Characterisation and effectiveness of bioactive compounds from selected medicinal plants used in the treatment of malaria in Zimbabwe.

By

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DECLARATION

I, **Zakio Makuvara** hereby declare that the dissertation/thesis, with the title; '**Characterisation and effectiveness of bioactive compounds from selected medicinal plants used in the treatment of malaria in Zimbabwe**' which I hereby submit for the degree of Doctor of Philosophy at the University of South Africa, is my own work and has not previously been submitted by me for a degree at this or any other institution.

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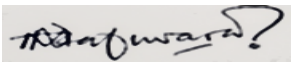
Student signature: 

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SUMMARY

Background: Significant investments and efforts in the fight against malaria have resulted in a notable reduction in new cases and deaths globally. An important challenge is the emergence of *Plasmodium falciparum* strains resistant to antimalarial drugs, affecting prophylaxis. Limited published information exists on the use of herbal medicine against malaria. This study aims to identify and scientifically evaluate medicinal plants used in malaria treatment in Zimbabwe.

Methodology: A comprehensive literature analysis and database mining were conducted to identify plants used in malaria treatment in Zimbabwe. Plant samples were air dried and exposed to ethanol, water, and ethyl acetate. The activities of plant extracts against *Plasmodium falciparum* and their toxicities against healthy erythrocytes were determined using modified Trager and Jensen (1976) method and MTT-based colorimetric assay. Crude extracts with selectivity index (SI) >7 were selected for phytochemical screening using qualitative and FTIR profiling. Plant extracts with SI >10 underwent further examination based on GC-MS analysis.

Results: A total of 61 plant species belonging to 30 families were identified, with *Fabaceae* being the most represented family, accounting for 17% of the species recorded. Five categories of indigenous practices used in malaria treatment, including herbal medicines, were identified. Twenty-seven plant extracts assessed were active against *Plasmodium falciparum*, with IC_{50} below 100µg/ml. The most active extracts were from *Tabernaemontana. elegans* (IC_{50} below 5 µg/ml). Most crude extracts (24/27) exhibited good selectivity (SI > 2). Bioactive compounds like terpenoids, cardiac glycosides, alkaloids, and tannins were present in all screened plant extracts. Nineteen phytochemicals were identified, including 9-Octadecenamide, cis-Vaccenic acid, 9, 12-Octadecadienoic acid (Z, Z) - and n-Hexadecanoic acid.

Conclusion: This study provides scientific evaluation of selected medicinal plants used against malaria treatment in Zimbabwe and identifies *T. elegans* as a promising source of antiplasmodial compounds. The strong antiplasmodial activity, selectivity, and diverse phytochemical composition of its stem bark extract highlight its potential as a source of lead compounds for antimalarial drug discovery. These findings contribute to the growing search for plant-derived antimalarial agents and provide a foundation for future isolation and characterisation of bioactive molecules.

Keywords: Antimalarial plants, *Plasmodium falciparum*, gas chromatography-mass spectrometry, phytochemicals, malaria, bioactive compounds, indigenous practices, selectivity index, toxicity tests, antiplasmodial activity, *T. elegans*

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LIST OF ABBREVIATIONS

GCMS	Gas Chromatography-Mass Spectrometry
FTIR	Fourier Transform Infrared
SI	Selectivity Index
IC_{50}	Half-maximal inhibitory concentration
CC_{50}	Cytotoxic concentration 50%
CQ	Chloroquine
ZINATHA	Zimbabwe National Traditional Healers Association
ASAQ	Artemisinin-based combinations including artesunate–amodiaquine
AL	Artemether–lumefantrine
DP	Dihydroartemisinin–piperaquine
ACT	Artemisinin-based combination therapy
CDC	Centre for Disease Control and Prevention
WHO	World Health Organisation
HBHI	High burden to high impact
LLINS	Long-lasting insecticidal nets
IRS	Indoor residual spraying
IVM	Integrated vector management
LSM	Larval source management
IPTi	Intermittent preventive treatment for infants
IPTp	Intermittent preventive treatment for pregnant women
SMC	Seasonal malaria chemoprevention
ACD	Active case detection
API	Annual Parasite Index
APD-APIs	Antimalarial Plant Derived Active Pharmaceutical Ingredient

CHAPTER 1: INTRODUCTION AND BACKGROUND OF STUDY

1.0 Background of the study.

1.1 Malaria: Biological account; Vector capacity and Distribution.

Malaria is an infectious vector-borne disease caused by Apicomplexa protozoa represented by several *Plasmodium* species (Mulaw et al., 2019). *Plasmodium* species are transmitted between hosts by mosquito bites, with hosts including human beings, rodents, simians, reptiles, and birds (Krettli et al., 2001; Lima, Rocha Silva, et al., 2015). Of the several species of the genus *Plasmodium*, only five infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi*. *Plasmodium vivax* and *Plasmodium falciparum* are the most common malaria pathogens, with the latter being the most deadly form, predominating in Africa (Ngarivhume et al., 2015; Mulaw et al., 2019). Studies confirm that 98% of malaria cases reported in Zimbabwe are due to *P. falciparum*, with the remaining 2% caused by *Plasmodium ovale* and *Plasmodium malariae* (Ngarivhume et al., 2015). Research conducted between 2013 and 2016 on the role of vectors in malaria distribution concluded that *Anopheles (An.) gambiae* s.l. and *Anopheles funestus* were the most widely distributed malaria vector species in Zimbabwe (President's Malaria Initiative Zimbabwe, 2018; President's Malaria Initiative Zimbabwe, 2020).

Despite significant investments and efforts in the fight against malaria, leading to fewer cases and deaths globally, malaria remains prevalent worldwide (Mugwagwa et al., 2015). Malaria is endemic in tropical and subtropical countries, with approximately 50% of the global population vulnerable to infection (Bekono et al., 2020; Mulaw et al., 2019; Quanquin et al., 2020; Tahghighi et al., 2020; Mugwagwa et al., 2015). A report from December 2019 revealed 228 million malaria cases in 2018, resulting in about 405,000 malaria-related deaths (Bekono et al., 2020). The highest malaria burden is in Africa, accounting for 93% of all malaria deaths globally (Mulaw et al., 2019). Children under five (Tahghighi et al., 2020) and expectant mothers are at the highest risk worldwide (Mulaw et al., 2019). In 2015, nearly twenty-eight million pregnant women in Africa were found to be at risk of malaria (Quanquin et al., 2020). Children under five accounted for 61% of all deaths in Africa (Mulaw et al., 2019).

1.1.2 Current malaria control interventions (successes and failures) and impacts of malaria on health and the economy.

The elimination and reduction of malaria align with the Global Technical Strategy for Malaria 2016–2030, aiming to eliminate malaria from at least 35 countries and reduce cases and mortality by at least 90% by 2030 (WHO, 2016). Current global malaria control strategies are based on three pillars guiding interventions to reduce malaria transmission. According to WHO (2016), the first pillar ensures universal access to malaria diagnosis, treatment, and prevention, while the second and third pillars focus on accelerating efforts for malaria elimination and achieving malaria-free status (WHO, 2020).

Globally, estimated malaria-related mortality and incidence were at 60% and 37%, respectively (Asnake et al., 2016). Despite remaining a health burden in endemic countries, malaria morbidity and mortality have declined over the past 15 years due to increased vector control, improved diagnosis, and treatment (Kemibala et al., 2020). Between 2010 and 2017, malaria incidence rates, cases, and deaths decreased by 18%, 20 million and 172,000, respectively (Abamecha et al., 2020). This decline is attributed to the introduction of artemisinin-based combination therapy (ACT), effective use and distribution of long-lasting insecticidal nets (LLINs), and consistent indoor residual spraying (IRS) (Figure 1.1) (Beavogui et al., 2020; Kemibala et al., 2020). Strategies like intermittent preventive treatment during pregnancy (IPTp) and seasonal malaria chemoprevention (SMC), and environmental management have also contributed significantly to the reduction in malaria-related deaths and cases (Gutman et al., 2020; Somé et al., 2020).

While some gains have been made in the combat against malaria, some challenges persist. For example accessibility of vector control strategies (**Figure 1.1**) is influenced by the cost of vector control products, a major impediment in implementing strategies to prevent insecticide resistance in malaria vectors and reduce residual malaria transmission (WHO, 2016). Effective implementation of vector control interventions requires accurate predictions of product requirements and is dependent on human resources, organisational structures, and infrastructure development (WHO, 2016). Despite extensive malaria control campaigns, approximately 60% of the population in malaria-infested areas still lack access to effective malaria interventions (Odufuwa et al., 2020).

Malaria exerts a significant economic toll globally, particularly in sub-Saharan Africa, where it remains a major public health and development challenge. According to the World Health Organization (WHO,

2023), malaria has led to annual losses of USD 12 billion in the African economy. These losses are primarily due to healthcare expenses and decreased productivity. In Zimbabwe, malaria has had a detrimental impact on economic productivity, especially in agricultural areas. Outbreaks during peak farming seasons result in absenteeism and reduced output (Ministry of Health and Child Care [MoHCC], 2021).

The Zimbabwean government allocates substantial resources each year to malaria prevention and treatment efforts, including indoor residual spraying, vector control, and public health campaigns. These direct costs are further compounded by long-term indirect consequences, such as lower educational attainment and decreased economic participation in heavily affected regions (Chitunhu & Musenge, 2018).

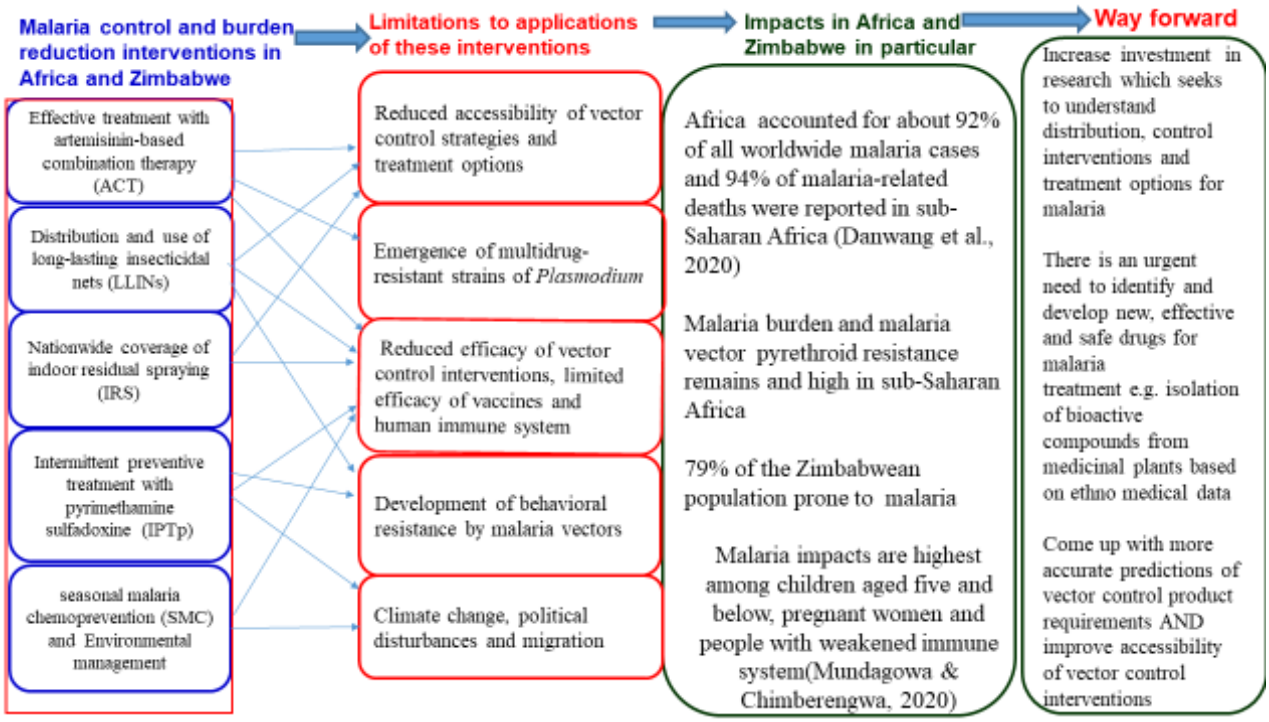

Figure 1.1 A synopsis of malaria control, treatment interventions, impacts, and mitigation in Zimbabwe.

1.1.3 Prevalence of malaria in Zimbabwe.

The prevalence of malaria in Zimbabwe is highest among those under five years of age, expectant mothers, and people with weakened immune systems, especially those living with HIV and AIDS (Chapu & Mgocheki, 2017). Although there was a marked decline in new malaria cases to 45 cases per 1000 per

year by 2010, malaria still poses a serious threat to public health in Zimbabwe (Manyangadze et al., 2017). It is estimated that about half of the Zimbabwean population lives in malaria risk areas (President's Malaria Initiative Zimbabwe, 2018; President's Malaria Initiative Zimbabwe Malaria Operational Plan Fiscal Year (FY), 2020).

Generally, transmission of malaria in Zimbabwe is based on seasons and directly linked to the geographic variation in rainfall patterns and topology, with the highest transmission manifesting between February and April (President's Malaria Initiative Zimbabwe Malaria Operational Plan FY, 2020; Manyangadze et al., 2017). Consistently, the country is subdivided into the northern and eastern low-lying areas associated with high malaria transmission, and the central and southwestern watershed that is higher than 1,200 meters above sea level, with reduced malaria transmission (President's Malaria Initiative Zimbabwe Malaria Operational Plan FY, 2020). In line with this transmission pattern, nearly 80% of annual malaria cases are confined to the northern and eastern provinces of Zimbabwe. The most impacted provinces are Mashonaland Central, Mashonaland North, and Manicaland, which amount to 30% of the country's total number of provinces (President's Malaria Initiative Zimbabwe, 2018; President's Malaria Initiative Zimbabwe Malaria Operational Plan FY, 2020). Nationally, annual malaria incidences per 1,000 population dropped significantly, from 153 in 2004 to 19 in 2018. Disturbingly, much progress was made before 2011, after which malaria incidences followed an uneven cyclical pattern ranging from 19 to 40 per 1000 population (President's Malaria Initiative Zimbabwe Malaria Operational Plan FY, 2020).

1.1.4 Challenges in the fight against malaria and the use of medicinal plants.

Containment of malaria is hampered by the emergence of multidrug-resistant strains of *Plasmodium*. Of particular importance is the development of antimalarial resistance by *Plasmodium* species, including *P. falciparum*, presenting a handful of challenges, including prophylaxis and limitation on the choice of drugs used (Silva et al., 2011). Antimalarial drugs such as chloroquine (CQ) and antifolates/sulfadoxine-pyrimethamine are no longer effective in most endemic areas (Bekono et al., 2020). Introduction of combination therapy has emerged, which involves combining artemisinin with several complementary drugs, including artemether-lumefantrine (AL), dihydroartemisinin-piperaquine (DP), and artesunate-amodiaquine (ASAQ) (Beavogui et al., 2020). Generally, artemisinin-based combination therapy (ACT) includes artemisinin or one of its derivatives and a complementary drug. The partner or complementary drugs include lumefantrine, piperaquine, mefloquine, amodiaquine, artemether, artesunate, and

pyronaridine (Pornputtapong et al., 2020; Wu et al., 2020). Although artemisinin-based combinations are the most effective antimalarial drugs, clinical resistance has been identified in all classes of antimalarial drugs, calling for further research into new antimalarial drugs (Bekono et al., 2020; Mulaw et al., 2019; Silva et al., 2011). In addition to antimalarial drug resistance, malaria prevalence remains high due to the evolution of malaria vectors and the emergence of resistance genes against the insecticide dichlorodiphenyltrichloroethane (Bekono et al., 2020).

Ethno-medicinal and ethno-botanical studies have laid a foundation for the discovery and development of novel drugs against infectious diseases (Qayum et al., 2016). Many countries worldwide, including those in Africa, are dependent on natural remedies and phytomedicines for their primary health care needs (Mulaw et al., 2019). Isolation of bioactive compounds from medicinal plants based on ethno-medical data is critical in drug discovery and development (Silva et al., 2011). Medicinal plants are rich sources of anti-malarial phytochemicals such as quinine and artemisinin. Antimalarial plants normally contain bitter secondary metabolites, mainly alkaloids and terpenoids. According to Bekono et al. (2020), approximately 80% of the population in most tropical countries and those in Africa are dependent on medicinal plants for the treatment of malaria and other infectious diseases. Despite the availability of standard antimalarial drugs in Africa, people in African countries continue to use plants for malaria treatment (Bekono et al., 2020), probably due to trust in indigenous knowledge systems, availability, and low costs. Generally, the plant parts used, method of preparation, and route of administration of plant medicines vary geographically (Qayum et al., 2016).

1.1.5 Evidence of high malaria burden due to malaria parasite and vector resistance in Africa.

Chloroquine was used as the first-line antimalarial treatment for uncomplicated malaria caused by *P. falciparum* in sub-Saharan Africa up to the early 2000s (Flegg et al., 2013). African countries such as Zambia, South Africa, Kenya, and Tanzania switched to sulfadoxine/pyrimethamine (SP) as the first-line treatment in response to chloroquine resistance (Mwendera et al., 2016). However, gene mutations in *P. falciparum* have led to treatment failures with SP (Desai et al., 2016). Despite this, regions burdened by malaria and where it is endemic still use SP for intermittent preventive treatment in pregnant women (Zhao et al., 2020). Recently, ACTs have become the primary drugs for malaria, with drugs like artesunate-

amodiaquine and artemether-lumefantrine being used in sub-Saharan Africa for the treatment of uncomplicated malaria (Conrad & Rosenthal, 2019; WHO, 2020).

The antimalarial drugs recommended by the WHO for the African region include artesunate-pyronaridine, dihydroartemisinin-piperaquine, and artesunate-mefloquine. Additionally, other antimalarials such as artemisinin-piperaquine, artesunate-sulfadoxine-pyrimethamine, arterolane-piperaquine, and artemisinin-naphthoquine are also in use in Africa (Conrad & Rosenthal, 2019). In cases where intravenous treatment is not possible, rectal artemether and artesunate, as well as intramuscular artesunate, have been administered (Kremsner et al., 2016). However, in African settings, intravenous quinine has always been the preferred treatment for severe malaria (Esu et al., 2014). Antimalarial preventive drugs like sulfadoxine-pyrimethamine and amodiaquine-sulfadoxine-pyrimethamine are used to prevent malaria during pregnancy and in children during the rainy seasons (Desai et al., 2018). For travellers visiting high malaria transmission countries in Africa, malaria chemoprophylaxis is primarily based on mefloquine, atovaquone-proguanil, and doxycycline (Shellvarajah et al., 2017).

Chloroquine resistance was first reported in 1978, and by 1983, it had spread to countries like Zambia, Sudan, Malawi, and Uganda (World Health Organisation, 2018). This led to the discontinuation of chloroquine-based malaria treatment in most African countries, with SP replacing it in countries like Malawi in the early 1990s (Severini & Menegon, 2015). Parasites resistant to sulfadoxine-pyrimethamine and chloroquine in Africa were believed to have originated from Southeast Asia. According to Ehrlich et al. (2020), there was a significant decrease in the prevalence of *Asn86Tyr*, *pfmdr*1 *pfcrtLys76Thr*, and *Asp1246Tyr* mutations in sub-Saharan Africa. This suggests an increase in lumefantrine-resistant parasites and a resurgence of chloroquine-sensitive parasites.

Chloroquine (CQ) resistance has been linked to mutations in the CQ-resistant transporter (*pfcrt*) gene and the multidrug-resistance gene 1 (*pfmdr*-1) in P. falciparum (Bin Dajem and AlQahtani, 2010). Resistance of malaria parasites to chloroquine was confirmed in Tanzania and Kenya as early as 1978, and by 1980, it was reported in West Africa (Roux et al., 2021). Antimalarial drug resistance in Africa has been attributed to factors such as ineffective use of antimalarial drugs and policy changes. For example, in Kenya, the implementation of IPTp and changes in first-line malaria treatment policy were followed by the emergence of DNA drug resistance markers (Hemming-Schroeder et al., 2018).

Although resistance to chloroquine was observed in African countries, chloroquine has remained an option for treating uncomplicated malaria caused by *P. falciparum*. In the early 1990s, resistance to chloroquine in *P. falciparum* was reported in the southern and eastern parts of Africa (Conrad & Rosenthal, 2019). However, the use of artemisinin-based combination therapy (ACT) as alternatives to chloroquine has led to the emergence of malaria parasites with *pfmdr*1 and *pfcrt* sequences in eastern parts of Zimbabwe. The shift in polymorphism after the introduction of ACTs has resulted in parasites that are sensitive to chloroquine (Hemming-Schroeder et al., 2018; Tumwebaze et al., 2017). Up to 90% of countries in sub-Saharan Africa have started using artemisinin-based combination therapy (ACT) for malaria treatment in response to SP and chloroquine resistance (Frosch et al., 2011). Reports of isolated cases of malaria parasites resistant to artemisinin have been noted in sub-Saharan Africa, but artemisinin-resistant parasites have not been established in sub-Saharan Africa (Lu et al., 2017). Though resistance of malaria parasites to piperaquine is limited in Africa, cases have been reported in Uganda (Conrad et al., 2014).

Resistance of malaria vectors to all insecticides recommended by the WHO was reported in Africa between 2001 and 2012. In countries like Zimbabwe, *An funestus* and *An gambiae* s.l. were found to be resistant to carbamates, pyrethroids, and organochlorines (Knox et al., 2015). The lack of progress in the fight against malaria has been exacerbated by widespread resistance of malaria vectors to insecticides like pyrethroids, organophosphates, and carbamates used in IRS and ITNs. In Burkina Faso, high malaria burden has been associated with vector resistance to pyrethroids (Toé et al., 2014). In addition to resistance to commonly used pesticides, behavioural resistance, such as resting outdoors, biting outdoors, and changes in biting times, has been observed in malaria parasites (Foy et al., 2023).

Resistance of *An funestus* to pyrethroids and carbamates has been reported in Africa (Choi et al., 2014; Cuamba et al., 2010). In African countries like Mozambique (Casimiro et al., 2006), *An Arabiensis* has shown reduced resistance to carbamates, while *An gambiae* s.s. has shown resistance to pyrethroids and DDT (Chanda et al., 2011). Insecticide resistance has been largely attributed to genetic changes due to metabolic resistance and mutations (Chanda et al., 2020). The invasion of *Anopheles stephensi* in African urban areas (Sinka et al., 2020) has hindered the fight against malaria. This malaria parasite can thrive in urban environments due to increased adaptability to breeding in highly polluted aquatic environments (Batra et al., 2001) and the ability to utilise purpose-built water storage reservoirs in urban areas (Thomas et al., 2016).

1.2 Statement of the problem

1.2.1 Malaria parasite and vector resistance

1.2.1.1 Malaria parasite resistance

The cases of chloroquine (CQ) resistance in Zimbabwe were first reported in 1984 in the Zambezi Valley. Before 1990, chloroquine resistance was confined to the Zambezi Valley (Makono & Sibanda, 1999). According to Mharakurwa & Mugochi (1994), out of the 83% of the chloroquine-resistant cases reported in Gokwe, only 3% responded to chloroquine treatment. Despite significantly high CQ failure rates, the widespread use of CQ continued until 2000 (Mlambo et al., 2007). Resistance to CQ by parasites led to the replacement of CQ with artemether-lumefantrine and sulfadoxine-pyrimethamine in 2008 and 2008, respectively. Up to 67% of sampled malaria parasites in 2003 in Mutasa District were resistant to CQ, which was associated with the CQ resistance transporter gene (*pfcrt*) (Ministry of Health and Child Welfare, Zimbabwe, 2007). However, in 2013, only 3% of samples had *pfcrt* mutants, and between 2017 and 2018, 100% of P. falciparum were *pfcrt* wild type. The presence of *P. falciparum* with the *pfcrt* wild type indicated the emergence of CQ-susceptible *P. falciparum* (Mharakurwa et al., 2021).

1.2.1.2 Malaria vector resistance.

Malaria vector resistance has significantly hampered malaria control efforts in Zimbabwe. For instance, pyrethroid resistance was reported in Mutasa District, Manicaland Province, Zimbabwe in 2014 (Wesolowski et al., 2022). The shift from pyrethroid-based IRS to organophosphate spraying in Zimbabwe was prompted by *An funestus* resistance to carbamates and pyrethroids (Kanyangarara et al., 2016; Choi et al., 2014). In 2014, insecticide resistance in *An funestus* was reported in Mutasa and Mutare Districts. *An arabiensis* samples collected from Chiredzi District, Masvingo Province, Zimbabwe, in the early 1980s were resistant to benzene hexachloride (Green, 1981). Additionally, resistance to permethrin and DDT was noted in *An arabiensis* from Gokwe District (Masendu et al., 2005) and (Munhenga et al., 2008). Malaria vectors in Zimbabwe, *An arabiensis* and *An funestus* has shown behavioural resistance and continues to spread malaria parasites even with efficient vector control methods. For example, *An. arabiensis* and *An gambiae* complex collected from Gokwe South District were exophilic, making malaria vector control difficult (Dandalo, 2007). Up to 16% of *An funestus* sampled from Mutasa and Mutare Districts had exophilic features, and 10% of those found indoors were on unsprayable surfaces (Sande et al., 2016). This presents a new dimension in the fight against malaria in Mutare and Mutasa Districts, Manicaland, Zimbabwe.

The use of benzene hexachloride in malaria control programs in Zimbabwe has resulted in the emergence of malaria vectors (*An arabiensis*) resistant to benzene hexachloride in Chiredzi District (Green, 1982). According to Munhenga et al. (2008), *An Arabiensis* from Gokwe was found to be resistant to Dichloro-Diphenyl-Trichloroethane (DDT) and permethrin. Apart from this, resistance to pyrethroids, carbamates, and organophosphates was noted among *An funestus* (Choi et al., 2014). Resistance of malaria vectors to insecticides in areas of high agricultural activities (Gwave, Gokwe (cotton production) (Munhenga et al., 2008), Burma Valley (tea and banana farming (PMI. |Africa IRS, 2013), Triangle Estates (Sugarcane production), Hippo Valley (Sugarcane production), and Honde Valley (tea and banana farming) (Munhenga et al., 2008) indicates that malaria vector resistance in Zimbabwe has been shaped by the use of insecticides in agricultural activities.

1.2.2 Limitations of vaccines and the human immune system in the fight against malaria.

Malaria vaccine research has evolved in response to a rise in malaria vector and drug resistance. In Africa, twenty-four malaria vaccines are being evaluated at 99 experimental sites (Bhagavathula & Elnour, 2016). Mosquirix™ (RTS, S), a vaccine designed against *Plasmodium falciparum*, received approval in the African region in 2015. Though the vaccine (Mosquirix™) is effective against *P. falciparum*, it was found to be ineffective against infant severe malaria in a Phase III study (Winskill et al., 2019; Dimala et al., 2018). However, WHO approved and recommended the use of Mosquirix among infants in Sub-Saharan Africa in 2021 (Kolawole et al., 2023). High prices, along with compliance and viability issues, may impede the acceptance of such malaria vaccines in Africa (Bhagavathula & Elnour, 2016).

The approval of the malaria vaccine, RTS, S (Mosquirix) in Africa was based on successful pilot studies in sub-Saharan African countries: Ghana, Kenya, and Malawi (Oladipo et al., 2022). Of the vaccinated infants (830,000) in the trials, above 30% and 40% reduction of admitted infants with severe malaria and malaria episodes were reported, respectively. Scaling up the malaria vaccine, however, encounters inherent problems associated with vaccination programs, which include distribution, declining immunity, and financing (Dimala et al., 2018). The reduced efficacy of Mosquirix from its original efficacy, which lies between 60% and 70%, has serious implications for the WHO target of producing a malaria vaccine that can protect over 75% of people in malarious regions by 2030. Although there are other malaria vaccines, such as R21 with efficacy above 70% (Datoo et al., 2021), their efficacy in malaria perennial

regions is yet to be explored. The capacity of vaccine production in Africa is extremely low, as local vaccine production depends on external support (Abiodun et al., 2021). Out of the 54 African countries, only 8 have vaccine-manufacturing companies, and only Senegal has been approved by the WHO to sell vaccines to other countries (Okereke et al., 2022). Local production of vaccines in Africa has been linked to poor investment in research, insufficient skills transfer, and the exodus of highly skilled professionals (Ekström et al., 2021).

1.2.3 Dearth of information on plants used against malaria in Zimbabwe.

There is limited published information on the utilisation of herbal medicine against malaria in Zimbabwe. Apart from this, the published information is mainly based on ethnobotanical surveys without scientific evaluation (Chapu & Mgocheki, 2017; Kazembe et al., 2012; Lukwa et al., 2001; Mbauya, 2015; Ngarivhume, Van, et al., 2015). However, many Zimbabweans, especially in rural areas, continue to utilise antimalarial plants for basic healthcare, despite the lack of documentation and regulation (Van Wyk & Prinsloo, 2018). The use of antimalarial plants in Zimbabwe is based on indigenous practices. Unfortunately, these antimalarial plants are principally undocumented and poorly integrated into the mainstream health sector (Maroyi, 2012; Ngarivhume, Van, et al., 2015; Usai et al., 2022). Information on the use of plants for the prophylaxis and treatment of malaria in Zimbabwe is scattered and not comprehensive. Ethnobotanical and pharmacological studies in Zimbabwe are still in the initial phases, and therefore, the exploitation of antimalarial plants in Zimbabwe is based on the experiences of communities. However, such practices are highly dynamic and evolve (Dhewa, 2008; Gureje et al., 2015).

Overall, literature synthesis indicates that malaria prevention and control mechanisms in Zimbabwe are increasingly challenged by vector and parasite resistance, and the limitations of current effective vaccines. Although indigenous medicinal plants in Zimbabwe remain widely used for malaria management, most studies have focused on ethnobotanical documentation with limited scientific evaluation of their efficacy, safety, and bioactive compounds. Consequently, the antiplasmodial activity of many Zimbabwean medicinal plants remains poorly understood. This study addresses this gap by combining ethnobotanical, antiplasmodial, cytotoxicity, and phytochemical analyses to identify promising plant-derived antimalarial candidates and support future drug discovery efforts.

1.3 Aim of the study

To characterise and evaluate the effectiveness of bioactive compounds from selected medicinal plants used in malaria treatment in Zimbabwe.

1.4 Specific objectives

- ❖ To identify plant species used in the treatment of malaria in Zimbabwe.
- ❖ To explore the indigenous practices (merits and demerits) in the fight against malaria in Zimbabwe.
- ❖ To examine the activity against *Plasmodium falciparum* and the toxicity of crude extracts from selected Zimbabwean antimalarial plants.
- ❖ To characterise bioactive compounds from selected medicinal plants used against malaria.

1.6 Research questions

1. Which indigenous plant species are utilised in management of malaria in Zimbabwe?
2. How do indigenous malaria control practices contribute to malaria prevention in Zimbabwe, and what are their merits and demerits?
3. Which bioactive compounds with potential antimalarial activity can be isolated and characterized from selected Zimbabwean medicinal plants?

1.7 Hypotheses

H_0: Crude extracts from selected Zimbabwean medicinal plants have no significant antiplasmodial activity against Plasmodium falciparum.

H_0: Crude extracts from selected Zimbabwean medicinal plants have no significant toxicity profiles against erythrocytes.

1.8 Scope and delimitations of the study

This study investigates medicinal plants used in the management of malaria in Zimbabwe, indigenous malaria management practices, and the antiplasmodial activity, toxicity, and bioactive compounds of selected plants. The study is limited to selected medicinal plants identified through literature surveys, with laboratory analyses restricted to *in vitro* evaluation of crude extracts and their bioactive constituents. Findings may not be generalisable to all antimalarial plants and clinical efficacy in humans.

1.9 Significance of the study

The outcomes of this study are expected to significantly contribute to the scientific body of knowledge on the utility of plants in antimalarial drug discovery and development. This study will allow the identification of bioactive compounds that can act as important lead molecular structures for possible anti-malarial drug development. The identified bioactive compounds may be used as markers for the standardisation of drug preparation, dosage, route of administration, and formulations of antimalarial drugs used in Zimbabwe. Bioactivity of anti-malarial plant extracts will provide preliminary scientific justification for the traditional uses of identified plant medicines. The current work will provide an important step towards the acceptance and development of alternative therapeutic agents against malaria. This research will give insight into the relationships between the use of plants as anti-malarial drugs and the efficacy of the plant extracts against *Plasmodium* species. Furthermore, the study would allow the preservation of indigenous knowledge about anti-malarial plants in Zimbabwe.

10.1 Layout of the thesis

The thesis is structured into five chapters: Chapter 1 (General introduction and background of the study), Chapter 2 (Literature review), Chapter 3 (Materials and methods), Chapter 4 (Data presentation, Analysis, and Discussion of results), and Chapter 5 (Conclusion and recommendations).

Chapter 1

This chapter summarises the background of the study and gives a detailed outline of the problem statement, aim, objectives, and significance of the study. A background of malaria as an infectious disease in Africa with a specific focus on Zimbabwe, is presented in this chapter. The use of plants, traditional medicines, and indigenous knowledge systems in the fight against malaria is outlined here.

Chapter 2

In this chapter, literature on the biology of malaria, history of malaria treatment, distribution and prevalence of malaria in Africa with special attention on Zimbabwe, indigenous practices in the fight against malaria in Zimbabwe, malaria and COVID-19, use of plants in the management of malaria, antimalarial plant bioactive compound characterisation, and steps towards malaria elimination in Zimbabwe was reviewed.

Chapter 3

This chapter summarises the methods that were applied in the identification of plants and other indigenous practices that are used in the fight against malaria. Additionally, the methods used in the extraction and characterisation of bioactive compounds are presented in this chapter.

Chapter 4

All the findings are analysed, summarised, and presented in this particular chapter. In this chapter, all the results are discussed.

Chapter 5

All conclusions drawn from the findings of the study are presented in this chapter. Moreover, recommendations and future directions are summarised in this chapter.

The following chapter is a literature survey on the prevalence of malaria in Zimbabwe and the efficacy of the implemented remedial and control measures. Special focus is given to indigenous plant-based practices that are believed to be remedial in the treatment of malaria.

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CHAPTER 2: LITERATURE REVIEW

2.0 Introduction

This chapter explores literature on the prevalence of malaria in Zimbabwe and the efficacy of the remedial and control measures that are used. Special attention is given to indigenous plant-based practices that are believed to be remedial and efficacious in the control of malaria in Zimbabwe. Part of the data obtained in this chapter has been published in an articles entitled "Malaria situation and elimination in Africa with specific focus on Zimbabwe: a systematic review" published in the Cogent Public Health, 11:1, 2376945, DOI: 10.1080/27707571.2024.2376945 and "Malaria control interventions: The role of indigenous practices in Zimbabwe." published in the Cogent Public Health, 11(1). https://doi.org/10.1080/27707571.2024.2347654 (See **Appendix 5 and 6**).

2.1 Biology of malaria

Malaria is a serious vector-borne and globally important infectious disease with significant public health impacts across many tropical and subtropical countries (Mulaw et al., 2019; Cowman et al., 2016). This disease is spread by *Plasmodia*-infected *Anopheles* mosquitoes, and five known *Plasmodium* species can infect and result in clinical symptoms (*P. falciparum*, *P. ovale*, *P. vivax*, *P. malariae*, and *P. knowlesi*) (Mulaw et al., 2019). Additionally, there are several *Plasmodium* species known to infect most mammals except humans, described in Africa as *P. vinckei*, *P. chabaudi*, *P. yoeli*, and *P. berghei* (Ogugua et al., 2019). The global distribution of malaria is closely linked to the complexity (Cowman et al., 2016) and multistage nature of the *Plasmodium* life cycle in two living hosts (Kahime et al., 2019). The pathogenesis of *Plasmodium* species is anchored in a highly complex life cycle that alternates between mosquitoes and vertebrates (Cowman et al., 2016).

Clinical symptoms of malaria vary depending on the type of *Plasmodium* species present. Among other symptoms, the central universal symptom in all forms of malaria is the manifestation of persistent or recurring fevers associated with shivering, sweating, jaundice, and sometimes anaemia (Nosten et al., 2022; Breeveld et al., 2012; Butler et al., 2010). Generally, the effects of *Plasmodium* species can be recognised through less specific symptoms such as malaise, gastrointestinal problems, neurological issues (dizziness, disorientation, confusion, and coma), and headaches (Nosten et al., 2022).

In response to the challenges posed by malaria globally, malaria control interventions were introduced (Ngatu et al., 2019). However, success with these malaria control strategies requires a deep understanding of malaria biology, novel antimalarial drugs, and vaccines (Cowman et al., 2016). The major challenge is the limited data and knowledge on the ability of symptomatic and asymptomatic human hosts to transmit the malaria parasite at different stages (Nosten et al., 2022; Breeveld et al., 2012). The lack of sufficient knowledge in this case stems from the complexity and lack of correlation between the parasite density of malaria parasites in humans and infectiousness to malaria vectors (mosquitoes) (Meibalan and Marti, 2017). The available data on strategies exploited by *Plasmodium* species for efficient transmission are still inconclusive. An in-depth understanding of malaria biology is crucial in developing new strategies and tools for malaria eradication and elimination (Meibalan and Marti, 2017). This chapter explores the cell biology of malarial parasites at different stages of development in female *Anopheles* mosquitoes and human vectors, as well as how different stages of *Plasmodium* species may affect infectivity.

2.1.1 Plasmodium life cycle

The complex life cycle of *Plasmodium* species is subdivided into three broad phases (**Figure 2.1**): the asymptomatic stage (exo-erythrocytic cycle), the symptomatic stage (erythrocytic cycle), and the infective stage (sporogenic cycle) (Cox-singh et al., 2010; Alano, 2015). This highly complex life cycle of *Plasmodium* species depends on a wide range of varying proteomes that allow adaptability of these parasites in different host sites (Khan, 2016; Bosson-vanga et al., 2015). *Plasmodium* species utilise their ever-changing proteome to survive in hepatocytes, female *Anopheles* mosquitoes' gut and salivary gland, erythrocytes, and the human bloodstream (Khan, 2016). Challenges are associated with identifying effective drugs that can target multiple sites within the host (Khan, 2016; Bosson-vanga et al., 2015). A wide range of clinical manifestations, including asymptomatic infection, uncomplicated, severe, and lethal malaria, have been linked to human malaria (Nosten et al., 2022; Butler et al., 2010). This spectrum of clinical cases depends on the complex interaction between *Plasmodium* species and both the human host and environmental factors (Kahime et al., 2019).

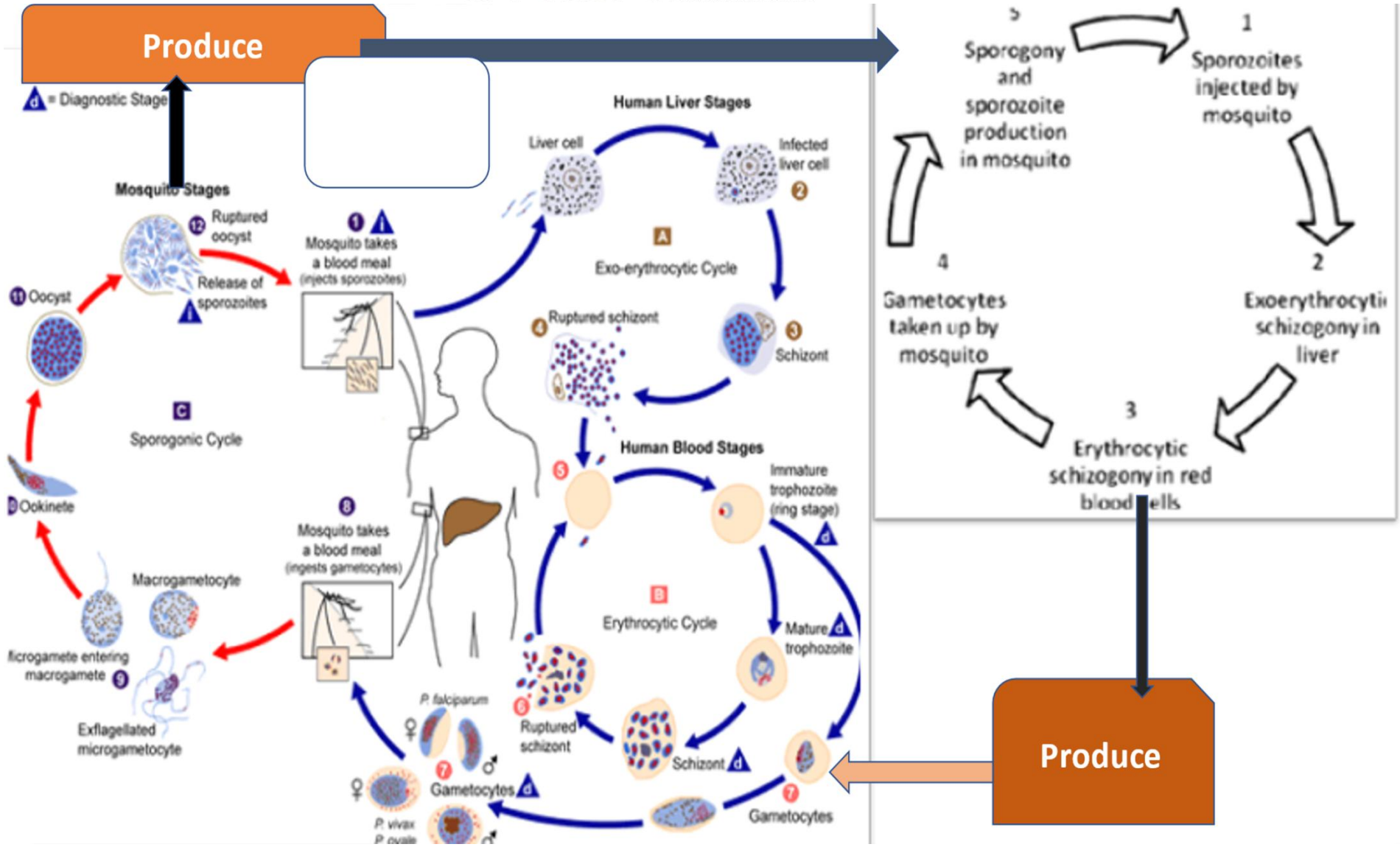

Figure 2.1 Life cycle of malaria parasite based on Cox-singh et al. (2010)

The *Plasmodium* parasite is characterised by a highly complex life cycle which alternates in terms of site (extracellular and intracellular) and type of reproduction (sexual and asexual) (Cox-singh et al., 2010). Female *Anopheles* mosquitoes initiate the life cycle of *Plasmodia* species by injecting infective parasites into the human body and other vertebrate hosts (Cox-singh et al., 2010; Chauhan et al., 2016; Meibalan & Marti, 2017; Rubio et al., 2016). The deposited sporozoites are carried within the bloodstream, passing through a large number of cells, including Kupffer cells, and finally infecting hepatocytes in the liver (Chauhan et al., 2016; Rubio et al., 2016). Sporozoites typically take about 30 minutes to move from the skin through a multitude of cells to hepatocytes (**Figure 2.1**).

For their survival, sporozoites extract nutrients from hepatocytes and develop into replicative schizonts that periodically release several infectious daughter merozoites (Meibalan and Marti, 2017; Cox-singh et al., 2010). Schizonts divide rapidly, forming thousands of merozoites within hepatocytes 5-8 days after their formation (Cox-singh et al., 2010). According to Chauhan et al. (2016), a single sporozoite can give rise to approximately forty thousand infective merozoites in hepatocytes. 2-3 days after merozoite formation, hepatocytes burst, releasing the merozoites into the blood plasma (Matthews et al., 2018). The merozoites infect uninfected red blood cells, and the cycle continues (Matthews et al., 2018; Rubio et al., 2016; Bosson-vanga et al., 2015; Cox-singh et al., 2010). In merozoite-infected erythrocytes, erythrocytic schizogony occurs, allowing the production of around 16 to 32 daughter merozoites from a single infectious merozoite (Chauhan et al., 2016; Alano, 2015). Infection of red blood cells is repeated several times, resulting in the development of malaria (Cox-singh et al., 2010).

Depending on the *Plasmodium* species, merozoites develop into trophozoites and subsequently into schizonts that can harbour up to 32 distinct merozoites in the liver (Matthews et al., 2018). A small percentage, ranging from 0.1% to 5%, of trophozoites develop into gametocytes, which circulate within the blood and are transmitted by female *Anopheles* mosquitoes when blood is sucked from infected human beings (Matthews et al., 2018; Rubio et al., 2016; Bosson-vanga et al., 2015). Gametocyte maturation time differs significantly depending on the *Plasmodium* species; for example, *P. falciparum* gametocytes mature after about 8 to 10 days, whereas *P. vivax* gametocytes take only up to 48 hours to develop and disappear after sexual maturation in 3 days

(Meibalan and Marti, 2017). In the female *Anopheles* mosquito midgut, *P. falciparum* mature gametocytes differentiate into female and male gametes (Meibalan and Marti, 2017; Alano, 2015). A decrease in temperature, a rise in pH, and an increase in the concentration of xanthurenic acid stimulate the differentiation of gametocytes into female and male gametes (Meibalan and Marti, 2017).

Although in most cases, merozoites develop into gametocytes, hepatic *P. ovale* and *P. vivax* remain in the erythrocytes as hypnozoites (Cox-singh et al., 2010). Notably, *Plasmodium* species, *including P. ovale* and *P. vivax*, are difficult to clear from human hosts due to the relapse of hypnozoites that remain in erythrocytes for many months after the initial red blood cell infection (Meibalan and Marti, 2017). The hypnozoites are linked to relapse several weeks after the primary infection (Cox-singh et al., 2010), and these relapses can even occur years after the initial infection (Rubio et al., 2016; Bosson-vanga et al., 2015). The gametocytes of *P. vivax* are microscopically detected in the human host circulation seven days after infection, presenting a serious challenge in *P. vivax* elimination.

A day after its formation, the zygote transforms into ookinetes that burrow into the midgut of mosquito walls and eventually transform into oocysts (**Figure 2.1**) (Meibalan and Marti, 2017; Alano, 2015). The maturation of *P. falciparum* oocysts takes 11–16 days, after which infectious sporozoites are released from these oocysts (Meibalan and Marti, 2017). Generally, ookinetes encyst and form oocysts that divide to produce about 1,000 sporozoites each. The oocysts normally rupture to release sporozoites after approximately 7 days, and these sporozoites move to the salivary glands of malaria vectors for onward transmission to vertebrate hosts during feeding (from blood) (**Figure 2.1**) (Meibalan and Marti, 2017; Alano, 2015; Lee et al., 2014). The chances of host infection during a blood meal are influenced by human, parasite, and mosquito factors (Alano, 2015; Lee et al., 2014). Critically, gametocyte development in humans maintains the transmission of malaria and presents a likely bottleneck in the life cycle of *Plasmodium* species. Based on evidence from literature, understanding gametocyte biology and the infectious stages in human hosts remains key in ablating malaria transmission (Meibalan and Marti, 2017; Lee et al., 2014).

The malaria pathology infection, as well as subsequent clinical symptoms, are asexual erythrocytic stages attributable (Alano, 2015; Lee et al., 2014). Precisely, malaria-related clinical symptoms are

a result of asexual erythrocytic parasites and parasite-derived toxins (Meibalan and Marti, 2017; Alano, 2015; Lee et al., 2014). The release of merozoites is accompanied by the introduction into the bloodstream of Plasmodium-derived toxins such as haemozoin pigment and glycosyl-phosphatidyl-inositol molecules (Rubio et al. 2016). The provocation of immune system cells, such as macrophages, by parasite-derived toxins stimulates the release of pyrogenic-inflammatory mediators and cytokines. An increase in cytokines and inflammatory mediators has a positive feedback effect on thermoregulation and therefore results in an increased body temperature, resulting in febrile malaria-induced episodes (Stanway et al., 2019; Rubio et al., 2016; Lee et al., 2014). On the other hand, the sexual stages of malaria parasites in human hosts are responsible for transmission to female *Anopheles* mosquitoes through the development of gametocytes (Bosson-vanga et al., 2015; Lee et al., 2014). To stop the transmission of *Plasmodium* species, measures should be taken to get rid of the sexual and asexual stages from the human host.

