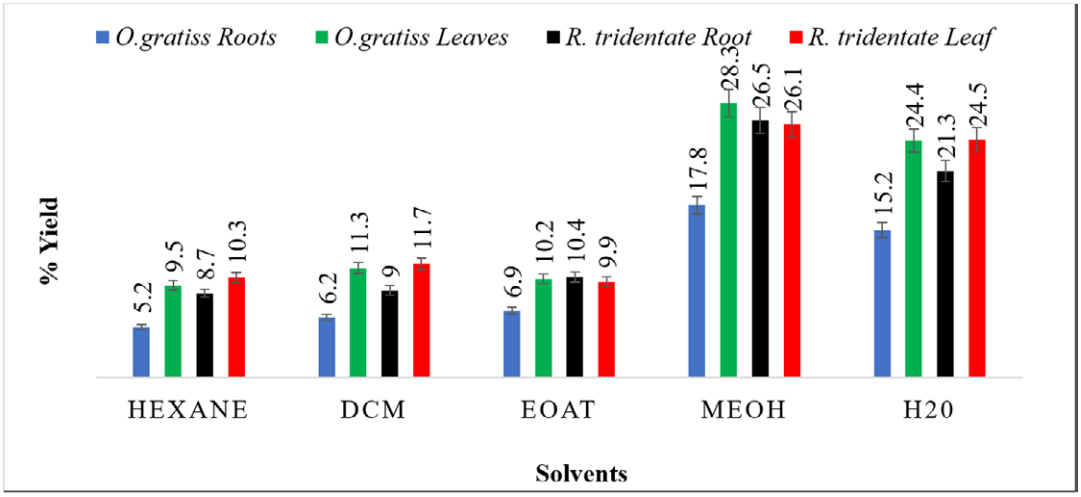
Table 4. Quantitative screening for Alkaloids, Carotenoids, Flavonoids, Glycosides, Phenols and Saponins of *O. Gratissimum* and *R. Tridentate* methanol extracts (µg/ml)

Phytochemicals	*O. gratissimum* **Extracts**		*R. tridentate* **Extracts**	
	Leaves	**Roots**	**Leaves**	**Roots**
Alkaloids	5.7 ± 0.0^c	6.2 ± 0.1^c	3.9 ± 0.1^c	1.3 ± 0.0^b
Carotenoid	0.1 ± 0.0^a	0.7 ± 0.1^a	1.8 ± 0.0^b	6.8 ± 0.2^c
Flavonoids	1.3 ± 0.2^b	1.8 ± 0.2^b	1.3 ± 0.0^b	0.8 ± 0.1^a
Glycosides	0.3 ± 0.1^a	$0.8 + 0.1^a$	0.2 ± 0.0^a	0.7 ± 0.0^a
Phenols	0.6 ± 0.2^a	0.6 ± 0.1^a	0.6 ± 0.0^a	0.4 ± 0.0^a
Saponins	0.5 ± 0.2^a	0.7 ± 0.1^a	0.4 ± 0.0^a	0.2 ± 0.0^a

Legend: *Same Letters Indicate there is No Significant Difference in Results along the Rows and Columns; Mean ± Standard Error; n =3*

4.5. Determination of % yields of *O. Gratissimum* and *R. Tridentate* extracts

The graph figure 2 shows the percentage (%) yield of *O. gratissimum* and *R. tridentate* solvents extractions of leaves and roots using five different solvents: hexane, Dichloromethane, Ethyl acetate, Methanol, and water. In *O. gratissimum*, the highest yield was observed in the leaves extract with Methanol (28.3%), followed by the roots Methanol extract (14.3%). The roots extract had the lowest yield, with hexane and Dichloromethane yielding 5.2% and 6.2%, respectively. In *R. tridentate*, the highest yield was observed in the root extract with Methanol (26.5%), followed by the leave's extracts with Methanol (24.5%).



DCM= Dichloromethane; EOAT= Ethyl acetate; MEOH= Methanol; H_2O= Water; Mean ± SEM (n=3)

Figure 2. Percentage (%) yield extracted from 100g *O. Gratissimum* and *R. Tridentate* solvent extraction.

