In vitro antiplasmodial activity, acute toxicity and phytochemical quantification of selected medicinal plants used in symptomatic management of malaria in eastern Uganda

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Abstract

Ethnopharmacological relevance: The emergence of artemisinin resistance alongside limited antimalarial access in Uganda has intensified the use of herbal medicines as complementary and alternative therapies. Whereas there is widespread indigenous use of *Zanthoxylum chalybeum, Albizia coriaria, Entada abyssinica, Maytenus senegalensis,* and *Kigelia Africana* in treatment of malaria, there was limited scientific evidence of efficacy and safety.

Aim of the study: This study assessed the *in vitro* antiplasmodial activity, acute toxicity, and phytochemical composition of the five plants so as to identify alternative sources of antimalarial drugs.

Materials and methods: Phytochemical screening and quantification was conducted on ethanolic extracts from dried samples using UV-Vis spectrophotometry. The acute toxicity testing was conducted in Wistar albino rats using limit dose test and the antiplasmodial efficacy evaluated using a 72-hour SYBR Green assay on *Plasmodium falciparum* laboratory and clinical isolates.

Results: Alkaloids, flavonoids, tannins, and terpenoids were present in all the samples. *Z. chalybeum* was the most potent, with IC50 values as low as 0.90 and 1.54 µg/ml against both chloroquine-sensitive (3D7) and resistant (Dd2) strains respectively. *Albizia coriaria* and *Entada abyssinica* also displayed moderate activity, with IC50s ranging from 30.26 to 101.26 µg/ml. Acute toxicity studies indicated that most extracts were relatively safe in Wistar albino rats, except for *A. coriaria* and *M. senegalensis*, which were moderately toxic with median lethal less than 2000mg/kg.

Conclusion: *Z. chalybeum*, *A. coriaria* and *E. abyssinica* are promising medicinal plants for developing novel and effective antimalarial drugs, especially against drug-resistant strains that warrant more phytochemical and pharmacological studies.

Keywords*: Medicinal plants, Malaria, Antiplasmodial activity, Phytochemicals, Drug resistance*

1. INTRODUCTION

Malaria is a treatable and curable infectious disease which remains a public health concern to most health care systems especially in Sub-Saharan Africa and bears over 90% of the disease burden (Oladipo et al., 2022). In 2022, there were 249 million malaria cases and 608,000 deaths (90% mortality from Africa) out of which more than half were from Sub Saharan Africa. With the COVID-19 pushing the health systems to focus on the pandemic, there was a 2% (+ 59,000) increase in malaria cases in 2022. Of the four Sub-Saharan African (SSA) nations that accounted for more than half of all malaria cases worldwide in 2022, Uganda (5%) was rated third by the World Health Organization (WHO, 2023). The endemicity of malaria in Uganda varies with geographical region, with eastern and northern Uganda being hotspot areas (Ocen et al., 2023). Other factors that influence the malaria patterns are rainfall patterns, temperature, altitude, season of the year and transmission intensity. Several strategies have been employed to combat the high burden of malaria in Uganda including vector control and chemoprevention. Additionally, Uganda also rolled-out a malaria vaccine for new born children in four doses at 6, 7, 8, and 18 months. While these strategies have shown an improvement in the reduction of malaria cases, the resistance of the mosquitoes to insecticides, emergence and spread of drug resistant *P. falciparum* parasites have undermined the realization of a malaria free Uganda by 2020 (WHO, 2023). Therefore, the need to discover novel antimalarial compounds against both sensitive and resistant *P. falciparum* is key in the fight against malaria (Chen, 2014; Clement et al., 2020; Irungu et al., 2023). With the antimalarial drugs such as quinine, artemether, dihydoartemisinin, and artesunate having a plant origin, medicinal plants provide a promising source of novel antimalarial molecules.

The indigenous use of herbal remedies in the management of malaria continues to gain momentum in Uganda due to their availability, affordability, perceived efficacy and safety (Gumisiriza et al., 2023; Tabuti et al., 2023; Tugume et al., 2016). In Tororo district, 76% of the study respondents reported to use herbal medicine as the first line option to treat malaria (Tabuti et al., 2023). *Z. chalybeum* (stem bark and rook bark), *A. coriaria* (stem bark), *E. abyssinica* (stem bark), *M. senegelensis* (leaves), and *K. Africana* (fruit) are widely used by the traditional medicine practitioners (TMP) in Eastern Uganda to prepare decoctions for treatment of malaria (Angupale et al., 2023; Clement et al., 2020; Tabuti et al., 2023). The number of TMP who reported using these plants were; *M. senegelensis (30)*, *Z. chalybeum* (9), *A. coriaria (2)*, *E. abyssinica* (not reported) and *K. Africana* (not reported) (Nabatanzi et al., 2020; Philip et al., 2017; Tabuti, 2008; Tabuti et al., 2023). But also in our previous study to document medicinal plants used in management of diabetes mellitus, these five plants were frequently mentioned to be used in treatment of malaria as an alternative indication (Obakiro et al., 2023). These plants are used either

singly in monoherbal formulations or combinations in polyherbal formulations. Although the doses of the herbal preparations are not standardized, usually 200 - 500ml of the decoction are orally administered three times daily for three to five days depending on the severity of malaria, age and weight of the patient with contraindication among pregnant mothers particularly in the second and third trimester (Angupale et al., 2023; Tabuti et al., 2023). These herbal medicines, prepared as needed and usually stored for not more than two weeks, have been reported to be relatively safe with minor side effects such as bitter taste, nausea, vomiting and abdominal discomfort (Angupale et al., 2023). In central Uganda, Western Uganda and Western Kenya, these plants have also been reported to be used in treatment of malaria (Angupale et al., 2023; Gumisiriza et al., 2023; Owuor et al., 2012). Apart from malaria, these plants are also useful in management of HIV/AIDS (Owor et al., 2024), inflammatory diseases (Schultz et al., 2020), diabetes mellitus (Obakiro et al., 2023), and tuberculosis (Bunalema et al., 2014). Apart from *Z. chalybeum* being a rare species in eastern Uganda, this plant is threatened due to its versatile medicinal properties and thus needs urgent conservation strategies.