The alignment of traditional plant use with scientific understanding of *Plasmodium* biology facilitates the validation of ethnomedical practices. Many in Africa, Asia, and South America have long used plant-based remedies for the treatment of malaria symptoms. For instance, traditional healers in several African countries have used plants such as *Cryptolepis sanguinolenta*, *Azadirachta indica*, and *Artemisia afra*, which have been shown to be good sources of antimalarial compounds such as cryptolepine, azadirachtin, and flavonoids, respectively (Wright et al., 2010; Clarkson et al., 2004). In the context of rising antimalarial resistance to current antimalarial drugs including artemisinin and its derivatives, the development of new, plant-derived antimalarial drugs becomes critically important (Dondorp et al., 2009; WHO, 2023). As the *Plasmodium* parasite develops resistance mechanisms, particularly in Southeast Asia and parts of Africa, relying only on synthetic compounds becomes increasingly unsustainable. Therefore, a strategy that fuses traditional knowledge systems with modern scientific evaluation of plant-based drugs offers sustainable malaria control and treatment.

2.2 History of malaria and plant-based malaria therapy

Malaria or a malaria-related illness has been documented for over 4,000 years, dating back to 2700 BCE. The Nei Ching and other Chinese medical publications documented the clinical symptoms of malaria (Yin, 2023; Benelli & Beier, 2017). By the beginning of the 4th century, information

regarding the biology of malaria was widespread in Greece, and Hippocrates defined the main symptoms of malaria. In another literature (The Compendium of Susruta), fever was identified as the primary symptom of malaria and was connected to the bites of various insects (CDC, 2017).

In 1880**,** Laveran from France was the pioneer scientist to identify parasites in the circulation of a malaria-suffering individual in Algeria (Kamiura & Scott Smith, 2013). The malaria parasites were studied by Camillo Golgi for the first time in 1886, who established that malaria exists in two distinct forms with different fever episodes. In addition to this, Camillo reported that the two forms of the distinct diseases were known to generate significantly varied quantities of emerging parasites at the maturity stage and that symptoms of fever occurred when red blood cells burst and introduce new parasites into the blood plasma (CDC, 2017).

Raimondo Filetti and Giovanni Batista Grassi were the ones who assigned the names *P. malariae* and *Plasmodium vivax* to parasites infecting and causing malaria in human beings in 1890. During the same time, Laveran had believed that there was one malarial parasite he named *Oscillaria malariae* (Keeling & Rayer, 2015). Laveran's work was revised and reviewed by William H. Welch (an American) in 1897; he named the infectious malaria parasite *P. falciparum* (CDC, 2017). In addition to the named malarial parasites, Stephens J. W. W (1922) gave a detailed account of yet another parasite (*P. ovale)*. After the description of *P. ovale* by John William Watson Stephens (1922), Robert Knowles and Biraj Mohan Das Gupta in 1931 identified another species (*P. knowlesi*), and the effect of this parasite was first documented in 1965 (Okoth, 2017).

The transmission pathway of *Plasmodium* between human beings was detailed by Giovanni Batista Grassi, Giuseppe Bastianelli and Amico Bignami (Singha, 2018; Okoth, 2017). To trace the transmission of malaria parasites, Giovanni Batista Grassi and his group of researchers collected mosquitoes (*Anopheles claviger*) and allowed them to feed on people suffering from malaria. Moreover, these researchers (Giovanni Batista Grassi and company) studied and demonstrated the complete sporogonic cycle of *Plasmodium falciparum, P. vivax*, and *P. malariae*. In 1899, malarial parasite-infected mosquitoes from Rome were allowed to bite two volunteers in London, and both of them developed malaria (CDC, 2017).

The spread and exposure to malaria increased as the British got into tropical regions of Africa, India, and the Caribbean. Malaria was among the most deadly tropical diseases of the eighteenth and early nineteenth centuries. The number of malaria-related deaths and deaths due to tropical diseases resulted in West Africa being nicknamed the "white man's grave". It was difficult to deal with malaria in the eighteenth and early nineteenth centuries since acute malarial fevers often resulted in a weakened and compromised immune system (CDC, 2017).

Treatment of malaria in China was based on the Qinghao plant (*Artemisia annua*), which was discovered during the 2nd century BCE and identified as the most important malaria treatment. Fifty-two remedies were also discovered in the Mawangdui Tomb. In the United States, the Qinghao plant (*Artemisia annua*) was recognised as annual or sweet wormwood (Yin, 2023; Benelli & Beier, 2017). In 340 CE, the bioactivity of Qinghao plant (*Artemisia annua*) extracts as anti-fever agents was first reported by Ge Hong. The bioactive compound artemisinin was isolated for the first time by researchers from China in 1971 (Keeling & Rayner, 2015). In the 17th Century, missionaries from Spain acquired information from the indigenous people of India about the medicinal plant barks that were used to treat fevers (Kamimura et al., 2018). The medicinal plant barks were then used to treat and cure fevers experienced by the Countess of Chinchón, wife of the Viceroy of Peru. Based on the geographical location of this bark, it was named Peruvian bark, and the tree was called Cinchona, named after the countess. The medicinal extract from Cinchona bark was the first known source of quinine (an antimalarial bioactive compound). Artemisinins from the Chinese Qinghao plant and quinine from the Peruvian bark are the most effective antimalarial drugs available (Kamimura et al., 2018; Keeling & Rayner, 2015).

In the 15th century, Juan Fragoso and Nicolas Monardes published information on Cinchona, and the malaria treatment in this case was widely valued by the South Americans. According to historical records, South American Indians used cinchona beverages to cure fevers and referred to these drinks as quinas. Additionally, Calancha of Lima (Peru) stated that quina (bark) powder was used to treat fevers and tertians (Yin, 2023; Benelli & Beier, 2017). In 1643, a new fever remedy (Jesuit's bark) was described in European medical literature and was named after the Jesuits who took and spread cinchona from Amazonia.

Cinchona bark was first used prophylactically in the late 17th century by Francesco Torti. Francesco Torti insisted on using heavy dosages of powdered bark immediately and multiple times when symptoms of malarial fever appeared (CDC, 2017). Pelletier and Caventou pioneered the extraction and separation of quinine from alkaloids in cinchona in 1820 (French chemist-pharmacists). The extraction of quinine from cinchona alkaloids led to a paradigm shift in malarial therapy towards single plant-derived compounds as therapies (CDC, 2017).

Quinine is a recognised active chemical derived from cinchona that is beneficial against malaria. Experiments on Cinchona officinalis bark led to the discovery of seven alkaloids, all known to have antimalarial activity (Keeling & Rayner, 2015). The cinchona alkaloids identified by the United States military during World War II contained 7 – 12% anhydrous quinine and 70 – 80% total anhydrous crystallisable cinchona alkaloids (CDC, 2017). Research conducted during World War II determined that quinine is an effective medicine for treating acute malaria, although it has significant adverse effects (nausea and blurred vision). In addition to quinine, two less hazardous bioactive compounds derived from cinchona have been identified: (1) cinchonine and (2) cinchonidine (Yin, 2023; Benelli & Beier, 2017). In other trials, a combination of quinine, quinidine, and cinchonine was found to be more effective against *P. falciparum*.

Studying the history of using plants to manage malaria opens up new pathways for discovering antimalarial drugs. Additionally, researching phytochemicals against malaria helps preserve valuable ecological and cultural resources, supports culturally sensitive healthcare, and combats drug resistance. Overall, integrating the historical use of plants in treating malaria with modern science is crucial for advancing both biomedical research and global health.

2.3 Distribution of malaria in Africa

In the African region, over 67% of deaths due to malaria were among those under the age of 5 years (WHO, 2021). In 2020, it was noted that the African region accounted for over 96% and 95% of all malaria-related deaths and malaria cases, respectively (WHO, 2021). Up to 50% of malaria deaths worldwide are accounted for in five African countries: Tanzania, Nigeria, the DRC, and Mozambique (Okokon et al., 2010). It was reported that only 15 countries in the WHO African region made significant progress or achieved the target of reducing malaria incidences by a

minimum of 40% (African Union, 2021). The countries that reduced incidences of malaria by 40% include Ethiopia, Ghana, Cabo Verde, Mauritania, and Gambia. However, the countries that have made significant progress include Eswatini, Togo, Rwanda, Kenya, and Equatorial Guinea. Notably, only two African countries recorded zero malaria-related deaths since 2018, and these are Cabo Verde and São Tomé & Príncipe (African Union, 2021; WHO, 2021). The WHO African region recorded 405,000 malaria-related deaths and up to 28 million cases of malaria in 2018, translating to 94% and 93% of global malaria deaths and cases, respectively (WHO, 2019).

Though there was a significant decrease in malaria incidences (by 17%) and death rates (by approximately 26%) in the African region between 2000 and 2013, in some African countries, the progress in the fight against malaria has significantly stalled, and in some cases reversed since 2014 (Mbacham et al., 2019). In 2017 alone, the WHO African region contributed to 92% of global malaria deaths and cases. Generally, a drop in malaria cases and deaths of 38% and 67%, respectively, was reported between 2000 and 2019 (WHO, 2020). However, in 2020, malaria case incidence sharply increased due to disruption in the fight against malaria in the wake of COVID-19 (WHO, 2021).

The number of malaria cases increased from 227 million to 241 million in the period between 2019 and 2020. However, a drop in malaria-related deaths (by 44%) was reported between 2000 and 2019, associated with a significant decrease in the death rate by 67% in the same period (WHO, 2021). According to Balakrishnan (2022), the contribution of African countries to global malaria is significantly high (Figure 2.2). For example, Nigeria, Mozambique, the DRC, and Tanzania contribute up to 53% of global malaria deaths. Contrary to this, some African countries have made significant progress in the fight against malaria. For instance, in 2019, Algeria was certified as malaria-free (Figure 2.2).

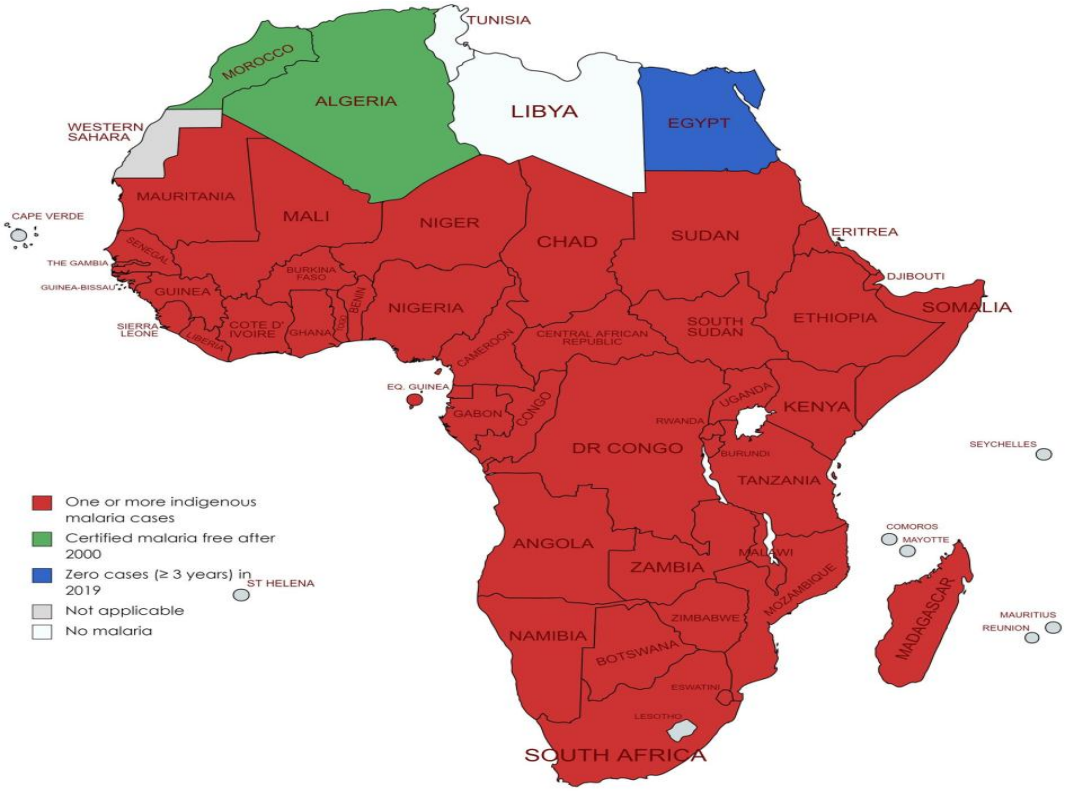

Figure 2.2 African countries: malaria cases distribution and current status in 2019 (Adapted from WHO database) (WHO, 2020).

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Although COVID-19 disrupted malaria control interventions in 2021, significant progress was made in the fight against malaria in the African Region during this period. For instance, in 2021, malaria-related deaths dropped slightly to 593,000 from 599,000 reported in 2020 (African Union, 2021). In countries such as Cabo Verde, malaria cases have been reduced to zero since 2018, and in several African countries (Ghana, Zimbabwe, Mauritania, Togo, South Africa, Ethiopia, and Sierra Leone), significant declines in malaria cases have been noted (WHO, 2022b). Notably, the drop in malaria cases was very high in three countries (Mauritania, Zimbabwe, and Ethiopia), with a percentage decrease between 68 and 71. Moreover, the number of malaria-related deaths decreased significantly in South Africa, Ethiopia, and Togo, with the highest decrease recorded in Ethiopia (68%) (WHO, 2022b). However, in most malaria-burdened African countries, the

decrease in malaria deaths and cases has stalled. In 2021, two countries in Africa (DRC and Nigeria) contributed 47% and 41% of malaria deaths and cases, respectively (WHO, 2021).

In 2018, the "High Burden to High Impact" (HBHI) initiative was launched to reduce the malaria burden in most malaria-burdened African countries, including Niger, DRC, Uganda, Burkina Faso, Nigeria, Mozambique, and Ghana (WHO, 2019). It has been shown that in the HBHI countries, malaria cases increased in the period from 2015 to 2021. The most impacted countries were DRC, Nigeria, and Uganda, as indicated by a 36%, 19%, and 25% increase in malaria-related deaths between 2015 and 2021, respectively (African Union, 2021; WHO, 2022). The only African countries that were able to meet the set GTS targets (2020) were Cabo Verde, Zimbabwe, Algeria, Ethiopia, and South Africa (WHO, 2022a). Although the HBHI was meant for ten highly malaria-burdened countries, small population countries with high malaria incidences such as Burundi, Somalia, and Guinea implemented HBHI interventions (WHO, 2019).

Understanding the distribution of malaria in Africa is crucial for guiding scientific research, clinical use of antimalarial phytochemicals, and conservation of antimalarial plants. Studying malaria prevalence allows public health experts and researchers to document identified antimalarial plants traditionally used in regions with high malaria incidence, aligning indigenous knowledge systems with the malaria burden. In summary, the study of malaria distribution in Africa, combined with research into the traditional use of antimalarial plants, provides a comprehensive framework for conserving, discovering, and using plant-based antimalarial remedies. This ensures that plant-based antimalarial drugs are culturally and geographically relevant, ecologically sustainable, and scientifically informed. The information on the distribution of malaria in Africa and the utilisation of plants in malaria treatment is particularly important in African settings, where both medicinal plant diversity and malaria prevalence are highly concentrated and interdependent.

2.4 Prevalence and contributing factors of malaria in Zimbabwe

In 2018, the World Health Organisation (WHO) reported that out of the 14 million Zimbabweans, 4 million were vulnerable to malaria and up to 1 million malaria cases were confirmed (Gwitira et al., 2020). Zimbabwe has 10 provinces, subdivided into sixty-five districts, of which 63 are rural. Malaria districts account for approximately 75% of the 65 districts, with 64% classified as high

malaria-burdened districts (National Statistical Agency, 2012). Malaria prevalence in Zimbabwe is affected by seasonal and geographical variations directly linked to rainfall patterns (Malaria Operational Plan FY, 2018; Sande et al., 2016). Zimbabwe's districts are separated into two zones characterised by low and heavy rainfall. Malaria transmission is lower in low rainfall areas such as parts of Matabeleland South Province (less than 700 mm) and higher in high rainfall areas such as Manicaland Province (more than 1,500 mm) (Malaria Operational Plan FY, 2018; Coleman et al., 2009). The prevalence of malaria in Zimbabwe is highest among children under the age of five, pregnant women, and people with weakened immune systems (Chapu & Mgocheki, 2017; Mosha et al., 2014). Generally, malaria transmission in Zimbabwe is seasonal and directly linked to the geographic variation in rainfall patterns and topology (Manyangadze et al., 2017). In line with this transmission pattern, nearly 80% of annual malaria cases are confined to the northern and eastern provinces of Zimbabwe. The most impacted provinces are Mashonaland Central, Mashonaland North, and Manicaland, which amount to 30% of the country's total number of provinces (Malaria Initiative Plan FY, 2020).

Malaria is a serious disease that affects individuals of all ages in Zimbabwe. According to a 2016 WHO report, 79% of Zimbabweans are susceptible to malaria. Additionally, in the same year, the number of reported malaria cases at the eastern borders of Zimbabwe was significantly high and contributed up to 82% of all recorded malaria cases (Malaria Initiative Plan FY, 2018). The malaria incidence was reported to be 139 per 1000 people in 2013. However, between 2012 and 2014, there was a significant increase in malaria-related fatalities (from 6.1% to 13.8%) (Malaria Operational Plan FY, 2018). Malaria-related mortality is reported year-round, even though disease transmission occurs mostly between November and April (Malaria Operational Plan FY, 2020). The annual number of malaria cases documented changes between 300,000 and 500,000, with a notable continuous decreasing trend recently (**Figure 2.5**). This success prompted a shift in emphasis from malaria control to elimination in several provinces, including Midlands and Matabeleland, coupled with the launch of pre-elimination of malaria in selected regions (Sande et al., 2017). However, there seems to be insufficient and inconclusive evidence that malaria incidences have substantially dropped. In 2019, for example, nearly 310,000 malaria cases (22 per 1000) were recorded across the country (Malaria Initiative Plan FY, 2020). This was a 19% rise in reported cases from the previous year, 2018, and this was associated with an increase in malaria deaths by up to 12.8%

from 2018 to 2019 (Malaria Operational Plan FY, 2022) (Figure 2.3). Additionally, in 2020, malaria occurrences in the country were abnormally high, with 135,585 recorded cases and 131 malaria-related fatalities as of April 2020, primarily from four provinces: Matabeleland South, Manicaland, Mashonaland East, and Masvingo (Malaria Operational Plan FY, 2022). Given the resources allocated to malaria control and eradication, as well as the Zimbabwean economy and policies, it is vital to assess whether malaria pre-elimination is realistic.

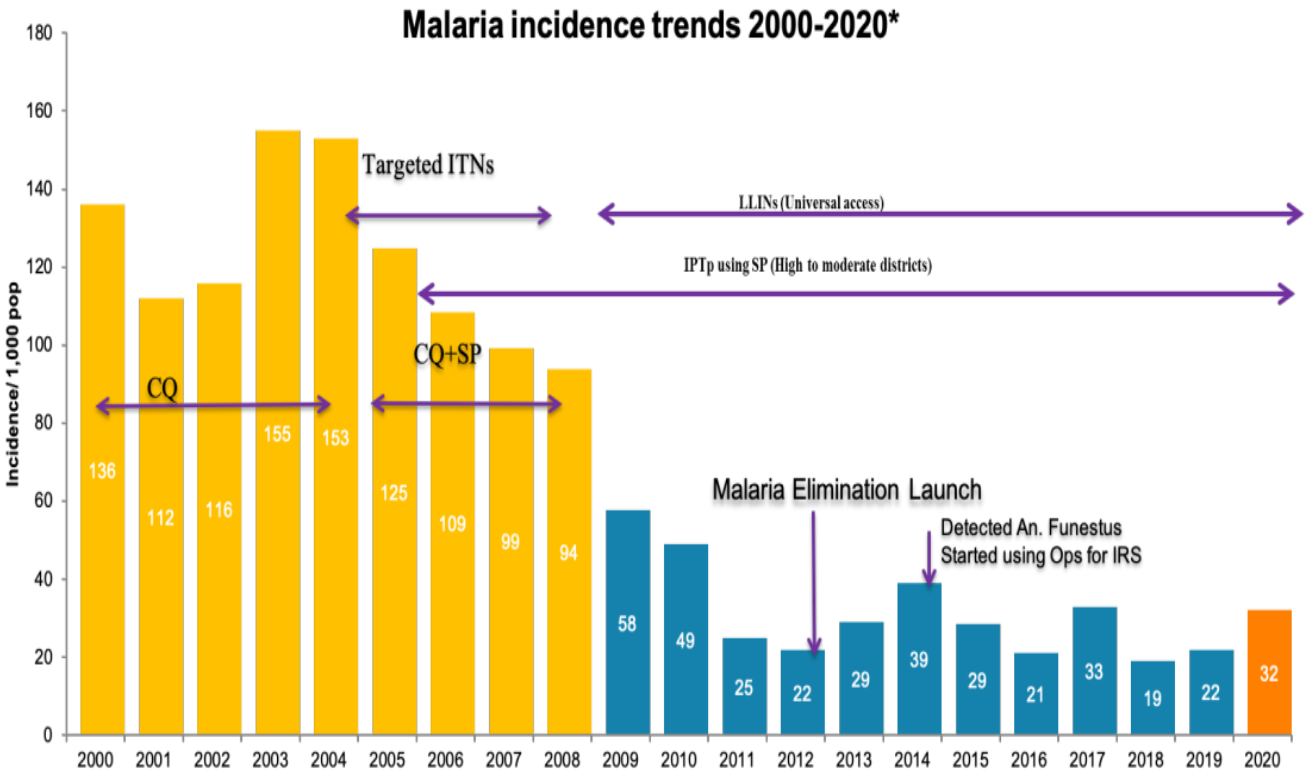


Figure 2.3 Malaria incidence trends from 2000 to 2020 in Zimbabwe and associated malaria control interventions.

Source: Zimbabwe District Health Information System 2 (Malaria Operational Plan FY, 2022). (***No Copyright:*** *You can copy, modify, distribute and perform the work*).

Malaria is an endemic disease in Zimbabwe and ranks among the top five causes of illness and death in the country. The Ministry of Health and Child Care (MOHCC) manages malaria through the National Malaria Control Program (NMCP), which implements various interventions such as case management, malaria vector control strategies, and behavioural modification communication

(Malaria Operational Plan FY, 2018). The transmission of malaria in Zimbabwe varies seasonally and geographically, and is closely linked to environmental factors like altitude and rainfall patterns in the country (Mundagowa & Chimberengwa, 2020). Districts located from the northwestern to the south-eastern boundaries of the nation are considered high malaria transmission zones, while districts in the central and south-western parts of the country have very low or no reported cases of malaria transmission (Figure 2.4) (Malaria Operational Plan FY, 2018). Malaria transmission is most prevalent during the rainy season, with temperatures ranging from 18 to 30 degrees Celsius. *Plasmodium falciparum* is the most common malaria pathogen, responsible for 98% of all confirmed malaria cases in Zimbabwe (Malaria Operational Plan FY, 2020). In areas heavily affected by malaria, transmission occurs throughout the year, with a peak in cases between November and April, during the rainy season.

Over the past 13 years, the Zimbabwe NMCP and the Roll Back Malaria Programme (RBMP) have made significant progress in the fight against malaria, leading to a shift in focus from prevention and control to eradication (Sande et al., 2017). These programs, managed centrally in Harare, are overseen by provincial and disease control managers who gather data from district health care teams. These teams work to coordinate malaria case management, monitoring, and epidemic response at the local level (Malaria Operational Plan FY, 2018). The Zimbabwean 2016–2020 National Malaria Strategic Plan directs the use of IRS in regions with an annual parasite index (API) of 5 or higher per 1000 inhabitants. Long-lasting insecticidal nets (LLINs) are distributed in areas with an API of 2–4 per 1000 people, and IRS is carried out annually as a routine strategy to contain and reduce malaria outbreaks (Malaria Operational Plan FY, 2022). The NMCP employs a dual approach to vector control, using both LLINs and IRS in malaria-endemic regions. In zones with moderate to high incidence, 51% of households have at least one ITN per two household members, and 85% have at least one ITN and are sometimes protected by IRS, indicating high levels of protection (Malaria Operational Plan FY, 2022).

Despite significant progress towards malaria eradication, periodic outbreaks continue to occur. For instance, Beitbridge District experienced a second malaria epidemic in 2017. Malaria eradication efforts face challenges from insecticide and anti-malarial drug resistance, varying vector behaviour, and the colonisation of new regions by malaria vectors (Gunda et al., 2016). Factors

such as changing climate, inconsistent funding, economic crises, political disruptions, and increased cross-border human migrations have all hindered malaria control programs, contributing to intermittent malaria epidemics across Zimbabwe (Mundagowa & Chimberengwa, 2020).

Table 2.1: Distribution of parasitologically confirmed malaria cases in Zimbabwe.

Province	Malaria cases	Percentage contribution
Manicaland	110 485	39
Mashonaland east	68 175	24
Mashonaland central	52 401	19
Other provinces	49 781	18

Source: Zimbabwe District Health Information System 2 (Malaria Operational Plan FY, 2018).

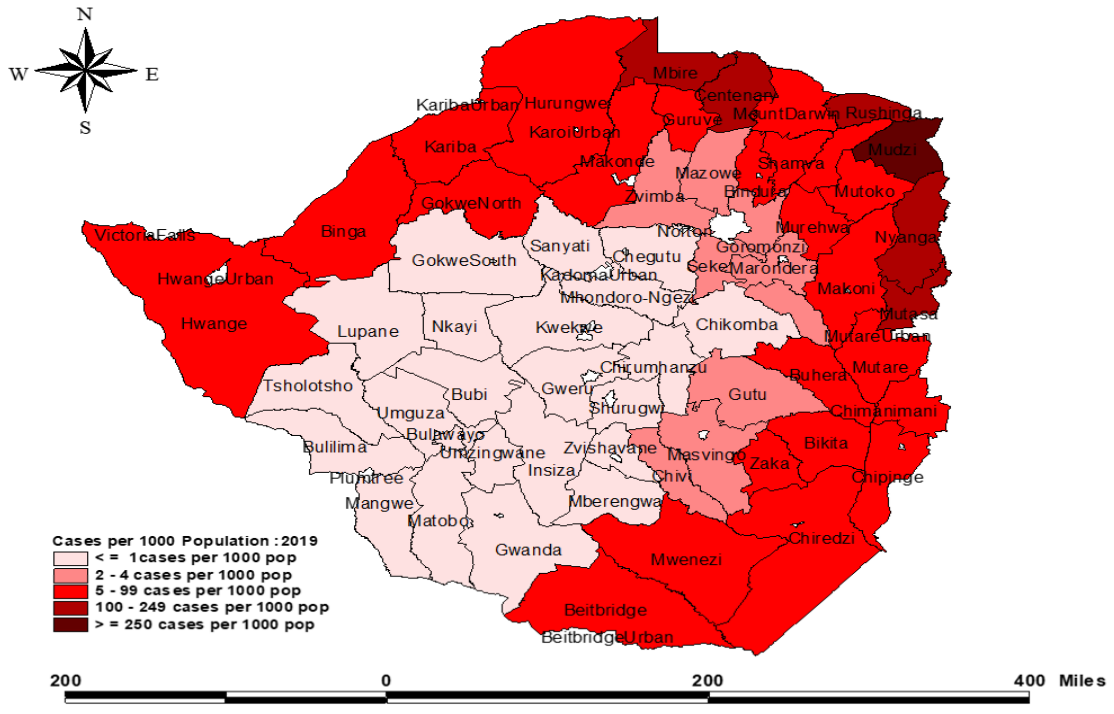


Figure 2.4 Malaria cases distribution in Zimbabwe.

Source: https://www.cdc.gov/globalhealth/countries/zimbabwe (***No Copyright:*** *You can copy, modify, distribute and perform the work*)

The study of malaria prevalence and its contributing factors in Zimbabwe is important for guiding the utilisation of plant-based antimalarial treatments and research. Although Zimbabwe is not among the highly burdened countries in sub-Saharan Africa, malaria remains a serious public health concern, particularly in low-lying and border regions such as Mashonaland Central, Manicaland, and Masvingo provinces (Ministry of Health and Child Care [MoHCC], 2022). Therefore, understanding the seasonal and spatial malaria prevalence, along with environmental and behavioral factors that contribute to its transmission, provides a basis for identifying and applying traditional plant-based remedies effectively and sustainably.

2.5 Malaria control interventions and their accessibility

2.5.1 Malaria control interventions

Malaria control interventions are summarised in **Figure 2.5**

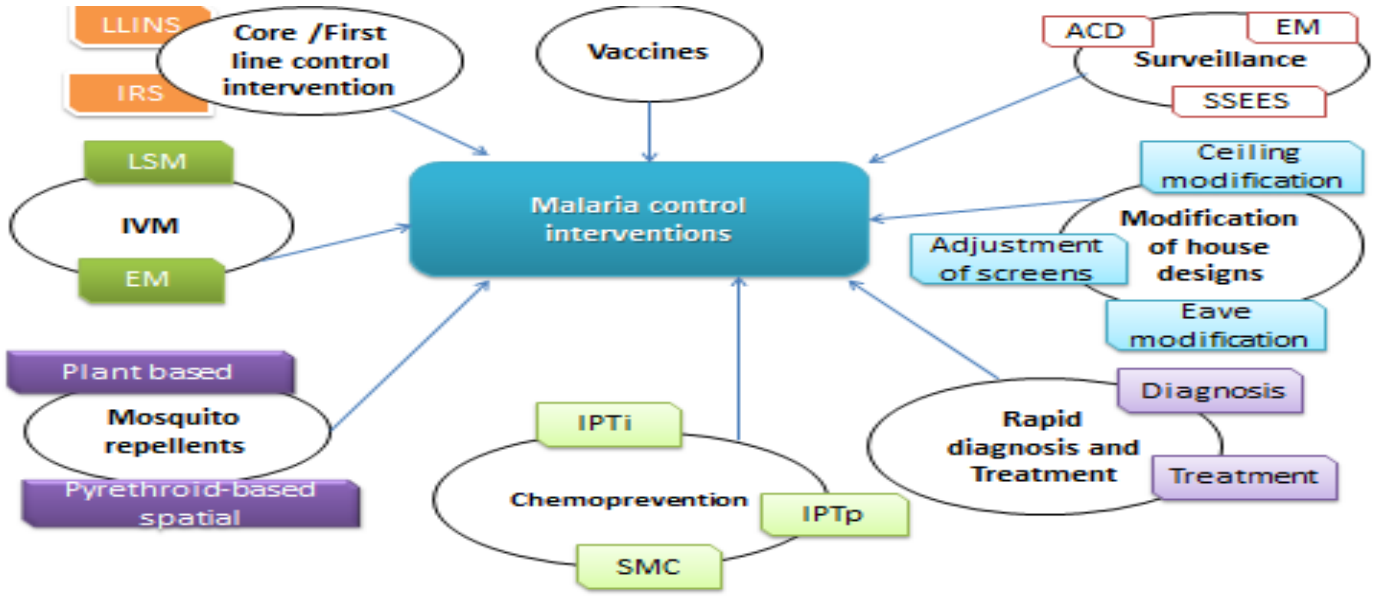


Figure 2. 5 Global malaria control interventions.

There are eight (8) malaria control interventions summarized in figure 2.4: (1) Core or first line control interventions (LLINS (Long-lasting insecticidal nets) and IRS (Indoor residual spraying)), (2) Vaccines, (3) IVM- integrated vector management (LSM (Larval source management) and EM (Environmental management)), (4) Mosquito repellents (Plant-based and essential oil repellents

and Pyrethroid-based spatial), (5) Chemoprevention (IPTi (Intermittent preventive treatment for infants), IPTp (Intermittent preventive treatment for pregnant women), SMC(seasonal malaria chemoprevention)), (6) Rapid Diagnosis and Treatment (Diagnosis and Treatment), (7) Modification of house designs (Ceiling and Eave modification, Adjustment of screens), (8) Surveillance (ACD (Active case detection), EM (Entomological surveillance) and SSEES (Surveillance systems for epidemics)).

2.5.2 Malaria control interventions and their accessibility in Africa

Most national malaria control interventions have advocated for universal accessibility of ITNs (WHO, 2017). Between 2011 and 2016, over 800 million ITNs were distributed across sub-Saharan Africa (Olapeju et al., 2018). This distribution led to an increase in the number of individuals sleeping under ITNs from 30% to 54% between 2010 and 2016. Notably, between 67% and 73% of the 663 million cases reported between 2000 and 2015 were prevented by ITNs in sub-Saharan Africa (World Health Organisation (WHO), 2017). There has been an expansion in the provision of ITNs in sub-Saharan Africa between 2000 and 2016. However, a slight decline in ITN coverage since 2017 was reported in Africa, with 65% and 43% of households owning one or more ITNs, and children under 5 years sleeping under ITNs in 2020, respectively (Balakrishnan, 2022). Although IRS and LLINs have been adopted in the fight against malaria in Africa, the coverage of these interventions is still below set targets (Allcock et al., 2018; Singh & Brown, 2013). It has been reported that the accessibility of IRS and LLINs has been hampered due to prohibitive costs in instances where distribution programs are not free (Ahorlu et al., 2019; Asingizwe et al., 2019) and a lack of availability (Allcock et al., 2018; Sangaré et al., 2012). However, adoption and uptake of IRS and LLINs have been limited by a lack of awareness campaigns on the role of LLINs in malaria prevention (Berthe et al., 2019; Diema et al., 2017), and side effects such as skin irritation and residual effects of insecticides (Magaço et al., 2019). Alternative uses of LLINs as curtains, fishing nets, and seedling protectors (Asingizwe et al., 2019) have been reported in Africa.

In Africa, ITNs have been attributed to a 68% decline in the prevalence of *Plasmodium falciparum* between 2000 and 2015 (Slater et al., 2020). Besides ITNs, seasonal malaria chemoprevention (SMC) and intermittent preventive treatment in pregnancy (IPTp) have been linked to a significant reduction in morbidity in African countries (Foy et al., 2023). For example, SMC has been applied in West Africa and has effectively reduced malaria morbidity and incidence by about 70% (WHO, 2022). Besides the common malaria control interventions such as IRS and LLINs, other methods

involving house modifications (screens, ceilings, and eaves) (Getawen et al., 2018; Rek et al., 2018; Snetselaar et al., 2017; Tusting et al., 2017), use of repellents (essential oils, spatial, and plants such as Lantana camara, Azadirachta indica, and Cymbopogon citratus) (Masalu et al., 2020; Tesfahuneygn, 2019), and the application of integrated vector management (environmental and larval source management) have been applied in Africa (Nalinya et al., 2022; Tusting et al., 2017).

In Southern African countries, including Zimbabwe, South Africa, and Eswatini, mortality due to malaria was significantly reduced by dichlorodiphenyltrichloroethane (DDT)-based indoor residual spraying (IRS) (Mabaso et al., 2004). The application of DDT-based IRS in Southern African countries started in the 1940s and was meant to control Anopheles funestus and the Anopheles gambiae complex (Nkya et al., 2022). Sub-Saharan Africa saw a 50% reduction in malaria cases and a 40% reduction in clinical disease from 2000 to 2015, primarily due to the extensive implementation of malaria prevention strategies, including indoor residual spraying (IRS) and insecticidal-treated nets (ITNs) (Oladipo et al., 2022). Apart from these vector control interventions, preventive malaria treatments (seasonal malaria chemoprevention (SMC) for under-five children, intermittent preventive treatment of infants (IPTi), and intermittent preventive treatment of pregnant women (IPTp)), treatment of malaria using Artemisinin combination therapy (ACT), case management, and the use of Mosquirix (malaria vaccine) have significantly reduced the impact of malaria in the sub-Saharan African region (Kesteman & Randrianarivelojosia, 2017; Pryce & Richardson, 2018). By 2020, up to 2.2 billion LLINs were distributed worldwide (Kesteman et al., 2017), and by 2015, approximately 67% of the sub-Saharan population had access to LLINs. In some instances, it was noted that LLINs were used for purposes other than malaria vector control. For example, in Tanzania, Mozambique, Nigeria, Rwanda, Uganda, and Cameroon, LLINs were used for fishing (Moon et al., 2016; Russell et al., 2015).

IRS has reduced the number of malaria cases by over 80% in Southern African countries (Yukich et al., 2022). However, the application of IRS in sub-Saharan Africa has recently been reduced, as reported in Senegal, Tanzania, Madagascar, and Zambia, where IRS application was reduced by 64%, 68%, 56%, and 63%, respectively (Oladipo et al., 2022). However, the implementation of preventive chemotherapy in Africa has increased significantly. For instance, in 2018, IPTp coverage increased in Madagascar, Nigeria, DR Congo, Madagascar, and Nigeria by 23%, 23.5%,

9.3%, 23.1%, and 22.5%, respectively (González et al., 2023). The implementation and universal accessibility of all these malaria control interventions are linked to investments towards malaria control. For example, up to $2.7 billion was made available for the fight against malaria in 2018, against the $8.1 billion required for universal access to malaria control interventions (WHO, 2019).

2.5.3 Malaria control interventions and their accessibility in Zimbabwe

The National Malaria Control Programme in Zimbabwe utilises vector control methods such as ITNs and IRS in districts heavily burdened by malaria (Gavi et al., 2021). In areas with moderate to high transmission rates, half of the households have at least one ITN for every two members, and 85% of households are protected by at least one ITN and IRS, providing a significant level of protection (Dube et al., 2016). For example, in Mutasa District, Manicaland Province, IRS and ITNs have been used as vector control strategies for 6 and 2 decades, respectively (Mharakurwa et al., 2021; Mharakurwa et al., 2013). In 2014, around 92% of the population in Mutasa District was covered by IRS (Sande et al., 2016), while access to ITNs was limited during that time. Between 2012 and 2017, it was observed that 70% of households had at least one ITN (Kanyangarara et al., 2018). Additionally, in districts where *An. Arabiensis*, *An. gambiae* complex, and *An. quadriannulatus*, are confirmed malaria vectors, IRS or Long-Lasting Insecticidal Nets (LLINs) have been implemented (Masendu et al., 2005; Sande et al., 2015). Malaria vectors identified in Zimbabwe include *Anopheles parensis*, *Anopheles leesoni, Anopheles confusus, An. Funestus*, and *Anopheles aruni* (Sande et al., 2016). *An. Funestus* was recognised as a problematic vector in Chiredzi and Mutasa Districts (Sande et al., 2015; Lukwa et al., 2014), although its distribution in Chiredzi District was limited due to IRS application (Masendu et al., 2005).

According to Kanyangarara et al. (2018), approximately 60.3% of households in Zimbabwe have one ITN for every two people, indicating a need to improve access, especially in malaria-burdened districts. Furthermore, the IRS was found to protect over 80% of the vulnerable population in Mutasa and Mutare Districts. IRS methods using Benzene and DDT have been in use since the 1940s (Pates & Curtis, 2005; Mabaso et al., 2004) and remain key interventions in malaria control in Zimbabwe. Notably, the implementation of the IRS in Zimbabwe aligns with the World Health Organisation's recommendation of achieving over 80% population coverage (Sande et al., 2016).

In Zimbabwe, IRS and ITNs are deployed in districts or areas with an Annual Parasite Index (API) of 5 and 2-4 per 1000 population, respectively (President's Malaria Initiative FY, 2020).

2.6 Indigenous practices and medical pluralism in the control and treatment of malaria in Zimbabwe

2.6.1 Indigenous practices

Several studies have outlined the traditional malaria-fighting strategies employed in Zimbabwe (Chapu & Mgocheki, 2017; Kazembe et al., 2012; Macherera et al., 2017; Mavhurume, 2016; Dhewa, 2008; Makundi et al., 2006). Traditional medicine is defined by the WHO as knowledge, techniques, and abilities based on concepts, values, and traditions unique to many societies that are used to improve health and avoid, detect, relieve, or cure physical and mental diseases (WHO, 2013). In Zimbabwe, traditional health care practices include herbal, wildlife, and mineral treatments, massage, mysticism, and other culturally distinct activities. Some of the indigenous practices against malaria are based on early warning and detection, and forecasting (Macherera et al., 2017). These malaria prevention methods mainly rely on weather variables such as rainfall and their possible impact on malaria outbreaks and transmission. According to Mabaso et al. (2006), the high yearly malaria incidence was accompanied by heavy rains in the period between 1988 and 1999. Besides rainfall, temperature was identified as one of the predictors of malaria prevalence and transmission. However, the uptake and implementation of these indigenous practices have been limited, and more emphasis has been given to scientific issues (Macherera et al., 2017).

In Zimbabwe, individuals from malaria hotspots have the potential to utilise early traditional warnings of malaria to prepare for outbreaks (Macherera et al., 2017). However, according to Soropa et al. (2015), indigenous knowledge systems are not only meant for local decision-making but also for researchers, planners, and managers to develop strategies that improve communities facing climate change and infectious diseases like malaria. It has been argued that while tools such as remote sensing, forecast warnings, and geographic information systems are crucial for predicting climate and malaria vulnerability, indigenous knowledge systems are simple, accurate, and more applicable in local communities (Glantz, 2009). Given that indigenous knowledge systems have evolved over a long period (Eyong, 2007), it is critical to integrate these systems with

scientific surveillance and warning systems to reduce vulnerability in rural communities to infectious diseases such as malaria and climate change (Jiri et al., 2016).

The application of indigenous knowledge systems in predicting rainfall (a predictor of malaria prevalence) in Zimbabwe has been revealed in several studies (Soropa et al., 2015; Risiro et al., 2012). The World Health Organisation has outlined potential malaria detection indicators and early warning systems (Thomson & Connor, 2001). In line with this, a study was conducted in Gwanda district, Matabeleland South, Zimbabwe, to assess potential malaria detection indicators and early warning systems used in communities (Macherera et al., 2017).

2.6.2 Medical pluralism and indigenous health practices in Zimbabwe and beyond

Medical pluralism, as described by Gabe et al. (2004), is a practice where different medical systems are adopted based on health beliefs and their ability to treat illnesses. Additionally, medical pluralism involves the integration of alternative and conventional biomedicines for a healthy society (Amzat & Razum, 2014). While indigenous practices have been combined with conventional biomedicines in treating and preventing malaria in Zimbabwe, there is limited published work indicating the direct application of medical pluralism in malaria prevention and treatment in Zimbabwe. The evidence of medical pluralism presented focuses on other diseases in Zimbabwe and beyond (Choguya, 2015; Kajawu et al., 2016; Mandizadza & Mandizadza, 2017; Moshabela et al., 2017; Robbins et al., 2021; Subedi, 2019; Wuthrich Grossenbacher et al., 2021).

Although both conventional and alternative medicines are applied in most societies, there is still reluctance in mainstreaming alternative/traditional medicine in national healthcare systems. For example, in Zimbabwe, traditional birth attendants, empowered to manage pregnancies and perform deliveries based on socio-cultural values and tradition, are excluded due to ambivalent government policies, leaving them at the periphery of healthcare provision (Choguya, 2015). In Tharus, Nepal, the link between medical pluralism or the use of indigenous medicine and state health policy was evaluated (Subedi, 2019). While the application of folk medicine, biomedicine, and scholarly traditional medicine is common, biomedicine is officially preferred. Medical pluralism in Nepal has been expanded by integrating scholarly traditional medicine into the mainstream healthcare system. Unfortunately, most indigenous medicines are outside state

regulation, and the legitimacy of indigenous care providers (healers) is still questioned (Subedi, 2019).

This practice of medical pluralism has been applied in many countries (Priya, 2012). For example, in Zimbabwe, Uganda, Tanzania, Kenya, South Africa, and Malawi, a study was conducted to establish medical pluralism among people living with HIV, revealing that medical pluralism was common, with people living with HIV using therapeutics from the biomedical health, traditional, and faith-based worlds (Moshabela et al., 2017). According to Grossenbacher et al. (2021), spiritual (religious) ontologies should be integrated into mainstream healthcare services and programs. However, there is a need to strengthen these ontologies to prevent compromised therapy and holistically support people living with HIV. In another study describing pathways to primary care for patients with common mental disorders in Harare, Zimbabwe, it was revealed that most patients sought help from biomedical and traditional care providers. While patients preferred biomedical care providers, in cases of treatment failure, traditional care providers were consulted (Patel et al., 1997).

In most societies, including Zimbabwe, people typically use a diverse range of therapeutic options, where one option can be used as a complement or an alternative. In a study conducted in Zimbabwe, Uganda, Tanzania, Kenya, South Africa, and Malawi, evidence was found of mixing therapeutics from faith-based, traditional, and biomedical healthcare providers. However, it was shown that medical pluralism in this case introduces mistrust among faith-based, traditional, and biomedical healthcare providers, delaying proper care for people living with HIV (Moshabela et al., 2017). In a study examining African Traditional Medical practices on mental disorders, it was reported that herbalists were the most preferred caregivers. African Traditional Medical practices, in this case, provided treatment in congruence with the cultural beliefs of patients, which was not true for biomedicine (Kajawu et al., 2016).

More often, therapeutics from different traditions can be combined to treat certain ailments and meet primary healthcare needs (Kalpagam, 2012; Bode & Hariramamurthi, 2014). For instance, cancer patients from Zimbabwe seek treatment from traditional health practitioners who use indigenous knowledge systems based on economic, cultural, and affective perspectives

(Mandizadza & Mandizadza, 2017). In a separate study in South Africa, most Traditional Health Practitioners are trained sangomas (traditional healers) and prophets (faith healers). While this reflects a pluralistic approach, it is not applicable across all societies, as some churches disregard traditional beliefs. Traditional Health Practitioners typically practice medical pluralism by combining traditional practices and Western medicines. Moreover, the use of therapeutics from different traditions has been demonstrated through a cross-cultural comparative study on pre-eclampsia in Zimbabwe, Haiti, and Ethiopia, concluding that apart from conventional biomedicine, traditional and faith caregivers should be engaged to move towards life-saving care and pre-eclampsia elimination (Robbins et al., 2021).