4.6. Discussion

The evaluation of extracts from *Ocimum gratissimum* and *Rhoicissus tridentate* presents significant insights into their potential as antimalarial agents, especially in the context of emerging resistance to conventional drugs like Artemether and Lumefantrine. The findings from this study align with existing literature that highlights the efficacy of plant-derived compounds in combating malaria (Lokole *et al.*, 2024). For instance, *O. gratissimum* demonstrated remarkable antiplasmodial activity, with methanol and water extracts achieving inhibition rates of 70.7% and 67.6%, respectively. These results corroborate previous studies indicating the potential of *O. gratissimum* as a source of bioactive compounds (Pandey, 2017). In contrast, the extracts from *R. tridentate* exhibited significantly lower antiplasmodial activity, with a maximum inhibition rate of only 36.1%. This disparity may be attributed to the differing phytochemical profiles, as noted by previous research, which has shown that the presence of specific active compounds can greatly influence antimalarial efficacy (Mamede *et al.*, 2020). The minimum inhibitory concentration (MIC) values obtained for *O. gratissimum*, although higher than those of standard antimalarial, still indicate substantial activity, particularly concerning the effects of solvent polarity and the bioactive compounds involved. Methanol and water, tend to extract bioactive compounds with stronger antimalarial effects. Methanol and water extracts from plants exhibited higher antiplasmodial activity (Bagavan *et al.*, 2011), similar to what we observed for *O. gratissimum* (OG) with MIC values of 20 µg/ml (D6, W2) and 22 µg/ml (D6) and 24 µg/ml (W2). These solvents are known to extract compounds like alkaloids, flavonoids, and glycosides, which have been widely recognized for their antiplasmodial activity in other plants as well (Doughari, 2012). In particular, flavonoids have been noted to inhibit the growth of *Plasmodium* species by interfering with parasite metabolism or cellular processes (Fotie, 2008). further supporting the role of polar solvents in extracting active compounds.

On the other hand, the higher MIC values observed for *R. tridentate* extracts (250 µg/ml for hexane and ethyl-acetate) are consistent with studies that report varying levels of activity in different plant species (Eichelbaum, 2016). Some plant species, particularly those with fewer secondary metabolites or those that contain less polar compounds, tend to show weaker antimalarial activity (Gurib-Fakim, 2006). *R. tridentate*'s higher MIC values suggest that it may either contain fewer active compounds or that the active compounds present are more challenging to extract with the solvents used. The less potent activity of *R. tridentate* extracts could also be attributed to a lower concentration of alkaloids or flavonoids, compounds that are often responsible for antimalarial activity, as observed in *O. gratissimum*.

Natural products may not always match synthetic drugs in potency, they can offer alternative therapeutic options, particularly in light of the growing resistance observed in *Plasmodium* spp. (Cheuka *et al.*, 2016). The lower toxicity of the methanol and water extracts of *O. gratissimum*, compared to the more toxic hexane and dichloromethane extracts, further underscores its safety profile, which is essential for any potential therapeutic application (Kodi, 2018). Phytochemical analysis revealed that *O. gratissimum* contains higher flavonoid content, which is well-documented for its antimalarial properties (Ouandaogo *et al.*, 2023). Conversely, *R. tridentate* had elevated carotenoid levels, which, while beneficial for general health, may not directly contribute to antimalarial activity. The highest alkaloid content in *O. gratissimum* roots at 6.2 ± 0.1 mg/g suggests the presence of alkaloids known for their biological activities (Oliveira *et al.*, 2009), further enhancing its potential as an antimalarial candidate. The yield percentages from methanol extraction being highest for both plants affirm the solvent's efficacy in extracting potent compounds. Methanol is advocated as an optimal solvent for extracting bioactive phytochemicals (Bhadange *et al.*, 2024). This study contributes to the growing knowledge addressing the urgent need for novel antimalarial therapies with the emergence of resistant parasite strains, the exploration of natural phytochemicals from medicinal plants offers a promising avenue for drug development. However, the sustainability of harvesting these plant products is a significant concern (Van Wyk & Prinsloo, 2018). As such, there is a critical need for biotechnological advancements to ensure the sustainable production of these valuable compounds (Fatima *et al.*, 2024). Implementing strategies such as tissue culture and genetic engineering could enhance the availability of these natural resources while minimizing ecological impact (Canter *et al.*, 2005).

5. CONCLUSION

This study evaluated the antimalarial potential of *Ocimum gratissimum* and *Rhoicissus tridentate* extracts, revealing that *O. gratissimum* showed superior antiplasmodial activity compared to *R. tridentate*. Methanol and water extracts of *O. gratissimum* achieved high inhibition rates (70.7% and 67.6%, respectively), whereas *R. tridentate* extracts, especially those from hexane, showed lower activity (36.1%). Despite the strong activity of *O. gratissimum*, its MIC values were higher than those of the standard antimalarial drug, indicating that higher doses are needed to achieve similar efficacy. Cytotoxicity testing revealed that methanol and water solvents extracts were safer, with lower cytotoxicity compared to other solvents extracts. Phytochemical analysis highlighted *O. gratissimum*'s roots higher flavonoid content and *R. tridentate*'s higher carotenoid levels in the roots. The study underscores *O. gratissimum* as a promising candidate for antimalarial development, though further dose optimization is required to enhance its efficacy and evaluate its safety suitability for In vivo therapeutic use.