The World Health Organization recommends that all traditional and complementary medicines should undergo rigorous scientific validation so as to enhance patient care and safety (WHO, 2019). Despite the widespread use of herbal remedies in the management of malaria in Uganda, there is a paucity of scientific data regarding the efficacy, safety, and phytochemical composition of several medicinal plants and the products themselves (Angupale et al., 2023). Unstandardized herbal products can pose a huge threat to the health of the public as they may cause potential toxicity due to inherent toxic phytochemicals, contaminants, and/or inappropriate dose administration (Clement et al., 2020; Obakiro et al., 2024). Apart from identifying potential lead compounds and enriching the compound libraries to support new antimalarial drug discovery programs, scientific validation increases the confidence among the traditional medicine practitioners and the public about their herbal medicine (Clement et al., 2020; Obakiro et al., 2023). Therefore, as the drive towards integration of traditional and modern medicines into the health care systems continues in different countries, more scientific validation studies are necessary to provide the required evidence about the efficacy and safety of the traditional medicine. This study therefore evaluated the *in vitro* antiplasmodial activity, acute toxicity and phytochemical composition of selected medicinal plants used in the management of malaria in Eastern Uganda.

2.0 Materials and Methods

2.1 Plant sample collection and identification

The medicinal plants investigated were selected based on their widespread use in management of malaria in Eastern Uganda, a malaria-endemic region and their high of frequency of citation in various published articles (Tabuti et al., 2023). These are *Albizia coriaria* Oliv. (Fabaceae) *Zanthoxylum chalybeum* Engl. (Rutaceae), *Entada abyssinica* DC. (Fabaceae), *Maytenus senegalensis* (Lam.) Exell (Celastraceae) *and Kigelia Africana* (Lam.) Benth (Bignoniaceae). Traditionally, a decoction of the individual plant parts is made by boiling in water and administered orally three times a day for 5 to 7 days. In this study, the samples were harvested from their natural habitats from selected districts in eastern Uganda depending on the species abundance during February 2023. These are *Zanthoxylum chalybeum* (root) and *Maytenus senegalensis* (stem bark) from Katakwi district, *A. coriaria* (stem bark) and *Entada abyssinica* (stem bark) from Pallisa district and *Kigelia Africana* (fruit) from Kapchorwa district (Fig. 1). Harvesting of the samples was done from mature and healthy plants using good harvesting practices and the guidance of the herbalists and a plant taxonomist (Dr. Carol Kawuma). Voucher specimens of *Zanthoxylum chalybeum* (OSB/001/2023), *A. coriaria* (OSB/002/2023), *Entada abyssinica* (OSB/003/2023), *Maytenus senegalensis* (OSB/004/2023), and *Kigelia Africana* (OSB/005/2023) were prepared and deposited at the Makerere University Herbarium, at the Department of Plant Sciences, Microbiology & Biotechnology for authentication and reference purposes. The plants were botanically identified and confirmed by Mr. Protase Rwabirondere. The harvested samples were packed in zip lock bags and transported to the Busitema University Natural Products Research and Innovation Centre laboratory for processing. The samples were reduced into small pieces by chopping and shade-dried at 25.0 ± 2.0°C for about 30 days. The air dried samples were pulverized using an electric grinder (NutriBullet® 600 Series). They were then labelled and stored at room temperature till further use.

2.2 Extraction

Ethanol (70%) was prepared by diluting analytical grade ethanol (Sigma - Aldrich (Germany) with distilled water. Three hundred (300g) of each of the dried pulverized samples were macerated in 1000ml of ethanol (70%) for 48 hours with intermittent shaking to obtain crude ethanolic extracts. The mixture was filtered using Whatman No. 1 filter paper to obtain a solution. Concentration of the resultant solution was done at 40 °C and low pressure (70 -100 mBar) using a rotary evaporator (DLAB: RE100-Pro). The concentrated crude extracts were dried to constant weight using a desiccator over anhydrous copper (II) sulfate, at room temperature. The extracts were stored in clean, labeled bottles in a refrigerator at 4°C until further use.

2.3 Phytochemical screening

Preliminary phytochemical screening was performed to determine the presence of triterpenes, saponins, tannins, flavones, phenols, and alkaloids in the extracts (Harborne, 1973; Koparde, 2017). Briefly, flavonoids were screened using the alkaline test, alkaloids using the Dragendorff's test, tannins by the ferric chloride test on a pre-heated sample, phenols by the ferric chloride test, triterpenes by the chloroform-sulphuric acid test, and saponins by the foaming test.

2.4 Quantification of different phytochemicals in the selected medicinal plant

The quantity of tannins, alkaloids, flavonoids, triterpenoids, and steroids of the five different plant extracts was analyzed using UV-VIS spectroscopy.

2.4.1 The total flavonoid content:

Total flavonoid content was measured using an aluminum chloride spectrophotometric assay and quercetin as a standard flavonoid. Briefly, 500µl of the plant extract (1 mg/mL) was transferred to a test tube and 1 ml of 5% sodium nitrate was added, then the mixture was incubated at room temperature for 5 minutes. After incubation, the mixture was treated with 1 ml of 10% $AlCl_3$ solution, followed by 1 ml of 1 M sodium hydroxide and 6.5 ml distilled water. The solution was mixed well, and the absorbance was recorded at 420 nm (after scanning for the maximum absorbance) against the blank spectrophotometer 607 UV-Visible Jenway. The content was determined from the calibration curve, which was obtained from a standard solution of quercetin (45.0, 22.5, 11.25, 5.63, 2.81 ⌈g/mL) prepared in 80% methanol. The concentration of flavonoids in the extracts was determined using an equation $y = 0.0417x - 0.0243$ and $R^2 = 0.9961$ obtained from a standard quecetin graph. The samples were analyzed in triplicate, and the total flavonoid content was expressed as quercetin equivalent (QUE) in milligrams per gram of dry extract.

2.4.2 Total tannin content

500 µL of plant extract (1 mg/ml in 80% methanol) was transferred to a test tube then 2 ml of 0.1M ferric chloride in 0.1M hydrochloric acid and 0.008M potassium ferricyanide were added to the extract. The resulting mixture was mixed well and the absorbance was measured at 650 nm within 10 minutes against the blank in a spectrophotometer 607 UV-Visible Jenway. The standard solution was prepared using tannic acid (1.0 – 0.2 mg/mL) in 80% methanol to generate the calibration curve. The concentration of tannin in the extracts was determined using an equation $y = 1.7935x + 1.2247$ and $R^2 = 0.9926$ obtained from a standard tannic acid graph. The samples were analyzed

in triplicate and the total tannins content was expressed as tannic equivalent (TAE) in milligrams per gram of dry extract.