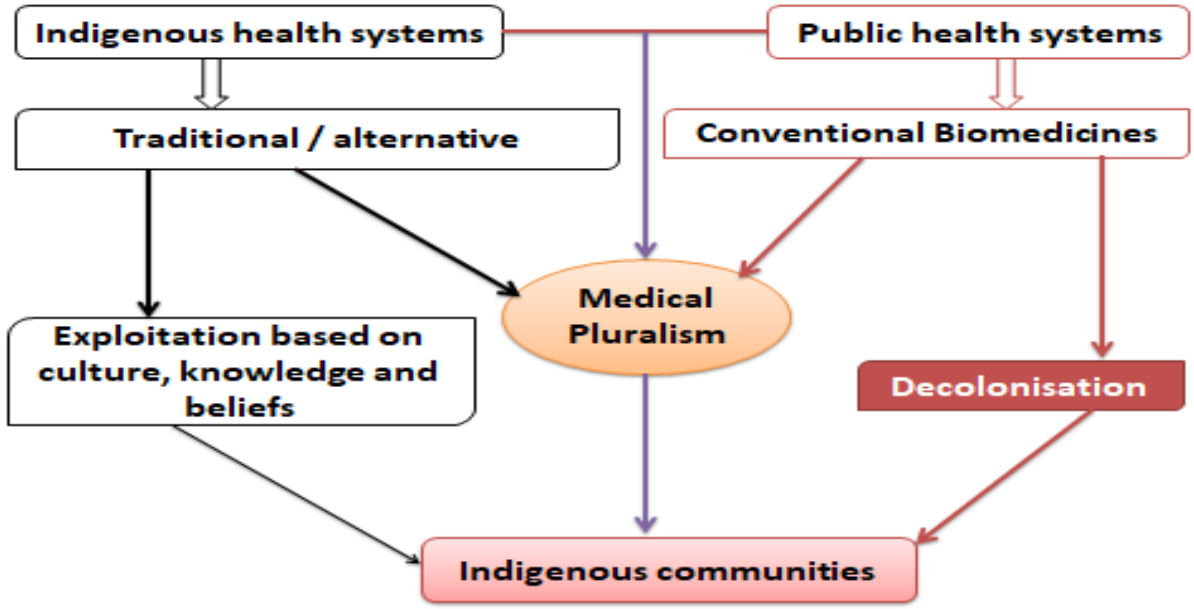


Figure 2. 6 Conceptual framework of the interaction of medical pluralism, indigenous health practices and decolonisation of public health.

2.7 Use of plants in the treatment of malaria in Zimbabwe

2.7.1 Use of therapeutic plants

Plants have been identified as important sources of active pharmaceutical ingredients (APIs) that have been utilised in the prevention and treatment of various infectious diseases, including malaria (Makuvara, 2022). Notably, over 25% of identified plant species are used in the global medical industry. In line with this trend, up to 65% of the world's population relies on plant extracts for

their primary health care (Mazid et al., 2012). Zimbabwe, part of the Southern Africa region, is a global centre of high species biodiversity and indigenousness (Shumba et al., 2009). With 66,888 known plant species, 3,666 of which are indigenous, approximately 80% of the Southern African population depends on plant-based remedies for basic primary health care (Shumba et al., 2009).

In Zimbabwe, most traditional knowledge about herbal medicine is held by medical practitioners, many of whom are spirit mediums. Spirit mediums argue that the knowledge of herbal medicines belongs to the spirits, not to them (Kazembe et al., 2012). Traditional healers believe that the knowledge of plant medicine is generational, passed on to successors when the traditional healer passes away. According to information from traditional healers, the knowledge that needs preservation is in the hands of non-spirit medium practitioners (Kazembe et al., 2012). Zimbabwe is fortunate to have non-spirit medium practitioners who keep records of medicinal plants and their effectiveness in treating certain ailments.

Historically, plants have been identified as important sources of antimalarial plant-derived APIs (APD-APIs), which have served as templates for the development of novel antimalarial drugs (Makuvara, 2023). The search for APD-APIs is based on previous work that identified quinine and artemisinin from Cinchona spp. and Artemisia annua, respectively (Asnaashari et al., 2016; Lima et al., 2015). These lead APD-APIs have been used as templates for antimalarial drugs, chloroquine, and artemether, respectively (Makuvara, 2023). Despite the knowledge of antimalarial plants in Zimbabwe, there is a lack of a comprehensive list of antimalarial plants used nationwide, along with information on their phytochemistry, pharmacological properties, and medicinal properties. Ethnobotanical and pharmacological studies on anti-malarial plants in Zimbabwe are still in their early stages, emphasising the need for further research.

Medicinal plants are an integral part of traditional medicine in Zimbabwe, with the treatment of malaria based on indigenous knowledge systems. To preserve information on indigenous practices and traditional medicines, the Traditional Medical Practitioners Act of 1981 was introduced (Shoko, 2018). The act aimed to integrate traditional medicine into the mainstream Zimbabwean health system (Ngarivhume et al., 2015). However, indigenous practices, such as the use of medicinal plants, are largely undocumented, unregulated, and poorly integrated into the

mainstream health sector (Ngarivhume et al., 2015). Medicinal plants, including antimalarial plants, are still being used for basic health care in rural Zimbabwe (Ngarivhume et al., 2015; Kazembe et al., 2012; Lukwa et al., 2001). Information on the use of antimalarial plants for the prevention and treatment of malaria in Zimbabwe is fragmented and not comprehensive enough to provide a full picture of the situation nationwide.

According to surveys and studies conducted in Zimbabwe, Elephantorrhiza goetzei (Harms), *Adansonia digitata*, *Toddalia asiatica (L.) Lam*, *Albizia amara*, *Dalbergia nitidula*, *Pavetta schumanniana F. Hoffm.*, *Lannea discolor*, *Brachylaena huillensis* O., *Harungana madagascariensis* Poir, *Moringa oleifera*, and *Vangueria infausta* are among the traditionally used plants in the management of malaria (Chapu and Mgochek, 2017; Ngarivhume et al., 2015; Mbauya, 2015; Kazembe et al., 2012; Lukwa et al., 2001). The main plant parts used in the treatment of malaria are roots, stem barks, and leaves. Extracts from Zimbabwean antimalarial plants are associated with various classes of APIs, including alkaloids, flavonoids, terpenoids, phenolic acids, and glycosides (Makuvara, 2023; Ngarivhume et al., 2015). Traditionally, the extracts from antimalarial plants' stem bark, root, and leaves are prepared through decoction, cold infusion, and hot infusion (Ezeani et al., 2022; Alebie et al., 2017).

Malaria control interventions including ITNs, rapid diagnostic tests (RDTs), IRS and antimalarial drugs have been critical in reducing malaria morbidity and mortality. However, the effectiveness and accessibility of these interventions significantly vary across regions and populations, particularly in rural settings. This variation underscores the need for studying plant-based antimalarial drugs as alternative treatment options, especially in instances where modern interventions are inconsistently accessed. The accessibility gap in malaria control interventions directly dictates the traditional use of medicinal plants against malaria.

In many sub-Saharan African countries, including Zimbabwe, rural populations often face constraints such as stock-outs of pharmaceuticals, long distances to health facilities, limited health literacy, and economic constraints (WHO, 2023; MoHCC, 2022). As a result, communities turn to indigenous herbal treatments as primary sources of antimalarial drugs. Although antimalarials such

as ACTs are effective, their limited availability, cost, and the threat of drug resistance make them insufficient as the ultimate antimalarial drug interventions.

In communities with significantly limited access to these therapies, locally available antimalarial plants can provide a culturally acceptable and cost-effective treatment alternative. Studying their usewith modern interventions allows for cost-effective treatment options and a holistic approach to malaria management. Notably, understanding accessibility issues helps in the identification of deficiencies in policy and healthcare delivery that traditional medicine often fills. For example, in malaria-endemic regions of Zimbabwe, the health system still relies in part on collaboration with traditional healers, especially where access to health centers is constrained. Evaluating plant use in such settings offers insights into trust in traditional knowledge, community resilience, and the potential for integration of validated herbal treatments into national malaria control interventions (WHO, 2003; Maroyi, 2013).

2.7.2 Herbal and other traditional medicines used in malaria control and treatment.

There is limited information on the use of herbal medicine against malaria in Zimbabwe. Additionally, the available information is mainly based on ethnobotanical surveys without scientific evaluation (Chapu & Mgochecki, 2017; Ngarivhume et al., 2015; Mbauya et al., 2015; Kazembe et al., 2012; Lukwa et al., 2001). Traditional medicine is one of the most accessible and widely available forms of therapy in Zimbabwe's resource-limited communities. The indigenous people have great experience in using traditional therapeutic drugs (Olsson et al., 2015; Dhewa, 2008). Orthodox medicinal practice in Zimbabwe is based on the experiences of communities within the framework of the native cultural context. However, the practice is dynamic and evolves situationally (Gureje et al., 2015; Dhewa, 2008). Zimbabwean traditional medical practitioners include herbalists, psychic therapists, midwives, fortune tellers, seers, and faith healers who design techniques and materials employing indigenous knowledge (Rupande, 2022). Despite the rising acceptance of ethnomedicine in Zimbabwe, much traditional knowledge remains unrecorded (Usai et al., 2022; Maroyi et al., 2012). However, documentation of traditional medicines is required to maintain knowledge, preserve it, and use it sustainably.

Traditional remedies are used by around 80% of people in underdeveloped nations since they cannot afford the costs associated with Western medicines and medical services (Dhewa, 2008). The uptake of traditional remedies is normally high because they are more accepted from a spiritual and cultural standpoint (Pan et al., 2014). The recording of traditional preventive and treatment medicine in Zimbabwe is a huge move in maintaining cultural customs, enabling community participation, and inclusion in developing traditional malaria control strategies.

Between 65 and 80% of the world's population relies on traditional remedies for their primary healthcare needs. According to the WHO, local, additional, and complementary therapy of top quality and safety offers various advantages (WHO, 2013). Ease of access, affordability, income level, education, effectiveness, and the non-availability of contemporary healthcare services were the prime causes behind the utilisation of Traditional medicines. Zimbabwean traditional medical practices exemplify the country's cultural diversity (Jidong et al., 2021; Rutto, 2005). Historically, Zimbabwean medicines are used for preventive care, therapy, and well-being enhancement. Traditional remedies have an important role in Zimbabwean healthcare systems, as evidenced by the country's healthcare policies (Dhewa, 2008; Razzak & Kellermann, 2002).

Traditional medicine has remained a source of pride for most Africans, particularly the Shona people of Zimbabwe (Bobo & Sukdaven, 2020; Mapira & Mazambara, 2013). According to statistics, 50% of the people in Africa utilise complementary therapies daily, with herbal remedies being by far the most popular. Because most of the medication is located near African populations, it is cheap and accessible (James et al., 2018).

The role of traditional healers was mainstreamed in Zimbabwe in 1980 with the formation of the Zimbabwe National Traditional Healers Association (ZINATHA) (Chakawa, 2015; Mposhi & Manyeruke, 2013). Zimbabwe has also been plagued by an influx of herbal medications from countries including Tanzania, China, and India, resulting in the spread of traditional remedies throughout the country (Fajinmi et al., 2017). Traditional medicine's influence in Zimbabwe is associated with the practices of traditional healers as the primary physicians (Evans, 2015). Herbal medicines are traditionally used for various purposes, including moral preservation, lucky charms, fertility, and spirituality issues (Novotna et al., 2020).

2.8 Isolation and characterisation of bioactive compounds from antimalarial plants

Bioactive compounds derived from plants play a key role in protecting plants against herbivores, pathogens, and parasites. Plant bioactive components are often referred to as phytochemicals (phyto- from Greek -phyto meaning 'plant') (Doughari, 2009; Tradit et al., 2011). The major classes of bioactive compounds from antimalarial plants such as Artemisia species include terpenoids, phenolic compounds, coumarins, alkaloids, sterols, and acetylenes (Brusotti, 2016). According to Makuvara 2023, potential antimalarial APIs from plants are classified into different classes, including flavonoids, glycosides, alkaloids, phenolic acids, and terpenoids. The basic steps in characterising bioactive compounds involve extraction (obtaining crude extracts), phytochemical screening (identifying different classes of bioactive compounds), antimicrobial activity, toxicity, selectivity index, isolation (obtaining pure compounds), bioactive compound characterisation (determining chemical structure), and clinical tests (Figure 2.7).

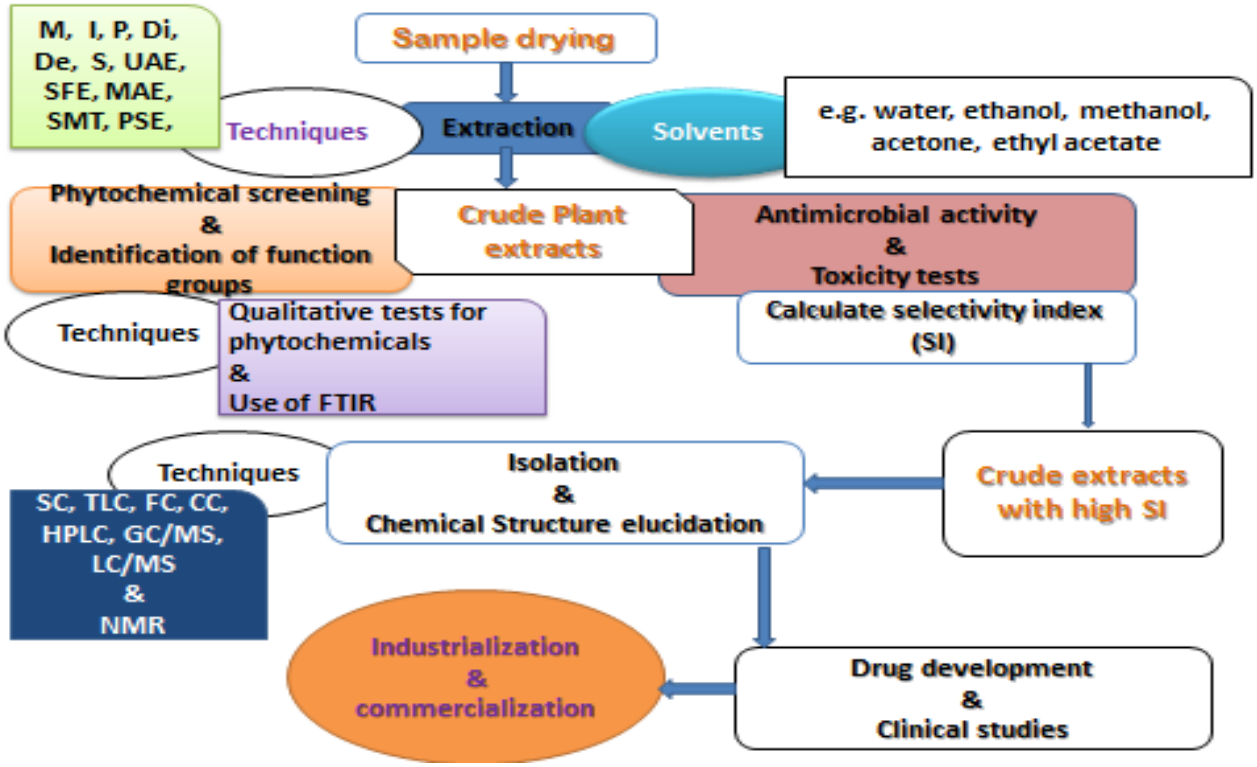
Extraction is a critical step in the characterisation of plant-based bioactive compounds as it allows further purification and chemical structure elucidation. Extraction involves washing and shade drying plant samples, homogenising dried plant specimens by grinding (Stephane et al., 2022; Thakker & Sun, 2021). A homogenous plant sample has a large surface area and efficiently interacts with the solvent during the extraction process (Brusotti et al., 2016; Tradit et al., 2011). Caution should be taken during extraction to prevent the loss of bioactive compounds. In ethnomedical studies, sample preparation should be based on traditional uses of selected samples (Thakker & Sun, 2021; Doughari et al., 2009). Plant sample extraction processes use various extraction solvents such as water, ethanol, methanol, acetone, and ethyl acetate (Brusotti et al., 2016). Solvent selection depends on the type of plant, the plant part from which extraction is carried out, the polarity of secondary metabolites (bioactive compounds) being targeted, and solvent availability (Abubakar & Haque, 2020). Extraction of lipophilic and hydrophilic bioactive compounds is based on non-polar (e.g., dichloromethane) and polar (e.g., methanol and ethyl acetate) solvents, respectively (Tradit et al., 2011).

The basic extraction methods used in the extraction of plant-based bioactive compounds (PBBCs) include maceration, infusion, decoction, percolation, and digestion (Abdullahi et al., 2020). Recent methods applied in the extraction of bioactive compounds are based on a variety of extraction

techniques, including Soxhlet, ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), surfactant-mediated techniques (SMT), pressurised solvent extraction (PSE), and hydrodistillation (Figure 2.7) (Nour et al., 2021; Alara et al., 2020). MAE is the most applied method due to reduced extraction time, efficient solvent utilisation, and reduction in sample degradation before further analysis (Raut et al., 2015).

After extraction, the crude extracts are screened for phytochemicals. Phytochemical screening is a preliminary step in identifying bioactive compound classes in plant extracts (Raut et al., 2015; Tradit et al., 2011). Techniques used in identifying secondary metabolites (bioactive compounds) are based on qualitative tests. These qualitative tests are instrumental in identifying flavonoids, saponins, terpenes, flavones, tannins, sterols, alkaloids, and cardiac glycosides (Kaur et al., 2022). Tools such as Fourier-transform infrared spectroscopy (FTIR) have been utilised in characterising bioactive compounds in crude extracts by identifying functional groups of compounds in crude plant extracts (Jomoh & Lin, 2019; Tradit et al., 2011; Doughari et al., 2009). In characterising the bioactive compounds in plant extracts, antimicrobial activity is evaluated. However, in crude extracts, there is a combination of phytochemicals that are variable in structure and polarity (Brusotti et al., 2016). This calls for further separation and purification of crude extracts so that individual biomolecules are identified.

Several techniques are used in the isolation and separation of bioactive compounds from crude plant extracts, including sephadex chromatography (SC), thin-layer chromatography (TLC), flash chromatography (FC), column chromatography (CC), and high-performance liquid chromatography (HPLC) (Mahato et al., 2019, Costa et al., 2012). Identification of bioactive compounds from purified samples can be done using liquid/gas chromatography coupled with mass spectrometry (L/GC/MS) (Tradit et al., 2011). The pure compounds identified after applying these chromatographic techniques are normally used in determining the structure of bioactive compounds. Bioactive compound structural elucidation is carried out using tools such as Nuclear Magnetic Resonance (NMR) spectroscopy (Martins et al., 2022). The general steps that are followed in the characterisation of plant-based bioactive compounds are summarised in **Figure 2.7**.



Extraction methods: maceration (M), infusion (I), decoction (De), percolation (P) and digestion(Di), Soxhlet (S), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), surfactant-mediated techniques(SMT), pressurised solvent extraction (PSE) and hydrodistillation (H). Techniques are used for isolation and separation: sephadex chromatography (SC), thin-layer chromatographic (TLC), flash chromatography (FC), column chromatography (CC), and high performance liquid chromatography (HPLC), liquid/gas chromatography coupled with mass spectrometry (L/GC/MS) and Nuclear Magnetic Resonance (NMR) spectroscopy.

Figure 2. 7 The general steps that are followed in the characterisation of plant-based bioactive compounds (PBBCs), drug development and commercialisation.

Given the increasing resistance of *Plasmodium falciparum* to conventional drugs such as chloroquine and, more recently, to artemisinin in some regions, there is an urgent need to explore new antimalarial agents, particularly from plant sources (Talisuna et al., 2012; WHO, 2023). Isolating bioactive compounds from traditional medicinal plants allows for the identification of pharmacologically active ingredients and the elimination of toxic or ineffective components. This is especially important when traditional preparations involve complex mixtures of phytochemicals whose individual roles are not well understood. Bioassay-guided isolation enables for the identification of compounds that are responsible for antimalarial activity and assess their potency, mechanism of action, dosage, synergistic effects and toxicity (Willcox & Bodeker, 2004; Newman & Cragg, 2020).

Moreover, characterization of bioactive compounds supports the development of standardized herbal products and the transition from traditional remedies to formally approved phytomedicines. Without isolation and chemical profiling, traditional remedies cannot be reliably reproduced and regulated, and this limits their clinical application. This process is crucial for ensuring quality control, safety, and efficacy, particularly when such products are considered for integration into national malaria treatment programs (WHO, 2003).

In addition, extraction, isolation and structural elucidation normally leads to identification of novel compounds that can serve as templates for synthetic modification and reproduction. Many natural antimalarial bioactive compounds are used in the production of synthetic pharmacologically active compounds with reduced toxicity, improved pharmacokinetics, and broader activity profiles. The modifications of natural plant based antimalarial compounds has contribute to effective antimalarial drug production and thereby mitigating the threat of antimalarial drug resistance (Kaur et al., 2009; Tona et al., 2004).

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CHAPTER 3: MATERIALS AND METHODS

3.0 Introduction

The four objectives covered in this Chapter are to identify plant species used in the treatment of malaria in Zimbabwe, to explore the indigenous practices applied against malaria in Zimbabwe, to examine the activity and toxicity of crude extracts of selected antimalarial plant species against *Plasmodium falciparum* and to characterise bioactive compounds from selected medicinal plants. The first two objectives are addressed under **Section 3.2**, the third objective under **Section 3.3** and the fourth objective under **Section 3.4**.

3.1 Ethical clearance

Ethical approval was sought from the UNISA-CAES Health Research Ethics Committee and permission to conduct the study in Zimbabwe was granted by the Ministry of Health and Child Care and the Ministry of Lands, Agriculture, Fisheries, Water and Rural Development, Department of Research & Specialist Services (DR&SS). The letters of approval and permission to conduct the study are attached in the Appendix section. All activities in this current study were conducted in line with well-established research integrity standards, ethical principles, and biosafety guidelines to ensure effective handling of all biological materials, protection of participants, transparency, reliability and accuracy of the research findings.

3.2 Objective 1: To identify plant species used in the treatment of malaria in Zimbabwe, and Objective 2: To explore the indigenous practices (merits and demerits) in the fight against malaria in Zimbabwe.

A hybrid technique that included quantitative and qualitative methodologies was applied. The application of this technique was based on a thorough analysis of literature and data mining from primary and secondary databases was used to identify plants used in the treatment of malaria in Zimbabwe. Using the Boolean search approach, literature was gathered from the University of South Africa (UNISA) library, Zimbabwean libraries, and electronic databases such as Web of Science, Google Scholar, SciELO, Science Direct, Scopus, and PubMed. The Boolean search methods in this instance expanded and narrowed the search by combining search strings with modifiers and operators such as OR, AND, and NOT. An overview of the representative search strings and Boolean search techniques applied in this study include: "Plants used AND/OR malaria", "Zimbabwean plants AND/OR malaria", "Malaria treatment", "Malaria prevalence

AND/OR Zimbabwe", "medicinal plants AND/OR Zimbabwe", and "Plants AND/OR malaria therapy", “Antimalarial plants AND/OR vitro studies”, “Antimalarial plants AND/OR in vivo studies”, “Zimbabwean indigenous practices AND/OR malaria”, “malaria treatment AND/OR Zimbabwean indigenous practices”, “Malaria prevention and indigenous knowledge systems in Zimbabwe”. This technique was crucial in retrieving relevant literature, although it was limited to publications in the English language and may have missed publications in other languages.

Consequently, data were gathered from published scientific articles, conference papers, theses, books, and grey literature. The individual data sources were qualitatively screened and evaluated based on the main objectives of the study. Due to limited information on plant species used and indigenous practices applied in the treatment of malaria in Zimbabwe before 2000 (based on preliminary search), the literature search in this study was limited to the period 2000 to 2024. After screening and analysis, publications on indigenous practices and plants utilised in malaria treatment in Zimbabwe were examined, critically reviewed, and the significant findings were summarised and tabulated. Notably, in this study, a quantitative review employing meta-analysis and bibliometric techniques was utilised. Focus was mainly on articles with relevant primary data and in order to ensure integrity and quality of this review, the PRISMA 2020 Checklist was used. The literature search yielded a total of over thirty six thousand (36,000) articles of which twenty five thousand one hundred and twenty five (25,125) were excluded as duplicates and up to one thousand and seventeen (1017) were excluded based on titles and abstracts. Only one thousand one hundredand three (1103) articles were retrieved and based on the exclusion criteria one hundred and eighty five (185) articles were included.

3.3 Objective 3: To examine the activity against *Plasmodium falciparum* and the toxicity of crude extracts from selected antimalarial plants.

The antiplasmodial activity and toxicity of crude extracts and characterisation of bioactive compounds from selected antimalarial plants are outlined in **Figure 3.1**

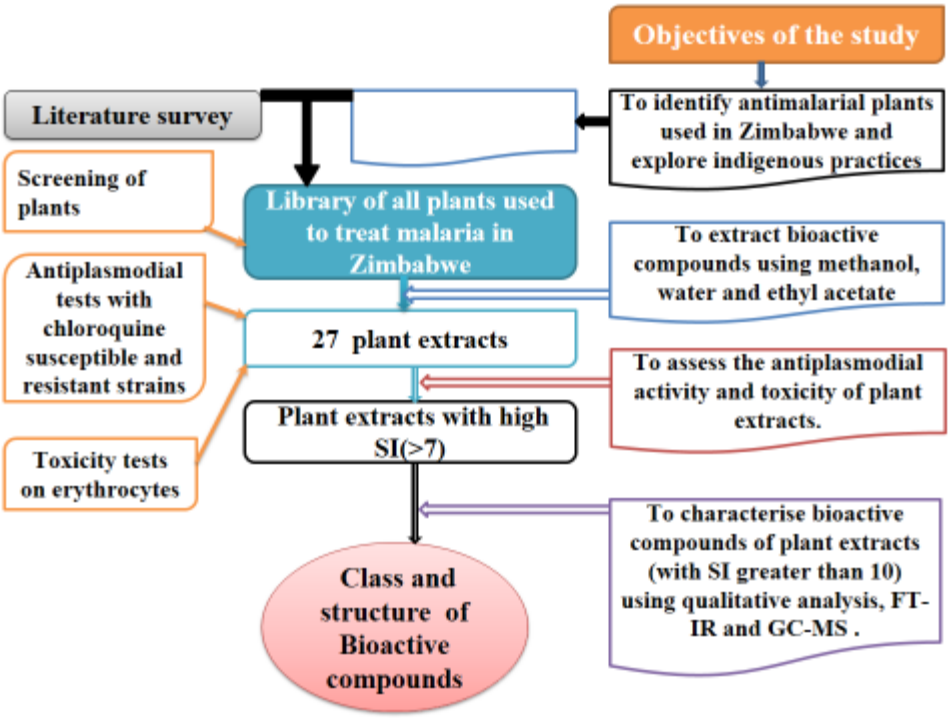

Figure 3.1 An outline of antimalarial plant identification to final antimalarial bioactive compounds.

3.3.1 Plant sample selection

For the purposes of this study, nine plants (*Tabernaemontana elegans* Stapf, *Diplorhynchus condylocarpon, Elephantorrhiza goetzei* (Harms), *Kirkia acuminata* Oliver, *Ficus ingens, Holarrhena pubescens* Wall. Ex G. Don, *Euclea. divinorum* Hiern, *Pterocarpus anglolensis, Garcinia huillensis*) were selected based on screening criteria: (1) existence in published work focusing on Zimbabwe, (2) lack or limited information on toxicity and activity against *Plasmodium* species (lack of pharmacological references), (3) indigenous to Zimbabwe, (4) accessibility of the plant species, and (5) presence of samples that can be collected sustainably. After screening, plants were collected from Masvingo and Zaka districts, Masvingo province, Zimbabwe, and were identified by a certified state botanist. The identified plants were processed, voucher numbered, and deposited in the National Herbarium in Harare, Zimbabwe.

3.3.2 Extraction and sample preparation

In this study, water, methanol, and ethyl acetate (purchased from Sigma-Aldrich Chemical Company and Merck, USA, and Germany) were used as solvents. The utilisation of solvents in extracting bioactive compounds from plants has been reported in several studies (Kifle et al., 2021; Zewdie et al., 2020; Palla et al., 2020; Noubissi et al., 2019; Afroz et al., 2019; Suleiman et al., 2017). The extraction process of phytochemicals depends on the solvent and extraction technique applied. Among other factors, solvent polarity and extraction method effectiveness influence phytochemical extraction (Aziz et al., 2021). Polar solvents, including methanol, ethanol, water, and ethyl acetate, are normally used in extracting polar bioactive compounds, such as phenols, flavonoids, alkaloids, and triterpenes. Water is frequently used as a solvent since it is polar, nontoxic, cheap, and nonflammable. Accordingly, the use of alcohol (e.g., methanol) and water has been reported to extract several polar compounds (Abubakar & Haque, 2020), with more than 84% of phytochemicals isolated and extracted using alcohols (Dhawan & Gupta, 2017).

The extraction technique employed in this study was maceration, a method commonly used by participants managing malaria in Zimbabwe. Maceration is among basic extraction methods, such as infusion and decoction (Abdullahi et al., 2020). Recently, methods like Soxhlet, supercritical fluid extraction, ultrasound-assisted extraction, surfactant-mediated extraction, and microwave-assisted extraction have been applied in extracting critical bioactive compounds (Oluwaseun et al., 2021; Alara et al., 2018; Raut et al., 2015).

Generally, extraction involves shade or air drying of plant samples after washing (Stephane et al., 2022; Thakker & Sun, 2021). The dried samples are then homogenised to increase the surface area and improve the interaction between the solvent and powdered samples (Brusotti, 2016; Tradit et al., 2011). In this study, nine plant samples (barks, roots, and leaves) were cleaned using deionised water. The cleaned materials (barks and leaves) were shade-dried in the air at 25ºC following a method by Dah-Nouvlessounon et al. (2023). Fresh plant samples can alternatively be sun-dried for at least seven days instead of shade-drying. The dried plant samples were ground into a homogenous sample using a laboratory pestle and mortar according to Suleiman et al. (2017).

The homogenised plant barks, roots, and leaves were macerated in distilled water, methanol, and ethyl acetate for three days at 25ºC. Maceration in this case was based on previous studies (Palla et al., 2020; Tagne et al., 2019; Tadesse et al., 2017). After maceration, the liquid sample was filtered on a number 1 filter paper (Merck, Whatman® membrane filters). For exhaustive extraction, the residue was macerated twice using fresh solvent. The aqueous extracts were concentrated by a rotary evaporator (Biobase, RE100-Pro with SHZ-D (III) water circulating vacuum pump, China) at a speed of 260 rpm and a temperature of 45°C. Concentrated extracts were then evaporated in an oven (Model-MIN0118, GENLAB) at 45°C to obtain a dried sample, which was refrigerated (Biobase, China) at a temperature of -20°C. The yield of the dried sample was determined following the method by Karim et al. (2021)

Generally, the selection of water, methanol, and ethyl acetate was based on their differing polarities and complementary capacities to extract a wide range of bioactive compounds. Water was included to reflect traditional medicinal preparations, while methanol and ethyl acetate facilitated the extraction of semi-polar and polar phytochemicals. Extraction conditions, including temperature, repeated extraction cycles, and maceration time were standardised based on established protocols to maximise extraction efficiency and preserve bioactive constituents.

3.3.3 Activity against *Plasmodium falciparum*

Chloroquine-sensitive *P. falciparum* (3D7 strain) (obtained from the National Health Research Institute, Harare, Zimbabwe) was maintained in culture following a modified method by Trager and Jensen (1976). All the chemicals used in the screening of extracts against P. falciparum were standard and of analytical grade (purchased from Sigma-Aldrich Chemical Company and Merck, USA, and Germany). The antiplasmodial screening of plant extracts was based on a modified method by Andrade-neto et al. (2007). Each of the 27 plant extracts was constituted in dimethyl sulfoxide (DMSO) to form a stock solution of 5.0 mg/ml. The stock solutions were further diluted with RPMI-1640 culture medium to concentrations between 0 to 100 µg/ml, and 20 µL of each of the diluted stock solutions was added to a well of a 96-well plate. Up to 180 µL of parasitised red blood cells (1% parasitemia and 3% hematocrit) was added to each well containing diluted plant extracts. All the antiplasmodial assays in this study were replicated three times. The negative

controls were based on a suspension containing 1% DMSO and RBCs only, whereas chloroquine was used as the positive control. The Plasmodia cultures with plant extracts were incubated for two days at 37°C under atmospheric conditions: oxygen (5%), carbon dioxide (5%), and nitrogen (90%). After 24 hours, parasitemia was examined based on Giemsa stain and microscopy, and the mean suppression of parasitemia was derived using the formula by Irabor et al. (2020).

$$Suppression\ of\ parasitaemia = \frac{Average\ \%\ parasitaemia\ in\ control\ -\ Average\ \%\ parasitaemia\ in\ test}{Average\ \%\ parasitaemia\ in\ control} \times 100$$

The activities of plant extracts against *Plasmodium falciparum* strains were expressed as IC_{50}. The mean and standard deviations of IC_{50} of each plant extract were calculated using data from the replicates. Mean IC_{50} (antiplasmodial activity) of different plant extracts were separated using one-way analysis of variance (ANOVA). The classification of in vitro antiplasmodial activities of plant extracts was done according to Rasoanaivo et al. (1992). The classification system criterion is indicated in **Table 3.1**

Table 3.1: Antiplasmodial activity based on Rasoanaivo et al., 1992

Classification number	**IC_{50}/ µg/ml**	**Activity**
1	<5	Very active
2	Between 5 and 50	Active
3	Between 50 and 100	Weakly active
4	>100	Inactive

3.3.4 Cytotoxicity tests

The cytotoxicity of twenty-seven (27) plant extracts was assessed using a modified colourimetric assay. Analytical grade chemicals from Sigma-Aldrich Chemical Company and Merck in the USA and Germany were utilised. The technique involved the use of 3-(4,5-dimethylthiazol-2-yl)-2,5-

diphenyl tetrazolium bromide (MTT) to screen the toxicity of plant crude extracts on healthy erythrocytes obtained from the National Health Research Institute in Harare, Zimbabwe (Appiah-Opong et al., 2011). The plant extracts, at concentrations ranging from 3 µg/ml to 120 µg/ml, were added in triplicate to 96-well microtiter plates (Kwansa-Bentum et al., 2019). To each well, 100µL of plant extracts and 100 µL of healthy erythrocytes were added. Control experiments were set up to exclude the influence of culture medium, plant extracts, and erythrocytes on optical densities. After 3 days of incubation at 37°C with 5% O_2 and CO_2, 20 µL of MTT (7.5 mg/ml) was added to each well and incubated for 120 minutes under the same conditions. Following incubation, 150 µL of culture media was discarded, and 200 µL of Triton X-100 in acidified isopropanol was added to the wells. The plates were then incubated in total darkness at 25°C for a day, and optical density readings were taken at 570 nm using a 96-well plate reader (Spectra Max 340, Molecular Devices Corporation, California, USA). The CC_{50} values were determined from dose-response curves.

3.3.5 Selectivity index (SI)

The selectivity index was used to determine the potential of each plant extract as an antiplasmodial drug. The SI value is calculated as

$$SI = \frac{CC50\ erythrocytes}{IC50\ P.falciparum}$$

An SI value greater than 10 indicates safety *in vitro* against *Plasmodium* species (Ondo et al., 2012). Plant extracts with an IC50 <10 µg/ml and SI value >10 are considered potent against *Plasmodium* species (Chaniad et al., 2022). Plant extracts were classified based on their SI values, with those equal to or greater than 2 considered antiplasmodial. Crude extracts with SI values of 7 and above underwent phytochemical screening, and extracts with SI >10 were further analysed using GC-MS. In this study, *T. elegans* bark extracts with the highest SI (>10) were selected for GC-MS analysis.

3.4 Objective 4: To characterise bioactive compounds from selected medicinal plants used against malaria

3.4.1 Qualitative phytochemical analysis.

Phytochemical screening is considered an important preliminary technique in the identification of active compound classes in plant extracts (Raut et al., 2015; Sasidharan et al., 2011). The screening of phytochemicals in crude extracts with an SI greater than seven (7) was carried out using standard protocols as described by Nortjie et al. (2022), Shaikh et al. (2020) and Banu & Cathrine (2015). All chemicals used for phytochemical screening were purchased from Sigma-Aldrich Chemical Company and Merck, USA, and they were of analytical grade. Ten (10) crude plant extracts were screened for terpenoids, saponins, cardiac glycosides, alkaloids, tannins, coumarins, flavonoids and anthraquinones following the froth test, Salkowski's test, ferric chloride test, Keller Killiani's test, Dragendorff's test, Shinoda's test, NaOH paper test and Borntrager's test. However, all these tests were slightly modified, and secondary metabolites (bioactive compounds) classes were qualitatively identified according to Shaikh and Patil (2020).

3.4.2 Identification of functional groups of phytochemicals.

Tools such as Fourier-transform infrared spectroscopy (FTIR*)* have been utilised in the characterisation of bioactive compounds in crude extracts through the identification of functional groups of compounds in crude plant extracts (Jomoh and Lin, 2019; Sasidharan et al., 2011; Doughari et al., 200). In this work, the functional groups of compounds in water, methanol and ethyl acetate *T. elagans* bark extracts were identified using FTIR spectroscopy (JASCO, FTIR6300, Japan). The identification and analysis of functional groups of phytochemicals followed modified techniques by Bolade et al. (2018). Sample preparation was based on a KBr salt pellet produced by combining the sample and KBr salt of FT-IR grade in the ratio 1:10. This was done considering that KBr salts do not absorb any light, and the pelleted samples absorb light. After sample pelleting, the sample was placed on a holder, and the FT-IR spectrum of each sample was noted from a wave number of 400 to 4000 cm^{-1}. The functional groups within bioactive compounds were then identified based on peaks on the FTIR spectrum (within the Infrared radiation region). Overall, the functional groups were separated according to their peak ratios.

3.4.3 Gas chromatography/mass spectrometry (GC-MS) analysis

One (1) plant extract with the highest selectivity index (>10) (*T. elegans* bark extract) was further examined using GC-MS analysis based on a method by Hema et al. (2010) with a few adjustments. The GC-MS profiling of bioactive compounds was performed on a GC system and GC-coupled with a mass spectrometer (GC Model: 7890B, MSD Model 5977A, Agilent Technologies, USA). For GC-MS detection, a 70 eV ionising energy was utilised. A 99.99% helium was used as a carrier gas at a flow rate of 1 ml/min through the whole analysis process and 2 µL injection volume with ion source and injector temperatures of 280°C and 250°C, respectively. Additionally, oven temperature followed this programme: 110°C (2 min), coupled with a 10°C increase for every minute until a temperature of 2000°C is reached, followed by a 5°C increase for every minute until a temperature of 280°C is attained, and finally 9 minutes at a temperature of 280°C. Each of the mass spectra was extracted at an ionising energy of 70 eV, at a scanning interval of 0.5 seconds and fragments from 45 to 450 Da. Moreover, GC was run for an overall total of 36 minutes and the quantity of components was determined as a percentage. The database of the National Institute Standards and Technology (NIST) was used in the identification of compounds. Peaks with 80% and above similarity with NIST libraries were selected and analysed. GC-MS analysis allowed for the molecular weight and structure of bioactive compounds in plant isolates to be ascertained.

3.5 Data presentation and analysis

Data from the literature survey, yields of crude extracts, and bioactive compound characterisation were summarised and presented on graphs, charts and tables. The collected data on IC_{50} and CC_{50} were obtained based on dose-response curves plotted in Excel, and the data were further analysed based on one-way Analysis of Variance (ANOVA) in Genstat 18.1 Copyright 2016. The means for data on extract yields, IC_{50} and CC_{50} were separated using the Tukey test, and significant differences only existed in cases where $P < 0.05$. Additionally, data from FT-IR and GC-MS were presented in tables and on graphs.

Overall, all experiments were conducted using standard validated methods, analytical-grade reagents, calibrated equipment, and controlled laboratory conditions in order to ensure scientific rigour. Antiplasmodial and cytotoxicity assays were performed in triplicate, with appropriate controls (negative and positive) to minimise bias and verify assay performance. Reproducibility

and reliability were enhanced through standardised procedures across all experimental stages. Statistical robustness was ensured through the application of one-way ANOVA followed by Tukey's post hoc test ($P < 0.05$), enabling objective comparison of treatment effects and strengthening confidence in the validity of the findings.

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CHAPTER 4: RESULTS, DATA ANALYSIS AND DISCUSSION

4.0 Introduction

This chapter focuses on the results obtained, data analysis and discussion of the findings. Data obtained are presented in tables, graphs and charts. All the findings are summarised and discussed in this chapter. The results from the study are subdivided into six categories: (1) Plants used in the management of malaria in Zimbabwe, (2) Herbal and other traditional medicines used in malaria control and treatment, (3) Plants selected for antiplasmodial activity and toxicity tests, (4) Percentage yield of water, methanol and ethyl acetate plant extracts, (5) Antiplasmodial activities against *Plasmodium falciparum* (3D7 strain) and toxicity of plant extracts against healthy erythrocytes' and (6) Characterisation of bioactive compounds from selected antimalarial plants

4.1 Plants used in the management of malaria in Zimbabwe.

According to a literature survey, *Elephantorrhiza goetzei* (Harms), *Adansonia digitata*, *Toddalia asiatica* (L.) Lam, *Albizia amara*, *Dalbergia nitidula*, *Pavetta schumanniana* F. Hoffm., *Lannea discolor*, *Brachylaena huillensis* O., *Harungana madagascariensis* Poir, *Moringa oleifera*, and *Vangueria infausta* (**Table 4.1**) are some of the traditionally used plants in the management of malaria in Zimbabwe (Maroyi, 2017; Chapu and Mgochek, 2017; Ngarivhume et al, 2015; Mbauya, 2015; Kazembe et al, 2012; Lukwa et al., 2001). It should be noted that there is limited information in the literature on plants used in the treatment of malaria in Zimbabwe. The main plant parts used in the treatment of malaria are roots, stem barks, and leaves. Extracts from Zimbabwean antimalarial plants are mainly associated with different classes of APIs, including alkaloids, flavonoids, terpenoids, phenolic acids, and glycosides. A total of sixty-one (61) plants used against malaria have been reported in Zimbabwe. However, one plant species was not identified up to the genus and species level. Of the 61 plant species, 14.3% are preventive, 3.2% are used in both treatment and prevention of malaria, and most of the plants (85.7%) are utilised as malaria treatment drugs. The identified plants are presented in **Table 4.1, 4.2, 4.3**

4.1.1 Plants used for preventative purposes.

Seven plant species used in the prevention of malaria in Zimbabwe (*Sclerocarya caffra*, *Dichrostachys cinerea*, *Cleome gynandra*, *Cucumis anguria*, *Adenia cissampeloides*, *Momordica foetida*, and *Momordica balsamina*) were identified from two different studies done in Zimbabwe

(Mbauya, 2015; Ngarivhume et al., 2015). Most of these plant species (3/7) were from the family *Cucurbitaceae* and in most cases leaf extracts were utilised as malarial preventive drugs. These findings are summarised in **Table 4.1.** *Sclerocarya caffra* (syn. *Sclerocarya birrea* subsp. *caffra*) stem bark decoction is traditionally as a prophylactic drug against malaria (Mbauya, 2015). Similarly, the *Tsonga* and *Zulu* in South Africa have utilised *Sclerocarya caffra* bark infusions treat fever (Mawoza, 2015). The use of *Sclerocarya caffra* to prevent malaria may be attributed to high concentration of phenolic compounds, tannins and flavonoids, which exhibit antioxidant and antimicrobial activity (Maroyi, 2013). Generally, it has been reported that these herbal medicines are administered based on knowledge from traditional healers (Chipiti et al., 2014). However, the current study therefore give emphasis on characterisation of bioactive compounds from selected antimalarial drugs. Information from traditional healers is characterised by absence of dosage standardization, reliance on subjective efficacy, and lack of toxicity data. This underscores the need for antimalarial scientific validation of *Sclerocarya caffra* extracts.

The root extracts of *Dichrostachys cinerea* have been utilised in the prevention of malaria in some parts of Zimbabwe (Mbauya, 2015). Beyond Zimbabwean boarders, *Dichrostachys cinerea* extracts have been exploited in the management of fevers across sub-Saharan Africa (Kakudidi et al., 2020). The antimicrobial and antiplasmodial activity is believed to be linked to presence of bioactive compound classes such as flavonoids, tannins, alkaloids and Saponins (Sanon et al., 2003). Similarly, the use of root and leaf extracts of *Adenia cissampeloides* in preventing malaria was attributable to presence of alkaloids, flavonoids, ribosome-inactivating proteins and glycosides (Olorunnisola et al., 2011). Leaf extracts of *Cleome gynandra*, *Cucumis anguria*, *Momordica foetida*, and *Momordica balsamina* are associated with a bitter taste and have been reported to prevent malaria (Ngarivhume et al., 2015; Mbauya, 2015). The leaves of these plants (*Cleome gynandra*, *Cucumis anguria*, *Momordica foetida*, and *Momordica balsamina*) have been incorporated into diets and therefore have contributed to nutritional value and are believed to prevent malaria. Notably, *Cleome gynandra* as been identified as a highly nutritious vegetable rich in iron, antioxidants, vitamins A and C, which may enhance immune function (Mibei et al., 2017). Additionally, the other three plant species (*Cucumis anguria*, *Momordica foetida*, and *Momordica balsamina*) are classified in the family *Cucurbitaceae* and they contain cucurbitacins and cucurbitane-type triterpenoids (Akinmoladun et al., 2020), which may contribute significantly to antimalarial activities of their leaf extracts. For instance, scientific evaluation of *Momordica charantia* leaf extracts revealed potent *in vitro* antiplasmodial activity (Freeman et al., 2019).

Table 4. 1: Plants used for preventative purposes.

No	Scientific name	Local name	Family	Part used	Use against malaria	References
1	*Sclerocarya caffra*	Mupfura	Anacardiacea	Stem bark	Prevention	Mbauya, 2015
2	*Dichrostachys cinera*	Mupangara	Fabaceae	Roots	Prevention	Mbauya, 2015
3	*Cleome gynandra*	Nyeve	Cleomaceae	Leaf	Prevention	Mbauya, 2015
4	*Cucumis anguria*	Mukakashango	Cucurbitaceae	Leaf	Prevention	Ngarivhume et al., 2015
5	*Adenia cissampeloides* (Planch ex Hook) Harms	Muore	Passifloraceae	Root/ Leaf	Prevention	Ngarivhume et al., 2015
6	*Momordica foetida* Schumach	Muchukubaba	Cucurbitaceae	Leaf	Prevention	Ngarivhume et al., 2015
7	*Momordica balsamina* L	Ngaka	Cucurbitaceae	leaf	Prevention	Ngarivhume et al., 2015

4.1.2 Plants used for both preventative and treatment purposes.

Based on findings from literature, four plants (*Aristolochia albida*, *Prunus persica*, *Cissampelos mucronata*, and *Capsicum annuum*) which are used for both prevention and treatment of malaria were reported. These results are summarised **Table 4.2.** Decoctions of *Aristolochia albida* tuber were used as part of multi-plant based remedies to treat chills and fevers (malaria-related symptoms). Although *Aristolochia albida* has been traditionally reported to prevent and treat malaria (Ngarivhume et al., 2015), the scientific evaluation of plant extracts have been linked to carcinogenicity and nephrotoxicity (Grollman & Shibutani, 2010). Based on this piece of evidence, the efficacy *Aristolochia albida* extracts may be overshadowed by potential toxicity if not properly dosed or prepared. Aristolochia species including *Aristolochia albida* are known for the presence of phytochemicals (aristolochic acids, alkaloids, and flavonoids) (Chen et al., 2019), which likely to contribute to the reported antiplasmodial and antimicrobial activity of this plant (Iqbal et al., 2017).

Though it has been reported that *Prunus persica* root and leaf extracts are used in decoctions or infusions for the prevention and treatment of malaria (Ngarivhume et al., 2015), there is no direct evidence from literature of antiplasmodial activity of this particular plant. On the contrary, plants such as *Cissampelos mucronata* which are used for malaria treatment and prevention (Ngarivhume et al., 2015), have been shown to have shown to have *in vitro* inhibitory effects against *Plasmodium falciparum* (Moshi et al., 2009). According to a study by Bafor et al., (2013), extracts from species of *Cissampelos* contain bisbenzylisoquinoline tannins, alkaloids, and flavonoids. Such phytochemicals have demonstrated antiplasmodial and antipyretic activities (Ancolio et al., 2002).