RECOMMENDATION

Based on the study, *Ocimum gratissimum* should be prioritized for antimalarial development due to its superior antiplasmodial activity and favorable safety profile, especially with methanol and water extracts. Methanol was the most effective solvent for extracting bioactive compounds from both *O. gratissimum* and *Rhoicissus tridentate*. Future research should continue using methanol and explore additional solvents to enhance extraction efficiency. It is important to evaluate the efficacy of different plant parts, as *O. gratissimum* roots have higher alkaloid content compared to leaves, while *R. tridentate* leaves show higher alkaloid levels than roots. Detailed analysis of

phytochemicals, like flavonoids and carotenoids, will help develop targeted antimalarial treatments. Non-polar solvents should be avoided due to higher cytotoxicity, with methanol and water recommended for safer extractions.

REFERENCES

Aje, O. O. (2023). *Inhibitory Effect of Some Flavones on Plasmodium falciparum Plasmepsin V Activity* (Master's thesis, Kwara State University, Nigeria).

Akhtar, N., & Mirza, B. (2018). Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. *Arabian Journal of Chemistry, 11*(8), 1223-1235. https://doi.org/10.1016/j.arabjc.2015.01.013

Alamgir, A. N. M., & Alamgir, A. N. M. (2018). Methods of Qualitative and Quantitative Analysis of Plant Constituents. *Therapeutic Use of Medicinal Plants and their Extracts: Volume 2: Phytochemistry and Bioactive Compounds* (pp. 721-804). https://doi.org/10.1007/978-3-319-92387-1_9

Alghamdi, J. M., Al-Qahtani, A. A., Alhamlan, F. S., & Al-Qahtani, A. A. (2024). Recent Advances in the Treatment of Malaria. *Pharmaceutics, 16*(11), 1416. https://doi.org/10.3390/pharmaceutics16111416

Alum, E. U. (2025). Sustainable harvesting of medicinal plants: balancing therapeutic benefits with environmental conservation. *Agroecology and Sustainable Food Systems, 49*(3), 380-385. https://doi.org/10.1080/21683565.2024.2421948

Anstey, N. M., Tham, W. H., Shanks, G. D., Poespoprodjo, J. R., Russell, B. M., & Kho, S. (2024). The biology and pathogenesis of vivax malaria. Trends in Parasitology, 40(7), 573-590. https://doi.org/10.1016/j.pt.2024.04.015

Bagavan, A., Rahuman, A. A., Kamaraj, C., Kaushik, N. K., Mohanakrishnan, D., & Sahal, D. (2011). Antiplasmodial activity of botanical extracts against *Plasmodium falciparum*. *Parasitology Research, 108*, 1099-1109. https://doi.org/10.1007/s00436-010-2151-0

Bekono, B. D., Ntie-Kang, F., Onguéné, P. A., Lifongo, L. L., Sippl, W., Fester, K., & Owono, L. C. (2020). The potential of anti-malarial compounds derived from African medicinal plants: a review of pharmacological evaluations from 2013 to 2019. *Malaria Journal, 19*, 1-35. https://doi.org/10.1186/s12936-020-03231-7

Bhadange, Y. A., Carpenter, J., & Saharan, V. K. (2024). A comprehensive review on advanced extraction techniques for retrieving bioactive components from natural sources. *ACS Omega, 9*(29), 31274-31297. https://doi.org/10.1021/acsomega.4c02718

Brookes, K. B., & Katsoulis, L. C. (2006). Bioactive components of Rhoicissus tridentata: A pregnancy-related traditional medicine. *South African Journal of Science, 102*(5), 267-272. https://hdl.handle.net/10520/EJC96537

Canter, P. H., Thomas, H., & Ernst, E. (2005). Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. *Trends in Biotechnology, 23*(4), 180-185. https://doi.org/10.1016/j.tibtech.2005.02.002

Cheuka, P. M., Mayoka, G., Mutai, P., & Chibale, K. (2016). The role of natural products in drug discovery and development against neglected tropical diseases. *Molecules, 22*(1), 58. https://doi.org/10.3390/molecules22010058

Cibulskis, R. E., Alonso, P., Aponte, J., Aregawi, M., Barrette, A., Bergeron, L., ... & Williams, R. (2016). Malaria: global progress 2000–2015 and future challenges. *Infectious Diseases of Poverty, 5*, 1-8. https://doi.org/10.1186/s40249-016-0151-8

De Silva, G. O., Abeysundara, A. T., & Aponso, M. M. W. (2017). Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *American Journal of Essential Oils and Natural Products, 5*(2), 29-32.