2.4.3 Total alkaloid content

The plant extracts were dissolved in Dimethyl sulfoxide (DMSO) to prepare a 1 mg/mL solution. 1 mL of 2 M HCl was added to 1 mL of the dissolved plant extracts and the resulting mixture was filtered. To 500µl of the filtrate in a separating funnel, 5 mL of 0.1% Bromocresol green (dissolved in methanol) was added followed by 5 mL of phosphate buffer (pH 6.6) and 5 ml of chloroform. The mixture was then vigorously shaken, and allowed to stand to allow separation of the layers. The lower layer was collected into a 10 mL volumetric flask. The process was repeated using 6, 7, and 8 mL of chloroform. The absorbance of the sample and standard (caffeine -1.0–0.2 mg/ml) was recorded at a wavelength of 470 nm against a blank in a spectrophotometer 607 UV-Visible Jenway. The concentration of alkaloid in the extracts was determined using an equation $y = 1.0665x + 0.9355$ and $R^2 = 0.995$ obtained from a standard caffeine graph. The total alkaloid content was expressed as milligram Caffeine equivalent per gram of extract (mg CAE/g). All the measurements were evaluated in triplicate.

2.4.4 Total steroids

500µl of plant extract (5 mg/ml) was placed in a test tube. 2 ml of 2M sulphuric acid and 2ml of ferric chloride (0.5% w/v) were added followed by 500µl of potassium ferricyanide solution (0.5% w/v). The mixture was heated in a water bath at 70^{o} C for 30 minutes with occasional shaking and then diluted to make up 10 mL with distilled water. The same procedure was repeated for the standard solution of Diosgenin (1.00 – 0.031 mg/ml) to create the calibration curve. The absorbance was measured at 780 nm against a blank using a spectrophotometer 607 UV-Visible Jenway. The concentration of steroid in the extracts was determined using an equation $y = 0.0012x + 0.2459$ and $R^2 = 0.9994$ obtained from a standard Diosgenin graph. Steroid concentration of plant extract was expressed as milligram Diosgenin equivalent (DSE) per gram of dry plant extract. All the measurements were evaluated in triplicate.

2.4.5 Total triterpenes

500µl of plant extract (1 mg/ml in chloroform) was placed in the test tube and kept in the water bath until dryness at 85^{o}C. 250µl vanillin solution (50 mg/ml, prepared in 85% acetic acid in methanol) was added followed by 500µl of concentrated sulphuric acid in that order. The mixture was then kept in the water bath at 60 ^{o}C for 30 minutes and then transferred to an ice bath followed by

addition of 2.5 ml of acetic acid. The resultant solution was then maintained under cooling for 20 minutes and additional 20 minutes under room temperature. A blank solution was similarly prepared and the same procedure was repeated for the standards using betullinic acid (0.50 – 0.031 mg/ml) as a standard which was dissolved in chloroform. The absorbance was measured at 548 nm against a blank in a spectrophotometer 607 UV-Visible Jenway to generate a calibration curve. The concentration of triterpene in the extracts was determined using an equation $y = 0.0022x + 0.8245$ and $R^2 = 0.999$ obtained from a standard betullinic acid graph. Triterpene concentration of plant extract was expressed as milligram betullinic acid equivalent (BTE) per gram of dry plant extract. All the measurements were evaluated in triplicate.

2.5 Acute toxicity determination

Acute toxicity was used to determine the immediate toxic effects and lethality order of all extracts using the OECD (Organization for Economic Co-operation and Development) guideline number 423 (2001). Healthy adult female nulliparous and non-pregnant wistar albino rats, 10 - 12 weeks of age weighing 150 - 220g were used in the experiment. Prior to experimentation, the animals were housed in standard wooden cages fitted with sawdust in a room maintained at temperatures of 22 ± 2^0C with alternate 12-hour cycles of light and darkness. The animals were fed on standard mice pencils and allowed free access to water for two weeks to allow for acclimatization. The animals were fasted for 24 hours prior to dosing. The animals were randomized into groups of three animals each and were given a limit dose of 2000mg/kg of plant extract by oral gavage. The rats were observed immediately after extract or distilled water administration; at 30 minutes, 60 minutes, 4 hours, and 24 hours after administration; and then once a day for the next 14 days for signs of behavioral changes (changes in hair, eyes, skin, mucous membrane, salivation, lethargy, diarrhea, and sleep) or mortality. If none of the animals died in this period, a repeat test was conducted with 5000mg/kg. If mortality was not observed at this dose, the extract was deduced to have a median lethal dose (LD_{50}) greater than 5000mg/kg and considered to be practically nontoxic. If at least one animal died from 2000mg/kg, then the LD_{50} can be estimated to be below 2000mg/kg. If, however mortality was not observed at 2000mg/kg but was observed at 5000mg/kg, a repeat test at 5000mg/kg was conducted. If mortality was observed at repeat test, then the LD_{50} was estimated to be between 2000 – 5000mg/kg and the substance considered to be slightly toxic.

2.6 Antiplasmodial activity

2.6.1 Preparation of extract stock solutions

This was done at the Infectious Diseases Research Collaboration (IDRC) Tororo Site. The samples were dissolved by weighing 100mg of paste or powder in 1 mL of Dimethyl sulfoxide (DMSO) and then 9 mL of distilled water was added. The suspension was incubated for 2 hours at 37^0 C, and the resultant solution completely dissolved, giving a stock solution of 10 mg/mL. The working solution was prepared fresh on the day of the assays.

2.6.2 The plasmodium strains

The plant extracts were evaluated for antiplasmodial activity against *P. falciparum* field isolates and laboratory strains MR4102 (3D7) and MR4156 (Dd2) (BEI Resources, Manassas, VA, USA). Laboratory strains were synchronized using a magnetic column (Miltenyi Biotec, Auburn, CA, USA) to obtain rings. The field isolate samples were centrifuged for 10 mins at RCF of 1500 rpm to remove the buffy coat. The pellet was washed three times with RPMI 1640 media (Thermo scientific, Waltham, MA, USA) and then suspended in complete medium consisting of RPMI 1640 with 25 mM HEPES, 24 mM $NaHCO_3$, 0.1 mM hypoxanthine, 10 µg/ml gentamicin, and 0.5% AlbuMAX II (Thermo Fisher Scientific, Waltham, MA, USA) to generate hematocrit of 50%.