In Zimbabwe, *Capsicum anuum* fruits has been utilised prophylactic and therapeutic drugs against malaria (Mbauya, 2015; Ngarivhume et al., 2015). However, evidence of direct antiplasmodial activity *Capsicum anuum* fruits extracts is limited. Of particular importance, are capsaicinoids, (the major phytochemicals in C. annuum), which were reported to have antimicrobial properties (Kraft et al., 2014) and fever-reducing effects.

Table 4. 2: Plants used for both preventative and treatment purposes.

No	Scientific name	Local name	Family	Part used	Use against malaria	References
1	*Aristolochia albida* Duch.	Ruzangariro	Aristolochiaceae	Tuber	Treatment & Prevention	Ngarivhume et al. 2015
2	*Prunus persica*	Mupirikisi	Rosaceae	Leaf/ root	Treatment & Prevention	Ngarivhume et al. 2015
3	*Cissampelos mucronata*	Chipombafodya	Menispermaceae	Tuber	Treatment	Maroyi, 2020
4	*Capsicum anuum*	Mhiripiri	Solanaceae	Fruit	Treatment & Prevention	Ngarivhume et al. 2015 Mbauya, 2015 Ngarivhume et al., 2015

	Kraft et al., 2014

4.1.3 Plants used purely for treatment purposes.

Literature from ethnobotanical surveys indicated the presence of 50 plant species used for malaria treatment in Zimbabwe. These species were identified in seven studies (Chapu and Mgochek, 2017; Maroyi, 2013; Ngarivhume et al., 2015; Mbauya, 2015; Munodawafa et al., 2013; Kazembe et al., 2012; Lukwa et al., 2001), most of which were ethnobotanical surveys lacking primary scientific evaluation of plant extracts. The majority of these species were identified in a study conducted in Manicaland Province, Zimbabwe by Ngarivhume et al. (2015). Additionally, most of these species belong to the families *Fabaceae*, *Rubiaceae*, *Apocynaceae*, and *Compositae*, with roots and stem barks being the most commonly utilised plant parts. Species such as *Tabernaemontana elegans*, *Terminalia sericea*, *Pavetta schumanniana*, and *Warburgia salutaris* were noted for their cultural and medicinal significance, as summarised in **Table 4.3.**

While most of the plants used for malaria treatment were identified in the study by Ngarivhume et al., (2015), the findings showcase the diverse array of antimalarial plants utilised in Zimbabwean traditional medicine. The prevalence of *Apocynaceae* and *Fabaceae* may be attributed to their abundance in Zimbabwe and richness in antiplasmodial phytochemicals like alkaloids, flavonoids, and terpenoids (Lukwa et al., 2001). Despite the valuable data on malaria treatment plants, there is a necessity for scientific evaluation, including toxicological tests. Plants like *Aristolochia heppii*, identified in this study, are known to contain aristolochic acids, which are carcinogenic and nephrotoxic (Maroyi, 2016). Without thorough scientific evaluation, such plants may pose more harm than benefit.

The prevalent use of roots and bark in species like *Strychnos potatorum*, *Elephantorrhiza goetzei*, *Warburgia salutaris*, and *Dalbergia nitidula* suggests a traditional emphasis on parts believed to contain antimalarial compounds. However, the reliance on roots and bark may present sustainability challenges, especially if harvesting methods are destructive (Maroyi, 2017). For instance, *Warburgia salutaris*, mentioned in this study, has been harvested destructively across southern Africa, leading to its endangerment (Maroyi, 2017). Conversely, species like *Zanha*

africana, *Adansonia digitata*, and *Moringa oleifera* offer renewable resources through their leaves and fruits, presenting more ecologically sustainable options.

While there is limited data on the pharmacological potency of the reported plants against malaria, some have shown bioactive potential in laboratory and clinical studies. For example, *Catharanthus roseus*, known for its alkaloids, is traditionally used against malaria, indicating the possibility of undiscovered antimalarial compounds beyond its anticancer properties (Ngarivhume et al., 2015). *Moringa oleifera* and *Carica papaya* are recognised for their nutritional and medicinal benefits, with increasing evidence of their antiplasmodial activity (Chapu & Mgochek, 2017). Similarly, *Aristolochia heppii and Holarrhena pubescens* contain antimicrobial alkaloids, though careful dosage control is necessary due to toxicity concerns. The global discovery of artemisinin from *Artemisia annua* underscores the importance of exploring traditional remedies for novel therapeutic breakthroughs (WHO, 2022).

Table 4. 3: Plants used purely for treatment purposes.

No	Scientific name	Local name	Family	Part used	Use against malaria	References
1	*Tabernaemontana elegans* Stapf	Muchenya	Apocynaceae	Root and stem bark	Treatment	Ngarivhume et al. 2015
2	*Diplorhynchus condylocarpon* (Müll.Arg.) Pichon	Mutowa	Apocynaceae	Bark (stem)	Treatment	Ngarivhume et al., 2015
3	*Pavetta schumanniana* F. Hoffm.	Muchena/ Murunganyama	Rubiaceae	Root	Treatment	Ngarivhume et al., 2015
4	*Terminalia sericea*	Mususu	Combretaceae	Bark (stem)	Treatment	Ngarivhume et al. 2015
5	*Adansonia digitata*	Muwuyu	Malvaceae	Bark (stem) Leaf	Treatment	Ngarivhume et al., 2015 Kazembe et al., 2012
6	*Toddalia asiatica* (L.) Lam.	Gato	Rutaceae	Root	Treatment	Ngarivhume et al. 2015

7	*Elephantorrhiza goetzei* (Harms)	Chiurayi, Mugudzuru, Ntorani	Leguminosae	Tuber Root	Treatment	Ngarivhume et al. 2015
8	*Strychnos potatorum* L.	Mudyambira, Mudyagudo	Loganiaceae	Root/ stem	Treatment	Ngarivhume et al., 2015
9	*Crossopteryx febrifuga* (Afzel. ex G. Dion) Benth.	Mukombegwa	Rubiaceae	Stem bark	Treatment	Ngarivhume et al. 2015
10	*Holarrhena pubescens* Wall. Ex G. Don	Masunungure	Apocynaceae	Root	Treatment	Ngarivhume et al. 2015
11	*Aristolochia heppii* Merxm	Chividze	Aristolochiaceae	root	Treatment	Ngarivhume et al. 2015
12	Catharanthus roseus (L.) G. Don	Muruwa	Apocynaceae	root	Treatment	Ngarivhume et al., 2015
13	*Senna septemtrionalis*	Mumwahuku	Leguminosae	root	Treatment	Ngarivhume et al., 2015
14	*Plumbago zeylanica L.*	Mhisepise	Plumbaginaceae	Root	Treatment	Ngarivhume et al. 2015
15	*Ocimum angustifolium* Benth.	Mufuranhema	Lamiaceae	Tuber	Treatment	Ngarivhume et al., 2015
16	*Baccharoides adoensis* (Sch. Bip. ex Walp.) H. Rob.	Chipenembe	Compositae	Leaf	Treatment	Ngarivhume et al., 2015
17	*Erythrocephalum zambesianum* Oliv. & Hiern	Muhloni	Compositae	Root	Treatment	Ngarivhume et al., 2015
18	*Carica papaya* L	Mupopo	Caricaceae	Root	Treatment	Ngarivhume et al., 2015
19	*Brachylaena huillensis* O.	Muphahla	Compositae	Root	Treatment	Ngarivhume et al., 2015
20	*Euclea natalensis*	Mushangura	Ebenaceae	Root	Treatment	Maroyi, 2013 Maroyi, 2017 Ngarivhume et al., 2015
21	-	Munyabangwa	Solanaceae	Root	Treatment	Ngarivhume et al., 2015

22	*Warburgia salutaris* (G. Bertol.) Chiov	Muranga	Solanaceae	Root	Treatment	Ngarivhume et al., 2015 Maroyi, 2013
23	*Amblyogonocarpus andongensis*	Muriranyenze	Fabaceae	Root	Treatment	Lukwa et al., 2001
24	***Albizia amara*** *(Roxb.) subsp.* ***sericocephala*** *(Benth.)*	Muora	Fabaceae	Fruits	Treatment	Kazembe et al., 2012 Lukwa et al., 2001
25	*Dalbergia nitidula*	Mudima or Murima	Fabaceae	Root	Treatment	Kazembe et al., 2012
26	*Vangueria tomentosa*	Munzviro or Munzvirwa	Rubiaceae	Leaf and Root	Treatment	Munodawafa et al., 2013 Kazembe et al., 2012
27	*Lannea discolour*	Mugan'acha	Anacardiaceae	Stem Bark and Roots	Treatment	Kazembe et al., 2012
28	*Lannea edulis*	Mutsambatsi/Tsombori	Anacardiaceae	Stem Bark and Roots	Treatment	Munodawafa et al., 2013 Kazembe et al., 2012
29	*Moringa oleifera*	Muzungi,/Muzungwi	Moringaceae	Stem bark and roots	Treatment	Chapu and Mgochek, 2017
30	*Tecomaria nyassae* (Oliv.) K.Schum.	Mununu	Bignoniaceae	Root bark	Treatment	Chapu and Mgochek, 2017
31	*Zanha africana*	Muchenya	Sapindaceae	Bark, leaves and roots	Treatment	Lukwa et al., 2001

32	*Solanum incanum* L	Munhundurwa	Solananceae	Roots, leaves	Treatment	Lukwa et al., 2001
33	*Kirkia acuminata* Oliver	Mubvumira	Kirkiaceae	Leaves and stem bark	Treatment	Maroyi, 2017 Maroyi, 2016
34	*Ficus ingens*	Muonde	Moraceae	Stem bark	Treatment	Mbauya, 2015
35	*Cassia abbreviate*	Muremberembe	Fabaceae	Root	Treatment	Mbauya, 2015
36	*Solanum panduriforme* E. May	Munhundurwa	Solananceae	Leaf	Treatment	Lukwa et al., 2001
37	*Harungana madagascariensis* Poir	Mukaranga	Guittiferae	Leaf/ root/ stem bark	Treatment	Lukwa et al., 2001
38	*Colophospermum mopane* (Benth.) J. Leonard	Mupane/ Musharu	Fabaceae	Leaf/ stem bark	Treatment	Lukwa et al., 2001
39	*Garcinia huillensis* Oliv.	Mutunduru	Guttiferae	Leaf and stem bark	Treatment	Lukwa et al., 2001
40	*Aloe greatheardii* Schoni (Liliaceae),	Gavakava	Liliaceae	Leaf	Treatment	Lukwa et al., 2001
41	*Pterocarpus angolensis* DC	Mutondo	Fabaceae	Stem bark	Treatment	Lukwa et al., 2001
42	*Terminalia sericea* DC	Mususu	Combretaceae	root	Treatment	Lukwa et al., 2001
43	*Aloe chabaudii* Schonl.	Gavakava	Liliaceae	leaf	Treatment	Lukwa et al., 2001
44	*Euclea divinorum* Hiern	Mubhununu/Mugarazvuru/ Mugurameno	Ebenaceae	Root/ Leaf	Treatment	Lukwa et al., 2001
45	*Bridelia cathartica*	Mutsvoritsvoto	Phyllanthaceae	Roots	Treatment	Mbauya, 2015
46	*Burkea Africana*	Mukarati	Fabaceae	Leaf and	Treatment	Mbauya, 2015

				stem bark		
47	*Tragia okanyua*	Mbawazani	Euphorbiaceae	Leaf	Treatment	Mbauya, 2015
48	*Azanza garkeana*	Mutohwe	Malvacea	Stem bark	Treatment	Mbauya, 2015
49	*Pterocarpus angolensis*	Mubvamaropa	Fabaceae	Stem bark	Treatment	Mbauya, 2015
50	*Afzelia quanzensis*	Mukamba	Fabaceae	Leaf and Stem bark	Treatment	Mbauya, 2015

NO - plant species number, - Absent/not found

4.1.4 The Families of documented plants used against malaria in Zimbabwe.

The plants identified in this literature survey are classified into 30 families. Out of these families, Fabaceae (17%) contributed the most to the families of plants used against malaria in Zimbabwe. Most of the families (27) contributed 5% or less, while only three families (Fabaceae (17%), Solanaceae (10%), and Apocynaceae (7%)) contributed more than 5%. The least recorded families had only a 2% contribution. However, the majority of the families reported in this study align with previous studies (Milliken et al., 2021; Omara, 2020; Shankar et al., 2012; Qayum et al., 2016; Ngarivhume et al., 2015; Titanji et al., 2008). It has been reported that most antimalarial plants belong to the Fabaceae family (Tajbakhsh et al., 2021; Omara, 2020; Alebie et al., 2017). The results are summarised in **Figure 4.1**

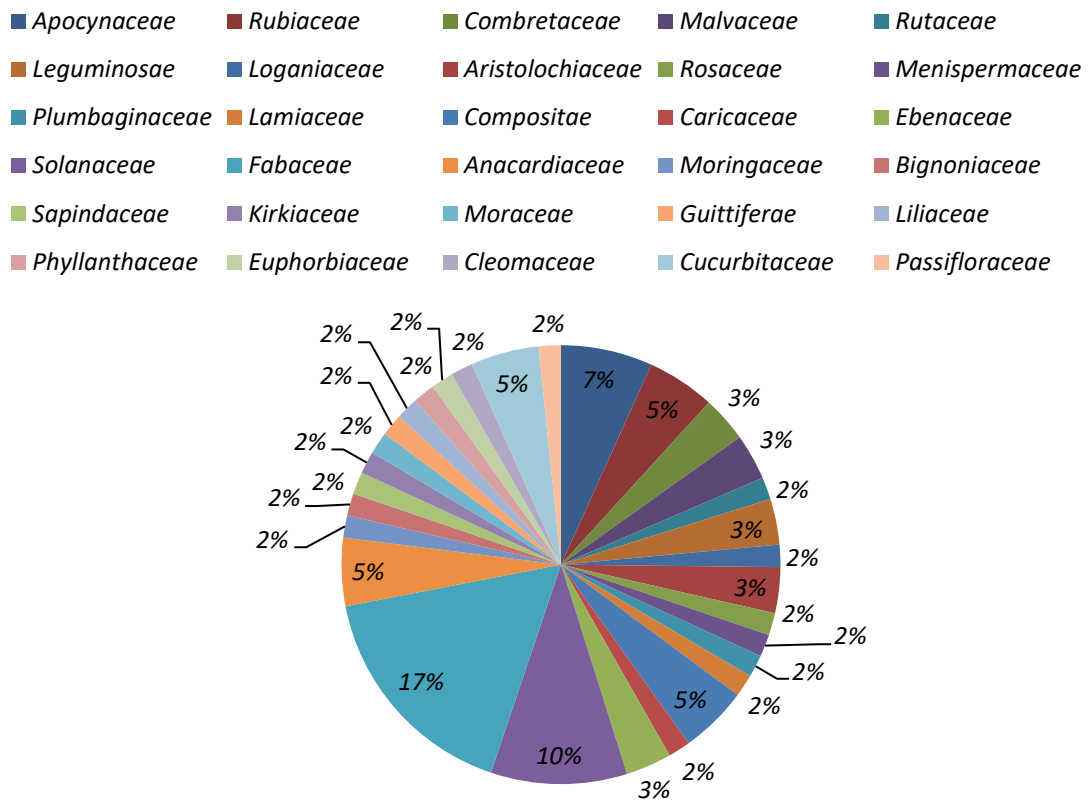


Figure 4.1 The proportion of families of plants used against malaria in Zimbabwe

4.1.5 Plant parts used against malaria in Zimbabwe.

Five parts (fruit, root, stem bark, leaves, and tuber) from plants are utilised in the management of malaria in Zimbabwe. The most widely used plant part is the root (41%), while the least used part is the fruit (2%). The stem bark and leaves are fairly utilised, as indicated by their percentages (24% and 27%, respectively) (Figure 4.2). Generally, the use of fruit, root, stem bark, leaves, and tuber in the management of malaria has been reported in other countries and several studies (Milliken et al., 2021; Omara, 2020; Aska et al., 2019; Qayum et al., 2016; Lima et al., 2015; Ngarivhume et al., 2015). The findings reported in this study corroborate with other research where plant roots were mainly utilised as antimalarials (Ngarivhume et al., 2015; Adebayo & Krettli, 2011). However, it has been reported that leaves are the most commonly used plant parts against malaria (Dkhil et al., 2021; Asafo-Agyei et al., 2019; Maroyi, 2013; Erinoso et al., 2012).

Although there is limited information on the use of fruits in the prevention and treatment of malaria in Zimbabwe, the use of fruits in the management of malaria outside Zimbabwe has been reported

(Tajbakhsh et al., 2021; Dkhil et al., 2021; Qayum et al., 2016; Erinoso et al., 2012; Titanji et al., 2008). The findings on plant parts used against malaria in Zimbabwe are presented in **Figure 4.2**.

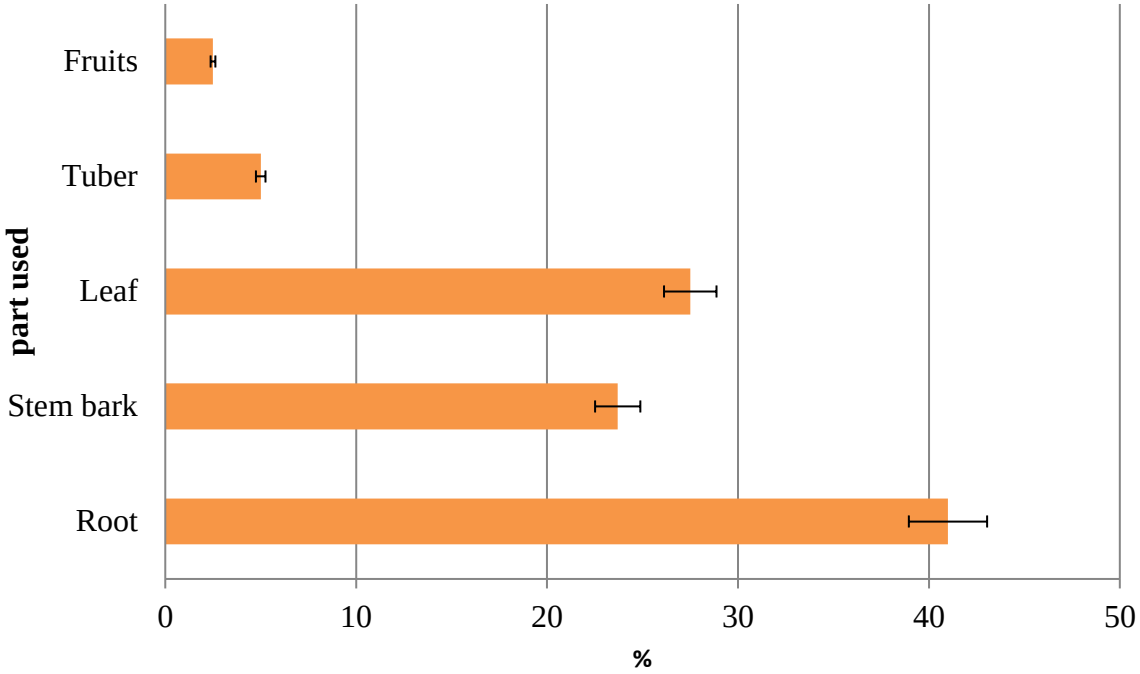


Figure 4.2 Plant parts used in the treatment and prevention of malaria in Zimbabwe.

4.1.6 Methods of antimalarial plant-based drugs preparation in Zimbabwe

Seven methods of preparing plant-based antimalarial remedies were identified in the literature: cold infusion, hot infusion, decoction, plant leaves cooked and consumed as relish, powder consumed in porridge, powder added to food or drink, and powder mixed with goat milk. The most commonly used methods for preparing antimalarial plant extracts traditionally are cold infusion (45%), decoction (24%), and hot infusion (18%). The remaining methods collectively account for 13%, with the least applied method at 1% (**Figure 4.3**).

The use of infusions (both cold and hot) in the preparation of plant-based drugs has been reported beyond the borders of Zimbabwe (Ezeani et al., 2022; Omara, 2020; Alebie et al., 2017). The use of decoction and infusion in preparing antimalarial plant extracts was also significantly pronounced in other studies (Omara, 2020; Alebie et al., 2017; Erinoso et al., 2012; Al-Adhroey et al., 2010).

For example, approximately 51% and 24% of plant extracts were prepared through decoction and infusion, respectively (Randrianarivelojosia et al., 2003). Although in this particular review infusion was the most applied method of antimalarial drug preparation, in most studies, decoction was reported as the most commonly used technique (Alebie et al., 2017; Mpiana et al., 2012; Dibong et al., 2011; Nguta et al., 2010). **Figure 4.3** provides a summary of the results.

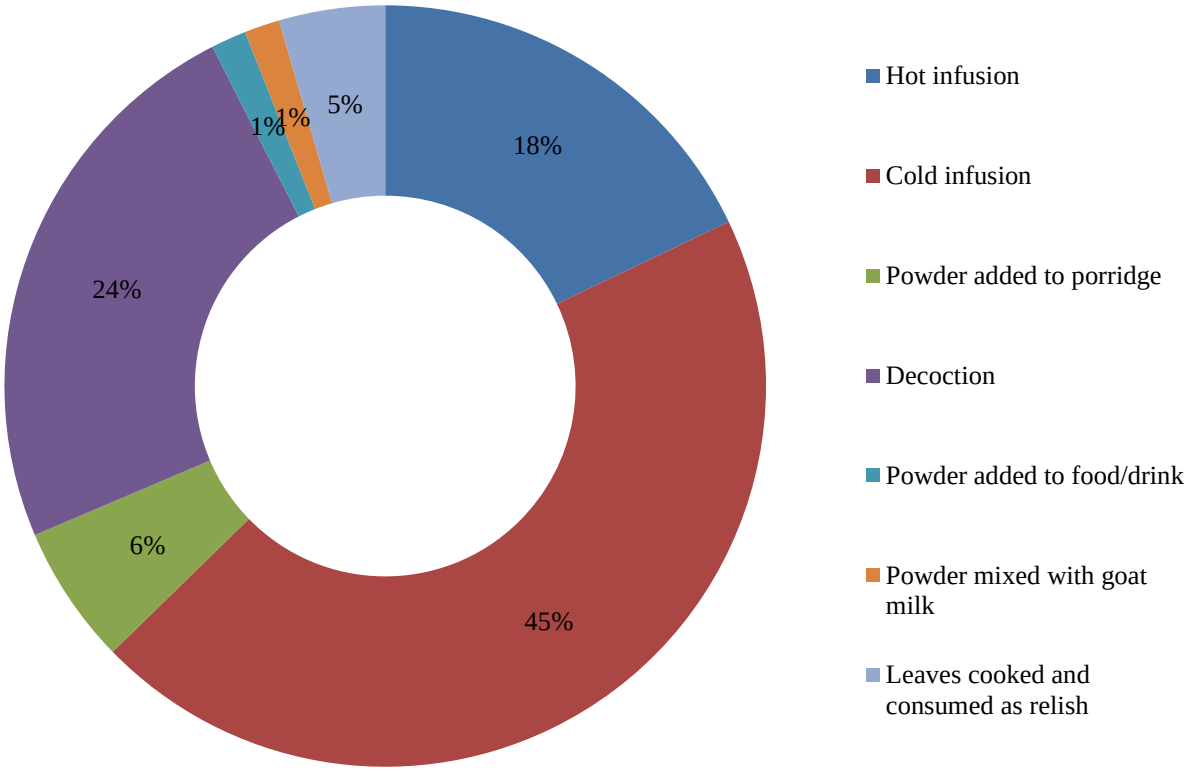


Figure 4. 3 Methods of preparation of antimalarial plant-based extracts

4.2 Indigenous practices exploited in the fight against malaria in Zimbabwe.

Based on data from a literature survey, five classes of indigenous knowledge practices utilized against malaria are: (1) the use of traditional environmental indicators, (2) the use of plant and other traditional medicines against malaria, (3) the use of red-hot granite rock and water, (4) spiritual malaria treatment based on faith and traditional healers, and (5) burning of dung and plants to deter malaria vectors.

4.2.1 Herbal and other traditional medicines used in malaria control and treatment.

Herbal and other traditional medicines used in malaria control and treatment have limited information in Zimbabwe. The available information is mainly based on ethnobotanical surveys without scientific evaluation (Chapu and Mgochecki, 2017; Ngarivhume et al., 2015; Mbauya et al., 2015; Kazembe et al., 2012; Lukwa et al., 2001). Traditional medicine is one of the most accessible and widely available forms of therapy in Zimbabwe's resource-limited communities. The indigenous people have great experience using traditional therapeutic drugs (Olsson et al., 2015; Dhewa, 2008). Traditional medical practitioners in Zimbabwe include herbalists, psychic therapists, midwives, fortune tellers, seers, and faith healers who design techniques and materials using indigenous knowledge (Rupande, 2022; Hlatshwayo, 2017). Despite the increasing acceptance of ethnomedicine in Zimbabwe, much traditional knowledge remains unrecorded (Usai et al., 2022; Maroyi et al., 2012). However, documentation of traditional medicines is necessary to maintain knowledge, preserve it, and use it sustainably. In Zimbabwe, the Traditional Medical Practitioners Act of 1981 attempted to incorporate indigenous medicines into mainstream healthcare systems, but failed due to a lack of financial and institutional support (Ngarivhume et al., 2015). Nevertheless, many residents in Zimbabwe, particularly in rural areas, continue to use traditional medicines for basic healthcare, despite the lack of documentation and regulation (Van Wyk and Prinsloo, 2018).

4.2.2 The use of traditional environmental indicators to control malaria.

Malaria incidence has been shown to strongly correlate with rainfall and temperature changes. In Zimbabwean communities, plant phenotypic plasticity and insects are mostly utilised markers in the prediction of malaria (Macherera et al., 2016). Additional malaria prediction markers exploited in Zimbabwe include apparent noise originating from mountains (thundering of mountainous regions) and specific ostrich behaviours. According to Macherera et al., (2016), there are five markers of how much rainfall will be received and hence the possibility of malaria outbreak: (1) Arthropod markers, (2) Bird predictors, (3) Animal attributes, (4) Plant phenology, and (5) Astronomical and meteorological signals (**Table 4.4**).

The insects recognised by residents of Gwanda district, Matebeleland South, Zimbabwe, mark the start of the rainy season and indicate whether the amount of rainfall is high or low. For example, an increased population of termites indicates the start of the rainy season (Macherera et al., 2016;

Chanza & de Wit, 2014; Risiro et al., 2012). Though such a marker does not reflect the quantity of the rain or the extent of malaria prevalence, it does herald the start of the malaria season. The appearance of massive white butterflies migrating from west to east in October indicates considerably high rainfall and hence a high malaria prevalence (Macherera et al., 2016).

Most bird actions predict whether the rainy season will be excellent or bad; however, a few species simply herald the start of the rainy season (Kupika et al., 2019; Musarandega et al., 2018; Chanza & de Wit, 2014; Risiro et al., 2012). The noise of ground hornbills from September to November, as well as the egg laying by the guinea fowls, indicate a favourable rain season and a possibility of an increase in incidences of malaria (Macherera et al., 2016). However, the appearance of huge numbers of species such as Swallows and Migratory birds in November indicates that the rainy season is approaching.

Wild animals have been recognised as reliable predictors of rainfall and malaria prevalence (Nyahunda & Tirivangasi, 2021). It was discovered that if wild animals have a large number of young ones, more rainfall would be obtained in the next season, leading to high malaria prevalence (Macherera et al., 2016). Furthermore, the passing of lions without interfering with the activities of domesticated animals or humans in the period starting from September to October signifies a favourable rainy upcoming season (Macherera et al., 2016). This shows that the likelihood of malaria transmission will be substantially high.

Plant phenology indicators are such as the absence and presence of leaves, fruits, and flowers, indicate high or low rainfall (**Table 4.4**) and hence intensity of malaria prevalence (Kupika et al., 2019; Mashoko, 2014). For example, if plants like *Lonchocarpus capassa* flowers in October and November, and *Boscia albitrunca* produces flowers early and numerous fruits in November, it indicates significantly high rainfall and therefore high malaria incidences. However, the presence of many fruits in plants such as *Acacia nigrescens* indicates low rainfall and drought is signified by the plant taking longer to produce new leaves (Macherera et al., 2016). In both cases, malaria prevalence is projected to be extremely low.

In Gwanda district, Matebeleland South, Zimbabwe, astronomical and meteorological signals (**Table 4.4**) such as clouds, humidity, and temperature variations are utilised to forecast rainfall patterns and the intensity of malaria transmission (Macherera et al., 2016). High rainfall and

malaria prevalence are indicated by the presence of a band all around the moon, finely distributed clouds, clouds all along the frontier, hot humidity interchanging with strong winds, gentle breeze changing directions that is east to west and sometimes west to east, increased frequency and intensity of whirlwinds in September through to October, and misty air during September and October (Macherera et al., 2016).

4.2.3 Burning of dung (cow or elephant) and plants to deter malaria vectors.

In most regions of Zimbabwe, hanging fresh leaves of *Ocimum canum* in the home efficiently provided mosquito bite protection. Besides hanging leaves, plants like *Azadirachta indica* and *Ocimum forskolin* have been burnt to generate repellent smoke that has considerably reduced human-mosquito bites (**Table 4.4**). Burning of plants to deter mosquitoes has been done in other African countries. In one trial conducted in Kenya, a combination of plant powders burnt directly produced smoke that repelled malaria vectors such as *Anopheles aegypti* (Wendimu & Tekalign, 2021).

A comprehensive survey in Chiredzi district, Masvingo, Zimbabwe (**Table 4.4**), indicated that most households burn dung (cow or elephant dung) and plant leaves to generate smoke, which repels mosquitoes (Chapu & Mgocheki, 2017). Several explanations have been proposed to explain how smoke fumigation of houses may change mosquito feeding behaviour. Plant and dung-derived smoke normally obscures human odours and carbon dioxide, which mosquitoes sense when a human being is close by (Moore & Lenglet, 2004). Additionally, smoke from plants normally interferes with mosquito receptors responsible for detecting chemicals by reducing water vapour in the air (Davis and Bowen, 1994). Furthermore, dung and plant smoke are associated with a wide range of organic compounds classified as insecticides, repellents and irritants (Moore & Lenglet, 2004). Such organic compounds include palmitic acid, capric and oleic acid.

Mosquito repellent herbs include *Chenopodiumam brosioides*, *Lippia javanica*, and *Chrysopogon nigratana*. To deter mosquitoes, dry pieces of plants such as *Spirostachys africana* are burnt, and glowing portions are utilised (**Table 4.4**) (Chapu & Mgocheki, 2017). This burning procedure is comparable to how mosquito coils are used nowadays. Elephant dung has historically been burnt and the smoke used for fumigation houses (Dubost et al., 2021, Maphane, 2018; Pennacchio et al., 2010). However, it should be emphasised that burning in poorly ventilated areas can expose

occupants to extraordinarily high amounts of indoor air pollution (Gordon et al., 2014). This is a common issue in low-income countries like Zimbabwe, particularly in rural regions where plants are abundant.

4.2.4 Spiritual malaria treatment based on faith and traditional healers.

Zimbabwe's Shona people, like monotheistic religions, have always believed in the power of God in the healing of diseases and general well-being (Kazembe, 2009). The connection between human beings with the metaphysical world is important before birth, during life, at death, and after death (Machingura, 2012). Among the Shona-speaking people of Zimbabwe, *mashavi* (wandering spirits) and *mhondoro* (wishful spirits) are powerful spirits in disease (e.g. malaria) prevention and treatment (Masaka & Makahamadze, 2013). Although *mashavi* and *mhondoro* are of critical importance in the *Shona* culture, their importance cannot supersede *vadzimu* in the everyday life of some Zimbabweans (Masaka & Makahamadze, 2013). As a result, Shona cosmology stresses the significance of *vadzimu* in terms of disease prevention and treatment, life and death. Zimbabweans also believe in taboos, which are important in illness prevention (Machingura, 2012). In some communities in Zimbabwe, the elderly (as well as departed descendants) are key in maintaining access to and inheritance of disease treatment and prevention through specific dreaming *(kurotswa*) and ceremonial divinations (*kusvikirwa*), in which the knowledge is disclosed as a gift (Masaka & Makahamadze, 2013).

According to informants, malaria, like other ailments, may be cured through spirituality based on the individual's level of commitment and faith. It is believed that nothing seems to be impossible with God (Kpobi & Swartz, 2018). Among other interventions, exorcism, anointed oil application, and the use of holy water have been reported to cure malaria. In Zimbabwe, the believers believe that malaria may be healed by pleading with God since it is thought that the disease is God's punishment (Treadwell, 2001). Additionally, some churches in Zimbabwe recommended that congregants drink plenty of water to avoid contracting the sickness, including malaria. Based on this notion, all forms of illnesses may be effectively cured with water treatment (Bosire et al., 2022; Hjelm & Mufunda, 2010).

4.2.5 The use of red hot granite rock and water (Steaming).

A red-hot stone is immersed in cold water and begins to boil. The patient and red hot stone are covered under a blanket or cloth (known as *kufukira* in Zimbabwe), causing the patient to sweat excessively (Chapu & Mgocheki, 2017). Steaming is commonly advised as an at-home treatment for upper respiratory tract infections. Heat, the most fundamental aspect of steaming, has a substantial influence on host immune response and pathogenicity, as well as activating responsive thermoregulation systems that could elevate or lower body temperatures to maintain equilibrium (Schieber & Ayres, 2016). Furthermore, raising the body temperature strengthens the second line of defence by generating thermal stress, which mimics feverish episodes (Schieber & Ayres, 2016).

Heat stress caused by steaming stimulates the immune system (Heinonen & Laukkanen, 2018). Cold exposure following heat stress enhances the immune system through a rise in natural killer cell number and activation, as well as quantities of interleukin 6 in blood (Brenner et al., 1999). Furthermore, reflux occurs in response to blood being redirected in the tissues, which aids in detoxification (Cochrane, 2004). Multiple cellular reactions are triggered by antispasmodic temperatures, including a highly complex gene expression regulation pathway, inflammatory processes, and the immune responses (Singh & Hasday, 2013). During steaming, heat shock proteins are produced and moved to cell membranes (Iguchi et al., 2012). The principal role of these proteins is to regulate and prevent damage in immune cells and the denaturation of proteins. Moreover, heat shock proteins contribute significantly to the activation of polymorphonuclear leukocytes, antigen presentation, dendritic cells recruitment and differentiation (Singh & Hasday, 2013)

Increased temperature caused by steam inhalation helps to improve cardiac health by influencing the autonomic nervous system, reducing blood pressure, inflammatory processes, and peroxidation. This, however, promotes and increases heart rate, blood volume, and perivascular blood circulation, and enhances cardiovascular health, serum lipid profile, and arteriolar regulation (Laukkanen & Kunutsor, 2019; Kunutsor et al., 2018; Heinonen & Laukkanen, 2018). Another advantage of thermal stress is the adjustment in blood pH produced by rapid breathing induced by hyperthermia, which is accompanied by pulmonary alkalosis (Tsuji et al., 2016). A summary of the results is presented in **Table 4.4**

Table 4.4: Indigenous practices in prevention and treatment of malaria in Zimbabwe.

Indigenous practice	Remarks on treatment /prevention /natural products used in Zimbabwe.	Applicability in Zimbabwean mainstream health systems	References
Herbal medicine	According to an ethnobotanical survey conducted in Manicaland Province, Zimbabwe, plants such as *Elephantorrhiza goetzei* (Harms), *Toddalia asiatica* (L.) Lam and *Pavetta schumanniana* F. Hoffm. In this particular study, *Aristolochia albida* and *Cassia abbreviata* were the most utilised herbal medicines against malaria.	As a method of preserving indigenous practices and traditional medicines, such as herbal medicines, the Traditional Medical Practitioners Act of 1981 was introduced (Shoko, 2018). This was meant to incorporate traditional medicine into the mainstream Zimbabwean health system. With proper scientific evaluation and screening, herbal medicines can be the basis of new antimalarial drugs that can be used beyond localised communities	Ngarivhume et al., 2015
	An ethnobotanical survey conducted in malaria hotspots of Zimbabwe revealed that the indigenous people are highly confident in herbal medicines, which are used against malaria. Some of the plants that are utilised in malaria-prone areas include *D. nitidula A. amara, V.*	Although herbal medicines are used for primary health care needs in Zimbabwe, the claim that combining these medicines with Western drugs improves efficacy is not always true. It	Kazembe et al., 2012

	infausta, A. digitata, and *L. discolour*.	depends on the type drug interactions which can be antagonistic, synergistic and additive. However, it has been concluded that some antimalarial plant-based drugs can compete with established drugs against malaria. This therefore, indicates that if plant based antimalarial drugs are scientifically evaluated especially for toxicity level can be applied beyond primary health in Zimbabwe.	
	In a survey conducted in Chiredzi district, Masvingo Province, Zimbabwe, antimalarial plants including *Adansonia digitata* roots, *Capsicum anuum* fruits, and *Warburgia salutaris* bark,	Results from this study indicated that plant remedies were combined with antimalarial drugs such as chloroquine. Although plant based antimalarial drugs are used traditionally in primary health care, there is need for assess their efficacy so that they are fully used in hospitals and readily in Zimbabwean pharmacies.	Chapu & Mgochek, 2017

	In Mola, Kariba district, Mashonaland West Province, Zimbabwe, some of the identified antimalarial plants include *Amblygonocarpus andongenis* (Olvi.), *Carica papaya* L. (Caricaceae), *Zanha africana* (Radlk.), *Solanum panduriforme*, *Diplorhynchus condylocarpon*, *Euclea divinorum* Hiern, *Aloe greatheardii* Schoni.	Antimalarial herbal medicines can be used as primary health care medicines, and with scientific evaluation on efficacy and toxicity, can be applied in the main health system.	Lukwa et al., 2001
	Pant species collected from several stations in Zimbabwe along the Harare-Nyamapanda road close to Marondera, Mashonaland East Province, Zimbabwe, were assessed for antiplasmodial activities. The targeted plants were *Cussonia spicata*, *Flueggea virosa*,*Vernonia colorata*, *Artemisia afra*, *Clutia hirsute*, *V. natalensis*, *Parinaricurate llifolia*, *Hymenodictyon floribundum*, and *Adenia gummifera*. Among all these plants, extracts from *Vernonia colorata* and *Artemisia afra* are active against both PoW and Dd2 (*Plasmodium* strain)	Based on the bioassays and identified *A. afra* and *V. colorata* extracts can be applied as antimalarial drugs. Although the IC50 values were less than 50µg/ml (1.1-12.6 µg/ml), there need to evaluate the toxicity of these extracts and to prescribe quantities that can be utilised. Moreover, *in vivo* assays and clinical tests should be carried out so that these herbal medicines are incorporated into the mainstream health system.	Kraft et al., 2003
	In a doctoral study conducted in Chikwanha-Muneri villages of Marange District, Manicaland Province, Zimbabwe, plants traditionally used to manage malaria were identified, and these include *Cassia abbreviate*,	Antimalarial herbal medicines can be used as primary health care medicines, and with scientific evaluation on efficacy and	Mbauya, 2015

	Bridelia cathartica, Burkea africana, Tragia okanyua, Pterocarpus angolensi and *Afzelia quanzensis*.	toxicity, can be applied in the main health system.	
The use of traditional environmental indicators to control malaria **Arthropod markers** **Bird predictors** **Animal attributes**	Insects identified in Gwanda district, Matabeleland South, Zimbabwe, for example, termites, were shown to mark the start of the rainy season. Additionally, massive white butterflies migrating from west to east in the month of October indicates considerably high rainfall.	Though termites do not reflect the quantity of the rain or the extent of malaria prevalence, it does herald the start of the malaria season. However, white butterflies in this case indicate high malaria prevalence. These markers can be applied in preparing for malaria outbreaks.	Macherera et al., 2016
Plant phenology **Astronomical and meteorological signals**	It has been shown that the noise of ground hornbills and the laying of eggs by guinea fowls from September to November indicate a favourable rainy season. However, Swallows and Migratory birds in November show the onset of the rainy season.	This is applicable in two ways since: (1) Early warning markers for the start of malaria and (2) Malaria incident intensity prediction.	Macherera et al., 2016.
	It was discovered that if wild animals have a large number of young ones and lions move past farmsteads without interfering with domesticated animals and humans in summer indicates more rainfall would be obtained in the next season.	This shows that the likelihood of malaria transmission will be substantially high.	Macherera et al., 2016
	Flowering of plants like *Lanchocarpus capassa* in October and November, and *Boscia albitrunca* in early and numerous fruits by November indicate significant rainfall in	Plant phenology indicators indicate the presence of high or low rainfall and,	Macherera et al., 2016

	that season. *Acasianigrescens* indicates low rainfall and drought if it takes longer to produce new leaves.	hence intensity of malaria prevalence.	
	In Gwanda district, Matebeleland South, Zimbabwe, astronomical and meteorological signals such as clouds, humidity, and temperature variations are utilised to forecast rainfall patterns.	In this case, humidity/rainfall and temperature can be used to predict malaria prevalence and intensity.	Macherera et al., 2016
The use of red-hot granite rock and water,	A red-hot stone is immersed in cold water and begins to boil. The patient and red hot stone are covered under a blanket or cloth (known as *kufukira* in Zimbabwe).	Although there is no published information linking this traditional method, it has been revealed that raising the body temperature strengthens the second line of defence by generating thermal stress, which mimics feverish episodes.	Chapu & Mgocheki, 2017
The burning of dung and plants to deter malaria vectors	A comprehensive survey in Chiredzi district, Masvingo, Zimbabwe, burning of dry pieces of plants such as *Spirostachys africana* and dung (cow or elephant dung) has been done to deter mosquitoes.	Evidence to suggest that smoke is an efficient repellent, and the practice of smoke fumigation of rooms to avoid biting mosquitoes is common. This is a preventive method that can be applied with precautionary measures to prevent indoor air pollution.	Chapu & Mgocheki, 2017.
	In most regions of Zimbabwe, hanging fresh leaves of *Chenopodium ambrosioides*, *Lippia javanica*, *Chrysopogon*	This is a preventive method that can be applied. Most plant-derived fumigants	Chapu & Mgocheki, 2017.

	nigratana, and *Ocimum canum* in the home efficiently provided mosquito bite protection.	are employed as mosquito repellents since they contain mosquito-repelling essential oils.	
Spiritual malaria treatment based on faith and traditional healers.	In Zimbabwe, the believers believe that malaria may be healed by pleading with God since it is thought that the disease is God's punishment. Additionally, some churches in Zimbabwe recommended that congregants to drink plenty of water to avoid contracting the sickness including malaria.	There is no scientific evidence to support the applicability of this traditional method in the Zimbabwean health systems.	Treadwell, 2001
	Among the Shona-speaking people of Zimbabwe, *mashavi* (wandering spirits), *mhondoro* (wishful spirits) and *vadzimu* are critical in disease prevention and treatment for some Zimbabweans.	There is no scientific evidence to support the applicability of this traditional method in the Zimbabwean health systems.	Masaka & Makahamadze, 2013
	In some communities in Zimbabwe, the elderly (as well as departed descendants) are key in maintaining access to and inheritance of disease treatment and prevention through specific dreaming *(kurotswa*) and ceremonial divinations (*kusvikirwa*), in which the knowledge is disclosed as a gift	There is no scientific evidence to support the applicability of this traditional method in the Zimbabwean health systems.	Masaka & Makahamadze, 2013

4.3 Plants selected for antiplasmodial activity and toxicity tests.

Based on a specified criterion in **chapter 3** ((1) existence in published work focusing on Zimbabwe, (2) lack or limited information on toxicity and activity against *Plasmodium* species (lack pharmacological references), (3) indigenous to Zimbabwe, (4) accessibility of the plant

species and (5) presence of sample which can be collected sustainably), a total of nine (9) plant species were selected from the 61 plant species identified in literature. The selected plants are indicated in **Table 4.5.**

Although there is limited information on the use of *Kirkia acuminata* against malaria**,** it has been noted that the plant has been exploited as a remedy against a number of ailments such as cholera, dysentery, toothache diarrhoea and fever (Maroyi, 2017and 2016). The use *Kirkia acuminata* against malaria maybe linked to its use in fever treatment.

Classes of bioactive compounds identified in crude extracts of *Elephantorrhiza goetzei* (Harms) include triterpenoids, stilbenoids, phenolic compounds, flavonoids, and tannins. The nu-butanol, water, ethyl acetate and dichloromethane of *E. goetzei* crude extracts were assessed for both antifungal and antibacterial capacity. Medicinal properties of *Elephantorrhiza goetzei*, which have been reported, include gastrointestinal treatment, treatment of sores and STIs (Maroyi et al., 2017; Ngarivhume et al., 2015; Grobler, 2012; Wanjala and Majinda, 2001).

Tabernaemontana elegans Stapf was reported to treat malaria (Ngarivhume et al., 2015), and according to Nzama (2023), Naidoo et al. (2021) and Mashilo (2020), this plant species was reported to treat venereal diseases, heart disease, tuberculosis and cancer.

Diplorhynchus condylocarpon (Müll.Arg.) Pichon is associated with bioactive compounds reported from this plant, including 14-hydroxy (-)-akuammicine, á-tocopherols, Condylocarpine and Nor-fluorocurarine (Bitwell et al., 2023), and these bioactive compounds could have contributed to the exploitation of the plant in the treatment of fever (Shopo et al., 2022), which is one of the major symptoms of malaria. It has been reported that *Diplorhynchus condylocarpon* aqueous, methanol, and dichloromethane root extracts were significantly active against *Plasmodium falciparum* (D10) (Malgrinia & Valentin, 2023). However, the study to confirm the activity against *Plasmodium falciparum* (D10) was done outside Zimbabwe.

Though there is limited information on the application of *Holarrhena pubescens* against malaria, the plant extracts from the seed and stem bark were found to contain 3-aminopregnans, canniness, steroidal alkaloids, for example, conarrhimine, holonamine and 12-hydroxyconessimine, triterpenes, e.g. ursolic acid and lupeol β-hydroxyhexad-ecanoate, sterols such as stigmasterol.

Furthermore, the extracts from this plant have been applied against diseases including diarrhea, bleeding piles, cholera, liver diseases, amoebic dysentery, anemia, stomach pain, jaundice, epilepsy, blood-based conditions, leprosy, chest infections, dyspepsia, and asthma (Singh et al., 2023; Zahara et al., 2020; Akbar & Akbar, 2020).

The bioactive compounds identified from the *F. ingens* stem bark and leaf extracts include (1) quercetin glycosides, e.g., arabinoside and quercetin-*O*-glucuronide, (2) phenolic acids, e.g., quinic and 3-*O*-caffeoylquinic, and (3) fatty acids, e.g., palmitic acid derivative. Additionally, leaf methanol-derived extracts and infusions have been shown to contain phenolic acids, hydroxycoumarin, flavonoids, methyl gallate, phenolic acid derivatives, 2-isopropylmalic, and coumaric (Sieniawska et al., 2022; Ogunlaja et al., 2022). The plant extracts have been utilised in the management of skin diseases, influenza, diarrhoea, tuberculosis, anaemia and STIs. Generally, the plant leaves have been used as a blood booster due to high iron content (Sieniawska et al., 2022; Ogunlaja et al., 2022; Mbauya, 2015).

The use of *Garcinia. huillensis* against malaria was reported in Zimbabwe. However, there is no scientific evidence reported that indicates its use in Zimbabwe. Based on studies done out of the country, extracts from *Garcinia species* were found to contain δ-selinene, β-caryophyllene, caryophyllene oxide and α-humulene valencene. Generally, the plant extracts are associated with substantial concentrations of xanthones, anthraquinones, benzophenones, tannins, flavonoids, steroids, phenolic and lactones (Padmalochana et al., 2013; Dibwe et al., 2012; Magadula & Suleimani, 2010). Traditionally, the plant bark extract has been utilised against diseases, including schistosomiasis. Additionally, fruit extracts have been used to reduce birth canal and ripe fruits have been eaten as an aphrodisiac (Maroyi, 2013; Dibwe et al., 2012; Magadula & Suleimani, 2010; Lukwa et al., 2001).