Doughari, J. H. (2012). *Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents* (pp. 1-33). Rijeka, Croatia: INTECH Open Access Publisher.

Eichelbaum, S. R. (2016). *Screening of plants for antibacterial properties: growth inhibition of Staphylococcus aureus by Artemisia tridentata* (Doctoral dissertation, Florida International University, USA).

Espíndola, M. R., Varotti, F. D. P., Aguiar, A. C. C., Andrade, S. N., & Rocha, E. M. M. D. (2022). *In vitro* assessment for cytotoxicity screening of new antimalarial candidates. *Brazilian Journal of Pharmaceutical Sciences, 58*, e18308. https://doi.org/10.1590/s2175-97902022e18308

Fatima, G., Magomedova, A., & Parvez, S. (2024). *Biotechnology and Sustainable Development*. Shineeks Publishers.

Fikadu, M., & Ashenafi, E. (2023). Malaria: an overview. *Infection and Drug Resistance, 16*, 3339-3347. https://doi.org/10.2147/IDR.S405668

Fotie, J. (2008). The antiprotozoan potential of flavonoids. *Pharmacognosy Reviews, 2*(3), 6. https://www.phcogrev.com/sites/default/files/PhcogRev-2-3-6.pdf

Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine, 27*(1), 1-93. https://doi.org/10.1016/j.mam.2005.07.008

Habibi, P., Shi, Y., Fatima Grossi-de-Sa, M., & Khan, I. (2022). Plants as sources of natural and recombinant antimalaria agents. *Molecular Biotechnology, 64*(11), 1177-1197. https://doi.org/10.1007/s12033-022-00499-9

Jensen, J. B. (2002). *In vitro* culture of *Plasmodium* parasites.

Malaria Methods and Protocols: Methods and Protocols (pp. 477-488).

Jha, A. K., & Sit, N. (2022). Extraction of bioactive compounds from plant materials using combination of various novel methods: A review. *Trends in Food Science & Technology, 119*, 579-591. https://doi.org/10.1016/j.tifs.2021.11.019

Kaou, A. M., Mahiou-Leddet, V., Hutter, S., Aïnouddine, S., Hassani, S., Yahaya, I., ... & Ollivier, E. (2008). Antimalarial activity of crude extracts from nine African medicinal plants. *Journal of Ethnopharmacology, 116*(1), 74-83. https://doi.org/10.1016/j.jep.2007.11.001

Khan, S., Chandramohan, K., & Husen, A. (2024). Diversity and Geographic Distribution of Some Antimalarial Plant Species. In *Antimalarial Medicinal Plants* (pp. 1-39). CRC Press.

Kigondu, E. V., Rukunga, G. M., Keriko, J. M., Tonui, W. K., Gathirwa, J. W., Kirira, P. G., ... & Ndiege, I. O. (2009). Anti-parasitic activity and cytotoxicity of selected medicinal plants from Kenya. *Journal of Ethnopharmacology, 123*(3), 504-509. https://doi.org/10.1016/j.jep.2009.02.008

Kingston, D. G., & Cassera, M. B. (2022). *Antimalarial Natural Products* (pp. 1-106). Springer International Publishing.

Kodi, P. (2018). *Antiplasmodial and toxicity activities and characterization of chemical compounds extracted from selected medicinal plants in Uganda* (Doctoral dissertation, Egerton University).

Kudamba, A., Kasolo, J. N., Bbosa, G. S., Lugaajju, A., Wabinga, H., Niyonzima, N., ... & Muwonge, H. (2023). *Phytochemical Profiles of Rhoicissus tridentata Harvested From the Slopes Elgon Sub-region, Uganda.* Research Square. https://doi.org/10.21203/rs.3.rs-3650181/v1

Lefebvre, T., Destandau, E., & Lesellier, E. (2021). Selective extraction of bioactive compounds from plants using recent extraction techniques: A review. *Journal of Chromatography A, 1635*, 461770. https://doi.org/10.1016/j.chroma.2020.461770

Lokole, P. B., Byamungu, G. G., Mutwale, P. K., Ngombe, N. K., Mudogo, C. N., Krause, R. W., & Nkanga, C. I. (2024). Plant-based nanoparticles targeting malaria management. *Frontiers in Pharmacology, 15*, 1440116. https://doi.org/10.3389/fphar.2024.1440116

Mamede, L., Ledoux, A., Jansen, O., & Frédérich, M. (2020). Natural phenolic compounds and derivatives as potential antimalarial agents. *Planta medica, 86*(09), 585-618. https://doi.org/10.1055/a-1148-9000

Monga, S., Dhanwal, P., Kumar, R., Kumar, A., & Chhokar, V. (2017). Pharmacological and physico-chemical properties of Tulsi (*Ocimum gratissimum* L.): An updated review. *Pharma. Innovation, 6*(4), 181-186.