2.6.3 Drug dilutions

Stock solutions of chloroquine (CQ) were dissolved in water (culture grade), while dihydroartemisinin (DHA), and test plant extracts were dissolved in dimethyl sulfoxide (DMSO). All stocks were diluted with RPMI 1640 to make the working solution. The working solution was then diluted in concentrations of 10,000, 5000, 2500, 1250, 625, 312.5, 156.25 and 78.125 µg/ml. The final concentration after addition of parasitized cells and donor cells to make 0.2 % parasitemia and 2 % heamatocrite in 96 well final volume of 200 µl, was 5000, 2500,1250,625, 312.5, 156.125, 78.125 and 39.06 These dilutions were then placed in a 96-well plate and serial diluted X3.

2.6.4 *In vitro* cultivation of *P. falciparum*

The antiplasmodial activity of plant extracts and standard antimalarials (CQ and DHA) was evaluated using a 72- hour growth inhibition assay with SYBR Green detection. Serial dilutions (factor of 3) of the extracts and drug stocks were made in complete media in 96 well microplates (50 µl per well), including drug free and parasite free control wells, with concentrations optimized to capture full dose response curves. Cultures were diluted with 200 µl per well at 0.2% parasitemia and 2% hematocrit of uninfected erythrocytes obtained from the local blood bank. Plates were

maintained at 5% CO_2, 5% O_2, and 90% N_2 for 72 h at 37°C in a humidified modular incubator (Billups Rothenberg, San Diego, CA, USA). After 72 h, well contents were re-suspended and 100 µL culture per well was transferred to black 96-well plates containing 100 µL per well SYBR Green lysis buffer (20 mM Tris, 5 mM EDTA, 0.008% saponin, 0.08% Triton X-100, and 0.2 µL/mL SYBR Green I (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), and mixed. Plates were incubated for 1 hour in the dark at room temperature, and fluorescence was then measured with a FLUOstar Omega plate reader (BMG LabTech, Cary, NC, USA; 485 nm excitation and 530 nm emission).

2.6.5 Data analysis

By using the raw data from the plate reader, the data from assay methods were analyzed with Prism Graphpad version 10.2.3 (San Diego, Clif) software. The counts were plotted against the logarithm of the drug concentration and nonlinear regression log dose curve (based on a sigmoidal dose response/variable slope equation) was applied to determine the drug concentration that caused 50% reduction from the maximum counts observed in the drug-free control wells (IC_{50}). The phytochemical screening data and the antiplasmodial activity data was presented in tables containing the means ± SD.

3.0 Results

3.1 Phytochemical screening

All the tested phytochemicals were present in the extracts except for emodols and anthracenosides which were absent in *E. abyssinica* and *K. Africana (Table 1)*. The most abundant phytochemicals in the extracts were alkaloids, flavonoids, saponins, tannins, terpenoids, steroids and glycosides.

Table 1: Phytochemical screening of stem bark extracts of the five plants

Phytochemical screened	Plant extract (70% ethanol)				
	Z. chalybeum	*A. coriaria*	*E. abyssinica*	*M. senegelensis*	*K. africana*
Saponins	+++	+++	+++	+++	+++
Tannins	+++	+++	++	+++	+++
Alkaloids	+++	+	+++	+	+
Coumarins	++	+	+	++	++
Steroids	+++	+++	++	+++	++
Glycosides	+++	+++	+++	+++	+
Terpenoids	++	+++	+	++	++
Flavonoids	+++	+++	++	+++	+++

Emodols	-	+++	-	+++	-
Anthracenosides	+++	+++	-	+++	-
Reducing sugars	++	+++	+++	+++	+++

Absent (-), weakly present (+), moderately present (++), strongly present (+++)

3.2 Quantification of phytochemicals from the different samples

The results indicated significant variability in the concentration of the selected phytochemicals compounds across the different species analyzed (table 2). For instance, *Z. chalybeum* showed high levels of tannins (35.22 mg TAE/g) and triterpenoids (42.00 mg BTE/g), while *A. coriaria* exhibited a notably high concentration of flavonoids (93.36 mg QUE/g) and steroids (192.08 mg DSE/g). In contrast, *E. abyssinica* had the highest tannin content (75.18 mg TAE/g) among the tested species.

Table 2: Relative quantities (mean ±SD) of different phytochemicals for selected medicinal plants analyzed.

Extract	Tannins (mg TAE/g)	Alkaloids (mgCAE/g)	Flavonoids (mgQUE/g)	Triterpenoids (mgBTE/g)	Steriods (mgDSE/g)
Z. chalybeum	35.22 ± 1.24	28.91 ± 2.08	42.00 ± 1.04	42.00 ± 1.04	32.36 ± 1.85
A. coriaria	11.06 ±1.24	38.29 ± 2.08	93.36 ± 0.91	93.36 ± 0.91	192.08 ± 2.78
E.abyssinica	75.18 ± 1.24	42.98 ± 2.08	30.13 ± 0.16	30.13 ± 0.16	93.47 ± 1.85
M. Senegalensis	38.01 ± 1.24	17.97 ± 2.08	51.83 ± 0.24	51.83 ± 0.24	69.86 ± 1.85
K. africana	84.47 ± 1.86	30.47 ± 3.13	65.30 ± 0.85	65.30 ± 0.85	39.31 ± 1.85

3.3 Acute toxicity results

With exception of *A. coriaria* and *M. senegelensis* which were moderately toxic, the rest of the plant species were relatively safe (Table 3). The acute toxicity signs observed included piloerection, excessive urination, hard breathing, lethargy, loss of righting reflex, coma and Death.