Though the scientific evaluation data on *P. anglolensis* against *Plasmodium falciparum* is limited, the plant extracts were found to contain significant concentrations of lup-20(29)-en-3β-ol, flavonoids, tannins and hexadecanoic acid (with antifungal and antibacterial capacity). Additionally, *P. anglolensis* extracts exhibited antimicrobial (against *S. typhimurium*, *E. coli* and *S. aureus*) and anti-inflammatory activities (Omara et al., 2022; Mbabazi et al., 2020; Santos et al., 2020; Bene et al., 2019). The plant root extracts have been used to relieve and treat toothache,

dermal ailments, oral and genital diseases, diarrhoea and cancer. However, fruits have been shown to treat skin diseases, abdominal pain, respiratory disorders, upsets, kidney infections and urine retention. The leaves of *P. anglolensis* have been used to treat tapeworm-based infections, gonorrhea, leprosy and syphilis. Overall, *P. anglolensis* has been used as an aphrodisiac, anti-snake venom, analgesic, antidiabetic, and antimycobacterial (all these properties require scientific evaluation) (Omara et al., 2022; Mbabazi et al., 2020; Muthaura et al., 2015; Lukwa et al., 2001).

E divinorum extracts have been revealed to contain bioactive compounds including alkaloids, tannins, saponins, steroids, terpenoids, flavonoids and glycosides (all these were identified in root extract). The methanol-based extracts of *E divinorum* were found to contain glycosides and polyphenols. Moreover, chloroform extracts were reported to contain triterpenoids (e.g. botulin, lupine and lupeol) and naphthoquinones (e.g. methyljuglone and diospyrin). Previous studies have indicated that *E divinorum* extracts have some properties, including contractile, diuretic, anti-giardial, antinociceptive, antiproliferative, renoprotective, proteolytic, antiprotozoal, antioxidant, molluscicidal, insecticidal, antimicrobial and anti-inflammatory (Omara et al., 2022; Girmaw & Engidawork, 2022; Mbabazi et al., 2020).

Table 4. 5: Plants selected for antiplasmodial activity and toxicity tests.

Identified Zimbabwean plant species	**Voucher number**	**Reported activity against *Plasmodium falciparum* and toxicity tests in Zimbabwe**	**Coordinates of locations where plants were collected**
K. acuminata	Makuvara Z, Marumure J 6	No reports	S20^{0}2’58.64676’’ E30^{0}48’6.6837’’
E. goetzei	Makuvara Z, Marumure J 10	No reports	S20^{0}9’3.4525’’ E31^{0}17’48.4256’’
E. divinorum	Makuvara Z, Marumure J 11	No reports	S20^{0}6’14.13108’’ E30^{0}51’15.7698’’

D. condylocarpon	Makuvara Z 12	No reports	S20^{0}6'7.86424'' E30^{0}51'40.8594''
H. pubescens	Makuvara Z 13	No reports	S20^{0}2'58.64676'' E30^{0}48'6.6837''
F. ingens	Makuvara Z 14	No reports	S20^{0}6'6.63804'' E30^{0}51'42.1783''
G. huillensis	Makuvara Z 16	No reports	S19^{0}57'3.67776'' E30^{0}46'7.04316''
P. anglolensis	Makuvara Z 17	No reports	S20^{0}6'11.19888'' E30^{0}50'16.33488''
T. elegans	Makuvara Z 19	No reports	S20^{0}9'3.4525'' E31^{0}17'48.42516''

4.4 Percentage yield of water, methanol and ethyl acetate plant extracts

4.4.1 Differences in percentage yields of crude extracts when solvents (methanol, ethyl acetate and water) were used on different plant species.

The percentage yields of the crude extractions after maceration in methanol were significantly different ($p < 0.05$). The quantity of *G. huillensis* leaf crude extract (14.59%) was significantly higher ($p < 0.05$) than the quantity of all other crude extracts. The lowest percentage yield was recorded for *D. condylocarpon* (3.6%), and therefore, the percentage yield for all nine plants when methanol was used as an extraction solvent ranged from 3.6% to 14.59%. Although there were significant differences between crude extracts when methanol was used, there were no significant differences ($p < 0.05$) between *H. pubescens* and *P. anglolensis* crude extracts (Table 4.4). The use of ethyl acetate as a solvent resulted in percentage yields of crude extracts ranging from 1.31% to 7.38%. Ethyl acetate-based crude extracts showed significant differences ($p < 0.05$) between plant species. However, no significant differences ($p < 0.05$) were noted between the yields of *K. acuminata* and *D. condylocarpon* extracts, and *H. pubescens* and *T. elegans* extracts, respectively.

Water crude extracts had significant differences ($p < 0.05$), and the yields in this instance ranged from 2.96% to 10.36%. The water extracts for *E. goetzei* and *E. divinorum*, and *P. anglolensis* and *T. elegans* were not significantly different ($p < 0.05$).

The use of methanol, water, and ethyl acetate as extraction solvents for antimalarial plants was reported in several studies (Chaniad et al., 2023; Annan et al., 2015; Adia et al., 2016; Lumpu et al., 2013; Shuaibu et al., 2008; Boyom et al., 2009; Waako et al., 2007). The results presented in **Table 4.6** and **Figure 4.4** (which shows that methanol was the best solvent for the selected antimalarial plants) were in line with findings by Muganga et al. (2014) and Mohd Abd Razak et al. (2014). Generally, it was indicated that the differences in yields of extracts from different solvents are a result of the polarity of different phytochemicals and solvents used (Pareck et al., 2017). The high yields recorded for crude extracts when methanol is used may be linked to the low polarity of methanol, which has allowed for the extraction of both non-polar and polar phytochemicals. However, aqueous extracts from *E. divinorum* were significantly higher than methanol extracts (**Figure 4.4**). This indicates that most phytochemicals in *E. divinorum* are soluble in water and, therefore, for this particular plant, water was the best solvent for obtaining extractable solids.

The application of maceration as an extraction technique was done in several studies (Palla et al., 2020; Tagne et al., 2019; Tadesse et al., 2017; Peace et al., 2013). The crude extract yields produced in this current study were low (less than 15%) (**Table 4.6**). These findings are in line with what was reported by Peace et al. (2013), who indicated that maceration produced the lowest yields compared to advanced extraction techniques. Although maceration is not effective, its application in this current study was based on the fact that it is simple, cheap, and traditionally applied in Zimbabwe.

Table 4. 6: Percentage yields of crude extracts when solvents (methanol, ethyl acetate and water) were used on different plant species.

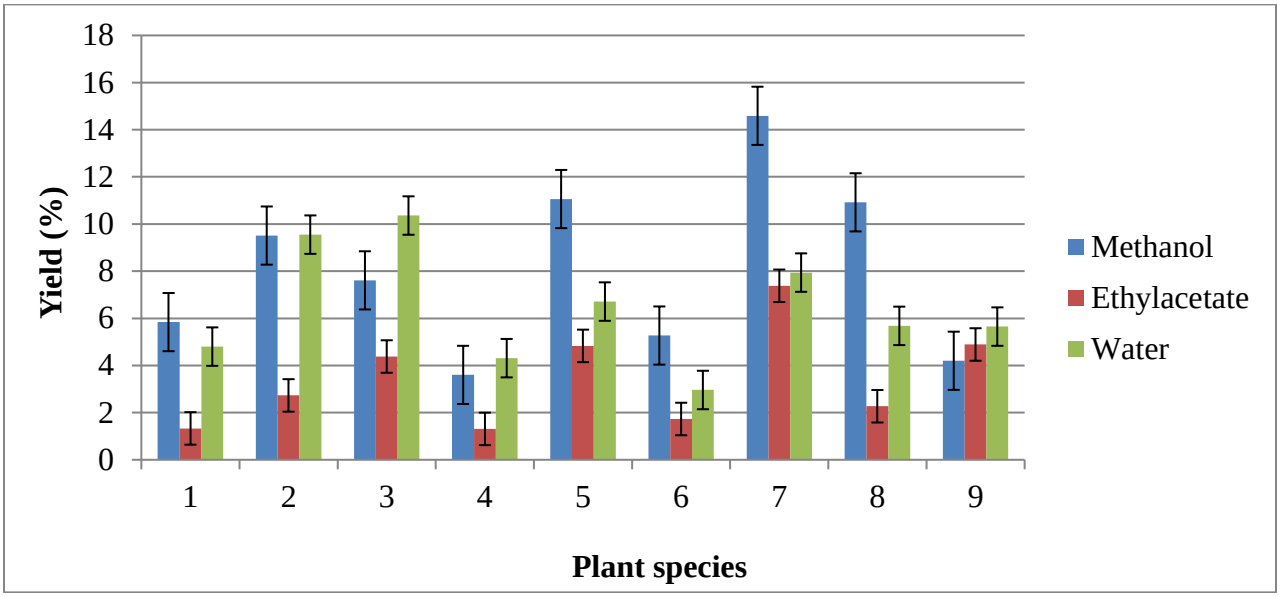
Plant	Yield (%)		
	Methanol	**Ethyl acetate**	**Water**
K. acuminata	5.84±0.08^{c}	1.33±0.13^{a}	4.80±0.05^{bc}
E. goetzei	9.51±0.10^{d}	2.73±0.58^{c}	9.55±0.62^{f}
E. divinorum	7.61±0.12^{e}	4.38±0.06^{d}	10.36±0.73^{f}
D. condylocarpon	3.60±0.27^{a}	1.31±0.07^{a}	4.31±0.09^{b}
H. pubescens	11.06±0.17^{f}	4.83±0.10^{d}	6.71±0.05^{d}
F. ingens	5.27±0.15^{c}	1.73±0.07^{ab}	2.96±0.06^{a}
G. huillensis	14.59±0.47^{g}	7.38±0.06^{e}	7.94±0.04^{e}
P. anglolensis	10.92±0.10^{f}	2.27±0.21^{bc}	5.68±0.22^{c}
T. elegans	4.20±0.10^{b}	4.89±0.13^{d}	5.65±0.07^{c}
Grand mean	**8.07**	**3.43**	**6.44**
LSD	**0.36**	**0.38**	**0.57**
cv%	**2.6**	**6.5**	**5.1**
p-value/F-value	**<.001**	**<.001**	**<.001**

LSD- least significant differences, cv%- coefficient of variation, percentage yields in the table were recorded as mean ± standard deviation (three replicates). The means with different letters (superscripts) in the same column are significantly different at $p< 0.05$.

4.4.2 Differences in percentage yields of crude extracts when different extraction solvents (methanol, ethyl acetate and water) were used on the same plant species.

Methanol was identified as the best extraction solvent for *K. acuminata*, *H. pubescens*, *G. huillensis*, and *P. anglolensis*. Methanol yields for these plants (*H. pubescens*, *G. huillensis*, and *P. anglolensis*) were significantly higher ($p<0.05$) than both ethyl acetate and water crude extract yields. Although methanol was the best extraction solvent for *K. acuminate* and *E. goetzei*, there were no significant differences ($p<0.05$) between methanol and water-based crude extracts.

Generally, ethyl acetate-based crude extracts were significantly lower ($p<0.05$) than both water and methanol crude extracts for *K. acuminata*, *E. goetzei*, *E. divinorum*, *D. condylocarpon*, *H. pubescens*, and *P. anglolensis*. There were no significant differences ($p<0.05$) between T. elegans crude extract yields for all solvents (methanol, ethyl acetate, and water) (**Figure 4.4**). Overall, maceration in methanol was the best, and ethyl acetate resulted in low yields of crude extracts. Water was a better solvent compared to ethyl acetate, as shown in **Figure 4.4**.



1-*K. acuminate*, 2-*E. goetzei*, 3-*E. divinorum*, 4-*D. condylocarpon*, 5-*H. pubescens*, 6-*F. ingens*, 7-*G. huillensis*, 8-*P. anglolensis*, 9-*T. elegans*. Each bar has an error bar, and non-overlapping error bars for each plant indicate significant differences ($p<0.05$).

Figure 4. 4 Percentage yield of crude extracts when water, methanol and ethyl acetate are used as solvents.

4.5 Antiplasmodial activities against *Plasmodium falciparum* (3D7 strain) and toxicity of plant extracts against healthy erythrocytes.

The twenty-seven plant extracts assessed for antiplasmodial activity and toxicity against healthy red blood cells were found to be active against *Plasmodium falciparum*, with an IC_{50} below 100µg/ml. However, the extracts most active against *P. falciparum* were those from *T. elegans*, with an IC_{50} below 5 µg/ml. Although all plant extracts were effective against *P. falciparum*, crude extracts from *F. ingens* were weakly active, with an IC_{50} between 50 to 100µg/ml. Overall, most plant extracts in this study had an IC_{50} below 50 µg/ml. In terms of selectivity, most crude extracts

(24/27) exhibited good selectivity against *P. falciparum*, with a Selectivity Index (SI) greater than 2. The lowest SI was recorded for crude extracts from *F. ingens*, which had an SI below 2. It was noted that the extracts from *T. elegans* had the best SI, with an SI >10 for each extract. Although most crude extracts were classified in the same class as the positive control (chloroquine), chloroquine had the lowest IC_{50} and highest SI. These findings are summarised in **Table 4.5**.

While the activity against *Plasmodium* species of the plant extracts assessed in this study has not been reported in Zimbabwe, the antiplasmodial activity of *T. elegans* was reported in South Africa (Bapela et al., 2014). The findings of this study are in line with what was reported by Bapela et al. (2014), who indicated that *T. elegans* extracts show antiplasmodial activity with an IC_{50} below 5 µg/ml. However, in this study, *P. falciparum* (3D7 strain) was used instead of *P. falciparum* (NF54).

Although there is limited information on the treatment of malaria using *Holarrhena pubescens* in Zimbabwe, the ethanol-based bark extracts assessed by Simonsen et al. (2001) had an IC_{50} of 28 mg/ml against *Plasmodium falciparum*. Additionally, the chloroform leaf extracts from *Holarrhena pubescens* leaves were shown to have an IC_{50} of 5.7 mg/ml against *Plasmodium falciparum* (Sinha et al., 2013). These findings were not in line with what is reported in this study, and this may be due to many factors, including the fact that the plants were collected in different locations and at different times.

The results recorded in this study are in line with those reported by Clarkson et al. (2004), who indicated that extracts from *E. elephantine*, which is close to *E. goetzei*, had significant antiplasmodial activity against *Plasmodium falciparum* with an IC_{50} between 26 and 28 µg/ml. However, the results were in contrast with what was reported for roots and leaves aqueous extracts, which had IC_{50} values above 100 µg/ml (Maphosa et al., 2009).

The antiplasmodial activity of *Euclea divinorum* was not reported in Zimbabwe. However, the results obtained in this study corroborate with what was previously reported outside Zimbabwe, where methanol-based *Euclea divinorum* root extracts showed antiplasmodial activity with an IC_{50}

of 37.5 µg/ml (Mothana et al., 2012). In a study conducted in Kenya, root bark methanol extracts of *Euclea divinorum* were found to have significantly good antiplasmodial activity against *Plasmodium falciparum* D6, with the IC_{50} between 6.9 and 12.4 µg/ml (Muthaura et al., 2015).

The data on the scientific evaluation of *K. acuminata* and *D. condylocarpon* extracts against *Plasmodium falciparum* is limited in the literature. However, in this study, plant extracts were found to be active against *Plasmodium falciparum*, with an IC_{50} between 16.02 to 24.86 µg/ml and 10.45 to 18.78 µg/ml. Although the findings of this study indicate that *Ficus ingens* bark extracts were not active against *P. falciparum*, high activity was reported in a study by Al-Musayeib et al. (2012), who recorded activity against *P. falciparum* with IC_{50} values of 8.4 µg/ml. Contrary to this, the SI value of 3.8, which was recorded, was almost close to the ones recorded in this study. The study on the antiplasmodial activity of *Ficus ingens* by Al-Musayeib et al. (2012) was done in the Arabian Peninsula region, and therefore, the differences in antiplasmodial activities noticed may be attributed to different locations from which samples were collected.

Antiplasmodial activity of *Garcinia huillensis* against *Plasmodium falciparum* reported in this study indicates that the extracts from this plant are active. These findings are in line with what was reported outside Zimbabwe by Omole et al. (2019), who isolated biflavanones from *Garcinia huillensis* and reported that the biflavanones had significant antiplasmodial activity against *Plasmodium falciparum* strain W2 and D6 with an IC_{50} less than 4µg/ml and less than 11 µg/ml but greater than 7µg/ml, respectively. These results indicate that the same plant extracts can result in different antiplasmodial activity against different *Plasmodium falciparum* strains.

The extracts of *P. anglolensis* were found to be moderately active, as indicated by an IC_{50} between 14 and 28 µg/ml. The activity of *P. anglolensis* extracts against *Plasmodium falciparum* was evaluated in other studies. For instance, it was found that *P. angolensis* extracts inhibited *Plasmodium falciparum* ATPase as well as the chaperone functions of Hsp70s. Additionally, the fractions from *P. angolensis* extracts showed antiplasmodial activity against 3D7 (Zininga et al., 2017)

Table 4. 7: Antiplasmodial activities against Plasmodium falciparum (3D7 strain) and toxicity of plant extracts against erythrocytes.

Plant species	Voucher number	Solvents	IC_{50}/ µg/ml	CC_{50}- µg/ml	Selectivity index	Classification based on IC_{50}	Selectivity against *P. falciparum*
K. acuminata	Makuvara Z, Marumure J 6	Water	17.34±0.87 ^{c}	123.67±2.89^{e}	7.13	Active	Good
		Methanol	24.86±0.56 ^{d}	120.86±3.44^{de}	4.86	Active	Good
		Ethyl acetate	16.02±0.87 ^{c}	98.89±3.45^{c}	6.17	Active	Good
E. goetzei	Makuvara Z, Marumure J 10	Water	26.81±0.66 ^{d}	97.99±2.33^{c}	3.65	Active	Good
		Methanol	29.89±0.98 ^{de}	110.87±2.68^{d}	3.71	Active	Good
		Ethyl acetate	30.02±0.44 ^{e}	123.90±3.89^{e}	4.13	Active	Good
E. divinorum	Makuvara Z, Marumure J 11	Water	35.61±0.23 ^{e}	130.90±2.46^{e}	3.68	Active	Good
		Methanol	34.78±0.78 ^{e}	145.89±2.33 ^{f}	4.19	Active	Good
		Ethyl acetate	28.67±0.88 ^{d}	129.98±3.78^{e}	4.53	Active	Good
D. condylocarpon	Makuvara Z 12	Water	18.78±0.21 ^{c}	123.67±4.22^{e}	6.59	Active	Good
		Methanol	16.79±0.15 ^{c}	98.76±3.11 ^{c}	5.89	Active	Good

		Ethyl acetate	10.45±0.11^{b}	110.89±2.33^{d}	10.61	Active	Good
H. pubescens	Makuvara Z 13	Water	10.41±0.02^{b}	89.90±1.34^{bc}	8.64	Active	Good
		Methanol	9.87±0.63^{b}	92.78±1.23^{c}	9.40	Active	Good
		Ethyl acetate	11.8±0.77^{b}	97.66±1.01^{c}	8.28	Active	Good
F. ingens	Makuvara Z 14	Water	76.85±0.34^{g}	120.10±2.33^{de}	1.56	Weakly active	Poor
		Methanol	89.70±1.26^{h}	125.78±2.18^{e}	1.40	Weakly active	Poor
		Ethyl acetate	67.92±1.74^{f}	90.86±1.91^{bc}	1.34	Weakly active	Poor
G. huillensis	Makuvara Z 16	Water	9.98±0.06^{b}	90.78±1.34^{bc}	9.10	Active	Good
		Methanol	14.78±0.17^{bc}	98.90±1.37^{c}	6.70	Active	Good
		Ethyl acetate	10.98±0.89^{b}	91.76±0.88^{bc}	8.34	Active	Good
P. anglolensis	Makuvara Z 17	Water	14.78±0.67^{bc}	80.6±0.91^{b}	5.45	Active	Good
		Methanol	23.86±0.88^{d}	91.25±1.20^{bc}	3.82	Active	Good
		Ethyl acetate	27.87±0.67^{d}	100.24±1.29^{c}	3.60	Active	Good

T. elegans	Makuvara Z 19	Water	4.82±0.15 [a]	78.89±0.94 [b]	16.37	Very active	Good
		Methanol	4.52±0.65 [a]	80.09±0.45 [b]	17.71	Very active	Good
		Ethyl acetate	4.71±0.18 [a]	68.78±0.91[a]	14.60	Very active	Good
Positive control	Chloroquine	-	1.02±0.12[a]	107.98±0.44 [d]	> 100	Very active	Good

IC50 and CC50 values are mean±standard deviation. The means with different letters (superscripts) in the same column are significantly different at $p < 0.05$.

4.6 Characterisation of bioactive compounds from selected antimalarial plants

4.6.1 Qualitative phytochemical analysis of crude extracts

Bioactive compounds such as terpenoids, cardiac glycosides, alkaloids, and tannins were present in all the screened plant extracts. Additionally, all extracts from *T. elegans* bark, ethyl acetate, and water extracts of *G. huillensis* had all the classes of bioactive compounds screened for. Saponins, coumarins, and anthraquinones were not found in all plant extracts, and the least common classes of bioactive compounds in the screened plants were coumarins. **Table 4.8** summarises the classes of phytochemicals in each plant extract.

In a study by Kaur et al. (2009), antimalarial compounds identified included quassinoids, alkaloids (such as bisbenzylisoquinoline and manzamine), flavonoids, coumarins, terpenes, and quinones. The plants that showed antimalarial activity in this current study had classes of compounds which were identified in many studies (Memvanga et al., 2015; Weathers et al., 2014; Van der Kooy & Sullivan, 2013; Dike et al., 2012). Most of the identified classes of antimalarial compounds were identified in other studies (Sieniawska et al., 2022; Ogunlaja et al., 2022; Zahara et al., 2020; Singh, 2018; Yang et al., 2018; Muthaura et al., 2015; Wanjala & Majinda, 2001; Moyo et al., 1999). However, in these studies, specific compounds in each class were identified. For instance, in a study by Muthaura et al. (2015), nitidine (alkaloid), cycloisobrachycoumarinone epoxide (coumarins), and acacetin (flavonoids) were identified from different antimalarial plants.

In each antimalarial plant assessed, more than one class of antimalarial bioactive compounds was identified (Table 4.6). This indicates that the antimalarial activity of these plants may be linked to a synergistic effect of these compounds. According to Weathers et al. (2014), flavonoids and terpenoids extracted from *A. annua* have shown a synergistic effect against malarial parasites. The presence of tannins in antimalarial and other medicinal plants has been reported by Mori et al. (2017). Alkaloids were identified in all the screened antimalarial plants in this current study, and it has been reported that alkaloids have antimalarial properties (Stary, 1998). Apart from alkaloids, flavonoids, which were identified in this current study, have been reported to have in vitro antimalarial properties, especially against *P. falciparum* (Chanphen et al., 2018). Plant extracts selected in this study were found to contain cardiac glycosides (**Table 4.8**), and these bioactive

compounds were found to have antimalarial, antioxidant, and anti-inflammatory activities (Han & Lee, 2017). All the plant extracts in this current study were extracted from woody trees and were based on bark. The use of antimalarial woody tree barks was in line with the findings by Muthaura et al. (2017), who indicated that most antimalarial plants are trees with stem bark

Table 4. 8: Qualitative phytochemical analysis of selected antiplasmodial crude extracts.

Plant species	Solvents	Saponins	Terpenoids	Cardiac glycosides	Alkaloids	Tannins	Coumarins	Anthraquinones	Flavonoids
K. acuminata	Water	+	+	+	+	+	-	+	+
D. condylocarpon	Ethyl acetate	-	+	+	+	+	-	+	+
H. pubescens	Water	+	+	+	+	+	+	+	-
	Methanol	_	+	+	+	+	_	_	+
	Ethyl acetate	+	+	+	+	+	+	_	+
G. huillensis	Water	+	+	+	+	+	+	+	+
	Ethyl acetate	+	+	+	+	+	+	+	+
T. elegans	Water	+	+	+	+	+	+	+	+
	Methanol	+	+	+	+	+	+	+	+
	Ethyl acetate	+	+	+	+	+	+	+	+

+ Present, - absent

4.6.2 Functional groups identification in *T. elegans* bark crude extracts.

4.6.2.1 FT-IR spectrum for *T. elegans* ethyl acetate bark extracts

The phytochemicals extracted from *T. elegans* bark using ethyl acetate showed significant bands at 3420.67, 2924.49, 2852.37, 1733.30, 1642.86, 1455.72, 1383.86, 1334.98, 1245.24, 1156.72, 1096.00, 1026.20, and 746.82 cm^{-1}, with the most notable peaks at 2924.49, 1733.30, and 1245.24 cm^{-1} (**Figure 4.5**). These bands indicate the presence of various functional groups, such as alkanes (which is characterised by bands in the range 2924–2853 cm^{-1}), amides I and II (with peaks between 1662 and 1627 cm^{-1}), carboxylic acids (indicated with a peak at 1733.38 cm^{-1}), C-O-C stretching groups (as indicated by wave number of 1026.20 cm^{-1} and 1245.24 cm^{-1}), -CH_3 groups (1370-1393 cm^{-1}), C-O (ester groups) (975-1200 cm^{-1}), Alkyl di-substitutions (with wave numbers ranging from 685 to 755 cm^{-1}), CH_3 groups with a rocking vibration (with peaks between1155–1165 cm^{-1}) indicating presence of organic phosphorus compound, and stretching group of alkanes (as indicated by a band at 1383.86 cm^{-1}) (Oyinloye et al., 2025; Ahda et al., 2023, Dwivedi et al., 2021; Pharmawati & Wrasiati, 2020; Ragavendran et al., 2011; Saravanakumar et al., 2016; Dias, 2005; Socrates, 2004).

According to Shaheen et al. (2022), peaks in the range of 3516.82 to 3152.48 cm^{-1} indicate the presence of amino groups. Therefore, the peak at 3420.67 cm^{-1} in this study shows that amino groups are present in the *T. elegans* bark extracts. The peaks observed in extracts from *Peganum harmala* indicated the presence of aliphatic compounds (C–H bend) at 2952.79 and 2846.44 cm^{-1}, similar to peaks observed at 2924.49 and 2852.37 cm^{-1} (**Figure 4.5**). Several peaks were observed between 1267.10 cm^{-1} and 1001.45 cm^{-1}, which, according to Shaheen et al. (2022), indicate the presence of aromatics, ketones, amides, and aldehydes. In this study, only one peak was found between 921.74 cm^{-1} and 615.19 cm^{-1}, indicating the presence of halogen compounds such as C-F, C-Cl, and C-Br.

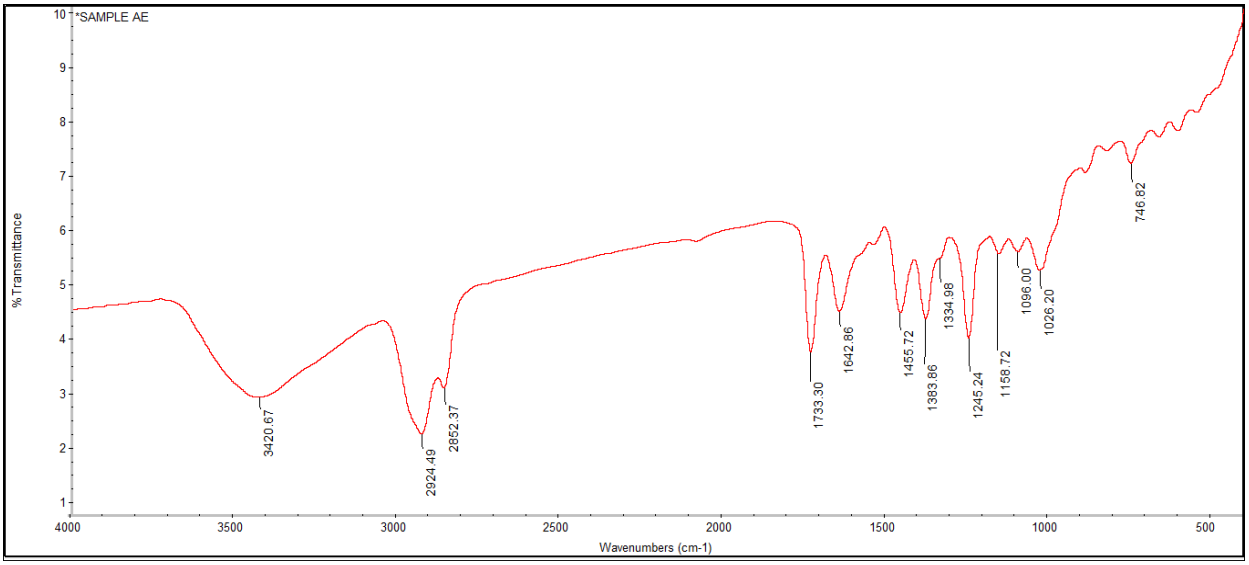


Figure 4.5 FT-IR spectrum for *T. elegans* ethyl acetate bark extracts.

4.6.2.2 FT-IR spectrum for *T. elegans* methanol bark extracts

The FTIR spectrum (**Figure 4.6**) recorded the presence of functional groups in the bioactive compounds based on the peaks in the infrared region. Generally, the spectrum showed significant peaks at 3388.79, 2927.68, 1732.23, 1639.78, 1456.13, 1384.81, 1245.71, 1049.28, 925.66, 748.96, and 600.65 cm^{-1}. According to Ahda et al. (2023), the -CH_2- functional group (wave number range 1430 to 1465 cm^{-1}) was identified from leaf extracts of *Orthosiphon stamineus*. This corroborates the findings of this study, where a peak was identified at 1456.13 cm^{-1} (within the range: 1430 to 1465 cm^{-1}). The most significant peaks identified in this study were at wave numbers 2927.68, 1732.23, 1639.78, 1384.32, 1245.71, and 1049.28 cm^{-1}. According to previous studies (Sahoo & Umashankara, 2023; Shaheen et al., 2022; Wongsa et al., 2020), these wave numbers correspond to O-H stretching vibration (free) (Hydroxyl group; characterized by wave numbers between 3500 and 3650 cm^{-1}), alkanes group (which is characterized by bands in the range 2924–2853 cm^{-1}), C=O stretching vibrations which are characteristic of carboxylic acids (indicated with a peak at 1732.23 cm^{-1}), C=C stretching vibration which indicates C=C conjugated with C=O group (1580–1660 cm^{-1}), C-CO-C stretching which is characteristic of a ketone (1249 cm^{-1} which is close to wave number 1245.71 cm^{-1} found with a peak in this particular study), C-C skeletal vibration which is characteristic of alkanes (925–930 cm^{-1}), -CH_3 group (1370-1393 cm^{-1}), and C-O (ester groups) (975-1200 cm^{-1}).

It was shown that peaks at 3398.30 cm-1 and 2926.66 cm^{-1} (which are close to the peaks observed in this study, 3388.79 and 2927.68 cm^{-1}) indicate the presence of O-H and N-H, respectively (Oyinloye et al., 2025; Wongsa et al., 2020; Santosh et al., 2015). These functional groups indicate the presence of alcohol, amines, amides, and amino acids in plant extracts. In a study where FTIR analysis of leaf extracts from *Ocimum sanctum* was carried out, peaks were reported between 2357.38 cm^{-1} and 778.31 $cm^{-1,}$ indicating the presence of C-H and C=O vibrations, bending, and stretching (Santosh et al., 2015). The peaks identified in this case were similar to those observed for *T. elegans* bark methanol extracts (**Figure 4.6**), which indicated the presence of alcohol, amino acids, and meta-substituted compounds. The findings of this study were similar to what was reported by Oyinloye et al. (2025), who indicated that *Solanum dasyphyllum* methanol extracts had peaks at 1047.4, 1390.3, 1602.8, and 3336.0 cm^{-1}. Based on these wave numbers, functional groups (C–H, C–O, C=C–C) which correspond to phenolic and aromatic compounds were identified. The presence of peaks in the region between 1400 and 900 cm^{-1} is an indication of the presence of C–O, C–H, P–O, and C–N bonds, which are characteristic of bands in this region (Oyinloye et al., 2025; Wongsa et al., 2020).

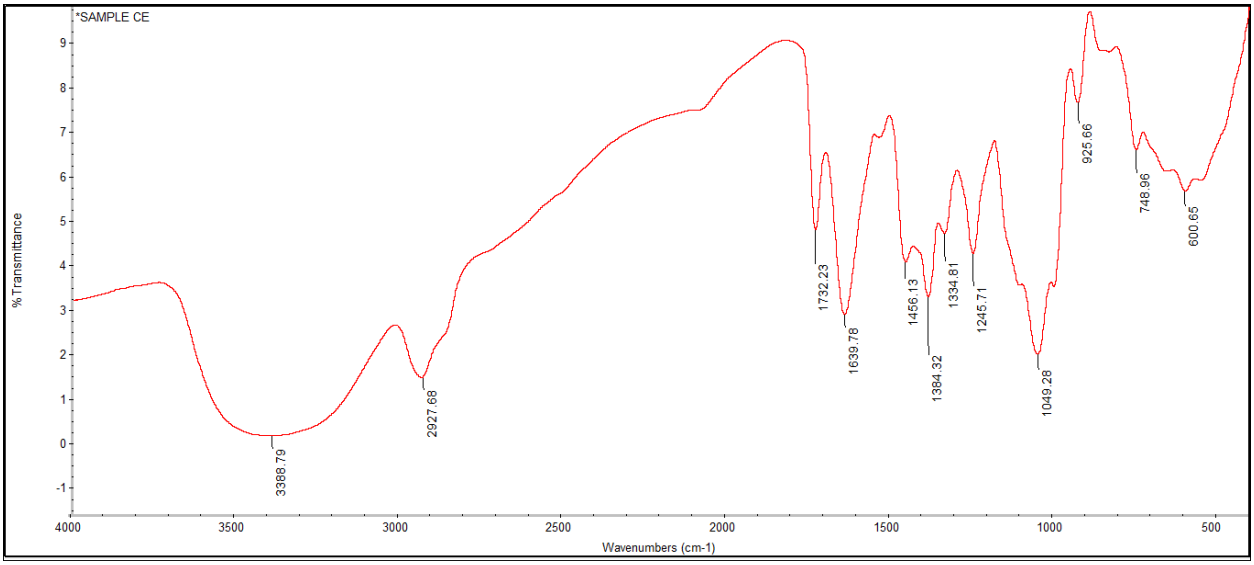


Figure 4. 6 FT-IR spectrum for *T. elegans* methanol bark extracts.

4.6.2.3 FT-IR spectrum for *T. elegans* aqueous bark extracts

The FTIR spectrum (**Figure 4.7**) recorded the presence of a few functional groups (only five) in the bioactive compounds from *T. elegans* aqueous bark extracts. The functional groups of bioactive compounds identified in this study had peaks at wave numbers: 3444.93, 1634.90, 1384.36, 1101.71, and 1033.09 cm^{-1}. The most significant peaks identified in this case were at wave numbers 1634.90 and 1384.36 cm^{-1}.

The presence of a significant peak at 1634.90 cm^{-1} indicates a C=C stretch group, which is characteristic of alkanes. This aligns with the findings of Dwivedi et al. (2021), who reported that bands at 1600–1680 cm^{-1} are due to the C=C group. The peak at 3444.93 cm^{-1} identified in this study is similar to previous findings, where a significant peak at 3436.0894 cm^{-1} indicated an OH broad group and the presence of hydroxyl compounds (Dwivedi et al., 2021).

According to the report on the FTIR spectrum of the antimalarial plant *Andrographis paniculata*, a C-N stretch group was identified at 1033.4677 cm^{-1}, corresponding to the presence of amines (Dwivedi et al., 2021). This aligns with the peak identified at a wave number of 1033.09 cm^{-1}. Additionally, the presence of functional groups corresponding to the wave number 1384.36 cm^{-1} indicates the presence of a secondary alcohol associated with C-H deformation vibration with a wave number between 350 and 1410 cm^{-1} (Pharmawati & Wrasiati, 2020; Socrates, 2004). The peak recorded at 1101.71 cm^{-1} in this study falls within the range of 975-1200 cm^{-1}, which was reported to be associated with C-O (ester group) (Ahda et al., 2023).

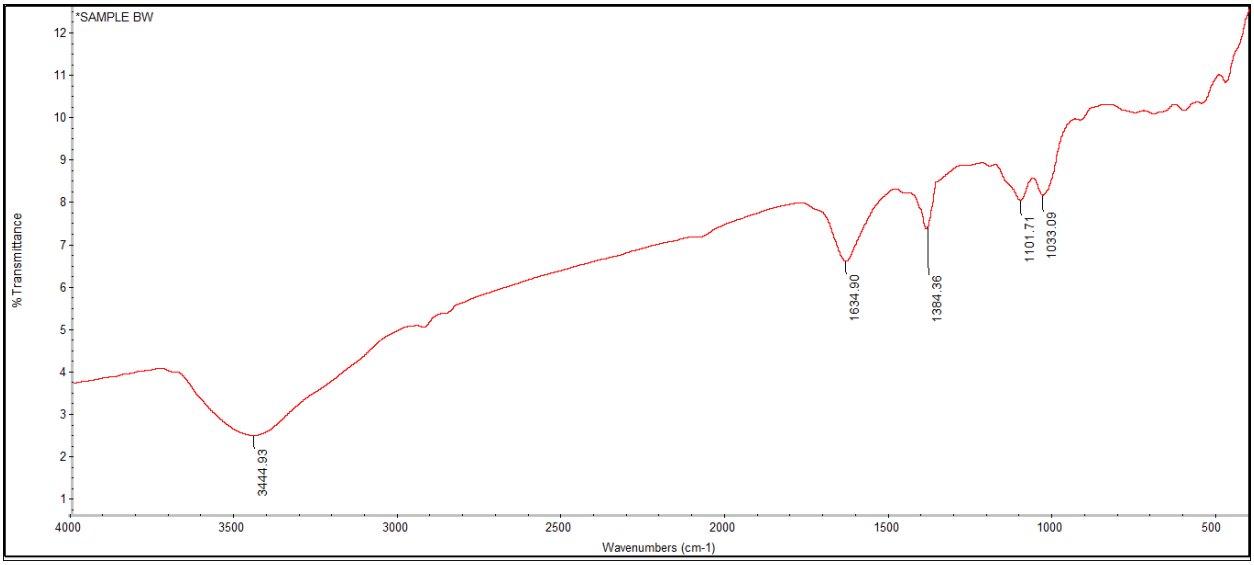


Figure 4. 7 FT-IR spectrum for *T. elegans* aqueous bark extracts.

The functional groups identified in this study suggest the presence of important bioactive compounds such as glycosides, flavonoids, terpenoids and phenolic compounds. The presence of functional groups characteristic of those reported in this current study is in line with what was reported by other researchers (Sahoo & Umashankara, 2023; Aziz et al., 2015; Siddiqu et al., 2007). Notably, the functional groups identified can be linked to the phytochemicals identified (**Table 4.6**) and show the presence of amines, carboxylic acids, amides, aliphatic compounds, polysaccharides, organic phosphorus compounds, halogens, organic hydrocarbons, and alkanes. Overall, the presence of reported compounds and associated functional groups may have contributed to the antiplasmodial activity of *T. elegans* bark ethyl acetate extracts.

4.6.3 GC-MS analysis

The GC-MS chromatogram of the ethyl acetate bark extract of *T. elegans* is shown in **Fig. 4.5**. The compounds identified in this current study were based on a comparison between the mass spectra of the phytochemicals with the spectra of known compounds deposited in the NIST library. Nineteen phytochemicals were identified (**Table 4.9**) and these include 9-Octadecenamide, (Z) - (peak area of 15058436; retention time of 23.3876 min), cis-Vaccenic acid (peak area of 18522022; retention time of 20.4886 min), 9,12-Octadecadienoic acid (Z, Z) - (peak area of 13041554; retention time of 20.3823 min), and n-Hexadecanoic acid (peak area of 12577039; retention time of 16.4836 min). The compound with the least peak area was 2,6-Difluorobenzoic acid, 4-

nitrophenyl ester (peak area of 6680; retention time of 8.0306 min). However, all the identified compounds had a matching score above 89 %, and these findings are summarised in **Table 4.9**.

9-Octadecenamide, (Z)- was identified in different extracts of medicinal and antimalarial plants (Muzahid et al, 2023; Makhubu et al., 2023; Ekasari et al., 2022; Tareq et al., 2020; Al-Shawi et al., 2020; Khan et al, 2019). This bioactive compound was analysed, and it was revealed that it is an amide compound associated with antibacterial and anti-inflammatory properties (Seanego et al, 2012). According to Gopalakrishnan & Udayakumar (2014), the plant extracts containing 9-Octadecenamide, (Z)- were active against many bacterial species, including *Enterobacter aerogenes* and *Staphylococcus epidermidis*. Up to almost 76 % of 9-octadecenamide was identified from ethyl acetate-based extracts of the plant *Diaporthe schini* (Dos Reis et al., 2019). Although hexadecanamide had a lower peak area compared to 9-Octadecenamide, (Z)-, it is in the same clas**s** as 9-Octadecenamide, (Z)-(These are fatty acid amides**).** Hexadecanamide was extracted and identified from *Saueda monoica* Forssk (Al-Shawi et al., 2020).

Cis-Vaccenic acid was identified from methanolic extracts of *Achillea filipendulina* (L.) leaves, and this phytochemical is a fatty acid which was reported to have anticarcinogenic activities. Moreover, cis-Vaccenic acid was shown to inhibit telomerase activity (Khan et al, 2019). Ekasari et al. (2022) extracted cis-Vaccenic acid from an antimalarial plant, *Sauropus androgynus* (L.) Merr and the plant leaf extracts were found to contain about 0.35% of cis-Vaccenic acid. In another study, cis-Vaccenic acid was isolated from an edible mushroom, *Lebopus beniensis* (Campi et al., 2023) and was reported in other mushrooms such as *Boletales* (Bhat et al., 2020). Several medicinal plants have been identified as sources of 9,12-Octadecadienoic acid (linoleic acid) (Ekasari et al., 2022; Nwonuma et al., 2022; Kalaimathi et al., 2022; Nazir et al., 2021), and this phytochemical has been identified as a strong antioxidant (Basiricò et al., 2017).

Many plant extracts with n-Hexadecanoic acid have been reported (Muzahid et al, 2023; Makhubu et al., 2023; Okpe et al., 2018), and it has been shown that this compound has antioxidant, anti-inflammatory, nematicide, and mosquito larvicide activities (Aparna et al., 2012; Rahuman et al., 2000). According to Akpuaka et al. (2013) and Oni et al. (2020), hexadecanoic acid is classified as a methyl ester, which is associated with significant antioxidant, antimicrobial, hypocholesterolemic, pesticide, insecticide and antiandrogenic properties. Additionally, it was reported that n-Hexadecanoic acid has 5-Alpha-reductase inhibitor and hemolytic activities (Oni

et al, 2020). Methanolic extracts from *Sterculia quadrifida* R.Br.(a medicinal plant normally used against hepatitis) were found to contain hexadecanoic acid, methyl ester (Forghani et al., 2025), and this particular bioactive compound was shown to have antimicrobial and antioxidant activity (Shaaban et al., 2021).

Although 11-Octadecenoic acid, methyl ester was not identified, compounds close to this bioactive compound, such as 9-Octadecenoic acid, methyl ester and Octadecanoic acid, methyl ester, were identified from extracts of *Haloxylon stocksii* (Boiss.) Benth (Rizvi et al., 2023). Octadecanoic acid, methyl ester and 9-Octadecenoic acid, methyl ester were reported to have antioxidant, antimicrobial and anti-inflammatory activities (Mohamad et al., 2018; Othman et al., 2015). 9, 12-Octadecadienoic acid, methyl ester was identified in leaf extracts of *Pistia stratiotes* L. (Gupta et al., 2023) and ethyl acetate bark extracts of *Jatropha cordata* (Jiménez-Nevárez et al., 2023).

The compound octadecanoic acid (known as stearic acid) was previously identified from the plant *Elaeagnus umbellata* (Nazir et al., 2021) and Cannabis extracts (Nwonuma et al., 2022). Octadecanoic acid has been found to have significant neuroprotective activities (Khabbush et al., 2017) and antioxidant potential (Wang et al., 2006). Additionally, octadecanoic acid was reported to be antibacterial and antifungal (Gopalakrishnan & Udayakumar, 2014). The antimicrobial compounds extracted from and identified in *Allium hookeri* root included tetradecanoic acid (Kim et al., 2015). Root extracts from *Allium hookeri* showed antimicrobial activities against several bacterial species such as *Micrococcus luteus*, *Salmonella typhimurium* and *Mycobacterium smegmatis* (Huang et al., 2011; Pu et al., 2010). It should be noted that tetradecanoic acid (myristic Acid) was identified from ethyl acetate leaf extracts of *Moringa oleifera*. Besides being antibacterial, tetradecanoic acid has cardioprotective capabilities and therefore lowers the chances of the development of heart diseases (Kalaimathi et al., 2022). Pentadecanoic acid is similar to 14-Pentadecanoic acid, which was extracted and identified in seed extracts of an antimalarial plant, *Ricinodendron heudelotii* (Imieje & Amafili, 2015).

Hexacosane was identified in ethyl acetate root extracts of the medicinal plant, *Sansevieria liberica* (Rukaiyat et al., 2015). Additionally, the presence of hexacosane in plant extracts has been reported by several other authors (Delica et al., 2023; El-Sayed et al., 2015; Hashem & Saleh, 1999; Ramaroson-Raonizafinimanana et al., 1997). It was reported that hexacosane is antimicrobial, and its antimicrobial activity was tested against bacterial species including *Salmonella typhi*, *Klebsiella*

pneumoniae, *Proteus vulgaris*, and Methicillin-resistant *Staphylococcus aureus* (Rukaiyat et al., 2015). In all the antimicrobial tests conducted, ethyl acetate root extracts showed significantly high antimicrobial activities. According to Marrufo et al. (2013), hexacosane in essential oils was found to be significantly active against *Staphylococcus aureus* and *Bacillus cereus*. The bioactive compound heneicosane was extracted from the antimalarial plant, *Plumbago zeylanica* L (Vanitha et al., 2020). Apart from this, the presence of heneicosane in plant extracts has been reported in several studies (Ebrahimabadi et al., 2016; Bülent Köse et al., 2016; Tharmaraj et al., 2015; Roy et al., 2010). According to Tharmaraj et al. (2015), *Plumbago zeylanica* L has been identified in India as a therapeutic plant with the potential to prevent atherosclerosis and inflammation. Additionally, the extracts from *Plumbago zeylanica* L were found to be antiplasmodial, antimicrobial, and hypolipidemic (Tharmaraj et al., 2015).