Nanyingi, M. O., Kipsengeret, K. B., Wagate, C. G., & Midiwo, J. O. (2009). *In vitro* and in vivo antiplasmodial activity of Kenyan medicinal plants. In Midiwo, J. O., Clough, J. (Eds.) *Aspects of African Biodiversity.*

Oliveira, A. B., Dolabela, M. F., Braga, F. C., Jácome, R. L., Varotti, F. P., & Póvoa, M. M. (2009). Plant-derived antimalarial agents: new leads and efficient phythomedicines. Part I. Alkaloids. *Anais da Academia Brasileira de Ciencias, 81*, 715-740. https://doi.org/10.1590/S0001-37652009000400011

Ong, E. S. (2004). Extraction methods and chemical standardization of botanicals and herbal preparations. *Journal of Chromatography B, 812*(1-2), 23-33. https://doi.org/10.1016/j.jchromb.2004.07.041

Ouandaogo, H. S., Diallo, S., Odari, E., & Kinyua, J. (2023). Phytochemical Screening and GC-MS Analysis of Methanolic and Aqueous Extracts of Ocimum kilimandscharicum Leaves. *ACS omega, 8*(50), 47560-47572. https://doi.org/10.1021/acsomega.3c05554

Ouattara, L. P., Sanon, S., Mahiou-Leddet, V., Gansané, A., Baghdikian, B., Traoré, A., ... & Sirima, S. B. (2014). *In vitro* antiplasmodial activity of some medicinal plants of Burkina Faso. *Parasitology research, 113*, 405-416. https://doi.org/10.1007/s00436-013-3669-8

Pandey, S. A. N. D. E. E. P. (2017). Antibacterial and antifungal activities of *Ocimum gratissimum* L. *Proteus, 25*(47), 56. http://dx.doi.org/10.22159/ijpps.2017v9i12.22678

Roux, A. T., Maharaj, L., Oyegoke, O., Akoniyon, O. P., Adeleke, M. A., Maharaj, R., & Okpeku, M. (2021). Chloroquine and sulfadoxine–pyrimethamine resistance in Sub-Saharan Africa—A review. *Frontiers in Genetics, 12*, 668574. https://doi.org/10.3389/fgene.2021.668574

Sato, S. (2021). *Plasmodium*—a brief introduction to the parasites causing human malaria and their basic biology. *Journal of physiological anthropology, 40*(1), 1.

Singha, B., Singh, V., & Soni, V. (2024). Alternative therapeutics to control antimicrobial resistance: a general perspective. Frontiers in Drug Discovery, 4, 1385460. https://doi.org/10.1186/s40101-020-00251-9

Stéphane, F. F. Y., Jules, B. K. J., Batiha, G. E. S., Ali, I., & Bruno, L. N. (2021). Extraction of bioactive compounds from medicinal plants and herbs. *Natural medicinal plants* (pp. 1-39). https://doi.org/10.5772/intechopen.98602

Van Wyk, A. S., & Prinsloo, G. (2018). Medicinal plant harvesting, sustainability and cultivation in South Africa. *Biological Conservation, 227*, 335-342. https://doi.org/10.1016/j.biocon.2018.09.018

Waiganjo, B., Moriasi, G., Onyancha, J., Elias, N., & Muregi, F. (2020). Antiplasmodial and cytotoxic activities of extracts of selected medicinal plants used to treat malaria in Embu

County, Kenya. *Journal of Parasitology Research, 2020*(1), 8871375. https://doi.org/10.1155/2020/8871375

Woodrow, C. J., & White, N. J. (2017). The clinical impact of artemisinin resistance in Southeast Asia and the potential for future spread. *FEMS microbiology reviews, 41*(1), 34-48. https://doi.org/10.1093/femsre/fuw037

World Health Organization. (2023). *WHO guidelines for malaria, 14 March 2023* (No. WHO/UCN/GMP/2023.01). World Health Organization. https://www.who.int/publications/i/item/9789240069297