Table 3: Acute toxicity signs and symptoms following oral administration of the ethanolic extracts of the plant samples

Plant species	Dose (mg/kg)	Mortality	Toxicity signs	Conclusion
Z. Chalybeum	2000	0/3	No observable sign of toxicity	Relatively safe
	5000	0/3	No observable sign of toxicity	
A. coriaria	2000	2/3	Piloerection, excessive urination, hard breathing, lethargy, loss of righting reflex and Death	Moderately toxic
E. abyssinica	2000	0/3	Reduced movement, lethergy but normal after 24 hours	Relatively safe
	5000	0/3	Reduced movement, lethargy but normal after 24 hours	
M. senegalensis	2000	1/3	Reduced movement, Piloerection, excessive urination, hard breathing, lethargy, and Death	Moderately toxic
K. africana	2000	0/3	No observable sign of toxicity	Relatively safe
	5000	0/3	Reduced movement, lethargy but normal after 24 hours	

3.4 Antiplasmodial activity

The ethanolic extracts of the tested plants showed statistically significant variation in the potency against different plasmodium strains compared to the standard antimalarial drug (Table 3). For the 3D7 strain, *Z. chalybeum* demonstrated the highest potency among all plant extracts that was comparable to dihydroartemisinin (P>0.05). The potency of the other extracts in the decreasing order was *A. coriaria, E. abyssinica,* and *M. senegalensis* and *K. Africana.* For the Dd2 and clinical isolate 1 strains, *Z. chalybeum* and *A. coriaria* demonstrated the highest potencies among all plant extracts that were comparable to dihydroartemisinin (P> 0.05). For the clinical isolate (2), *A. coriaria* demonstrated the highest potency among all plant extracts that was comparable to dihydroartemisinin (P>0.05). The potency of the other extracts in the decreasing order was *Z. chalybeum, E. abyssinica, M. senegalensis* and *K. Africana.* Collectively, these findings revealed that *Z. chalybeum* and *A. coriaria* extracts possess strong antiplasmodial activity across sensitive, resistant, and clinical *P. falciparum* strains (Fig.1), with their potency approaching that of standard drugs while *K. Africana* has weak antiplasmodial activity against all the strains.

Table 3: Shows the mean inhibitory concentrations (IC_{50}) of the selected medicinal plant ethanolic extracts against *Plasmodium falciparum* strains.

Plant Extract /drug	IC_{50} (ug/ml)			
	3D7	Dd2	Isolate 1	Isolate 2
Zanthoxylum chalybeum	0.90± 0.22	1.54±0.47	15.39±0.26	60.81±8.35#
Albizia coriaria	30.29± 1.76#	27.02± 3.26	16.49±1.21	13.89±4.99
Entada abyssinica	47.73± 5.87#	37.5± 3.35#	30.26±4.04#	44.49±7.87#
Maytenus Senegalensis	72.39± 9.96#	101.26± 59.83#	41.66 ±14.96#	58.73± 4.35#
Kigelia Africana	578.73± 458.41#	359.58± 124.33#	656.55±90.58#	386.95 ±9.69#
Chloroquine	9.83± 1.97	867.91± 1341.79#	9.20± 1.30	12.15± 1.54
Dihydroartemisinin	3.13± 4.07	2.325± 1.36	3.48± 1.32	6.12± 2.25

#p-value<0.05 as compared to dihydroartemisinin, IC_{50} presented as Mean ± SD

4.0 Discussion

Medicinal plants have evolved complex biosynthetic pathways that produce a diverse array of secondary metabolites primarily for defense purposes against predators, pests and pathogens. These phytochemicals such as alkaloids, flavonoids, tannins, and terpenoids contribute to the

plant's survival and environmental adaptation. Their concentration, however, can vary significantly depending on species genetic makeup, soil composition, climate, altitude, and other environmental factors (Inbathamizh & Padmini, 2013; Kaggwa et al., 2023). In this study, all plant specimens were collected from the same geographical area with relatively similar environmental conditions, which strongly suggests that observed differences in phytochemical content among species are primarily due to innate genetic differences and soil chemistry variations (Moomin et al., 2023). Chemotherapy of malaria continues to be threatened by the increasing resistance of *Plasmodium falciparum* to frontline antimalarial agents like artemisinin-based combination therapies (ACTs) that reduces their efficacy making them less effective (Agaba et al., 2024). However, medicinal plants have historically been invaluable in providing lead compounds, such as quinine and artemisinin that have revolutionized malaria treatment (Tabuti et al., 2023). The continued bioprospecting of medicinal plants remains a promising strategy as it offers immerse source of novel bioactive compounds capable of combating resistant strains and potentially reducing adverse effects associated with synthetic drugs.

The *in vitro* antiplasmodial activity of extracts or compounds can be categorized depending on their inhibitory concentration values as strong (IC_{50} < 10 µg/ml), moderate (IC_{50} = 10 - 50 µg/ml), slightly active (IC_{50} = 50 - 100 µg/ml) and weak (IC_{50} > 100 µg/ml) (Dolabela et al., 2008). According to this criteria, *Z. chalybeum*, *A. coriaria* and *E. abyssinica* were the most potent among the tested plants, displaying strong to moderate antiplasmodial activity against the chloroquine-sensitive 3D7 strain and the chloroquine-resistant Dd2 strain. This consistent high antiplasmodial activity across strains indicates broad-spectrum activity suggesting their potential as robust sources of antimalarial drug candidates. Alkaloids like skimmianine, chelerythrine, nitidine, candicine, tembetarine and other compounds like Fagaramide isolated from *Z. chalybeum* have demonstrated high antiplasmodial activity against chloroquine-resistant *P. falciparum* and moderate activity against the chloroquine sensitive *P. falciparum* parasites with IC_{50} values ranging from (2.85 to 16.6 µg/mL) (Adia et al., 2016; Bbosa et al., 2014). Like in this study, extracts of *Z. chalybeum* harvested from central Uganda exhibited similar IC_{50} values (ranging from 0.57 to 4.25 µg/ml) against *Plasmodium falciparum* NF54 & FCR3 strains (Adia et al., 2016). The ether and methanol leaf extracts of *Z. chalybeum* had EC_{50} values of 13.39 and 8.10 µg/ml against wild-type *P. falciparum* isolates (Bbosa et al., 2014). Like in this study, *Z. chalybeum* harvested in Rwanda exhibited higher potency with IC_{50} values ranging from 4.2 to 6.2 µg/ml against the 3D7 strain (Muganga et al., 2010). *Albizia coriaria* contains saponins like coriariosides, flavonoids, and phenolic compounds such as Atranorin, which have previously demonstrated antimalarial potential (Moomin et al., 2023; Ntie-Kang et al., 2014). In a related study, the dichloromethane stem bark extract of *A. coriaria* exhibited

moderate antiplasmodial activity with IC_{50} values ranging from 6.798 to 10.679 µg/ ml against P. *falciparum* strains W2 and D6 (Owuor et al., 2012).In contrast to our study where the *M. senegelensis* extracts were slightly active, the methanolic root bark extracts of *M. senegalensis* harvested in Kenya had IC_{50} values ranging from 4.7 to 9.8 µg/ml against P. *falciparum* D6, W2 (Muthaura et al., 2017). Similarly, the ethyl acetate and ethanolic extract from stem bark and root bark of *M. senegalensis* harvested in Tanzania showed strong activity, with IC_{50} values ranging from 0.16 to 2,05 µg/ml against *P. falciparum* K1 strain respectively (Malebo et al., 2009). Our findings for *E. abyssinica* resonate with previous reports that also identified this extract as having moderate to weak antiplasmodial activity (Waiganjo et al., 2020).