In this study, phthalates, including benzenedicarboxylic acid, bis(2-ethylhexyl), 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl), and dibutyl phthalate, were extracted and identified from *T. elegans*. These phthalates were also identified in natural organisms, including medicinal plants (Javed et al., 2022; Mahmud et al., 2022; Sirwani et al., 2022; Enenebeaku et al., 2021; El-Sayed et al., 2012; Habib & Karim, 2009). The activity of dibutyl phthalate (also known as Bis(2-ethylhexyl) phthalate) from *Procera gigantea* flower extracts was assessed against *Escherichia coli*, *Shigella dysenteriae*, *Clostridium perfringens*, *Shigella sonnei*, *Pseudomonas aeruginosa*, and *Shigella shiga* (El-Sayed et al., 2012; Habib & Karim, 2009). Besides being antibacterial, bis(2-ethylhexyl) phthalate has shown antifungal activity, especially against *Aspergillus flavus* (Habib & Karim, 2009). According to Habib & Karim (2012) and Habib & Karim (2009), dibutyl phthalate from *Dictyandra arborescens* root methanol-based extracts was found to have antitumor and antimicrobial activities. More importantly, dibutyl phthalate extracted from *Streptomyces* sp. H11809 showed antiplasmodial activity against the human malaria parasite *Plasmodium falciparum* 3D7 (Pf 3D7) (Mahmud et al., 2022). Additionally, bis(2-ethylhexyl) phthalate identified from *Lactiplantibacillus plantarum* has been shown to have some larvicidal and antibacterial activities (Javed et al., 2022).

Other phthalates, such as 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl), were found to be anticancer, and this was confirmed by preventing the proliferation of MCF-7 and HepG2 cancer

cells (Mahmud et al., 2022). In this study, the 1,4-benzenedicarboxylic acid, bis(2-ethylhexyl), which was identified, is closely related to 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester, which was isolated and identified from twigs of the plant *Thevetia peruviana*. The bioactive compound 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester has been identified as a candidate for microbial allergies, arthritis, and cancer treatment (Save et al., 2015).

Plants have been a very important source of bioactive compounds. For example, cyclopentasiloxane, decamethyl- was identified in an antimalarial plant, *Sauropus androgynus* leaf methanol extracts (Ekasari et al., 2022) and 2,6-difluorobenzoic acid, 4-nitrophenyl was extracted from *Fimbristyis miliacea* (Abdullah et al., 2022) and *Trigonella foenum-graecum* L. (Sirwani et al., 2022).

The bioactive compounds classified as fatty acids (such as n-hexadecanoic acid and octadecanoic acid) and amines (including 9-octadecenamide (Z)) were identified from some antimalarial plants. For instance, n-hexadecanoic acid, 9,12-octadecadienoic acid, ethyl ester Octadecadienoic acid, and 9,12-octadecadienoic acid (Z,Z)- were identified from an antimalarial plant *Ricinodendron heudelotii* seed extracts (Imieje & Amafili, 2015). These amides and fatty acids are believed to contribute to the antimalarial activities reported in this study. Most of the identified bioactive compounds in this study are essential oils and have been utilised in many traditional drugs to treat various diseases, including malaria (Dagli et al., 2015). The compounds such as n-hexadecanoic acid, 9,12-octadecadienoic acid (Z,Z)-, hexadecanoic acid, and 9-octadecenamide, (Z)-, extracted from the leaves of Olea europaea were reported to be antimicrobial (Syed et al., 2022). Notably, antimicrobials are sometimes antimalarial, and therefore, the reported antimalarial activity in this study may be linked to this. Additionally, *Jatropha cordata* ethyl acetate bark extracts were reported to be antimicrobial, and among the compounds identified were fatty acids, including 9,12-octadecadienoic acid, n-hexadecanoic acid, and octadecanoic acid (Jiménez-Nevárez et al., 2023)

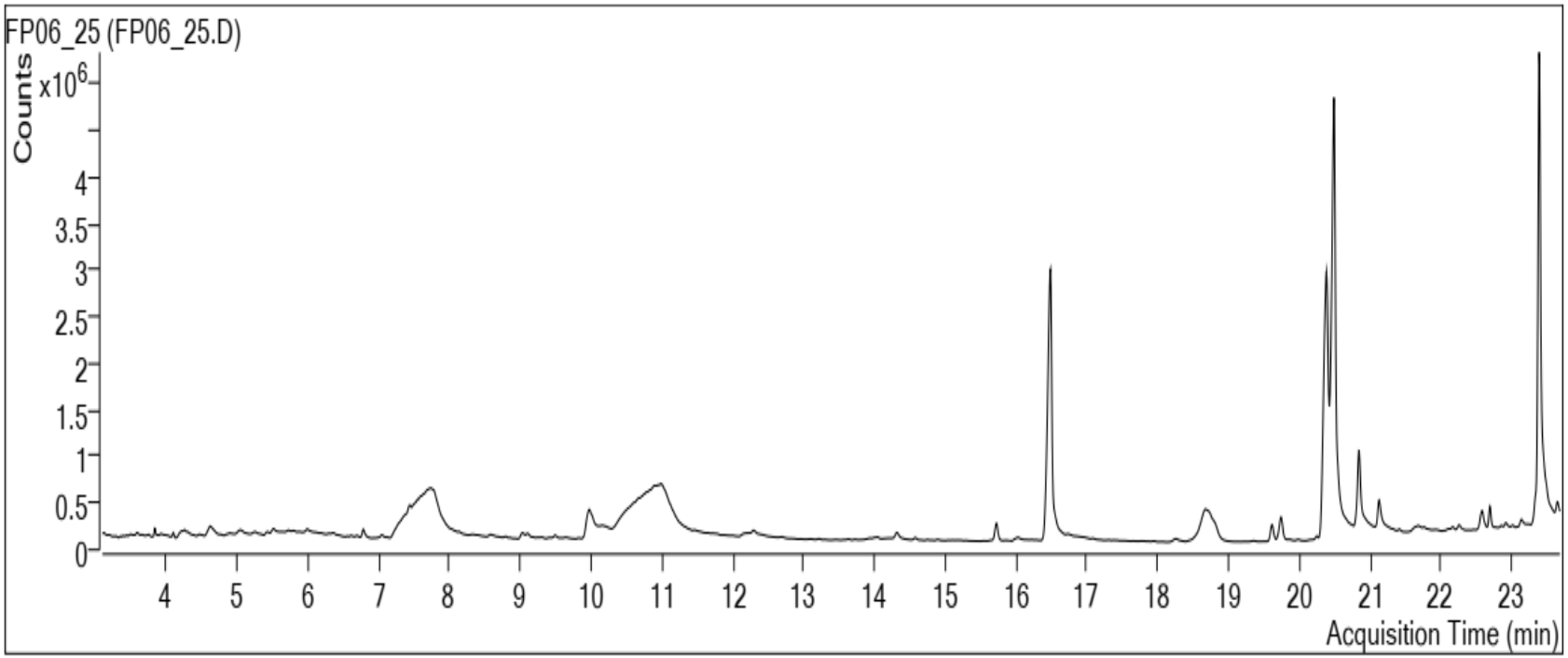


Figure 4. 8 GC-MS Chromatogram of *T. elegans* ethyl acetate bark extract.

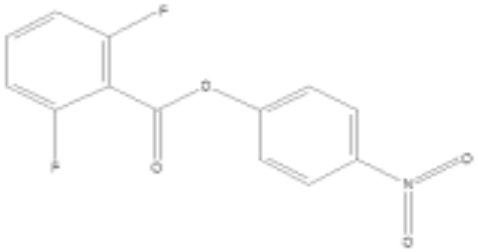
Table 4. 9: GC-MS bioactive compounds from ethyl acetate bark extract from *T. elegans*.

S. NO	RT	Name of compound	Molecular formulae	Peak area	Matching Score
1	6.5600	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$	13462	92.9
2	8.0306	2,6-Difluorobenzoic acid, 4-nitrophenyl ester	$C_{13}H_7F2NO_4$	6680	90.4
3	12.2982	Tetradecanoic acid	$C_{14}H_{28}O_2$	149795	90.2
4	14.3189	Pentadecanoic acid	$C_{15}H_{30}O_2$	273589	93.2
5	14.5779	1,2-Benzenedicarboxylic acid, bis(2-	$C_{16}H_{22}O_4$	62521	92.4
6	15.7227	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	559685	97.1
7	16.4836	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	12577039	98.2
8	16.5454	Dibutyl phthalate	$C_{16}H_{22}O_4$	206498	91.9
9	18.6757	Hexacosane	$C_{26}H_{54}$	4544993	93.8
10	19.6145	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	521500	95.8
11	19.7429	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	776742	94.1
12	20.3823	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	13041554	98.0
13	20.4886	cis-Vaccenic acid	$C_{18}H_{34}O_2$	18522022	98.5
14	20.8438	Octadecanoic acid	$C_{18}H_{36}O_2$	2407385	96.7

15	21.1260	Hexadecanamide	$C_{16}H_{33}NO$	1069217	90.0
16	21.6760	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)	$C_{24}H_{38}O_4$	412729	93.4
17	22.5807	Hexacosane	$C_{26}H_{54}$	686063	95.8
18	22.6918	Heneicosane	$C_{21}H_{44}$	480247	95.0
19	23.3876	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	15058436	96.5

Structure of Bioactive Compounds Identified from Ethyl Acetate Extracts of *T. elegans*

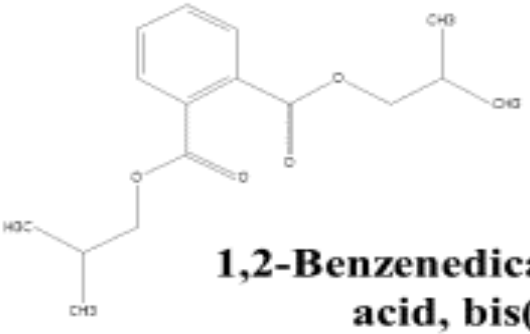
Cyclopentasiloxane, decamethyl-



2,6-Difluorobenzoic acid, 4-nitrophenyl ester

Tetradecanoic acid

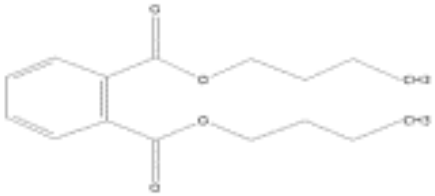
Pentadecanoic acid



1,2-Benzenedicarboxylic acid, bis(2-

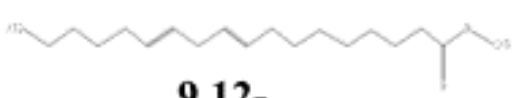
Hexadecanoic acid, methyl ester

n-Hexadecanoic acid



Dibutyl phthalate

Hexacosane

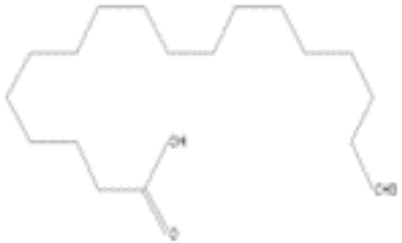


9,12-Octadecadienoic acid, methyl ester

11-Octadecenoic acid, methyl ester

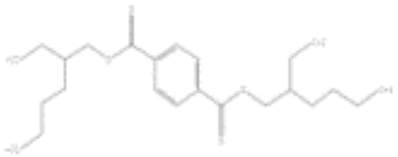
9,12-Octadecadienoic acid (Z,Z)-

cis-Vaccenic acid



Octadecanoic acid

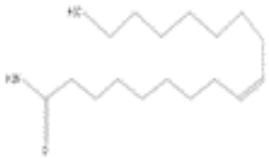
Hexadecanamide



1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)

Hexacosane

Heneicosane



9-Octadecenamide, (Z)-

Figure 4. 9 Structure of Bioactive Compounds Identified from Ethyl Acetate Extracts of *T. elegans*

The antiplasmodial activity reported in this current study is likely to be due to a wide range of classes of phytochemicals reported previously including alkaloids, flavonoids, tannins, terpenoids and phenolic compounds. These phytochemicals can inhibit the growth of *Plasmodium falciparum* through a number of mechanisms including, haem detoxification, oxidative stress, nucleic acid and protein synthesis and interference with essential metabolic pathways of *Plasmodium falciparum* (Ginsburg & Deharo, 2011; Cowan, 1999; Batista et al., 2009). The significant activity of *Tabernaemontana elegans* could be attributed to its phytochemical profile rich in alkaloids while activities of *Garcinia huillensis*, *Euclea divinorum* and *Elephantorrhiza goetzei* may be due to synergistic effects of flavonoids, phenolic compounds and terpenoids (Wink, 2012; Muthaura et al., 2015). Notably, extraction yields obtained in this study were not consistently correlated with antiplasmodial activity, suggesting that biological efficacy is determined by the nature of bioactive compounds and their potency rather than crude extract quantity alone. Similar observations have been reported by Cos et al., (2006), who indicated that highly active compounds may occur in low concentrations within crude extracts. This highlights significance of phytochemical characterisation in antimalarial drug discovery.

The results from this study should be interpreted within the context of quite number of limitations. The current study evaluated crude extracts against a single *P. falciparum* strain (3D7), and toxicity assessment was limited to healthy erythrocytes. Furthermore, the active compounds responsible for the observed effects were not isolated, and phytochemical composition may vary according to geographical location, environmental conditions, seasonality, and plant developmental stage (Pandey & Tripathi, 2014; Cos et al., 2006). Based on these limitations, further studies involving *in vivo* efficacy studies, bioassay-guided fractionation, comprehensive toxicity assessments, and mechanistic investigations are recommended to validate the therapeutic potential of indigenous medicinal plants used to treat malaria in Zimbabwe.

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CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Identified plant species based on literature survey.

A variety of plants are used against malaria in Zimbabwe, but there is limited scientific evaluation of these plants. A literature survey identified a total of sixty-one (61) antimalarial plants used in the management of malaria in Zimbabwe. These plants are classified into 30 different families, with most belonging to the *Fabaceae* family (17%). The main plant parts used in malaria management are roots (41%), leaves (27%), and stem bark (24%). These plant parts are prepared into plant-derived medicines using traditional methods such as cold infusion (45%), decoction (24%), and hot infusion (18%). While there is no comprehensive list of antimalarial plants used in Zimbabwe, there is evidence of plant use against malaria. However, there is a need to conduct a nationwide survey to identify all plants used against malaria in Zimbabwe and scientifically evaluate those that are most commonly used. The protection of medicinal plant species with promising antimalarial properties should be prioritised, as growing demand may result in overharvesting. Promoting sustainable harvesting and gardening techniques can assist to preserve these rich resources for future generations.

5.2 Indigenous practices exploited in the fight against malaria in Zimbabwe.

In this work, five classes of indigenous practices used in the prevention and management of malaria were identified and evaluated. According to the findings in the literature, there is a lack of published data on this particular subject. However, the identified indigenous knowledge systems applied in malaria management are based on: (1) herbal and other traditional medicines for treatment and prevention, (2) traditional environmental indicators for predicting malaria prevalence, (3) red-hot granite rock and water for treating malaria symptoms, (4) burning plants and dung to prevent mosquitoes, and (5) treating malaria spiritually based on traditional and faith healers. Information on the applicability and efficacy of these methods in Zimbabwe is limited. However, herbal medicines have been used in the development of antimalarial drugs, and burning dung and plants have been exploited in the development of mosquito repellent creams and coils that disrupt the transmission of Plasmodium species. Practices such as steaming and spirituality have not been scientifically evaluated in terms of malaria management and treatment, so the application of these knowledge systems lacks a scientific basis. Overall, the application of indigenous knowledge practices in the management of malaria in Zimbabwe is inconclusive.

Therefore, it is recommended that these practices be scientifically evaluated through comprehensive studies. In addition to this literature survey, nationwide surveys should be conducted to gain more insights into the identified and other existing indigenous malaria control and treatment methods.

5.3 Percentage yield of water, methanol and ethyl acetate plant extracts.

The percentage yields of the crude extracts after maceration in water, methanol, and ethyl acetate ranged from 1.31% (*D. condylocarpon* bark crude extract) to 14.56% (*G. buchananii* leaf crude extract). Generally, the percentage yields of crude extracts were low but sufficient for further analysis. Additionally, ethyl acetate-based crude extracts were significantly lower ($p<0.05$) than both water and methanol crude extracts for *K. acuminata*, *E. goetzei*, *E. divinorum*, *D. condylocarpon*, *H. pubescens*, and *P. anglolensis*, showing significant differences ($p<0.05$). Overall, maceration in methanol was the best, and ethyl acetate resulted in the lowest yields of crude extracts. Water was a better solvent compared to ethyl acetate. Based on the findings and previous studies, it has been noted that maceration can be exploited in the extraction of crude plant-based extracts. However, other methods are much better than maceration, especially when the initial plant sample is small (less than 10g). The use of methanol as a solvent and maceration as an extraction method is recommended.

5.4 Antiplasmodial activities and toxicity of crude plant extracts.

The twenty-seven plant extracts assessed for antiplasmodial activity and toxicity against healthy red blood cells had IC_{50} values below 100µg/ml. However, plant extracts that were most active against *P. falciparum* were those from *T. elegans* with an IC_{50} below 5 µg/ml. Although all plant extracts were effective against *P. falciparum*, crude extracts from *F. ingens* were weakly active, as indicated by IC_{50} between 50 to 100µg/ml. In terms of selectivity, 88.89% (24/27) of crude extracts had good selectivity against *P. falciparum*, based on SI greater than 2. It was noted that the extracts from *T. elegans* had the best SI, indicated by SI >10 for each extract. Although most crude extracts were classified in the same class as the positive control (chloroquine), chloroquine had the lowest IC_{50} and highest SI. Although 88.89% of plant extracts can be used against malaria, it is recommended that *T. elegans*, *D. condylocarpon*, *H. pubescens*, and *G. buchananii* be further screened against *Plasmodium falciparum* of different variants to confirm the activity noted in this

study. Overall, the antiplasmodial activities of the plant extracts recorded in this study were reported in other studies. However, the studies were done outside Zimbabwe. Notably, in Zimbabwe, the antiplasmodial activity of these plants is reported here for the first time. Further studies on *T. elegans* extracts' activity against *Plasmodium falciparum* should be done, and phytochemical screening of these extracts will provide further details on the properties of these extracts.

5.5 Characterisation of bioactive compounds from selected antimalarial plants

5.5.1 Qualitative phytochemical analysis of crude extracts.

Bioactive compounds such as terpenoids, cardiac glycosides, alkaloids, and tannins were present in all the screened plant extracts. Additionally, all extracts from *T. elegans* bark, ethyl acetate, and water extracts of *G. buchananii* had all the classes of bioactive compounds screened for. Saponins, coumarins, and anthraquinones were not found in all plant extracts, and the least common class of bioactive compounds in screened plants was coumarins. Further work should be done to quantify the bioactive compounds identified, and the effect of the age of the plant, the season when samples were collected, and the sample preparation methods on the availability and quantity of bioactive compounds should be evaluated.

5.5.2 Functional groups identification in crude extracts.

The functional groups identified in this study infer the presence of important bioactive compounds such as glycosides, flavonoids, terpenoids, and phenolic compounds. Notably, the functional groups identified can be linked to the phytochemicals identified and show the presence of amines, carboxylic acids, amides, aliphatic compounds, polysaccharides, organic phosphorus compounds, halogens, organic hydrocarbons, and alkanes. Overall, the presence of reported compounds and associated functional groups may have contributed to the antiplasmodial activity of *T. elegans* bark ethyl acetate extracts. However, there is a need to isolate the compounds and assess the efficacy of the functional groups and associated compounds against *Plasmodium falciparum* strains.

5.5.3 GC-MS analysis.

Nineteen phytochemicals were identified, including 9-Octadecenamide, (Z) - (peak area of 15058436; retention time of 23.3876 min), cis-Vaccenic acid (peak area of 18522022; retention

time of 20.4886 min), 9,12-Octadecadienoic acid (Z, Z) - (peak area of 13041554; retention time of 20.3823 min), and n-Hexadecanoic acid (peak area of 12577039; retention time of 16.4836 min). The compound with the least peak area was 2,6-Difluorobenzoic acid, 4-nitrophenyl ester (peak area of 6680; retention time of 8.0306 min). The bioactive compounds classified as fatty acids (such as n-hexadecanoic acid and octadecanoic acid) and amines (including 9-octadecenamide (Z)-) were identified from some antimalarial plants. These amides and fatty acids are believed to contribute to the antimalarial activities reported in this current study. Most of the identified bioactive compounds in this particular study are essential oils, and these have been utilised in some traditional drugs to treat a variety of diseases, including malaria.

Results from this current study revealed that ethyl acetate, methanol, and aqueous extracts of *T. elegans* stem bark were not toxic to healthy red blood cells and possess antiplasmodial activities. The plant extracts with the highest antimalarial activities were obtained from *T. elegans*. Notably, the phytochemicals identified from *T. elegans* might have contributed to the reported antimalarial activity in this current study. Although several bioactive compounds were identified, these compounds must be isolated to understand more biological activities. The presence of nine different classes of bioactive compounds identified in this study and nineteen individual compounds identified through GC–MS analysis provides scientific evidence for the antiplasmodial activity exhibited by the stem bark of *T. elegans*. This indicates that *T. elegans* presents a promising source of novel antimalarial drug templates. The results reported in the current study justify the traditional pharmacological use of *T. elegans* in Zimbabwe against malaria and other ailments.

Appendix

Appendix 1: Ethical clearance from UNISA

UNISA | university of south africa

UNISA-CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 09/06/2020

NHREC Registration # : REC-170616-051
REC Reference # : 2020/CAES_HREC/103_FR
Name : Mr Z Makuvara
Student #: 64008916

Dear Mr Makuvara

Decision: Ethics Approval from 04/06/2020 to 31/05/2025

Researcher(s): Mr Z Makuvara
zmakuvara@gmail.com

Supervisor (s): Prof SR Magano
magansr@unisa.ac.za; 011-471-3649

Working title of research:

Characterisation and effectiveness of bioactive compounds from selected medicinal plants used in the treatment of malaria in Zimbabwe

Qualification: PhD Life Science

Thank you for the application for research ethics clearance by the Unisa-CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is granted for five years, **subject to submission of the relevant permission letters and yearly progress reports. Failure to submit the progress report will lead to withdrawal of the ethics clearance until the report has been submitted.**

The researcher is cautioned that fieldwork may not commence until such time as the COVID-19 lockdown has been lifted.

Due date for progress report: 31 May 2021

Please note the points below for further action:

1. Permission from some of the relevant institutions and community leaders remain outstanding. These must be obtained and submitted to the committee before data collection may commence.

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

2. The researcher indicates that an interpreter will be used for data collection. Does this mean that the data collection instruments will be translated before distribution, or that an interpreted will be present when the questionnaires are administered? He is cautioned that the interpreter must sign a confidentiality agreement.
3. The researcher indicates in section 4.9 of ethics application form 1 that "researcher and community members" will collect the data. What is meant by this? What data will be collected by community members? The researcher is cautioned that any field assistants must sign a confidentiality agreement.
4. Unisa has a standard consent form that must be used to obtain consent from participants. The researcher may not use any other consent form and is requested to submit the corrected draft consent form to the Committee for record purposes. The forms are provided on the college website: https://www.unisa.ac.za/sites/corporate/default/Colleges/Agriculture-&-Environmental-Sciences/Research/Research-Ethics
5. The researcher will be working with parasites that cause malaria, and is cautioned to take the necessary precautions when doing so.
6. More detail is required on the statistical analysis that will be applied – what will be measured by the t-test and chi-squares? How will the dose-response curve be developed? How many means will there be for IC50?

*The **medium risk application** was **reviewed** by the UNISA-CAES Health Research Ethics Committee on 04 June 2020 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

The proposed research may now commence with the provisions that:

1. The researcher will ensure that the research project adheres to the relevant guidelines set out in the Unisa Covid-19 position statement on research ethics attached.
2. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
3. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the Committee.
4. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
5. Any changes that can affect the study-related risks for the research participants, particularly in terms of assurances made with regards to the protection of

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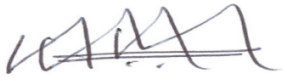
participants' privacy and the confidentiality of the data, should be reported to the Committee in writing, accompanied by a progress report.

6. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.
7. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data require additional ethics clearance.
8. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

Note:
*The reference number **2020/CAES_HREC/103** should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

Yours sincerely,



Prof MA Antwi
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Prof SE Mini
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Appendix 2: Proposal approval letter

UNISA university of south africa

Department of Life and Consumer Sciences
School of Agriculture and Life Sciences
College of agriculture and Environmental Sciences
Private Bag X6
Florida
1710

To: Z Makuvara (Student no: **640-089-16**)

Subject: Outcome of your research proposal

It gives me great pleasure to inform you that your PhD research proposal titled:" **Characterisation and effectiveness of bioactive compounds from selected medicinal plants used in the treatment of malaria in Zimbabwe**" has been approved.

Comments and suggested improvements were provided by the review committee. These comments will be communicated to you by your supervisor.

Good luck with the rest of your studies.

Best regards

Lebelo. Date 5/03/2020

Prof SL Lebelo
COD: Department of Life and Consumer Sciences

Appendix 3: Permission letter from Ministry of Health and Child Care

Telephone: +263-4-730011

ZIMBABWE

Reference:
Ministry of Health and Child Care
P O Box CY1122
Causeway
HARARE

10 January 2019

Zakio Makuvara
Great Zimbabwe University
P O Box 1235
MASVINGO

Attention: Mr Z Makuvara

RE: REQUEST TO CONDUCT A HEALTH RELATED RESEARCH IN ZIMBABWE

Your letter received on 9 January 2019 refers.

The Ministry of Health and Child Care would not have an objection to your intended study provided you get clearance from the National Institute of Health Research and the National Malaria Control Programme. By way of this letter you are further referred to the above agencies for supervision from the Institute of Health Research please contact **Mr Newman Handireketi-0773841024**, and for the National Malaria Programme- **Dr Joseph Mberi – 0772263525**.

Wishing you success in your endeavour.

SECRETARY FOR HEALTH & C/C
(PRINCIPAL DIRECTOR CURATIVE SERVICES)
10 JAN 2019
P.O. BOX CY 1122, CAUSEWAY
ZIMBABWE

MR S Makarawo
PRINCIPAL DIRECTOR – CURATIVE SERVICES
for: SECRETARY FOR HEALTH AND CHILD CARE
/pc

Appendix 4: Permission letter from the Ministry of Health and Child Care and the Ministry of Lands, Agriculture, Fisheries, Water and Rural Development, Department of Research & Specialist Services (DR&SS)

MINISTRY OF LANDS, AGRICULTURE, FISHERIES, WATER AND RURAL DEVELOPMEN
DEPARTMENT OF RESEARCH & SPECIALIST SERVICES (DR&SS)

All Communications to be addressed to
"THE HEAD"

Telephone: 263-242-708938
E-mail: nhbg@drss.gov.zw
Fax:

NATIONAL HERBARIUM & BOTANIC GARDEN
Box A889
Avondale, Harare, Zimbabwe

24 November 2021

Great Zimbabwe University
P.O. Box 1235
Masvingo
Zimbabwe
22 November 2021

PERMISSION TO COLLECT PLANT SPECIES FOR RESEARCH ON PLANTS USED IN THE TREATMENT OF MALARIA IN ZIMBABWE

Mr. **Zakio Makuvara**, a lecturer and researcher at Great Zimbabwe University, is a doctoral student at the University of South Africa focusing on antimalarial plants in Zimbabwe. The title of his thesis is "**Characterisation and effectiveness of bioactive compounds from selected medicinal plants used in the treatment of malaria in Zimbabwe**."

For this study, plants will be selected based on specific screening criteria: (1) existence in published works focusing on Zimbabwe, (2) lack or limited information on toxicity and activity against Plasmodium species (lack of pharmacological references), (3) indigenous to Zimbabwe, (4) accessibility of the plant species, and (5) presence of samples that can be collected sustainably.

We kindly request your assistance and permission to allow Mr. Makuvara to collect plants used in the treatment of malaria in Zimbabwe. No trees will be uprooted or destroyed. The plant parts collected will be used for scholarly research purposes only, and any information obtained will be shared with the relevant stakeholders.

Yours sincerely

C. Chapano (Mr.)
Head
National Herbarium and Botanic Garden,
0773 209 095 / 0714 025 235

Appendix 5: Research paper on "Malaria control and treatment: the role of indigenous practices in Zimbabwe"

Taylor & Francis
Taylor & Francis Group

Cogent Public Health

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Malaria control and treatment: the role of indigenous practices in Zimbabwe

Zakio Makuvara, Solomon Ramagoai Magano & Grace Mugumbate

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NEW PUBLIC HEALTH | REVIEW ARTICLE

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Malaria control and treatment: the role of indigenous practices in Zimbabwe

Zakio Makuvara[a,b], Solomon Ramagoai Magano[a] and Grace Mugumbate[c]

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REVIEWING EDITOR
Joe Thomas, Institute of Health and Management, Australia

SUBJECTS
Epidemiology; Microbiology; Pharmacology

1. Introduction

Malaria is still a major public health problem in Zimbabwe, impacting people of all ages and posing a threat to over 50% of the population (Manyangadze et al., 2017). Nonetheless, the World Health Organization's (WHO) 2016 report on malaria found that there was a higher likelihood of parasitic infection among 79% of the population. Malaria incidence in 2013 was 139 per 1000 people at risk, a considerable decrease from the reported rate of 139 cases per *1000* inhabitants in 2010 (Manyangadze et al., 2017). Despite the fact that the Roll Back Malaria Program and Zimbabwe National Malaria Control Program have helped to lower malaria

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ABSTRACT

Beside the use of conventional medicines, Zimbabweans rely on native malaria control and treatment practices. This mini-review therefore seeks to identify and assess applicability of Zimbabwean indigenous practices in the fight against malaria. This work is based on evaluation of literature and data was retrieved from databases such as the Web of Science, Google Scholar, Scopus, PubMed and Research Gate. Five categories of indigenous practices used in the fight against malaria were identified and these are (1) use of herbal and other traditional medicines in malaria, (2) use of traditional environmental indicators, (3) the use of red-hot granite rock and water, (4) burning of dung and plants to deter malaria vectors and (5) spiritual malaria treatment based on faith and traditional healers. Beyond these traditional practices, an overview of medical pluralism, indigenous health practices and decolonisation of public health is presented. It has been shown that herbal medicines are applicable in the fight against malaria. However, burning of plants and dung can be applied in the production of mosquito coils to prevent mosquito bites. Additionally, steaming is just limited to the management of fever and spirituality has no scientific basis on malaria management. Despite the application of indigenous practices in the fight against malaria in Zimbabwe, there is inconclusive evidence on their applicability in the main stream health systems. A comprehensive study should be conducted in order to scientifically evaluate the applicability of these indigenous practices.

IMPACT STATEMENT

Despite the efforts made in the fight against malaria in Zimbabwe, this disease is still one of the most common vector-borne diseases in the country. In addition, to conventional malaria treatment, indigenous practices have been applied in the fight against malaria in Zimbabwe. Beyond the traditional practices, the used use of different approaches in the treatment of malaria (medical pluralism) and decolonisation of mainstream health practices are presented. Among the traditional practices, it has been shown that herbal medicines burning of dung are applicable in the fight against malaria. However, other practices such as, steaming and spirituality lack substantial scientific basis on malaria management. Despite the application of indigenous practices in the fight against malaria in Zimbabwe, there is inconclusive evidence on their applicability in the main stream health systems.

occurrences over the past 13 years, a spike in the mortality rate attributed to malaria, from 6.1% in 2012 to 13.8% in 2014, has been noted (Mundagowa & Chimberengwa, 2020). Resistance of the malaria vectors to insecticides and parasite resistance to anti-malarial medications are the main causes of Zimbabwe's unusually high malaria prevalence, which is consistent with global trends (Gunda et al., 2016). Additionally, altering vector behaviour, climatic change, a lack of finance, the collapse of the economy, political unrest and migration are all factors that contribute to malaria transmission instability and periodic outbreaks in Zimbabwe (Mundagowa & Chimberengwa, 2020).

Artemisinin is not widely available in Zimbabwe and other sub-Saharan African nations, which is correlated with low production levels, relatively high costs, complicated dosage and a lack of clinical expertise with artemisinin-based combinations (Kazembe et al., 2012). Due to the lack of affordable and accessible effective antimalarial medications and innovations for malaria control, the effects of malaria in Zimbabwe were exacerbated (Kazembe et al., 2012; Mugwagwa et al., 2015). Infectious illnesses like malaria continue to be a problem for public health despite advances in our understanding of microbes and the pathogenic control of those pathogens. The unavailability of vaccines and antimicrobial resistance are often the two biggest obstacles to the elimination malaria in Zimbabwe (Mayer et al., 2009). Due to all of these obstacles, it is necessary to find and create innovative and reliable systems to fight against malaria.

A number of studies have outlined the traditional malaria-fighting strategies employed in Zimbabwe (Chapu & Mgocheki, 2017; Dhewa, 2008; Kazembe et al., 2012; Macherera et al., 2017; Makundi et al., 2007; Mavhurume, 2016). Traditional medicine is defined by WHO as knowledge, techniques and abilities based on concepts, values and traditions unique to many societies that are used to improve health and avoid, detect, relieve, or cure physical and men- tal diseases (WHO, 2013). In Zimbabwe, traditional health care practices include herbal, wildlife and mineral treatments, massage, mysticism and other culturally distinct activities. Some of the indigenous practices against malaria are based on early warning and detection and forecasting (Macherera et al., 2017). Such malaria prevention methods mainly relying on weather variables such as rainfall and their possible impact on malaria outbreak and transmission. According to Mabaso et al. (2006), the high yearly malaria incidence was accompanied by heavy rains in the period between 1988 and 1999. Besides

rainfall, temperature was identified has one of predictors of malaria prevalence and transmission. However, the uptake and implementation of these indigenous practices has been limited and more emphasis has been given to scientific issues (Macherera et al., 2017).

In Zimbabwe, individuals from malaria hotspots have the potential to exploit malaria early traditional warning to prepare for malaria outbreaks (Macherera et al., 2017). However, according to Soropa et al. (2015), indigenous knowledge systems are not only meant for decision making at local level, but for researchers, planners and managers to develop strategies which improve communities in the face of climate change and infectious diseases such as a malaria. It has been argued that although tools such as remote sensing, forecast warning and geographic information system are critical in predicting climate and malaria vulnerability, indigenous knowledge systems are simple, accurate and more applicable in local communities (Glantz, 2009). Considering that indigenous knowledge systems have evolved over a long period of time (Eyong, 2007), it is critical to integrate indigenous knowledge systems and scientific surveillance and warning systems in order to reduce vulnerability rural communities to infectious diseases such as malaria and climate change (Jiri et al., 2016).

The application of indigenous knowledge systems on prediction of rainfall (one predictor of malaria prevalence) in Zimbabwe was revealed in a number of studies (Risiro et al., 2012; Soropa et al., 2015). The World Health Organisation has outlined potential malaria detection indicators and early warning systems (Thomson & Connor, 2001). In line with this, a study was conducted in Gwanda district, Matabeleland South, Zimbabwe to assess potential malaria detection indicators and early warning systems used in communities (Macherera et al., 2017). Generally, there is still limited published information on the role of traditional practices in the fight against malaria. Therefore, this paper aims to explore the role of indigenous practices in malaria control.

2. Materials and methods

The current study used a hybrid technique that included quantitative and qualitative methodologies. Based on the Boolean search approach, literature was retrieved from University of South Africa (UNISA) library, Zimbabwean libraries and electronic databases such as Web of Science, Google Scholar, SciELO, Science Direct, Scopus and PubMed. Boolean search

methods in this particular instance extended and restricted the search through combining search strings with modifiers and/operators such as OR AND and NOT. An overview of the representative search strings and Boolean search technique applied in this study are summarised: 'Indigenous practices AND/OR malaria in Zimbabwe', 'Zimbabwean indigenous knowledge AND/OR malaria', 'Traditional AND Malaria treatment in Zimbabwe', 'Malaria prevalence AND/OR Zimbabwean herbal medicine', 'traditional early warning AND/OR malaria in Zimbabwe' and 'Traditional Medical Practitioners Act AND/OR malaria in Zimbabwe'. This particular technique was instrumental in retrieving relevant literature. However, this was confined to publications in the English language only and therefore might have missed publications in other languages.

Accordingly, data was retrieved from published scientific articles, conference papers, theses, books and grey materials. The individual data sources were qualitatively screened and evaluated based on the main objective of the study. Due to limited information on indigenous practices used in the management of malaria in Zimbabwe before 2000, the literature search in this particular study was limited to the period 2000 to 2023. After screening and anal- ysis, publications on indigenous practices used in the treatment of malaria in Zimbabwe were examined, critically reviewed, and the important findings were summarised and tabulated.

3. Results and discussion

There is a dearth of published information on indigenous practices applied against malaria. This study therefore, outlines indigenous practices in the control and treatment of malaria under the following sub headings: (1) the use of herbal and other traditional medicines in malaria control and treatment, (2) the use of traditional environmental indicators to control malaria, (3) the use of red hot granite rock and water, (4) the burning of dung and plants to deter malaria vectors and (5) spiritual malaria treatment based on faith and traditional healers (Figure 1).

3.1. Herbal and other traditional medicines used in malaria control and treatment

There is limited information the use of herbal medicine against malaria in Zimbabwe. Additionally, the available information is mainly based on ethnobotanical surveys without scientific evaluation (Chapu &

Mgocheki, 2017; Kazembe et al., 2012; Lukwa et al., 2001; Mbauya, 2015; Ngarivhume et al., 2015). Traditional medicine is one of the most accessible and widely available forms of therapy in Zimbabwe's resource-limited communities. The indigenous people have a great experience of using traditional therapeutic drugs (Dhewa, 2008; Olsson et al., 2015). Orthodox medicinal practice in Zimbabwe is based on experiences of communities in the framework of the native cultural context. However, the practice is dynamic and evolves over time situationally (Dhewa, 2008; Gureje et al., 2015). Zimbabwean traditional medical practitioners include herbalists, psychic therapists, midwives, fortune tellers, seers and faith healers who design techniques and materials employing indigenous knowledge (Hlatshwayo, 2017; Rupande, 2022). Despite the rising acceptance of ethnomedicine in Zimbabwe, much traditional knowledge remains unrecorded (Usai et al., 2022). However, documentation of traditional medicines is required in order to maintain knowledge, preserve and use them in a sustainable manner.

Traditional remedies are used by around 80% of people in underdeveloped nations since they cannot afford costs associated with western medicines and medical services (Dhewa, 2008). The uptake of traditional remedies is normally high due to the fact that they are more accepted from a spiritual and cultural standpoint (Ferngren, 2016; Pan et al., 2014). The recording of traditional preventive and treatment medicine in Zimbabwe is a huge move in maintaining cultural customs, enabling community participation and inclusion in developing traditional malaria control strategies.

In Zimbabwe, the Traditional Medical Practitioners Act of 1981, attempted to incorporate traditional medicine into the national healthcare system, failed to contribute significantly due to a lack of institutional and financial support (Ngarivhume et al., 2015). However, many residents in Zimbabwe, particularly in rural areas, continue to utilise traditional medicines for basic health care, despite lack of documentation and regulation (Van Wyk & Prinsloo, 2018). Between 65 and 80% of the world's population relies on traditional remedies for their primary healthcare needs. According to the WHO, local, additional and complementary therapy of top quality and safety offer various advantages (WHO, 2013). Ease of access, affordability, income, level of education, effectiveness and non-availability of contemporary healthcare services were the prime causes behind the utilisation of Traditional medicines. Zimbabwean traditional medical practices exemplify the country's cultural diversity

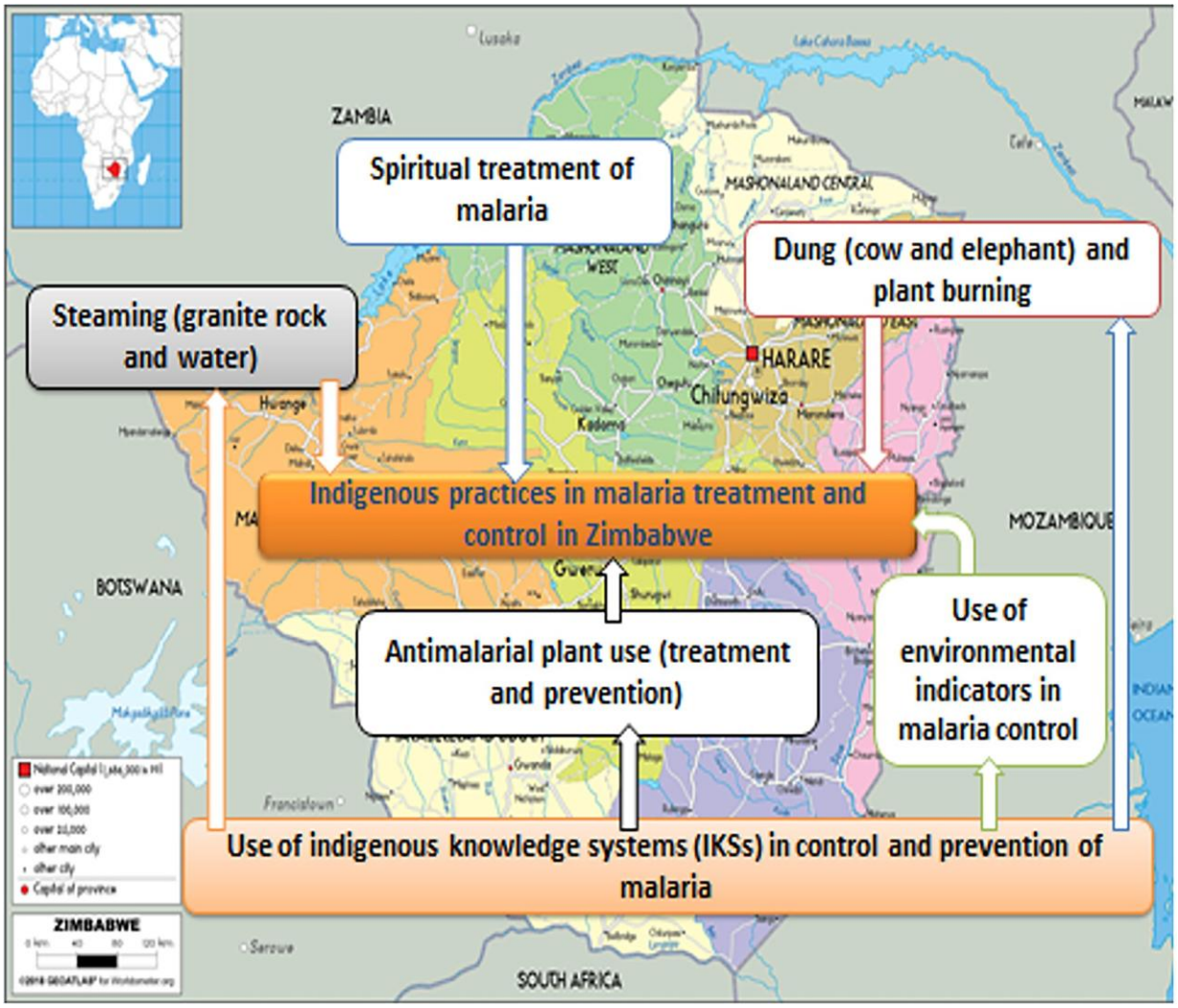

Figure 1. A theoretical framework of indigenous practices in the control and treatment of malaria in Zimbabwe.

(Jidong et al., 2021; Rutto, 2005). Historic Zimbabwean medicines are used for preventive care, therapy and well-being enhancement. Traditional remedies have an important role in Zimbabwean healthcare systems, as evidenced by the country's health-care policies (Dhewa, 2008; Razzak & Kellermann, 2002).

Traditional medicine has remained a source of pride for most Africans, particularly the Shona peo- ple of Zimbabwe (Bobo & Sukdaven, 2020; Mapira & Mazambara, 2013). According to statistics, 50 percent of the people in Africa utilise complementary thera- pies on a daily basis, with herbal remedies by far the most popular. Because most of the medication is located in close proximity to the African populations, makes it cheap and accessible (James et al., 2018).

The role of traditional healers was for the first time mainstreamed in Zimbabwe in 1980, with the formation of the Zimbabwe National Traditional Healers Association (ZINATHA) (Chakawa, 2015;

Mposhi et al., 2013). Zimbabwe has also been plagued by an influx of herbal medications from countries including Tanzania, China and India, resulting in the spread of traditional remedies throughout the country (Fajinmi et al., 2017). Traditional medicine's influence in Zimbabwe is inextricably associated with the practices of the traditional healer as the primary physician. Herbal medicines are utilised traditionally for a variety of purposes, including moral preservation, lucky charms, fertility and spirituality issues (Novotna et al., 2020).

3.2. The use of traditional environmental indicators to control malaria

Malaria incidence has been shown to strongly correlate with rainfall and temperature changes. In Zimbabwean communities, plant phenotypic plasticity and insects are mostly utilised markers in the

prediction of malaria (Macherera & Chimbari, 2016). Additional malaria predication markers exploited in Zimbabwe include apparent noise originating from mountains (thundering of mountainous regions) and specific ostrich behaviours. According to Macherera and Chimbari (2016), there are five markers of how much rainfall will be received and hence the possibility of malaria outbreak: (1) Arthropod markers, (2) Bird predictors, (3) Animal attributes, (4) Plant phenology and (5) Astronomical and meteorological signals (Table 1).

The insects recognised by residents of Gwanda district, Matebeleland South, Zimbabwe, mark the start of the rainy season and whether the amount of rainfall is high or low. For example, the presence of a large number of termites indicates the start of the rainy season (Chanza & de Wit, 2014; Macherera & Chimbari, 2016; Risiro et al., 2012). Though such a marker does not reflect the quantity of the rain or the extent of malaria prevalence, it does herald the start of the malaria season. The appearance of massive white butterflies migrating from west to east in the month of October indicates considerably high rainfall and hence a high malaria prevalence (Macherera & Chimbari, 2016).

Most bird actions predict whether the rainy sea- son will be excellent or bad; however a few spe- cies simply herald the start of the rainy season (Chanza & de Wit, 2014; Kupika et al., 2019; Musarandega et al., 2018; Risiro et al., 2012). The noise of ground hornbills from September to November as well as the laying of eggs by guinea fowls, indicate a favourable rainy season and a high malaria incidence (Macherera & Chimbari, 2016). However, the appearance of huge numbers of species such as Swallows and Migratory birds in November indicates that the rainy season is approaching.

Wild animals have been recognised as reliable predictors of rainfall and malaria prevalence (Nyahunda & Tirivangasi, 2021). It was discovered that if wild animals have a large number of young ones, more rainfall would be obtained in the next season, leading to high malaria prevalence (Macherera & Chimbari, 2016). Furthermore, lions passing by the farmsteads without attacking domesticated animals or humans during the months of September and October signify of a favourable rainy upcoming season (Macherera & Chimbari, 2016). This shows that the likelihood of malaria transmission will be substantially high.

Plant phenology indicators are based on the pres- ence or absence of leaves, fruits and flowers, which

indicate the presence of high or low rainfall (Table 1) and hence intensity of malaria prevalence (Kupika et al., 2019; Mashoko, 2014). For example, if plants like *Lanchocarpus capassa* flowers in October and November and *Boscia albitrunca* produces flowers early and numerous fruits by November, there will be a significant rainfall in that season and malaria will be a major problem. However, the presence of many fruits in plants such as *Acasia nigrescens* indicates low rainfall and drought is signified by the plant taking longer to produce new leaves (Macherera & Chimbari, 2016). In both cases, malaria prevalence is projected to be extremely low.

In Gwanda district, Matebeleland South, Zimbabwe, astronomical and meteorological signals (Table 1) such as clouds, humidity and temperature variations are utilised to forecast rainfall patterns and the intensity of malaria transmission (Macherera & Chimbari, 2016). High rainfall and malaria prevalence are indicated by the presence of a band all around the moon, finely distributed clouds, clouds all along the frontier, hot humidity interchanging with strong winds, gentle breeze from the east to the west and then from the west to the east, increased frequency and intensity of whirlwinds during September and October and misty air during September and October (Macherera & Chimbari, 2016).