Phytochemical screening revealed that these plants contained alkaloids, flavonoids, tannins, and terpenoids. It's therefore highly likely that the observed antiplasmodial activity of these plants are due to high total alkaloids, flavonoid and saponins contents. However further investigations are required to identify the real compounds in these plants responsible for the antimalarial activities. Alkaloids and flavonoids have been reported to interfere with parasite metabolic pathways such as inhibiting DNA synthesis, disrupting membrane integrity, and interfering with parasite enzyme functions leading to reduced replication and survival (Okello et al., 2023). The difference in the antiplasmodial activity observed in the different studies could be attributed to the different strains and solvents used as well as the region of plant collection.

Kigelia africana displayed weak activity, with IC_{50} values exceeding 300 µg/ml across both strains. This suggests limited efficacy as an antimalarial agent when used alone in monoherbal formulations. However, since several traditional medicine practitioners use combinations of medicinal plants to prepare herbal formulations for malaria (polyherbal formulations), the role of *K. africana* could be further investigated. Considering the fact that it's the fruit that is used, it probably could work as an excipient to improve the organoleptic properties of the formulations but also work synergistically in presence of other phytochemicals.

Acute toxicity determination helps to estimate the immediate toxicity associated with administration of substances when taken in a single high dose within 24 hours. *A. Coriaria* and *M. senegalensis* were moderately toxic to the animals implying that they could potentially cause toxicity to human. Therefore, they should be used cautiously in traditional and complementary medicines. Toxicity of plant extracts could be due to inherent poisonous chemicals synthesized by metabolic pathways for plant defense (Obakiro et al., 2024). It's therefore important to conduct further studies to determine what chemicals are responsible for the observed toxicity in these plants. Previous studies have also reported that *A. coriaria* and *M. senegelensis* extracts were toxic to cell lines,

animals and humans (Obakiro et al., 2024; Oloya et al., 2022). The rest of plant species were relatively safe and hence suitable for further exploration for novel molecules.

5. Conclusion

The ethanolic extracts of *Zanthoxylum chalybeum (root bark)*, *Albizia coriaria (stem bark)* and *Entada abyssinica (*stem bark*)* possess good *in vitro* antiplasmodial activity against both drug sensitive and resistant *P. falciparum*. The antiplasmodial activity is due to abundant phytochemicals present in these plants particularly alkaloids, flavonoids, terpenoids and saponins. Future research should focus on isolating active compounds and elucidating their antiplasmodial mechanisms of action as well as understanding the potential interactions when the different extracts are combined.

Declarations

Ethics approval

The study protocol was reviewed and approved by Cure Children's Hospital Uganda Research and Ethics Committee (CCHUREC/11/020) and registered with the Uganda National Council of Science and Technology (HS1222ES).

Consent for publication

Not applicable

Availability of Data and Materials

The raw data supporting the findings of this study can be obtained from the corresponding author upon request.

Competing interests

The authors confirm that there is no conflict of interest related the publication of this paper.

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Authors' Contribution

SBO, KK, PW, MA, YG and ROO conceptualized the idea, wrote the proposal and applied for funding. MC, CN, OM, MR, LTW, JH, MA, AN and SO conducted experiments and collected the data under the supervision of DK, ROO, SBO and MO. KK, MO, SO and ROO analyzed the data.

CN, SBO, KK, AN, DK and JH wrote the initial draft of the manuscript which was reviewed by all the authors. All the authors read and approved the final manuscript.

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List of abbreviations

ACTs – Artemisinin Combination Therapies
CQ – Chloroquine
DHA – Dihydroartemisinin
DMSO - Dimethyl sulfoxide
DNA – Deoxyribonucleic acid
IC50 - Inhibitory concentration at 50%
LD50 – Median Lethal Dose
SSA – Sub-Saharan Africa
WHO – World Health Organization

References

Adia, M. M., Emami, S. N., Byamukama, R., Faye, I., & Borg-Karlson, A. K. (2016). Antiplasmodial activity and phytochemical analysis of extracts from selected Ugandan medicinal plants. *Journal of Ethnopharmacology*, *186*, 14–19. https://doi.org/10.1016/j.jep.2016.03.047

Agaba, B. B., Travis, J., Smith, D., Rugera, S. P., Zalwango, M. G., Opigo, J., Katureebe, C., Mpirirwe, R., Bakary, D., Antonio, M., Khalid, B., Ngonzi, J., Kamya, M. R., Kaleebu, P., Piot, P., & Cheng, Q. (2024). Emerging threat of artemisinin partial resistance markers (pfk13 mutations) in Plasmodium falciparum parasite populations in multiple geographical locations in high transmission regions of Uganda. *Malaria Journal*, *23*(1), 330. https://doi.org/10.1186/s12936-024-05158-9

Angupale, J. R., Tusiimire, J., & Ngwuluka, N. C. (2023). A review of efficacy and safety of Ugandan anti-malarial plants with application of RITAM score. *Malaria Journal*, *22*(1), 1–19. https://doi.org/10.1186/s12936-023-04486-6

Bbosa, G., Mwebaza, N., Lubega, A., Musisi, N., Kyegombe, D. B., & Ntale, M. (2014). Antiplasmodial Activity of Leaf Extracts of Zanthoxylum chalybeum Engl. *British Journal of Pharmaceutical Research*, *4*(6), 705–713. https://doi.org/10.9734/bjpr/2014/6528

Bunalema, L., Obakiro, S., Waako, J. P., & Tabuti, J. R. S. (2014). Knowledge on plants used traditionally

in the treatment of tuberculosis in Uganda. *Journal of Ethnopharmacology*, *151*, 999–1004.

Chen, C. (2014). Development of antimalarial drugs and their application in China: A historical review. *Infectious Diseases of Poverty*, *3*(1), 1–10. https://doi.org/10.1186/2049-9957-3-9

Clement, O. A., Anthony, A. E., Félicien, M. K., Mercy, G. T., Hedmon, O., Anke, W., Casim, U. T., & Patrick, E. O. (2020). A review for selecting medicinal plants commonly used for malaria in Uganda. *African Journal of Pharmacy and Pharmacology*, *14*(9), 347–361. https://doi.org/10.5897/ajpp2020.5182

Dolabela, M. F., Oliveira, S. G., Nascimento, J. M., Peres, J. M., Wagner, H., Póvoa, M. M., & de Oliveira, A. B. (2008). In vitro antiplasmodial activity of extract and constituents from Esenbeckia febrifuga, a plant traditionally used to treat malaria in the Brazilian Amazon. *Phytomedicine*, *15*(5), 367–372. https://doi.org/10.1016/j.phymed.2008.02.001

Gumisiriza, H., Olet, E. A., Mukasa, P., Lejju, J. B., & Omara, T. (2023). Ethnomedicinal plants used for malaria treatment in Rukungiri District, Western Uganda. *Tropical Medicine and Health*, *51*(1). https://doi.org/10.1186/s41182-023-00541-9

Harborne, J. (1973). *Phytochemical methods* (pp. 49–188). Chapman and Hall Ltf.

Inbathamizh, L., & Padmini, E. (2013). Effect of geographical properties on the phytochemical composition and antioxidant potential of Moringa oleifera flowers. *Bio Med Rx.*, *1*(3), 239–247.

Irungu, B., Okari, E., Nyangi, M., Njeru, S., & Koech, L. (2023). Potential of medicinal plants as antimalarial agents: a review of work done at Kenya Medical Research Institute. *Frontiers in Pharmacology*, *14*(October), 1–16. https://doi.org/10.3389/fphar.2023.1268924

Kaggwa, B., Munanura, E. I., Kyeyune, H., Anywar, G., Okella, H., Ajayi, C. O., Wangalwa, R., Mulangwa, J., Sesaazi, C. D., Bagoloire, L. K., Tolo, C. U., Kamba, P. F., & Ogwang, P. E. (2023). Evaluation of catechin , lupeol , and betulinic acid as markers for the chromatographic quality Control of Albizia coriaria raw materials ; an experimental study. *Journal of Medicinal Plants Research*, *17*(5), 180–200. https://doi.org/10.5897/JMPR2022.7285

Koparde, A. A. (2017). Phyto Active Compounds From Herbal Plant Extracts: Its Extraction, Isolation and Characterization. *World Journal of Pharmaceutical Research*, *6*(8), 1186–1205. https://doi.org/10.20959/wjpr20178-8958

Malebo, H. M., Tanja, W., Cal, M., Swaleh, S. A. M., Omolo, M. O., Hassanali, A., Séquin, U., Hamburger, M., Brun, R., & Ndiege, I. O. (2009). Antiplasmodial, anti-trypanosomal, anti-leishmanial and cytotoxicity activity of selected Tanzanian medicinal plants. In *Tanzana Journal of Health Research* (Vol. 11, Issue 4).

Moomin, A., Russell, W. R., Knott, R. M., Scobbie, L., Mensah, K. B., Adu-Gyamfi, P. K. T., & Duthie, S. J. (2023). Season, storage and extraction method impact on the phytochemical profile of Terminalia ivorensis. *BMC Plant Biology*, *23*(1), 1–18. https://doi.org/10.1186/s12870-023-04144-8

Muganga, R., Angenot, L., Tits, M., & Frédérich, M. (2010). Antiplasmodial and cytotoxic activities of Rwandan medicinal plants used in the treatment of malaria. *Journal of Ethnopharmacology*, *128*(1),

52–57. https://doi.org/10.1016/j.jep.2009.12.023

Muthaura, C., Keriko, J., Mutai, C., Yenesew, A., Heydenreich, M., Atilaw, Y. M., Gathirwa, J. W., Irungu, B. N., & Derese, S. (2017). Antiplasmodial, Cytotoxicity and Phytochemical Constituents of Four Maytenus Species Used in Traditional Medicine in Kenya. *The Natural Products Journal*, *7*(2), 144–152. https://doi.org/10.2174/2210315507666161206144050

Nabatanzi, A., Nkadimeng, S. M., Lall, N., Kabasa, J. D., & McGaw, L. J. (2020). Ethnobotany, phytochemistry and pharmacological activity of kigelia africana (Lam.) benth. (bignoniaceae). *Plants*, *9*(6), 1–29. https://doi.org/10.3390/plants9060753

Ntie-Kang, F., Onguéné, P. A., Lifongo, L. L., Ndom, J. C., Sippl, W., & Mbaze, L. M. A. (2014). The potential of anti-malarial compounds derived from African medicinal plants, part II: A pharmacological evaluation of non-alkaloids and non-terpenoids. *Malaria Journal*, *13*(1). https://doi.org/10.1186/1475-2875-13-81

Obakiro, S. B., Kiyimba, K., Lukwago, T. W., Lulenzi, J., Owor, R. O., Andima, M., Hokello, J. F., Kawuma, C., Nantale, G., Kibuule, D., Anywar, G., Waako, P., & Gavamukulya, Y. (2023). Ethnobotanical study of plants used in management of diabetes mellitus in Eastern Uganda. *Phytomedicine Plus*, *3*(4), 100486. https://doi.org/10.1016/j.phyplu.2023.100486

Obakiro, S. B., Kiyimba, K., Owor, R. O., Andima, M., Lukwago, T. W., Kawuma, C., Gavamukulya, Y., Nabatanzi, A., Kibuule, D., Kato, C. D., Anywar, G., & Waako, P. (2024). Acute and subacute toxicity profile of ethanolic stem bark extract of Albizia coriaria Welw. ex Oliv. in Wistar albino rats. *Toxicology Reports*, *12*(November 2023), 178–185. https://doi.org/10.1016/j.toxrep.2024.01.005

Ocen, E., Opito, R., Tegu, C., Oula, A., & Olupot-Olupot, P. (2023). Severe malaria burden, clinical spectrum and outcomes at Apac district hospital, Uganda: a retrospective study of routine health facility-based data. *Malaria Journal*, *22*(1), 1–8. https://doi.org/10.1186/s12936-023-04761-6

Okello, D., Gathirwa, J., Wanyoko, A., Komakech, R., Chung, Y., Gang, R., Omujal, F., & Kang, Y. (2023). Comparative antiplasmodial activity, cytotoxicity, and phytochemical contents of Warburgia ugandensis stem bark against Aspilia africana wild and in vitro regenerated tissues . *Journal of Plant Biotechnology*, *50*, 97–107. https://doi.org/10.5010/jpb.2023.50.013.097