3.3. Burning of dung (cow or elephant) and plants to deter malaria vectors

In most regions of Zimbabwe, hanging fresh leaves of *Ocimum canum* in the home efficiently provided mosquito bite protection. Besides hanging of leaves, plants like *Azadirachta indica* and *Ocimum forskolin* have been burnt to generate repellent smoke that has considerably reduced human-mosquito bite (Table 1). Burning of plants to deter mosquitoes has been done in other African countries. In one trial conducted in Kenya, a combination of plant powders burnt directly, produced smoke that repelled malaria vectors such as *Anopheles aegypti* (Wendimu & Tekalign, 2021).

A comprehensive survey in Chiredzi district, Masvingo, Zimbabwe (Table 1), indicated that most households burn dung (cow or elephant dung) and plant leaves to generate smoke which repel mosquitoes (Chapu & Mgocheki, 2017). Several explanations have been propounded to explain how smoke fumigation of houses may change mosquito feeding behaviour. Plant and dug derived smoke normally obscure human odours and carbon dioxide, which mosquitoes sense when a human being is close by

Table 1. Indigenous practices in prevention and treatment of malaria in Zimbabwe.

Indigenous practice	Remarks on treatment/ prevention /natural products used in Zimbabwe	Applicability in Zimbabwean mainstream health systems	References
Herbal medicine (*most of the herbal medicines are taken orally as powders, cold and hot infusions from plant roots, leaves, barks and fruits. However, there is limited information on the dosage used*)	According to an ethnobotanical survey conducted in Manicaland Province, Zimbabwe, plant such as *Elephantorrhiza goetzei* (Harms), *Toddalia asiatica* (L.) Lam and *Pavetta schumanniana* F. Hoffm. In this particular study, *Aristolochia albida* and *Cassia abbreviata* were the most utilised herbal medicines against malaria.	As method of preserving indigenous practices and traditional medicines such has herbal medicines the Traditional Medical Practitioners Act of 1981 was introduced. This was meant to incorporate traditional medicine into the main stream Zimbabwean health system. With proper scientific evaluation and screening herbal medicines can be the basis of new antimalarial drugs that can be used beyond localised communities.	Ngarivhume et al. (2015)
	An ethnobotanical survey conducted in malaria hotspots of Zimbabwe revealed that the indigenous people are highly confident in herbal medicines which are used against malaria. Some of the plants that are utilised in malaria prone areas include *D. nitidula A. amara*, *V. infausta A. digitata* and *L. discolour*.	Although herbal medicines are used for primary health care needs in Zimbabwe, the claim that combining these medicines with western drugs improve efficacy is not always true. It depends on the type drug interactions which can be antagonistic, synergistic and additive. However, it has been concluded that some antimalarial plant-based drugs can compete with established drugs against malaria. This therefore, indicates that if plant based antimalarial drugs are scientifically evaluated especially for toxicity level can be applied beyond primary health in Zimbabwe.	Kazembe et al. (2012)
	In a survey conducted in Chiredzi dristict, Masvingo Province, Zimbabwe, antimalarial plants including *Adonsia digitata* roots, *Capsicum anuum* fruits and *Warburgia salutaris* bark,	Results from this study indicated plant remedies were combined with antimalarial drugs such as chloroquine. Although plant based antimalarial drugs are used traditionally in primary health care, there is need for assess their efficacy so that they are fully used in hospitals and readily in Zimbabwean pharmacies.	Chapu and Mgocheki (2017)
	In Mola, Kariba district, Mashonaland West Province, Zimbabwe, some of the identified antimalarial plants include *Amblygonocarpus andongenis* (Olvi.), *Carica papaya* L. (Caricaceae), *Zanha africana* (Radlk.), *Solanum panduriforme*, *Diplorhynchus condylocarpon*, *Euclea divinorum* Hiern, *Aloe greatheardii* Schoni.	Antimalarial herbal medicines can be used as primary health care medicines and with scientific evaluation on efficacy and toxicity can be applied in the main health system.	Lukwa et al., (2001)
	Pant species collected from a number of stations in Zimbabwe along Harare-Nyamapanda road close to Marondera, Mashonaland East Province, Zimbabwe, were assessed for antiplasmodial activities. The targeted plants were *Cussonia spicata*, *Flueggea virosa*, *Vernonia colorata*, *Artemisia afra*, *Clutia hirsute*, *Vernonia colorata*, *V. natalensis*, *Parinari curatellifolia*, *Hymenodictyon floribundum* and *Adenia gummifera*. Among all these plants, extracts from *Vernonia colorata* and *Artemisia afra* active against both PoW (chloroquine-sensitive *Plasmodium* strain) and Dd2 (*chloroquine-resistant Plasmodium*).	Based on the bioassays and identified *A. afra* and *V. colorata* extracts can be applied as antimalarial drugs. Although the IC50 values were less than 50 µg/ml (1.1–12.6 µg/mL), there need to evaluate toxicity of these extracts and to prescribe quantities that can be utilised. Moreover, *in vivo* assays and clinical tests should be carried so that these herbal medicines are incorporated into mainstream health system.	Kraft et al., 2003

In a doctoral study conducted in Chikwanha-Muneri villages of Marange District, Manicald Province, Zimbabwe plants traditionally used to manage malaria were identified and these include *Cassia abbreviate*, *Bridelia cathartica*, *Burkea Africana*, *Tragia okanyua*, *Pterocarpus Angolensi* and *Afzelia quanzensis*.	Antimalarial herbal medicines can be used as primary health care medicines and with scientific evaluation on efficacy and toxicity can be applied in the main health system.

(*Continued*)

Table 1. Continued.

Indigenous practice	Remarks on treatment/ prevention /natural products used in Zimbabwe	Applicability in Zimbabwean mainstream health systems	References
The use of traditional environmental indicators to control malaria Arthropod markers Bird predictors Animal attributes Plant phenology	Insects identified in Gwanda district, Matabeleland South, Zimbabwe, for example termites were shown to mark the start of the rainy season. Additionally, massive white butterflies migrating from west to east in the month of October indicates considerably high rainfall.	Though termites do not reflect the quantity of the rain or the extent of malaria prevalence, it does herald the start of the malaria season. However, white butterflies in this case indicate high malaria prevalence. These markers can be applied in preparing for malaria outbreaks.	Macherera and Chimbari (2016)
Astronomical and meteorological signals	It has been shown that noise of ground hornbills and laying of eggs by guinea fowls from September to November indicates a favourable rainy season. However, Swallows and Migratory birds in November indicate that the rainy season is approaching.	This is applicable in two ways since: (1) Early warning markers for start of malaria and (2) Malaria incident intensity predication.	Macherera and Chimbari (2016)
	It was discovered that if wild animals have a large number of young ones and lions passing by the farmsteads without attacking domesticated animals or humans during the months of September and October, then more rainfall would be obtained in the next season.	This shows that the likelihood of malaria transmission will be substantially high.	Macherera and Chimbari (2016)
	Flowering of plants like *Lanchocarpus capassa* in October and November and *Boscia albitrunca* in early and numerous fruits by November indicate significant rainfall in that season. *Acasia nigrescens* indicates low rainfall and drought if it takes longer to produce new.	Plant phenology indicators indicate the presence of high or low rainfall and hence intensity of malaria prevalence.	Macherera and Chimbari (2016)
	In Gwanda district, Matebeleland South, Zimbabwe, astronomical and meteorological signals such as clouds, humidity and temperature variations are utilised to forecast rainfall patterns.	In this case humidity/rainfall and temperature can be used to predict malaria prevalence and intensity.	Macherera and Chimbari (2016)
The use of red-hot granite rock and water,	A red hot stone is immersed in cold water and begins to boil. The patient and red hot stone are covered under a blanket or cloth (known as *kufukira* in Zimbabwe).	Although there is limited information linking this traditional method to the fight against malaria, it has been revealed that raising the body temperature strengthens the second line of defence by generating thermal stress, which mimics feverish episodes.	Chapu and Mgocheki (2017)
The burning of dung and plants to deter malaria vectors	A comprehensive survey in Chiredzi district, Masvingo, Zimbabwe, burning of dry pieces of plants such as *Spirostachys africana* and dung (cow or elephant dung) has been done to deter mosquitoes.	Evidence to suggest that smoke is an efficient repellent and the practice of smoke fumigation of rooms to avoid biting mosquitoes is common. This is a preventive method that can be applied with precautionary measures of preventing indoor air pollution.	Chapu and Mgocheki (2017)
	In most regions of Zimbabwe, hanging fresh leaves of *Chenopodium ambrosioides*, *Lippia javanica*, *Chrysopogon nigratana* and *Ocimum canum* in the home efficiently provided mosquito bite protection.	This is a preventive method that can be applied. Most plant derived fumigants are employed as mosquito repellents since they contain mosquito repelling essential oils.	Chapu and Mgocheki (2017)
Spiritual malaria treatment based on faith and traditional healers.	In Zimbabwe, the believers believe that malaria may be healed by pleading with God since it is thought that disease is God's punishment. Additionally, some churches in Zimbabwe recommended congregants to drink plenty of water to avoid contracting the sickness including malaria.	There is no scientific evidence to support applicability of this traditional method in the Zimbabwean health systems.	Treadwell (2001)
	Among the Shona speaking people of Zimbabwe, *mashavi* (wandering spirits) and mhondoro (wishful spirits) Although *mhondoro* and *mashavi* are important, they are not as important as vadzimu in everyday life of some Zimbabweans	There is no scientific evidence to support applicability of this traditional method in the Zimbabwean health systems.	Masaka and Makahamadze (2013)

	In some communities in Zimbabwean, the elderly (as well as departed descendants) are key in maintaining access to and inheritance of disease treatment and prevention through specific dreaming *(kurotswa*) and ceremonial divinations (*kusvikirwa*), in which the knowledge is disclosed as a gift	There is no scientific evidence to support applicability of this traditional method in the Zimbabwean health systems.	Masaka and Makahamadze (2013)

Moore et al. (2006). Additionally, smoke from plants normally interferes with mosquito receptors responsible for detecting chemicals through reducing water vapour in air (Davis & Bowen, 1994). Furthermore, dung and plant smoke is associated with a wide range organic compounds classified as insecticides, repellents and irritants (Moore et al., 2006). Such organic compounds include palmitic acids and capric, oleic.

Mosquito repellent herbs include *Chenopodium ambrosioides*, *Lippia javanica* and *Chrysopogon nigratana*. To deter mosquitoes, dry pieces of plants such as *Spirostachys africana* are burnt and glowing portions are utilised (Table 1) (Chapu & Mgocheki, 2017). This burning procedure is comparable to how mosquito coils are used nowadays. Elephant dung has historically been burnt and the smoke used for fumigation houses (Dubost et al., 2021; Maphane, 2018; Pennacchio et al., 2010). However, it should be emphasised that burning in poorly ventilated areas can expose occupants to extraordinarily high amounts of indoor air pollution. This is a common issue in low-income countries like Zimbabwe, particularly in rural regions where plants are abundant. It recommended that toxicity levels of the plant or animal waste to be burnt be assessed in order to prevent intoxication of people.

3.4. Spiritual malaria treatment based on faith and traditional healers

Zimbabwe's Shona people, like monotheism religions have always believed in the power of God in healing of diseases and general well-being (Kazembe, 2009). The connection between human beings with the metaphysical world is important before birth, during life, at death and after death (Machingura, 2012). Among the Shona speaking people of Zimbabwe, mashavi (wandering spirits) and mhondoro (wishful spirits) are powerful spirits in disease (eg, malaria) prevention and treatment (Masaka & Makahamadze, 2013). Although mhondoro and mashavi are important, they are not as important as vadzimu in everyday life of some Zimbabweans (Masaka & Makahamadze, 2013). As a result, Shona cosmology stresses the significance of vadzimu in terms of disease prevention and treatment, life and death. Zimbabweans also believe in taboos, which are important in illness prevention (Machingura, 2012). In some communities in Zimbabwean, the elderly (as well as departed descendants) are key in maintaining access to and inheritance of disease treatment and

prevention through specific dreaming (kurotswa) and ceremonial divinations (kusvikirwa), in which the knowledge is disclosed as a gift (Masaka & Makahamadze, 2013).

According to informants, malaria, like other ailments, may be cured through spirituality based on the individual's your level of commitment and faith. It is believed that nothing seems to be impossible with God (Kpobi & Swartz, 2018). Among other interventions exorcism, anointed oil application and use of holy water have been reported to cure diseases malaria included. In Zimbabwe, the believers believe that malaria may be healed by pleading with God since it is thought that disease is God's punishment (Treadwell, 2001). Additionally, some churches in Zimbabwe recommended congregants to drink plenty of water to avoid contracting the sickness including malaria. Based on this notion, all forms of illnesses may be effectively cured with water treatment (Bosire et al., 2022; Hjelm & Mufunda, 2010).

3.5. The use of red hot granite rock and water (steaming)

A red hot stone is immersed in cold water and begins to boil. The patient and red hot stone are covered under a blanket or cloth (known as kufukira in Zimbabwe), causing the patient to sweat excessively (Chapu & Mgocheki, 2017). Steaming is commonly advised as an at-home treatment for upper respiratory tract infections. Heat, the most fundamental aspect of steaming, has a substantial influence on host immune response and pathogenicity, as well as activating responsive thermoregulation systems that could really elevate or lower body temperatures to maintain equilibrium (Schieber & Ayres, 2016). Furthermore, raising the body temperature strengthens the second line of defence by generating thermal stress, which mimics feverish episodes (Schieber & Ayres, 2016).

Heat-stress caused by steaming stimulates the immune system (Heinonen & Laukkanen, 2018). Cold exposure following heat stress enhances immune system through an increase in NK cell number and activation, as well as levels of IL-6 in blood (Brenner et al., 1999). Furthermore, reflux occurs in response of blood being redirected in the tissues, which aids in detoxification (Cochrane, 2004). Multiple cellular reactions are triggered by antispasmodic temperatures, including a complex genetic regulation of the heat stress response pathway, inflammatory processes and immune response activation (Singh & Hasday, 2013). During steaming, heat shock proteins are produced and moved to cell membranes (Iguchi

et al., 2012). The principal role of these proteins is to regulate and prevent damage in immune cells and denaturation of proteins. Moreover, heat shock proteins contribute significantly in the activation of polymorphonuclear leukocytes, antigen presentation, dendritic cells recruitment and differentiation (Singh & Hasday, 2013).

Increased temperature caused by steam inhalation helps to improve cardiac health by influencing the autonomic nervous system, reducing blood pressure, inflammatory processes and peroxidation. This, however, promotes and increases in heart rate, blood volume and perivascular blood circulation and enhancing cardiovascular health, serum lipids profile and arteriolar regulation (Heinonen & Laukkanen, 2018; Kunutsor et al., 2018; Laukkanen & Kunutsor, 2019). Another advantage of thermal stress is the adjustment in blood pH produced by rapid breathing induced by hyperthermia, which is accompanied by pulmonary alkalosis (Tsuji et al., 2016).

4. Medical pluralism, indigenous health practices and decolonisation of public health

4.1. Medical pluralism and indigenous health practices in Zimbabwe and beyond

Medical pluralism is a practice where different medical systems are adopted based on health beliefs and their ability to treat illnesses (Figure 2). Additionally, medical pluralism involves the integration of alternative and conventional biomedicines for a healthy society (Amzat et al., 2014). Although the reported indigenous practices (Table 1) have been combined with convention biomedicines in treatment and prevent of malaria in Zimbabwe, there is limited published work which indicates direct application of medical pluralism in malaria prevention and treatment in Zimbabwe. In line with this, the presented evidences of medical pluralism focus on other diseases in Zimbabwe and beyond (Choguya, 2015; Grossenbacher et al., 2021; Kajawu et al., 2016; Mandizadza, 2016; Moshabela et al., 2017; Robbins et al., 2021; Subedi, 2019).

Although, both conventional and alternative medicines are applied in most societies, it has been noted that there is still reluctance in mainstreaming alternative/traditional medicine in national health care systems. For instance in Zimbabwe, although based on sociocultural values and tradition, traditional birth attendants are empowered to manage pregnancies and perform deliveries, the ambivalence of government policies has excluded them and they are at the periphery of healthcare provision (Choguya, 2015). In Tharus, Nepal the link between medical pluralism or use of indigenous medicine and state health policy was evaluated (Subedi, 2019). Though the application of folk medicine, biomedicine and scholarly traditional medicine is common, officially biomedicine is the most preferred. The medical pluralism in Nepal has since been widened by integrating scholarly traditional medicine into mainstream health care system. Unfortunately, most indigenous medicines are outside state regulation and the legitimacy of indigenous care providers (healers) is still questioned (Subedi, 2019).

This practice of medical pluralism has been applied in many countries (Priya, 2012). For example,

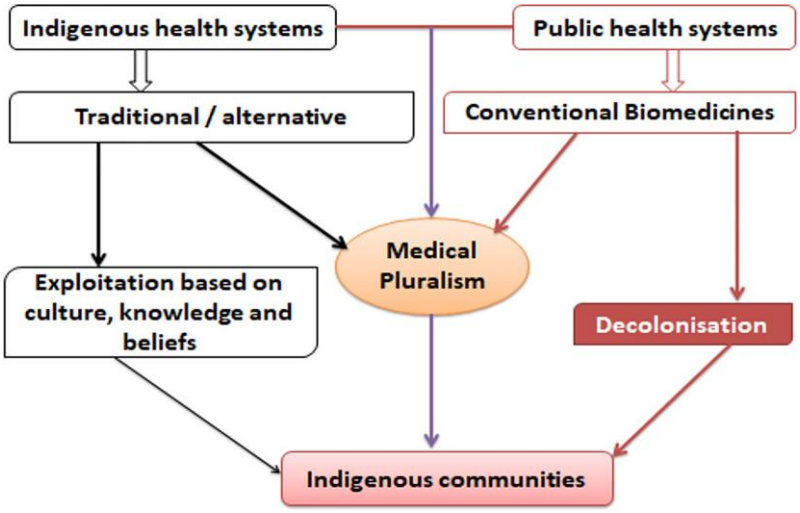

Figure 2. Conceptual framework of the interaction of medical pluralism, indigenous health practices and decolonisation of public health.

in Zimbabwe, Uganda, Tanzania, Kenya, South Africa and Malawi, a study to establish medical pluralism among people living with HIV was conducted and it was revealed that medical pluralism was common where people living with HIV used therapeutics from the biomedical health, traditional and faith-based worlds (Moshabela et al., 2017). According to Grossenbacher et al. (2021), spiritual (religious) ontologies are supposed to be integrated into main health care services and programs. However, there is need to strengthen these ontologies to prevent compromised therapy and holistically support people living with HIV. In another study to describe the pathways to primary care for patients with common mental disorders in Harare, Zimbabwe, it was revealed that most patients seek help from biomedical and traditional care providers. Although patients preferred biomedical care providers, in cases of treatment failure traditional care providers were consulted (Patel et al., 1997).

In most societies including Zimbabwe people normally use a diverse of therapeutic options where one option can be used as a complement or an alternate. In one study conducted in, Zimbabwe, Uganda, Tanzania, Kenya, South Africa and Malawi, there was evidence of mixing therapeutics from faith-based, traditional and biomedical healthcare providers. However, it was shown that medical pluralism in this case introduces mistrust among faith-based, traditional and biomedical healthcare providers and delays proper care of people living with HIV (Moshabela et al., 2017). In a study to examine African Traditional Medical practices on mental disorders, it was reported that herbalists were the most preferred care givers. The African Traditional Medical practices in this case provided treatment which was in congruency with cultural beliefs of patients and this was not true for biomedicine (Kajawu et al., 2016). More often, therapeutics from different traditions can be combined (Figure 2) in order to treat certain ailments and meet their primary health care needs. For instance, cancer patients from Zimbabwe seek treatment from traditional health practitioners which make use of indigenous knowledge systems based on economic, cultural and affectional perspectives (Mandizadza, 2016). In a separate study done in South Africa, most Traditional Health Practitioners are trained *sangomas* (traditional healers) and prophets (faith healers). Although this reflects pluralistic approach, it was not applicable across all societies since some churches disregard traditional beliefs. It has been shown that Traditional Health Practitioners normally practice medical pluralism through

combining traditional practices and Western medicines (Figure 2) (Galvin et al., 2023). Moreover, use of therapeutics from different traditions has been demonstrated through a cross-cultural comparative study on pre-eclampsia in Zimbabwe, Haiti and Ethiopia. From this study, it was concluded that apart from conventional biomedicine, traditional and faith caregivers should be engaged in order to move towards a life-saving care and pre-eclampsia elimination (Robbins et al., 2021).

4.2. Indigenous health practices and decolonising public health

Although indigenous health practices (Table 1) are experimental, systematic and empirical, they are localised and based on indigenous knowledge of human interactions, plants and animals in a specific location. This therefore, implies that indigenous health practices are situated, holistic, take into consideration nonhuman and human life forms (Stewart-Harawira, 2008). Notably, indigenous health practices take the human world as inseparable from nature and therefore, decolonisation process of pub- lic health should be independent of human conse- quences (Adams, 2016). On the other hand, public (global) health is based on Western conceptions of health which are basically anchored on biomedical, individual and human entities (Hindmarch & Hillier, 2022). In line with Barnabe (2021), public (global) health systems are modelled to suit the dominant society without contextualised indigenous knowledge systems. This implies that global/public health disregards the critical roles of indigenous cultural practices in maintaining wellness and health. However, according to Affun-Adegbulu and Adegbulu (2020), there is a shift towards decolonisation of global health as indicated by an increase in publications, conferences and seminars inclined to subject area recently.

In order to decolonise global health, a *Two-Eyed Seeing* approach by Mi'kmaq Elders Albert and Murdena Marshall can be adopted as a way of integrating indigenous knowledge systems/health practices and Western science (Martin, 2012). Decolonisation (Indigenisation) of public health is believed to enable the analysis of cultural practices and historical events that may have been lost during the transformation of indigenous to global health (Anderson, 2014). Accordingly, this decoloni- sation process requires empowerment, involvement and collaboration of leadership and indigenous communities (Barnabe, 2021). Public health can be

decolonised can be achieved through partnerships in global health education. In order to achieve this, the educators must incorporate local communities in needs assessments, encouraging cultural safety and promoting positive deviance. However, this calls for local and foreign partners to come up with moral framework which take into account ethical diversity. It should be noted that in some instances indige- nous health practices have been overlooked. For example, in an analysis on women health activism in Zimbabwe by Gumbonzvanda et al. (2021), it is has been found that the *Nhanga* (Zimbabwe local prac- tice) has represented emotions, narrative and culture which are normally overlooked in public health sys- tems. Such indigenous practices should be should be included in public health systems since they promote transformation at global, community and individual (Gumbonzvanda et al., 2021). For such indigenous health practices and global health interventions to benefit indigenous people there is need for congruency (Aubel & Chibanda, 2022). However, global health strategies are hampered by incongruity between cultural practices and public health systems. Therefore, the decolonisation of public health should be based on the restructuring of interventions and research in order to capture the roles, resources and structure of indigenous Global South cultures.

Indigenous health practices can be globalised through decolonisation of academic publishing spaces. According to Khan (2022), journals involved in global health research and report publishing should provide opportunities for publications from alternative health systems and epistemic standpoints. In line with this, public health decolonisation should occur at ontological and epistemic levels and allows for historicising and depoliticising health through leadership, knowledge and paradigm shift (Büyüm et al., 2020). However, funding should be increased in order to support cultural based health programs and research (Aubel & Chibanda, 2022). Additionally, global health research journals should at all times try to downplay traditional evidentiary hierarchy (Khan, 2022).

5. Conclusion and recommendations

In this work five classes of indigenous practices used in prevention and management of malaria were identified and these are methods were evaluated. According to the findings in literature, there is paucity of published data on this particular subject.

However, the identified indigenous practices applied in the fight against malaria are (1) herbal and other traditional medicines for treatment and prevention (2) traditional environmental indicators for prediction of malaria prevalence (3) red-hot granite rock and water for treatment of malaria symptoms (4) burning of dung and plants to deter malaria vectors and (5) spiritual malaria treatment based on faith and traditional healers. Information on the applicability and efficacy of these methods in Zimbabwe is limited. However, herbal medicines have been used in the development of antimalarial drugs and burning of plants and dung can be applied in the production of mosquito coils to prevent mosquito bites. Practices such as steaming and spirituality are just limited to the management of fever and has no scientific basis on malaria management respectively. Despite the application of indigenous practices in the fight against malaria in Zimbabwe, there is inconclusive evidence on their applicability in the main stream health systems. Although there is evidence of medical pluralism involving indigenous health practices in Zimbabwe, there is dearth of published information on direct application of medical pluralism in the fight against malaria. Therefore, it is recommended that indigenous health practices are scientifically evaluated through comprehensive studies. Beyond this literature survey, nationwide surveys should be done to gain more insights into the identified and other existing indigenous malaria control and treatment methods. Additionally, future studies should focus on applicability of medical pluralism and decolonisation of public health in the fight against malaria.

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Appendix 6: Research paper on Malaria situation and elimination in Africa with specific focus on Zimbabwe: a systematic review

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Malaria situation and elimination in Africa with specific focus on Zimbabwe: a systematic review

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ABSTRACT

Malaria is an infectious disease caused protozoa in genus *Plasmodium.* In spite of the efforts made in the fight against malaria, this tropic infectious disease is still one of the most common vector borne disease in the WHO African region. Therefore, this systematic review focuses on current malaria control interventions, treatment options and elimination in Africa with specific focus on Zimbabwe. The literature was searched in electronic databases such as PubMed, MEDLINE, ClinicalTrials.gov, DOAJ, Europe PubMed Central, Web of Science and Google Scholar. Furthermore, the literature search was expanded to include reference lists in peer-reviewed scientific publications. Some of the key phrases chosen in the literature search were 'malaria control interventions', 'Zimbabwe and malaria', 'Malaria treatment', 'Malaria prevalence in Zimbabwe', 'Malaria prevalence in Africa', 'malaria and Africa', and 'Africa and malaria therapy'. In this study, 185 articles were reviewed and literature was summarized in line with the objectives of the study. Based on literature survey, it was noted that intensification of malaria control interventions and treatment has led to a remarkable decline in malaria morbidity and mortality. However, malaria remains a public health concern in most African countries including Zimbabwe. This has been attributed to the (1) development of physiological and behavioral resistance among malaria vectors in response to insecticides overuse, (2) development of resistance in *P. falciparum* to antimalarial drugs, (3) migration of malaria tolerant and positive individuals from malaria endemic areas to settings where malaria is less common, (4) emergence of genetically distinct malaria parasites which has limited the development of an effective malaria vaccine and protective immunity.

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Malaria situation and elimination in Africa with specific focus on Zimbabwe: a systematic review

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Introduction

Malaria is an infectious vector borne disease caused by Apicomplexan protozoans of genus of *Plasmodium* (Mulaw et al., 2019). Plasmodia species are transmitted between human beings through mosquito bites. However, the other vertebrate secondary hosts for *Plasmodium* species include simians, rodents, birds, and reptiles (Krettli et al., 2001; Lima et al., 2015). Of the several species of *Plasmodium*, only five species infect humans and these are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malar- iae* and *Plasmodium knowlesi*. *Plasmodium vivax* and *Plasmodium falciparum* are the most common malaria pathogens and the latter is the most deadly form that dominates in Africa (Klooster et al., 2015; Mulaw et al., 2019). It has been confirmed that 98% of malaria cases reported in Zimbabwe are as a result of *P. falciparum* and the remainder of the cases, about 2%, are caused by *Plasmodium ovale* and *Plasmodium malariae* (Klooster et al., 2015). According to the research carried out between 2013 and 2016, it was concluded that *Anopheles (An.) gambiae* s.l. and *Anopheles funestus* were the most widely distributed malaria vector spe- cies in Zimbabwe (President's Malaria Initiative, 2018; President's Malaria Initiative, Zimbabwe, 2020)

A lot of investments and efforts in the fight against malaria have led to a significant reduction in malaria cases and malaria related deaths globally. However, in spite of these efforts, malaria is still the

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most common vector borne disease worldwide (Mugwagwa et al., 2015). Malaria is endemic in tropical and subtropical countries and approximately 50% of the world's population is at risk of infection (Bekono et al., 2020; Mulaw et al., 2019; Quanquin et al., 2020; Tahghighi et al., 2020, Mugwagwa et al., 2015). According to a report released in April, 2024, there were 249 million malaria cases in 2022 coupled with about 608, 000 malaria related deaths in 85 countries (Cohen, 2024). The malarial burden is high- est in Africa, accounting for 93% of all malaria deaths recorded globally (Mulaw et al., 2019). Up to 80% of Children under the age of five died due to malaria and pregnant women exposed to malaria can con- tributed significantly to neonatal and maternal deaths (Cohen, 2024). In 2015 alone, nearly 28 mil- lion pregnant women in sub-Saharan Africa were at risk of malaria (Quanquin et al., 2020). According to Mulaw et al., 2019, children aged less than 5 years accounted for 61% of all deaths in Africa.

Although malaria remains a health burden in endemic countries, malaria morbidity and mortality declined over the last 15 years due to increased vector control, improved diagnosis, and treatment (Kemibala et al., 2020). In 2018, malaria incidence rate, cases and number of deaths dropped by 18%,

20 million and 172,000 between 2010 and 2017, respectively (Abamecha et al., 2020). The decline in malaria cases and the related deaths is attributed to the introduction of prompt and effective treatment with artemisinin-based combination therapy (ACT), distribution and use of long-lasting insecticidal nets (LLINs) and nationwide coverage of indoor residual spraying (IRS) (Beavogui et al., 2020; Kemibala et al., 2020). Moreover, strategies such as intermittent preventive treatment with pyrimethaminesulfadoxine in the second and third trimesters of pregnancy (IPTp) and seasonal malaria chemoprevention (SMC) for children less than 5 years of age and environmental management have contributed significantly to the decline of malaria related deaths and cases (Gutman et al., 2020; Somé et al., 2020).

Accessibility of vector control strategies is influenced by the cost of vector control products and this is a major impediment in the implementation of strategies to prevent and manage insecticide resistance and to reduce residual transmission of malaria (Global Technical Strategy for Malaria 2016–2030, 2016; Winskill et al., 2020). Vector control interventions can be well implemented if countries come up with more accurate predictions of vector control product requirements. Accessibility of vector control interventions are also underpinned on human

resource, organizational and infrastructural development (Global Technical Strategy for Malaria 2016–2030, 2016). Despite extensive malaria control intervention campaigns, approximately 60% of the population in malaria-infested settings is still to gain access to effective malaria interventions (Odufuwa et al., 2020).

Containment of malaria is hampered by the emergence of multidrug-resistant strains of *Plasmodium* (Figure 1). Of particular importance is the development of resistance by *Plasmodium falciparum*, and this presenting a handful of challenges including prophylaxis and limitation on the choice of drugs used (Verde et al., 2011). Antimalarial drugs such as chloroquine (CQ) and antifolates/sulfadoxine-pyrimethamine are no longer effective in most endemic areas (Bekono et al., 2020). Introduction of combination therapy has emerged which involves the use of artemisinin-based combinations including artesunate–amodiaquine (ASAQ), artemether–lumefantrine (AL), and dihydroar- temisinin–piperaquine (DP)(Beavogui et al., 2020). Generally, artemisinin-based combination therapy (ACT) includes artemisinin or one of its derivatives and a complimentary drug. The partner or complimentary drugs include lumefantrine, piperaquine, mefloquine, amodiaquine,artemether, arteether, artesunate and pyronaridine (Pornputtapong et al., 2020; Wu et al., 2020). The adoption of artemisinin-based combination therapy (ACT) since 2001 has reduced the impact of malaria globally. However, recently the development of resistance in *P. falciparum* to ACT has been reported and this has emerged to be a major impediment in the control and elimination of malaria (Figure 1) (Wu et al., 2020). Resistance of *P. falciparum* to ACT was initially detected in western Cambodia six years after the introduction of ACT as the first line of malaria treatment and since then ACT induced resis- tance of *P. falciparum* has spread to all countries of the Greater Mekong Sub-region (GMS) (Wu et al., 2020). In Africa, resistance of *P. falciparum* to ACT has been in countries such as Uganda, Rwanda and Eritrea. However, resistance to ACT partner therapies is yet to be confirmed in the continent (WHO, 2022b). Unfortunately, there is limited reported data and extensive studies should be done to assess efficacy of ACT in Africa.

Elimination and reduction of malaria is in line with the Global Technical Strategy (GTS) for Malaria 2016–2030, that targets the elimination of malaria from at least 35 countries and reduction of malaria cases and mortality by at least 90% by 2030 (Global Technical Strategy for Malaria 2016–2030, 2016). Current global malaria control strategies are anchored on three

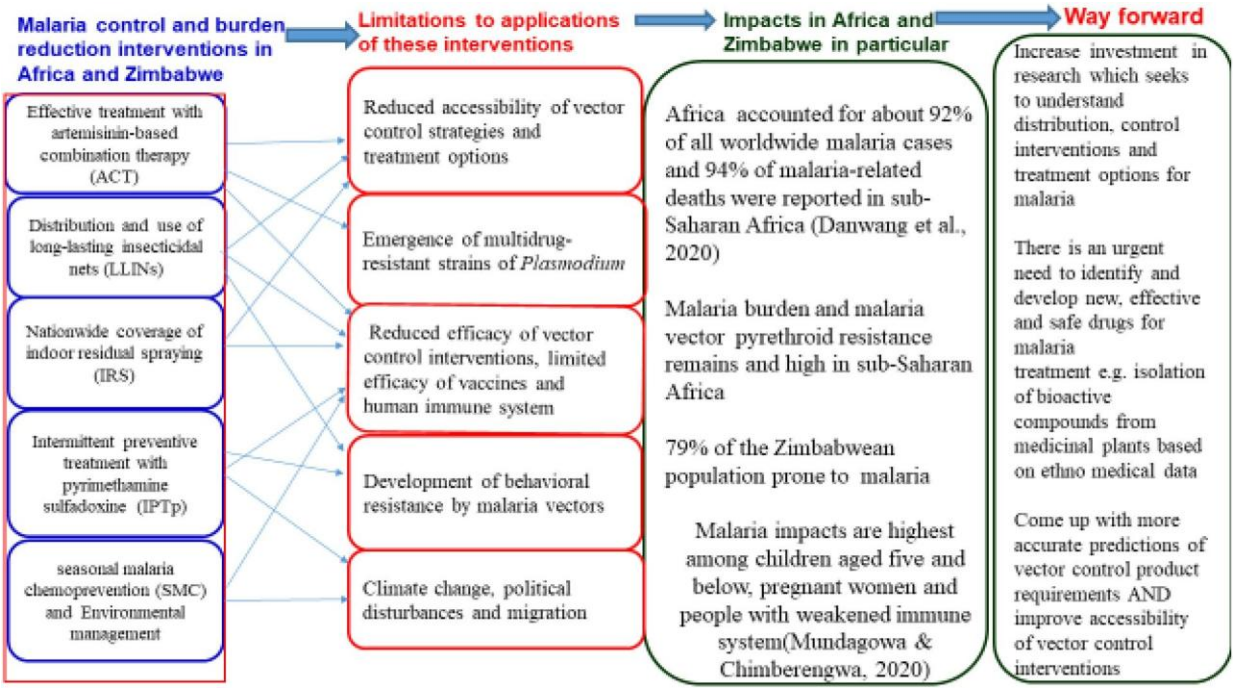

Figure 1. A synopsis of malaria control, treatment interventions, impacts, and mitigation in African countries including Zimbabwe.

pillars associated with elements that direct global endeavors in reducing malaria transmission. According Global Technical Strategy for Malaria 2016–2030, (2016), the first pillar ensures universal access to malaria prevention, diagnosis and treatment. The second and the third pillars involve acceleration of efforts towards elimination and attainment of malaria free status through transformation of malaria surveillance, respectively (World Health Organization, 2020; Premaratne et al., 2019).

Materials and methods

Literature analysis and data mining from primary and secondary databases were applied in order to extract relevant data. The literature was searched in scholarly electronic databases such as PubMed, MEDLINE, ClinicalTrials.gov, DOAJ, Europe PubMed Central, Web of Science and Google Scholar. Furthermore, the literature search was expanded to include reference lists in peer-reviewed scientific publications (snowballing literature review), government reports, reports from international organisations, literature from international bodies, dissertations, theses, and conference proceedings.

The literature search in this current study was based on (1) Boolean search using 'OR', 'AND NOT', 'AND', and 'NOT'. Additionally, qualitative analysis of the evidence was applied. Notably, the Boolean

search in this particular instance was categorized into two basic levels: (a) general search based on general information about 'Malaria situation and elimination in Africa with specific focus on Zimbabwe' and (b) specific objective search which focused on specific sub-sections: (1) The current state of malaria prevention and control, as well as their accessibility (in Africa with specific focus in Zimbabwe), (2) Antimalarial medications, resistance to antimalarial drugs, and the use of medicinal herbs in malaria therapy (in Africa with specific focus in Zimbabwe),

(3) Malaria vector resistance to currently available control strategies (in Africa with specific focus in Zimbabwe), (4) Limitations of vaccines and human immune system in the fight against malaria (in Africa with specific focus in Zimbabwe), (5) Evidence of high malaria burden in Africa (in Africa with specific focus in Zimbabwe), (6) Malaria in the face of COVID-19 outbreak (infectious disease co-infection) (in Africa with specific focus in Zimbabwe), (7) Prevalence and contributing factors of malaria in Zimbabwe, (8) Steps to malaria elimination in Africa with specific focus on Zimbabwe.

A summary of the search strings used in this study include 'malaria control interventions', 'Zimbabwe and malaria', 'Malaria treatment', 'Malaria prevalence in Zimbabwe', 'Malaria prevalence in Africa', 'malaria and Africa', and 'Africa and malaria therapy'. 'Malaria and COVID 19 in Africa', 'malaria

elimination and Africa', 'malaria elimination and Zimbabwe', 'COVID 19 and malaria in Zimbabwe', 'antimalarial drugs and resistance', 'resistance of malaria vectors and Zimbabwe or Africa'.

In order to determine relevance to the study, the retrieved articles were assessed by reviewing their abstracts. The articles focusing on 'Malaria situation AND/OR elimination in Africa AND/OR Zimbabwe were then downloaded and fully reviewed. The results of each article were synthesized and summarized in line with the specific objectives (inclusion). The articles with primary data, accessible full texts and prepared in English language were considered in this case. Articles which were associated with the fol- lowing traits were excluded (1) lack of critical pri- mary data, research done out of Africa, literature including other diseases and work not adequately addressing the objectives of the study. Focus was mainly on articles with relevant primary data and in order to ensure integrity and quality of this review paper, the PRISMA 2020 Checklist was used and data summarised in a PRISMA flow diagram.

Results

Summary of exclusion and inclusion criteria based PRISMA 2020 checklist

The literature search yielded a total of over thirty six thousand (36,000) articles of which twenty five thousand one hundred and twenty five (25,125) were excluded as duplicates and up to one thousand and seventy (1017) were excluded based on titles and abstracts (Figure 2). Only one thousand one hundred and three (1103) articles were retrieved and based on the exclusion criteria one hundred and eighty five

(185) articles were included. Results for systematic literature search is summarised in Figure 2.

The current state of malaria prevention and control, as well as their accessibility

In Africa

Most national malaria control interventions have advocated for universal accessibility of ITNs (WHO, 2017) and in period 2011 to 2016, over 800 million ITNs were distributed across sub-Saharan Africa (Olapeju et al., 2018). This has translated to an increase in number of individuals sleeping under ITNs from 30 to 54% between 2010 and 2016.

Notably, between 67 and 73% of 663 million cases reported between 2000 and 2015 were prevented by ITNs in sub-Saharan Africa (World Health Organisation

(WHO, 2017). There has been expansion in the provision of ITNs in sub-Saharan Africa between 2000 and 2016. However, a slight decline in coverage ITNs since 2017 was reported in Africa and, 65and 43% of households in Africa owned one or more ITN(s), and children below 5 years slept under ITNs in 2020, respectively (Balakrishnan, 2022). Although IRS and LLINs have been adopted in the fight against malaria in Africa, the coverage of these interventions is still below set targets (Allcock et al., 2018; Russell et al., 2015; Singh et al., 2013). It has been reported that accessibilityof IRS and LLINs have been hampered due to prohibitive costs in instances where distribution programmes are not free (Asingizweet al., 2019; Ahorlu et al., 2019), and lack of availability (Allcock et al., 2018; Sangaré et al., 2012). However, adoption and uptake of IRS and LLINs has been limited by lack of awareness campaigns on role of LLINs in malaria prevention (Bertheet al., 2019; Diemaet al., 2017; Allcock et al., 2018), and side effects such as skin irri- tation and residual effects of insecticides (Magaço et al., 2019). Alternative uses of LLINs as curtains, fishing nets, and seedlings protectors (Asingizwe et al., 2019) have been reported in Africa.

In Africa, ITNs have been attributed to 68% decline in prevalence of Plasmodium falciparum in period 2000–2015 (Slater et al., 2020). It should be noted that besides ITNs, seasonal malaria chemoprevention (SMC) and intermittent preventive treatment in pregnancy (IPTp) have been linked to significant reduction in morbidity in African countries (Foy et al., 2023). For example, SMC has been applied in West Africa and have effectively reduced malaria morbidity and incidence by about 70% (WHO, 2022a). Besides the common malaria control interventions such as IRS and LLINs, other methods involving modification of house (screens, ceilings, and eaves) (Reket al., 2018; Getawen et al., 2018; Tusting et al., 2017; Snetselaar et al., 2017; Gimnig and Slutsker, 2009), use of repellents(essential oils, spatial, and plants for example Lantana camara; Azadirachta indica and Cymbopogoncitratus) (Masalu et al., 2020; Tesfahuneygn & Gebreegziabher, 2019) and applica- tion of integrated vector (environmental and larval source management) have been applied in Africa (Nalinya et al., 2022).

In Southern Africa, countries including Zimbabwe, South Africa and Eswatini, mortality due to malaria was significantly reduced by dichlorodiphenyltrichloroethane (DDT) based indoor residual spraying (IRS) (Mabasoet al., 2004). The application of DDT based IRS in Sothern African countries started in the 1940s and was meant to control Anopheles funestus and

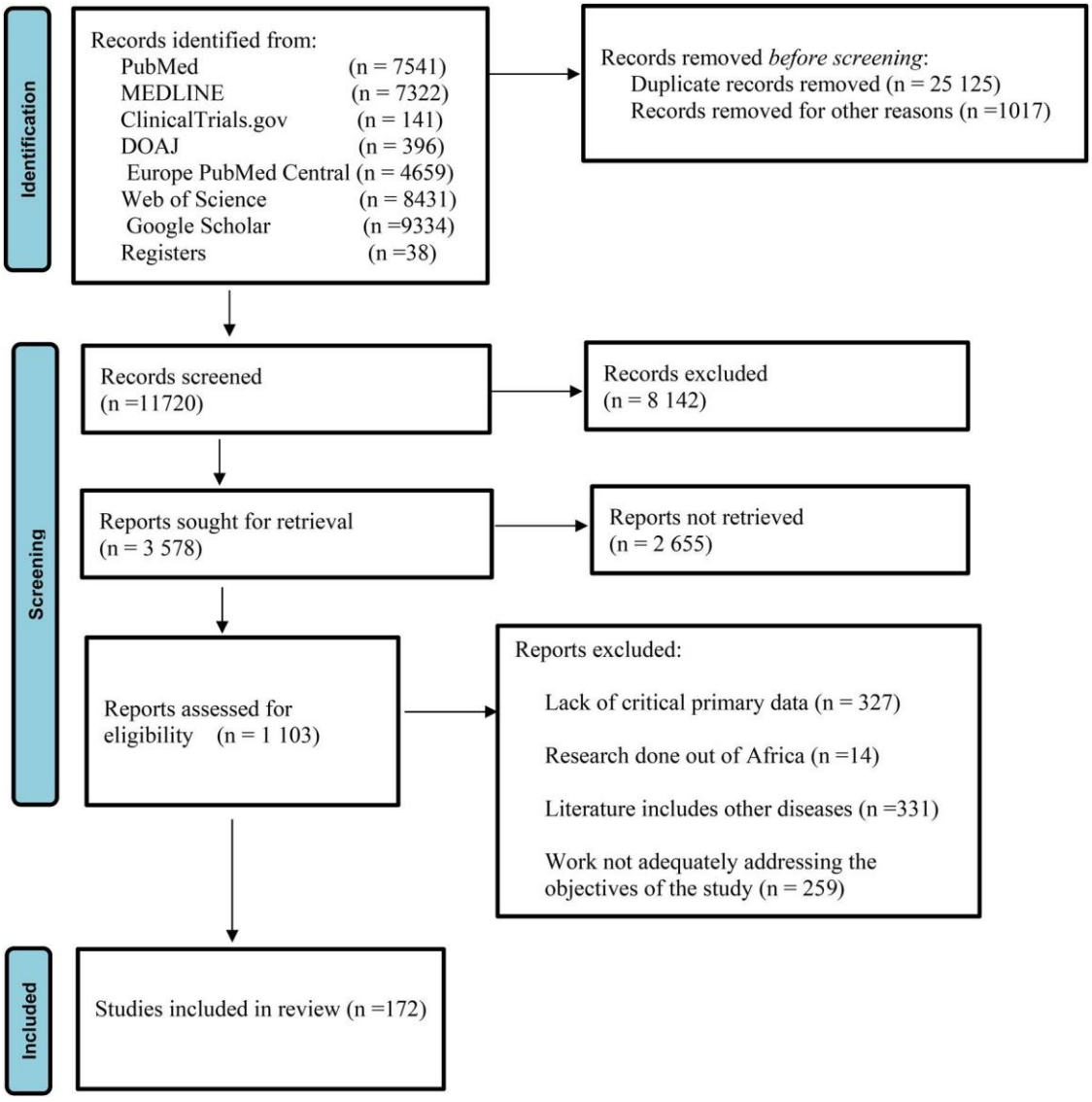

Figure 2. PRISMA flow diagram based on PRISMA 2020 checklist.

Anopheles gambiae complex (Nkya et al., 2022). Sub-Saharan Africa saw a 50% reduction in malaria cases and a 40% reduction in clinical disease from 2000 to 2015, owing primarily to the extensive implementation of malaria prevention strategies including indoor residual spraying (IRS), and insecticidal treated nets (ITNs) (Oladipo et al., 2022). Apart from these vector control interventions, preventive malaria treatment (seasonal malaria chemoprevention (SMC) for under-five children, intermittent preventive treatment of infants (IPTi), and intermittent preventive treatment of pregnant women (IPTp)), treatment of malaria using Artemisinin combination therapy (ACT), and case management have significantly reduced the impact of malaria in the sub Saharan African region (Pryceet al., 2018, Kesteman et al., 2017).

IRS has reduced number of malarial cases by over 80% in southern Africa countries (Yukichet al., 2022). However, the application of IRS in sub Saharan has recently been reduced and this has been reported in Senegal, Tanzania, Madagascar, and Zambia, where IRS application was reduced by 64, 68, 56 and 63%, respectively (Oladipo et al., 2022). However, the implementation of prevention chemotherapy in Africa has increased significantly. For instance, 2018, IPTp coverage increased in Madagascar, Nigeria, DR Congo, Madagascar and Nigeria by 23, 23.5, 9.3, 23.1, and 22.5%, respectively (González et al., 2023). The implementation and universal accessibility of all these malaria control interventions is linked to investments towards malaria control. For example, up to $2.7 billion was availed for the fight against

malaria in 2018 against $8.1 billion which was required for universal access to malaria control interventions (WHO, 2019).

In Zimbabwe

The National Malaria Control Programme in Zimbabwe uses vector control (ITNs and IRS) in malaria bur- dened districts (Gavi et al., 2021). In regions with moderate to high transmission, half of the house- holds possess a minimum of one ITN for any two members of the household, and 85 percent of house- holds have at least one ITN and are protected by IRS, indicating a significant degree of protection (Dube et al., 2019). For instance, in Mutasa District, Manicaland Province, IRS and ITNs were applied as vector control strategies for 6 and 2 decades, respec- tively (Mharakurwa et al., 2013). In 2014, approxi- mately 92% of the population in Mutasa District was covered by IRS (Sande et al., 2016), and during this period, access to ITNs was limited. Between 2012 and 2017, it was noted that 70% of households had least one ITN (Kanyangarara et al., 2018). Additionally, with the confirmation of *An. arabiensis, An. gambiae complex* and *An. quadriannulatus* as malaria vectors in Zimbabwe, IRS or LLINs have been applied in Malarious distrcts (Sande et al., 2015; Masendu et al., 2005). Malaria vectors reported in Zimbabwe include *Anopheles parensis*, *Anopheles leesoni*, *Anopheles con- fuses*, *An. funestus*, *Anopheles aruni* (Sande et al., 2016). In Chiredzi and Mutasa Districts, *An. funestus* was identified as one of the problematic vectors (Sande et al., 2015; Lukwa et al., 2014). However, *An. funestus* was not widely distributed in Chiredzi dis- trict due to IRS application (Masendu et al., 2005).

According to Kanyangarara et al., 2018, the num- ber of households with one ITN per two people in Zimbabwe was approximately 60.3%. These findings indicate that there is still need to improve accessibil- ity especially malaria burdened districts. Moreover, it was noted that IRS offered protection to more than 80% of malaria vulnerable population in Mutasa and Mutare Districts. Benzene and DDT based IRSs were from 1940s (Pates and Curtis, 2005, Mabaso et al., 2004), and currently are among the malaria control interventions in Zimbabwe. Notably, IRS implementa- tion in Zimbabwe was in line with recommendation by WHO which targets population coverage of over 80% (Sande et al., 2016). However, in Zimbabwe, IRS and ITNs are applied in districts or areas associated with annual parasite index (API) of 5 and 2–4 per 1000 population, respectively U. S. President's Malaria Initiative, 2020).

Antimalarial medications, resistance to antimalarial drugs, and the use of medicinal herbs in malaria therapy

In Africa

The treatment of choice for uncomplicated, severe malaria, and expectant mothers are distinct. Chloroquine has been used as first-line antimalarial for uncomplicated malaria caused by *P. falciparum* in sub-Saharan up to early 2,000 (Flegg et al., 2013). African countries such as Zambia, South Africa, Kenya and Tanzania switched to first line malaria treatment based on sulfa- doxine/pyrimethamine (SP) in response to chloroquine resistance (Mwendera et al., 2016). However, *P. falci- parum* gene mutations have resulted in SP treatment failures (Desai et al., 2015). Recently, ACTs have been considered has the primary drug for malaria and in sub-Saharan Africa antimalarial drugs such as artesunate- amodiaquine and artemether-lumefantrine have been used in uncomplicated malaria treatment (Conrad and Rosenthal, 2019; WHO, 2018).

The antimalarial drugs recommended by the WHO African region include artesunate-pyronaridine, dihydroartemisinin-piperaquine and artesunate meflo- quine. Apart from these antimalarial drugs, antimalarials such as artemisinin-piperaquine, artesunate-sulfadoxine- pyrimethamine, arterolane-piperaquine and artemisinin- naphthoquine are in use in Africa (Mhamilawa et al., 2020; Conrad and Rosenthal, 2019). In cases where intravenous treatment was impossible, rectal arte- mether and artesunate, and intramuscular artesu- nate, have been administered (Kremsner et al., 2016). However, in African settings, intravenous quinine has always been used in the treatment of severe malaria (Esu et al., 2014). Antimalarial preventive drugs including sulfadoxine-pyrimethamine and amodiaquine-sulfadoxinepyrimethamine are used to prevent malaria during pregnancy and in children during rain seasons (Desai et al., 2018, Kayentao et al. 2013). For travellers visiting high malaria transmission African countries, malaria chemoprophylaxis is mainly based on meflo-quine, atovaquone-proguanil and doxycycline (Shellvarajah et al., 2017).

Chloroquine resistance was first reported in 1978 and this type of resistance had spread in countries such as Zambia, Sudan, Malawi and Uganda by 1983 (WHO, 2013). This has resulted in discontinuation of chloroquine based malaria treatment in most African countries and replacement with sulfadoxine/pyrimethamine (SP) in countries such as Malawi in the early 1990s (Severini and Menegon, 2015). It has been reported that parasites resistant to sulfadoxine-pyrimethamine and chloroquine in Africa originated

from Southeast Asia. According to Ehrlich et al., (2020), there was a significant decrease in *Asn86Tyr*, *pfmdr1 pfcrtLys76Thr* and *Asp1246Tyr* prevalent in sub-Saharan Africa. This indicates an increase in the prevalence of lumefantrine resistant parasites and resurgence of chloroquine sensitive parasites.

Chloroquine resistance was implicated to mutations in the CQ-resistant transporter (*pfcrt*) gene and multidrug-resistance gene 1 (*pfmdr-1*) in *P. falciparum* (Bin Dajem & Al-Qahtani, 2010). The resistance of malaria parasites to CQ was confirmed with CQ treatment in Tanzania and Kenya as early as 1978 and by 1980 it was reported in West Africa (Roux et al., 2021). Among other factors, antimalarial drug resistance in Africa has been linked to ineffective use of antimalarial drugs and policy changes. For instance, in Kenya, it was reported that the implementation of IPTp and first-line malaria treatment policy has been followed by the emergence of DNA drug resistance markers (Hemming-Schroeder et al., 2018).

Although resistance to chloroquine was noted in African countries, chloroquine has remained as a ther- apy option for uncomplicated malaria caused by *P fal- ciparum* (Kgoroebutswe et al., 2020). In the early 1990s, *P falciparum* resistance to chloroquine was reported in southern and eastern part of Africa (Conrad and Rosenthal, 2019). However, use of ACTs as alternatives of chloroquine, has resulted in the emergence of malaria parasites with *pfmdr1* and *pfcrt* sequences in the eastern parts of Zimbabwe. The reported shift in polymorphism after the introduction of ACTs has resulted in parasites that are sensitive to chloroquine (Hemming- Schroeder et al., 2018; Tumwebaze et al., 2017). Up to 90% of countries in sub-Saharan Africa had started using artemisinin-based combination therapy (ACT) for malaria treatment in response to SP and CQ resistance (Frosch et al., 2011). Disturbingly, reports of isolated cases of malaria parasites resistant to artemisinin have been noted in sub-Saharan Africa. However, artemisinin resistant parasites are yet to be established in sub Saharan Africa (Lu et al., 2017). Although the resistance of malaria parasites to piperaquine is limited in Africa, it was reported in Uganda (Conrad et al., 2014).

In Zimbabwe

The cases of chloroquine (CQ) resistance in Zimbabwe were first reported 1984 in Zambezi Valley. Before 1990 chloroquine resistance was confined to Zambezi Valley (Makono and Sibanda, 1999) and according to Mharakurwa and Mugochi, (1994), out of the 83% of the chloroquine resistant cases reported in Gokwe, only 3% responded to chloroquine treatment.

Although CQ failure was significantly high, the widespread use CQ continued until 2000 (Mlambo et al., 2007). Resistance to CQ by parasites has resulted in replacement CQ with artemether-lumefantrine and sulfadoxine-pyrimethamine in 2008 and 2008, respec- tively. Up to 67% of sampled malaria parasites in 2003 in Mutasa District were resistant to CQ and this was associated with CQ resistance transporter gene (pfcrt) (Ministry of Health and Child Welfare (Zimbabwe), 2007). However, in 2013, only 3% of samples had *pfcrt* mutants, and between 2017 and 2018, 100% of *P. falciparum* were *pfcrt* wild type. The presence of *P. falciparum* with *pfcrt* wild-type indi- cated the emergence of CQsusceptible *P. falciparum* (Mharakurwa et al., 2021).

The use plants against malaria was reported in Sub-Saharan African countries. Although in most African countries people have continued to use plants against malaria without ethnobotanical stud- ies, in countries such as Guinea, Kenya, Nigeria and Uganda studies were conducted (Chinsembu, 2015). The use of plants including *Cassia singueana* against malaria in African countries such as Tanzania, Nigeria Burkina Faso and Kenya has been reported (Hiben et al., 2016). Apart from *Cassia singueana*, *Azadirachta indica* has been used in the management of malaria in Ivory Coast, Kenya, Sudan, Ghana, and Madagascar (Muthaura et al., 2011). According to Carraz et al., (2006), *Strychnopsis thouarsii* is a malaria prophylactic plant in Madagascar. Antimalarial plants have been used in the management of malaria in Zimbabwe. According to Ngarivh et al. (2015), such as *Aristolochia albida*, *Elephantorrhiza goetzei* (Harms), *Cassia abbreviate, Toddalia asiatica* (L.) Lam and *Pavetta schumanniana* F. Hoffm have been utilized in Manicaland Province, Zimbabwe.

Malaria vector resistance to currently available control strategies

In Africa

Resistance of malaria vectors to all insecticides recommended by WHO was reported in Africa between 2001 and 2012. In countries such as Zimbabwe, *An. funestus* and *An. gambiae*s.l were found to be resistant to carbamates, pyrethroids and organochlorines (Knox et al., 2014). The stagnation in the gains made against malaria has been promoted by widespread malaria vector resistance to insecticides (e.g. pyrethroids, organophosphates, carbamates) used in IRS and ITNs. For instance, in Burkina Faso high malaria burden has been linked to vector resistant to

pyrethroids (Toé et al., 2014). Apart from resistance to commonly used pesticides, behavioral resistance (e.g. resting outdoors, biting outdoors, and changing of biting at times) has been noted in malaria parasites (Foy et al., 2023).

Resistance of *An. funestus* to pyrethroids and carbamates was reported in Africa (Choi et al., 2014, Cuamba et al., 2011). In African countries including Mozambique (Casimiro et al., 2006), *An. arabiensis* was found to have reduced resistance to carbamates and *An. gambiae*s.s. with resistance to pyrethroids and DDT were detected (Chanda et al., 2011). Resistance to insecticides was largely linked genetic changes due to metabolic resistance and mutations (Gogue et al., 2020; Chanda et al., 2020).

The establishment and invasion of *Anopheles stephensi* in African urban areas (Sinka et al., 2020), has resulted in the fight against malaria to be stalled. This malaria parasite (*Anopheles stephensi*) can proliferate successfully in urban areas and this is linked to increased adaptability of the parasite to breeding in highly polluted aquatic environments (Batra et al., 2001) and ability to make use of purpose-built water storage reservoir in urban areas (Thomas et al., 2016). Additionally, *A. stephensi* is insecticide resistant to insecticides used in IRS. For example in a study con- ducted by Teshome et al., (2023), it was indicated that *A. stephensi* is resistant to a number of insecti- cides used in IRS and LLIN.

In Zimbabwe

Malaria vector resistance has significantly hampered malaria control efforts in Zimbabwe. For instance, pyrethroid resistance was reported in Mutasa District, Manicaland Province, Zimbabwe in 2014 (Wesolowski et al., 2022). The shift from pyrethroids based IRS to organophosphates spraying in Zimbabwe was prompted by *An. funestus* resistance to carbamates and pyrethroids (Kanyangarara et al., 2016; Choi et al., 2014).

In 2014, insecticide *An. funestus* was reported in Mutasa and Mutare Districts. *An. arabiensis* samples collected from Chiredzi District, Masvingo Province, Zimbabwe in the early 1980 were resistance to benzene hexachloride (Green, 1981). In addition to this, resistance to permethrin and DDT was noted in *An. arabiensis* from Gokwe District (Masendu et al., 2005) and (Munhenga et al., 2008). Malaria vectors in Zimbabwe, *An. arabiensis* and *An. funestus,* have shown behavioral resistance and continued to spread malaria parasites even if there are efficient vector control methods. For example, *An. arabiensis* and *An.*

gambiae complex collected from Gokwe South District were exophilic and therefore making malaria vector control difficult. Up to 16% of *An. funestus* sampled from Mutasa and Mutare Districts had exophilic features and 10% of those found indoors were on unsprayable surfaces (Sande et al., 2016). This therefore, presents a new dimension in fight against malaria in Mutare and Mutasa Districts, Manicaland, Zimbabwe.

The use of benzene hexachloride in malaria control programmes in Zimbabwe has resulted in the emergence of malaria vectors (*An. arabiensis*) which were resistant to benzene hexachloride in Chiredzi district. According to Munhenga et al., (2008), *An. arabiensis* from Gokwe was found to be resistance to DDT and permethrin. Apart from this, resistance to pyrethroids, carbamates, and organophosphates was noted among *An. funestus* (Choi et al., 2014). Resistance of malaria vectors to insecticides in areas of high agricultural activities (Gwave, Gokwe (cotton production) (Munhenga et al., 2008), Burma Valley (tea and banana farming) (PMI 2014), Triangle Estates (Sugarcane pro- duction), Hippo Valley (Sugarcane production) and Honde Valley (tea and banana farming) (Munhenga et al., 2008) indicates that malaria vector resistance in Zimbabwe has been shaped by the use of insecticides in agricultural activities.

Limitations of vaccines and human immune system in the fight against malaria

Although people in malaria-endemic areas progressively develop a natural immune response against del- eterious effects of malaria, this type of immunity is not sterilizing and *Plasmodium* infections associated with malaria tolerance may still occur throughout the life- time of immune individuals (Nhama et al., 2020). Intriguingly, these immune tolerant individuals may be reservoirs for malaria transmission as they migrate to settings where malaria is less common (Dwomoh et al., 2020). Individuals in areas where malaria preva- lence is low remain equally naive and susceptible to malaria once they get infected and irrespective of their age. The development of an effective malaria vaccine and protective immunity is limited by the emergence of genetically distinct malaria parasites in natural pop- ulations. Genetic variability among *Plasmodium* species is as result of high rate of genetic recombination that takes place during the sexual stages in female *Anopheles* mosquitoes. The net effect of this phenom- enon is transmission of multiple strains simultaneously and reduction of an antibody–mediated parasite

inhibition (Abamecha et al., 2020). Even though people from malaria endemic areas are immune to multiple

P. falciparum strains due to a series of exposure, genetic diversity of malaria parasite is one of the major contributing factors responsible for the slow acquisition of natural immunity against malaria (Abamecha et al., 2020).

In Africa

Malaria vaccine research has evolved in response to a rise in malaria vector and drug resistance. In Africa, twenty-four malaria vaccines are being evaluated at

99 experimental sites (Bhagavathula et al., 2016). Mosquirix™ (RTS, S), a vaccine designed against *P. falciparum*, received approval in the African region in 2015. Though the vaccine (Mosquirix™) is effective against *P. falciparum*, it was found to be ineffective against infant severe malaria in a Phase III study (RTS. S Clinical Trials Partnership, 2014). Based on research findings which indicated that RTS,S/AS01 has significantly low effectiveness protecting infants against malaria, WHO recommended the RTS,S/AS01 for children aged 5 months above. However, WHO approved and recommended the use of Mosquirix among infants in Sub-Saharan Africa in 2021 (Kolawole et al., 2023). The approval of malaria vaccine, RTS, S (Mosquirix) in Africa was based on successful pilot studies in subSaharan African countries: Ghana, Kenya and Malawi (Oladipo et al., 2022; WHO, 2021b).

RTS, S/AS01 was assessed in children and infants when given as a 3-dose vaccine associated with a booster dose. It was noted that the third dose of RTS,S/AS01 was 31.1 and 55.8% effective in protecting infants (6–12 weeks) and children (5–7 months) against the initial and only episode associated with clinical malaria within a period of one year, respectively (RTS. S Clinical Trials Partnership, 2015). Aside from the 3-dose vaccine regime, WHO has recommended a 4-dose vaccine regime. Based on findings from a phase 3 investigations, it was concluded that RTS, S/AS01 efficacy among children who were given 4-doses was 36% in 4 years (RTS. S Clinical Trials Partnership, 2015). Generally, it was shown that the efficacy of RTS, S/AS01 against clinical malaria decreased with an increase in the follow-up period for both infants and children and dosage systems (3- and 4-dose vaccine regimens). However, the decrease efficacy with an increase in follow-up period was faster in the 3-dose dose vaccine regime than 4-dose group (RTS. S Clinical Trials Partnership, 2015). Recently, data from a trial conducted in in west

Africa, based on a seven-dose regimen of RTS,S/AS01 indicated an efficacy of 73 and 58% over a follow – up period of 1 and 5 years, respectively (Datoo et al., 2024).

Of the vaccinated infants (830,000) in the trials conducted in Ghana, Kenya and Malawi, above 30 and 40% reduction of admitted infants with severe malaria and malaria episode were reported, respectively (Oladipo et al., 2022). The reduced efficacy of Mosquirix from its original efficacy which lies between 60 and 70% has serious implications on WHO target of producing malaria vaccine which can protect over 75% of people in malarious regions by 2030. Although there other malaria vaccines such as R21 with efficacy above 70% (Datoo et al., 2021), their efficacy in malaria perennial regions is yet to be explored. However, based on data from a 3 phase trial (conducted in Burkina Faso) of Matrix-M (R21), it was shown that R21 has an efficacy of 75% and 67% at seasonal and standard sites among participants of age 5 to 36 months over period of 1 year, respectively (Datoo et al., 2024). Generally, it was revealed that when efficacy was followed up after every 3 months over a period 12 months, a decline was noted and finally maintained at above 60% in the last quarter of the year (Datoo et al., 2024). The data from this trial was consistent with findings from a phase 2b trial at which was done in Burkina Faso, where the efficacy of R21 was 76% (follow up period of 1 year) (Datoo et al., 2021) and 77% over a period of 2 years using a 4-dose R21 regimen for children (age: 5–17 months) (Datoo et al., 2022). Following these clinical trials, R21 was approved for use in Nigeria and Ghana (WHO, 2024).

High prices, along with compliance and viability issues, may impede the acceptance of such malaria vaccines in Africa (Bhagavathula et al., 2016). According to Maxmen, (2021), RTS,S vaccine is characterized by a modest efficacy and requires complex regime of doses and sufficient funding. Scaling up malaria vaccine, however, encounters inherent problems associated with vaccination programs, which include distribution, declining immunity, and financing (Dimala et al., 2018). Generally, there is really limited dose supply available for RTS, S in Africa. The capacity of vaccine production in Africa is extremely low as local vaccine production depends on external support (Abiodun et al., 2021). Out of the 54 African countries, only 8 have vaccine-manufacturing companies and only Senegal has been approved by WHO to sell vaccines to other countries (Okereke et al., 2022). Local production of vaccines in Africa has been linked to poor investment in research,

insufficient skills transfer and exodus of highly skilled professionals (Ekström et al., 2021). Though this infor- mation can apply to on RTS, S and R21 vaccines, it should be noted that this pertains to COVID-19 vac- cines. Notably, there are significant differences in GMP production for an mRNA vaccine (COVID-19 vaccines) and malaria (RTS, S and R21) vaccines.

Evidence of high malaria burden in Africa

In the African region over 67% of deaths due to malaria were among those under age of 5 years (WHO, 2021a). In 2020, it was noted that the WHO African region accounted for over 96% and 95% of all malaria related deaths and malaria cases, respectively (WHO, 2021a). Up to 50% malaria deaths Worldwide are accounted for in five African countries: Tanzania, Nigeria, DRC and Mozambique (Okokon et al., 2010). It was reported that only 15 countries in the WHO African region made significant progress or achieved the target of reducing malaria incidences by a minimum of 40% (African Union, 2021). The countries that reduced incidences of malaria by 40% include Ethiopia, Ghana, Cabo Verde, Mauritania, and Gambia. However, the countries that have made significant progress include Eswatini, Togo, Rwanda, Kenya and Equatorial Guinea. Notably, only two African countries recorded zero malaria related deaths since 2018 and these are CaboVerde and Sao Tome & Principe (African Union, 2021; WHO, 2021a). The WHO African region recorded 405,000 malaria related deaths and up to 28 million cases of malaria casesin 2018 and this trans- lated to 94% and 93% of global malaria deaths and cases, respectively (WHO, 2019).

Though there was a significant decrease of malaria incidences (by 17%) and deaths rates (by approximately 26%) in the African region between 2000 and 2013, in some African countries the progress in the fight against malaria has significantly stalled and in some cases reversed since 2014 (Mbacham et al., 2019). In 2017 alone, the WHO African region contributed to 92% of global malaria deaths and cases. Generally, a drop in malaria cases and deaths of 38 and 67%, respectively, were reported between 2000 and 2019 (WHO, 2020). However, in 2020, malaria case incidence sharply increased due to disruption in the fight against malaria in wake of COVID-19 (WHO, 2021a).

The number of malaria cases increased from 227 to 241 million in the period 2019 and 2020. However, a decrease in the number of malaria related death (by 44%) was reported between 2000 and 2019, and this was associated by significant decrease in death

rate by 67% in the same period (WHO, 2021a). According to Balakrishnan, (2022), contribution of African countries to global malaria is significantly high. For example, Nigeria, Mozambique, DRC, and Tanzania contribute up to 53% of global malaria deaths. Contrary to this, some African countries have significantly made progress in the fight against malaria. For instance, in 2019, Algeria was certified as malaria free. Generally, malaria cases are reported in many parts of Africa (Figure 3).

Although COVID-19 disrupted malaria control interventions in 2021, significant progress was made in the fight against malaria in the African Region in this period. For instance, in 2021 malaria related deaths dropped slightly to 593,000 from 599,000 reported in 2020 (African Union, 2021). In countries such as Cabo Verde malaria cases were reduced to zero since 2018 and in quite a number of African countries (Ghana, Zimbabwe, Mauritania, Togo, South Africa, Ethiopia, and Sierra Leone) significant decline in malaria cases have been noted (WHO, 2022a). Notably, the decrease in the number of malaria cases was very high in three countries (Mauritania, Zimbabwe and Ethiopia), with percentage decrease between 68 and 71. Moreover, the number of malaria related deaths decreased significantly in South Africa, Ethiopia and Togo, with highest decrease recorded in Ethiopia (68%) (WHO, 2022a). However, in most malaria burdened African countries the decrease in malaria deaths and cases has stalled. In 2021, two countries in the African region (DRC and Nigeria) contributed 47 and 41% of malaria deaths and cases, respectively (WHO, 2021a).

In 2018, the 'high burden to high impact' (HBHI) initiative was launched in order to reduce malaria burden in most malaria burdened African countries including Niger, DRC, Uganda, Burkina Faso, Nigeria, Mozambique and Ghana (WHO, 2019). It has been shown that in the HBHI countries, malaria cases increased in the period 2015–2021. The most impacted countries were DRC, Nigeria and Uganda as indicated by 36, 19 and 25% increase in malaria related deaths between 2015 and 2021, respectively (African Union, 2021; WHO, 2022a). Aside from the WHO African region missing the GTS set targets (2020), African countries such as Mauritania, Cabo Verde, Gambia, Zimbabwe, Gambia, Algeria, Ghana, Ethiopia, Rwanda, and South Africa were able to reducing malaria incidences by 40% or more (WHO, 2022a). Although the HBHI was meant for ten highly malaria burdened countries, small population countries with high malaria incidences such Burundi, Somalia and Guinea implemented HBHI intervention (WHO, 2019).

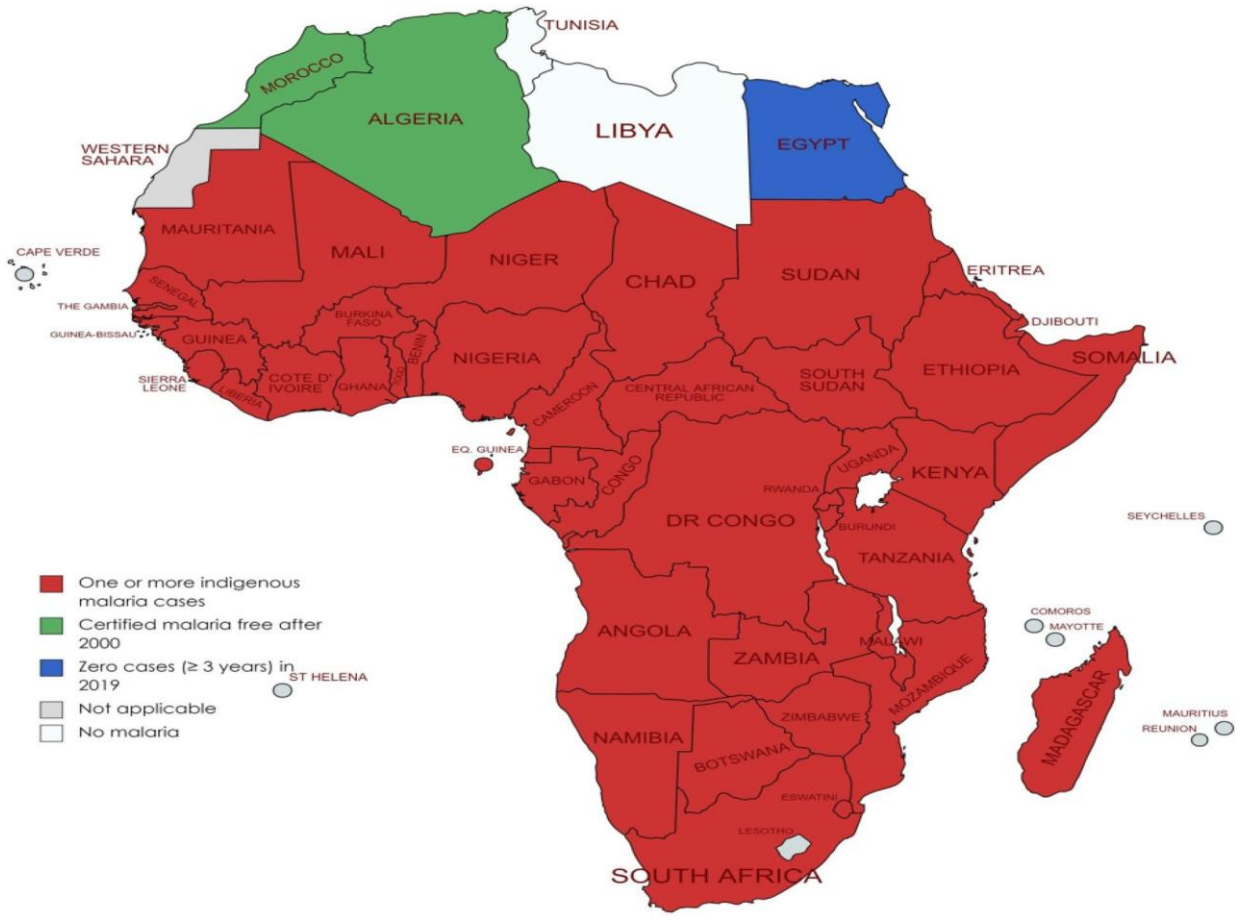

Figure 3. African countries: malaria cases distribution and current status in 2019 (Adapted from WHO database) (WHO, 2020). (No Copyright: You can copy, modify, distribute and perform the work, even for commercial purposes, all without asking permission).

Malaria in the face of COVID-19 outbreak (infectious disease co-infection)

Africa

COVID-19 seriously disrupted malaria control interven- tions in African countries and the most impacted were the HBHI countries. Due to COVID-19, African countries were faced with three major hurdles: (1) reducing the instantaneous consequences of COVID-19 on health, (2) minimizing interruptions to vital ser- vices, and (3) maintaining a healthy population in the midst of more generalized economic problems. Consequently, the progress achieved before the implementation of HBHI intervention was reversed and this has interrupted the rolling out of the HBHI approach. It was noted that in 2021, countries includ- ing Mozambique, Burkina Faso and Cameroon were yet to bounce back to pre-COVID-19 progress (in 2019) (WHO, 2022a). According to WHO (2020), the disruptions in the fight against malaria as a result of COVID-19 has contributed significantly to an increase in malaria related deaths of approximately 49,000. As

result of this, COVID-19 has negatively impacted on economies of several malaria burdened nations and significantly threatened the delivery of malaria prevention and treatment resources (Gao et al., 2023). The measures which were implemented to fight against COVID-19 in African countries impacted negatively on progress made against malaria. For instance, lockdowns reduced availability and accessibility of malaria control interventions (Park et al., 2023). In order to prevent the spread of COVID-19, measures including movement restrictions, quarantining, cur- fews, travel bans, and lockdowns were introduced (Patlolla et al., 2022). The implementation of these interventions is believed to have resulted in resur- gence of malaria (Mahajan et al., 2021). Of the 12 and 5% increase in malaria related deaths and incidence rate reported 2020, respectively, most of them were from the WHO African region and has a result of dis- ruptions associated with COVID-19 (Shah et al., 2015). Apart from the identified factors, malaria vaccine hesitancy is a potential barrier in the fight against malaria. For instance, it was noted that one of the

factors which influence malaria vaccination programs includes acceptability of the vaccines such as RTS, S/ AS01. Additionally, it was indicated that acceptability is a function of vaccine side effects concerns, quality of healthcare services and community engagement (Mumtaz et al., 2023). In a study which was done in Tanzania, Ghana and Nigeria, the acceptance rate of RTS,S vaccine ranged from 92.5 to 96.3%. Although acceptance rate was high, the reasons which were identified for not accepting the malaria vaccines include issues around multiple injections, efficacy profile, safety concerns, and low awareness (Sulaiman et al., 2022). RTS, S/AS01 receptivity was identified to be anchored on inadequate awareness, fear of side effects associated with RTS, S/AS01, inefficient vaccination services delivery and poor quality of health services (Dimala et al., 2018).

In Zimbabwe

The malaria cases recorded in Zimbabwe (from the beginning of year up to June 2020) were in excess of 30,000 as compared to those recorded in 2019, 2018 and 2017 (in the same period) (Gavi et al., 2021). Additionally, malaria related deaths recorded up to June 2020 (350) were significantly higher the yearly totals recorded in the year 2019 and 2018 (311 and 232, respectively). It was concluded that high malaria deaths and cases reported up to June 2020 coincided with the beginning of COVID-19 (Gavi et al., 2021). Of all the 62 Zimbabwean districts, 11 had more than expected incidences of malaria up to June 2020 and this indicated possibility of malaria outbreak. Among these 11 districts three are in malaria burdened prov- inces (Manicaland, Masvingo, Mashonaland East and Mashonaland Central) (Gavi et al., 2021). In districts such as Gweru, Centenary, Mazowe, Hwedza, and Makonde the reported mortality due to malaria was more than three times the expected malaria mortality up to June 2020 (Gavi et al., 2021).

Prevalence and contributing factors of malaria in Zimbabwe

In 2018 alone, WHO reported that out of the 14 million Zimbabweans, 4 million were vulnerable to malaria and up to 1 million malaria cases were confirmed (Gwitira et al., 2020). Zimbabwe is subdivided into 10 provinces, which are further subdivided into sixty-five districts, of which 63 are rural. Malaria dis- tricts account for approximately 75% of the 65 dis- tricts, with 64% classified as high malaria burdened districts (Zimbabwe National Statistical Agency, 2012).

Malaria prevalence in Zimbabwe is affected by seasonal and geographical variations that are directly linked to rainfall patterns (US President's Malaria Operational Plan FY, 2018; Sande et al., 2016). Zimbabwe's districts are separated into two zones characterized by low and heavy rainfall. Malaria transmission is lower in low rainfall areas such as parts of Matabeleland South Province (less than 700 mm) and higher in high rainfall areas such as Manicaland Province (more than 1,500 mm) (US President's Malaria Operational Plan FY, 2018; Coleman et al., 2009). Prevalence of malaria in Zimbabwe is highest among children under the age of five, pregnant women and people with weakened immune system (Chapu & Mgocheki, 2017; Mosha et al., 2014). Generally, malaria transmission in Zimbabwe is seasonal and directly linked to the geographic variation in rainfall patterns and topology (Manyangadze et al., 2017). In line with this transmission pattern, nearly 80% of annual malaria cases are confined to the northern and eastern provinces of Zimbabwe. The most impacted provinces are Mashonaland Central, Mashonaland North, and Manicaland (Table 1), these amounts to 30% of the country's total number of provinces (US President's Malaria Initiative Plan, 2020).

Malaria is a serious disease that affects individuals of all ages in Zimbabwe and according to a 2016 WHO report, 79% of Zimbabweans are susceptible to malaria. Additionally, in the same year, the number of reported malaria cases at the eastern borders of Zimbabwe was significantly high and contributed up to 82% all recorded malaria cases (President's Malaria Initiative, 2018). The malaria incidence was reported to be 139 per 1000 people in 2013. However, between 2012 and 2014, there was a significant increase in malaria-related fatalities (from 6.1 to 13.8%) (US President's Malaria Operational Plan FY, 2018). Malaria-related mortality is reported year round, despite the fact that disease transmission occurs mostly between November and April (US President's Initiative, 2016). The annual num- ber of malaria cases documented changes between 300,000 and 500,000, with a notable continuous decreasing trend recently (Figure 4). This success prompted a shift in emphasis from malaria control

Table 1. Distribution of parasitologically confirmed malaria cases in Zimbabwe.

Province	Malaria cases	Percentage contribution
Manicaland	110,485	39
Mashonaland east	68,175	24
Mashonaland central	52,401	19
Other provinces	49,781	18

Source: Zimbabwe District Health Information System 2 (US President's Malaria Operational Plan FY, 2018).

to elimination in a number of provinces including Midlands and Matabeleland, coupled with the launch of pre-elimination of malaria in selected regions (Sande et al., 2017). However, there seems to be insufficient and inconclusive evidence that malaria incidences have substantially dropped. In 2019, for example, nearly 310,000 malaria cases (22 per 1000) were recorded across the country (Figure 4). (President's Malaria Initiative, Zimbabwe, 2020). This was a 19% rise in reported cases of the previous year 2018, and this was associated with an increase in malaria deaths by up to 12.8% from 2018 to 2019 (US Pesident's Malaria Operational Plan FY, 2022) (Figure 4). In addition, in 2020, malaria occurrences in the country where abnor- mally high with 135,585 recorded cases and 131 malaria related-fatalities as of April 2020, primarily from four provinces: Matabeleland South, Manicaland, Mashonaland East and Masvingo (US President's Malaria Operational Plan FY, 2022). In light of the resources allocated to malaria control and eradication, as well as the Zimbabwean economy and policies, it is vital to assess if malaria pre-elimination is realistic.

Malaria is an endemic disease in Zimbabwe and is among the country's top five causes of illness and death. The Ministry of Health and Child Care (MOHCC) controls malaria through the National Malaria Control Program (NMCP), which employs several interventions such as case management, malaria vector control strategies, and behavioral modification communication (US Pesident's Malaria Operational Plan FY, 2017). The transmission of malaria in Zimbabwe is seasonally and geographically variable and is positively correlated to environmental factors such as altitude and rainfall fluc- tuations in the country (Mundagowa and Chimberengwa, 2020). Districts in the northwestern to the southeastern boundaries of the nation are classi- fied as high malaria transmission zones, whereas dis- tricts in the central and southwestern parts of the country are being associated with very low or lack of malaria incidences and transmission (Figure 5) (US President's Malaria Operational Plan FY, 2018). Malaria transmission is most common during the rainy season, when temperatures range between 18 and 30 degrees Celsius. Plasmodium falciparum is the most common malaria pathogen, accounting for 98 percent of all confirmed malaria incidences in Zimbabwe (US President's Malaria Operational Plan FY, 2019, 2018). In malaria-burdened areas, transmission occurs all year, with most of incidences occurring between or soon after November to April rainy season.

In the past 13 years, the Zimbabwe NMCP and the Roll Back Malaria Programme (RBMP) have had significant gains in fighting against malaria, leading to a shift in focus from malaria prevention and control to

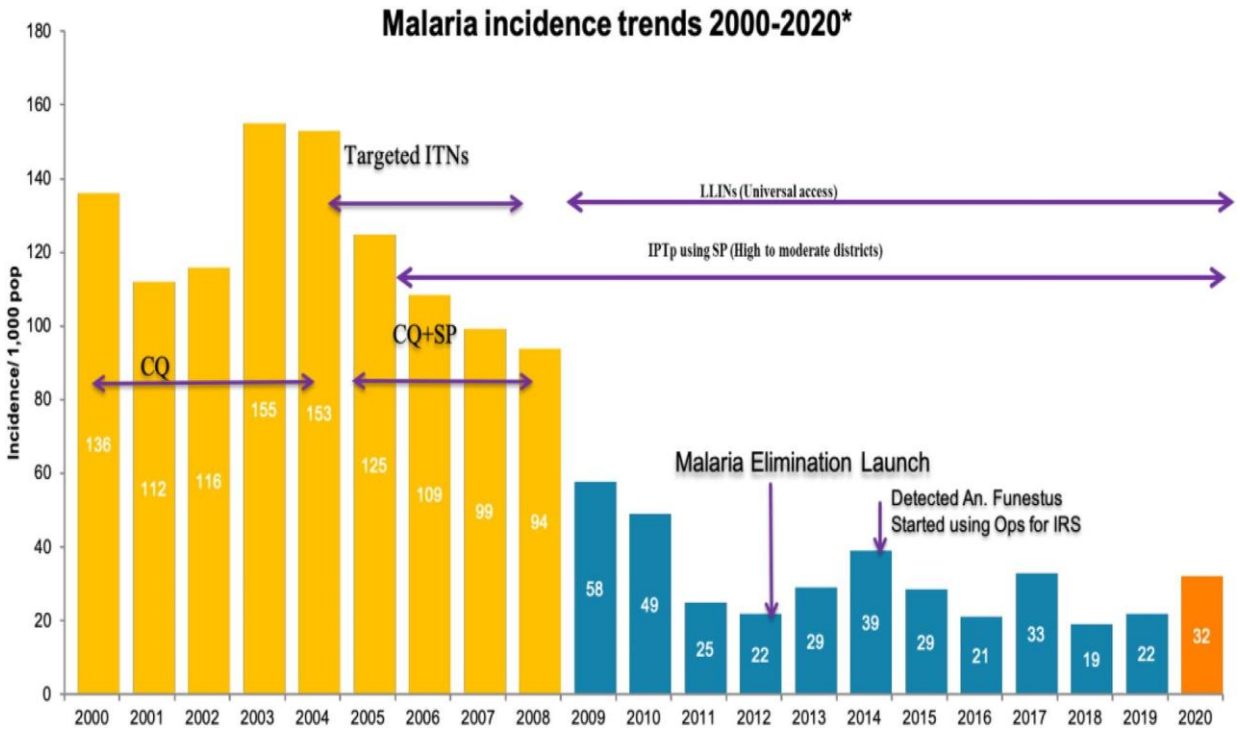

Figure 4. Malaria incidence trends from 2000 to 2020 in Zimbabwe and associated malaria control interventions. *Source*: Zimbabwe District Health Information System 2 (US President's Malaria Operational Plan FY, 2022). (No Copyright: You can copy, modify, distribute and perform the work).

eradication (Sande et al., 2016, 2017). The two pro- grams, whose administration is centralized in Harare, are guided by provincial and disease control managers who obtain data from district health care teams, whose main aim is to coordinate malaria case management, monitoring, and epidemic response at the local scale (US President's Malaria Operational Plan FY, 2017). The Zimbabwean 2016–2020 National Malaria Strategic Plan directs the deployment of IRS in regions with an annual parasite index (API) of 5 or higher per 1000 inhabitants. LLINs are distributed in locations with an API of 2–4 per 1000 people and the IRS intervention is a routine annual strategy in Zimbabwe, carried out to promote malaria outbreak containment and abatement (US President's Malaria Operational Plan FY, 2022). The NMCP uses a double strategy towards vector control, deploying both ITNs and IRS to malaria endemic regions. In moderate- to - high incidence zones, 51% of homes possess at least one ITN per two household members, and 85% own at least one ITN and are some- times protected by IRS, indicating excellent protection levels (US President's Malaria Operational Plan FY, 2022). Despite tremendous progress in achieving malaria eradication, some periodic outbreaks continue to reemerge. For example, Beitbridge District has seen a second malaria epidemic within a year, in 2017. Malaria eradication efforts have been hampered by malaria vector and pathogen insecticide and anti-malarial drug resistance, varied vector behavior, and malaria vector new regions colonization (Gunda et al., 2016). Changing climate, inconsistent funding, economic crisis, political disruptions, and growing cross-border human migra- tions have all hampered the malaria control programs (Gwitira et al., 2018; Mundagowa & Chimberengwa, 2020). These variables have aided intermittent malaria epidemics across Zimbabwe.

Steps to malaria elimination in Africa with specific focus on Zimbabwe

After the introduction of the E-8 initiative, malaria inci- dence status in eight provinces of Zimbabwe was assessed in line with WHO guidelines (WHO, 2007). The classification of provinces on malaria status and prog- ress on elimination were based on this criterion: (1) pre-elimination, (2) reintroduction prevention, (3) con- solidation and control, and (4) elimination (WHO, 2007). In the year 2009, the Elimination 8 (E-8) initiative was launched by Southern African Development Community (SADC) in order to facilitate zero malaria transmission by 2020 in countries such as South Africa, Namibia, Botswana and Eswatini (frontline countries) (SARN, 2010).

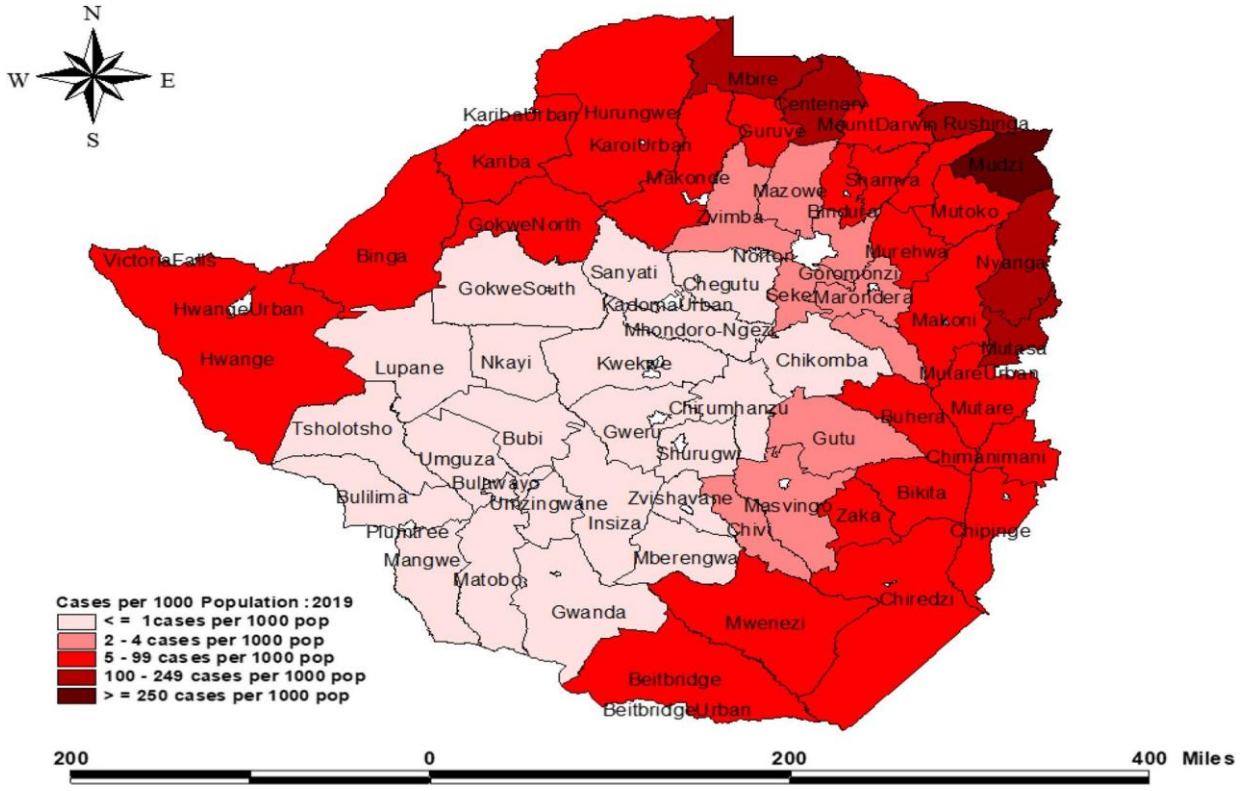

Figure 5. Malaria cases distribution in Zimbabwe. *Source*: https://www.cdc.gov/globalhealth/countries/zimbabwe (No Copyright: You can copy, modify, distribute and perform the work).

Following application these cateria, Matabeleland South was the first province implement malaria pre-elimination phase in 2013 (Sande et al., 2016). In 2015, Midlands, Mashonaland West Provinces and Matabeleland North were promoted to focus on activ- ities under malaria pre-elimination phase. However, the rest of provinces (Mashonaland Central, Masvingo, Manicaland and Mashonaland East) continue to focus on the consolidation and control phase, due to high malaria incidences (Sande et al., 2016).

Under E-8 initiative, countries such as Zambia, Angola, Zimbabwe and Mozambique were classified as second line states which were expect to eliminate malaria by 2030 (SADC, 2018). The introduction of E-8 by SADC was in line with the remarkable decline in malaria incidences between 2000 and 2010. This sig- nificant decline in malaria cases was due to vector control and better case management (SADC, 2018). According to WHO (2021a), Africa is likely to miss its target of eliminating malaria by 2030 as indicated by failure to reduce both malaria mortality and inci- dences by 40% in 2020.When compared to 2019, the number of malaria deaths in 2020 increased by 68 953 and this has been attributed to the state of health emergence as a result of COVID-19 (WHO, 2021a).

Conclusion

The major malaria control interventions applied in Sub-Saharan Africa are IRS and ITNs. Apart from IRS and ITNs, chemoprevention (SMC) for under-five children, IPTi, and IPTp, treatment of malaria using Artemisinin combination therapy (ACT), case management, and use of Mosquirix (malaria vaccine) were applied in the sub Saharan African region. The accessibility of these malaria control interventions in most African countries including Zimbabwe is still below the set global and national targets. Additionally, due to vector (to carbamates, pyrethroids and organochlorines) and parasite resistance (to antimalarial drugs such as chloroquine, and sulfadoxine/pyrimethamine) the fight against malaria has stalled and progress made in previously has been reversed in some African countries. The contribution of WHO African region to global malaria incidences is significantly (above 92%). In African countries including Zimbabwe, malaria is most devastating among children under the age of five, pregnant women and people with weakened immune system, especially those living with HIV and AIDS. Generally, malaria transmission in African countries such as Zimbabwe is seasonal and directly linked to the geographic

variation in rainfall patterns and topology. Africa is likely to miss its target of eliminating malaria by 2030 as indicated by failure to reduce both malaria mortality and incidences by 40% in 2020. However, the introduction of E-8 by SADC has resulted in a remarkable decline in malaria incidences between 2000 and 2010. Furthermore, the WHO African region introduced the HBHI initiative in order to reduce malaria burden in ten most malaria burdened African countries. The E-8 and HBHI initiatives have been hampered by COVID-19, which disrupted provision and accessibility of malaria control interventions through introduction of measures including movement restrictions, quarantining, curfews, travel bans, and lockdowns. Overall some African countries are moving towards malaria elimination (for example Cabo Verde, Sao Tome & Principe, Zimbabwe, Algeria, Ethiopia, and South Africa), yet some are still far from meeting certain targets (including Niger, DRC, Burkina Faso and Nigeria.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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