Oladipo, H. J., Tajudeen, Y. A., Oladunjoye, I. O., Yusuff, S. I., Yusuf, R. O., Oluwaseyi, E. M., AbdulBasit, M. O., Adebisi, Y. A., & El-Sherbini, M. S. (2022). Increasing challenges of malaria control in sub-Saharan Africa : Priorities for public health research and policymakers. *Annals of Medicine and Surgery*, *81*(August). https://doi.org/10.1016/j.amsu.2022.104366

Oloya, B., Namukobe, J., Ssengooba, W., Afayoa, M., & Byamukama, R. (2022). Phytochemical screening, antimycobacterial activity and acute toxicity of crude extracts of selected medicinal plant species used locally in the treatment of tuberculosis in Uganda. *Tropical Medicine and Health*, *50*, 16. https://doi.org/10.1186/s41182-022-00406-7

Owor, R. O., Kawuma, C., Nantale, G., Kiyimba, K., Obakiro, S. B., Ouma, S., Lulenzi, J., Gavamukulya, Y., Chebijira, M., Lukwago, T. W., Egor, M., Musagala, P., Andima, M., Kibuule, D., Waako, P., &

Hokello, J. (2024). Ethnobotanical survey and phytochemistry of medicinal plants used in the management of HIV/AIDS in Eastern Uganda. *Heliyon*, *10*(11), e31908. https://doi.org/10.1016/j.heliyon.2024.e31908

Owuor, B. O., Ochanda, J. O., Kokwaro, J. O., Cheruiyot, A. C., Yeda, R. A., Okudo, C. A., & Akala, H. M. (2012). In vitro antiplasmodial activity of selected Luo and Kuria medicinal plants. *Journal of Ethnopharmacology*, *144*(3), 779–781. https://doi.org/10.1016/j.jep.2012.09.045

Philip, K., Elizabeth, M., Cheplogoi, P., & Samuel, K. (2017). Ethnobotanical Survey of Antimalarial Medicinal Plants Used in Butebo County, Eastern Uganda. *European Journal of Medicinal Plants*, *21*(4), 1–22. https://doi.org/10.9734/ejmp/2017/35368

Schultz, F., Anywar, G., Wack, B., Leah, C., & Garbe, L. (2020). Ethnobotanical study of selected medicinal plants traditionally used in the rural Greater Mpigi region of Uganda. *Journal of Ethnopharmacology*, *256*(August 2019), 112742. https://doi.org/10.1016/j.jep.2020.112742

Tabuti, J. R. S. (2008). Herbal medicines used in the treatment of malaria in Budiope county, Uganda. *Journal of Ethnopharmacology*, *116*(1), 33–42. https://doi.org/10.1016/j.jep.2007.10.036

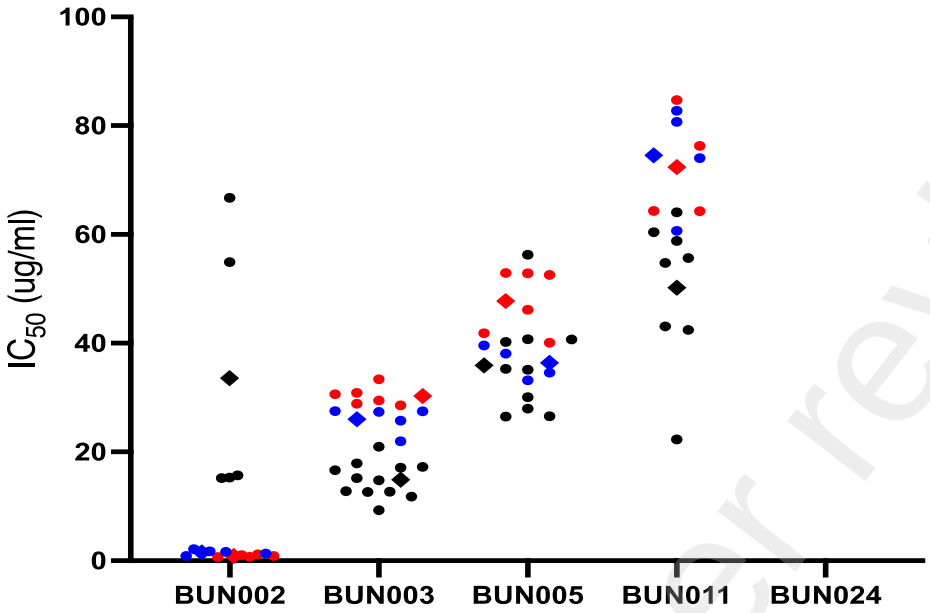
Tabuti, J. R. S., Obakiro, S. B., Nabatanzi, A., Anywar, G., Nambejja, C., Mutyaba, M. R., Omara, T., & Waako, P. (2023). Medicinal plants used for treatment of malaria by indigenous communities of Tororo District , Eastern Uganda. *Tropical Medicine and Health*, *51*(34), 1–14. https://doi.org/10.1186/s41182-023-00526-8

Tugume, P., Kakudidi, E. K., Buyinza, M., Namaalwa, J., Kamatenesi, M., Mucunguzi, P., & Kalema, J. (2016). Ethnobotanical survey of medicinal plant species used by communities around Mabira Central Forest Reserve, Uganda. *Journal of Ethnobiology and Ethnomedicine*. https://doi.org/10.1186/s13002-015-0077-4

Waiganjo, B., Moriasi, G., Onyancha, J., Elias, N., & Muregi, F. (2020). Antiplasmodial and Cytotoxic Activities of Extracts of Selected Medicinal Plants Used to Treat Malaria in Embu County, Kenya. *Journal of Parasitology Research*, *2020*. https://doi.org/10.1155/2020/8871375

WHO. (2019). WHO Global report on traditional and complementary medicine 2019. In *World Health Organization*.

WHO. (2023). *World Health Organization-World malaria report 2023 -- spread view*.



BUN002: *Z. chalybeum*, BUN003: *A. coriaria*, BUN005: *E. abyssinica* BUN011: *M. senegelensis,* BUN024: *K. Africana; The IC_{50} of BUN024 were too high above the acceptable limit.*

Figure. 1 shows the Inhibitory concentrations (IC_{50}) of the different extracts against different *P. falciparum* strains