



## ***In vivo* Antimalarial Activities of Five Ugandan Medicinal Plants on *Plasmodium berghei* in Mice**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. Author COA managed the literature searches, methodology, investigation, data analysis and writing of the original draft. Authors PEO and AAE conceptualized the study, designed the methodology and edited the manuscript for publication, as the supervisor and co-supervisor, respectively. Authors HO and JO contributed to the animal study, data analysis and manuscript drafting while author AR contributed to the validation and manuscript drafting. Authors AW and CUT contributed to the project administration, literature searches and data analysis. All authors read and approved the final manuscript.

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### **ABSTRACT**

**Aim:** Medicinal plants have played an important role in the treatment of different ailments including malaria in developing countries particularly in Africa. This study has evaluated the antimalarial activities of *Azadirachta indica* A. Juss (Meliaceae), *Cymbopogon citratus* Stapf. (Poaceae),

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*Moringa oleifera* Lam. (Moringaceae), *Tithonia diversifolia* (Hemsl) A. Grey (Asteraceae) and *Vernonia amygdalina* Del. (Asteraceae) which are commonly-used for malaria treatment in Uganda.

**Study Design:** This is an experimental laboratory report on antimalarial activities of some Ugandan medicinal plants for subsequent profiling in an herbal pharmacopoeia and eventual drug development.

**Place and Duration of Study:** The Animal Research Facility and the Clinical and Research Laboratory, Faculty of Medicine, Mbarara University of Science and Technology, Uganda, between July 2019 and March 2020.

**Methodology:** The antimalarial activity of the hot infusion of each leaf was evaluated on chloroquine-sensitive *Plasmodium berghei* ANKA-infected mice using 4-day test at 100 – 400 mg/kg with chloroquine (10 mg/kg) and artemether-lumefantrine (4 mg/kg) as positive controls; and distilled water as negative control. The observed haematological responses of the animals were determined with an automated haematometer.

**Results:** The results showed dose-dependent activities in the animals treated with the extract of each plant leaf in varying degrees. Thus, *V. amygdalina* and *T. diversifolia* showed the highest antimalarial activities with the chemosuppression values of 75% and 66% at 400 mg/kg, respectively. The results of *V. amygdalina*, *T. diversifolia* and *M. oleifera*, extracts gave the lowest ED<sub>50</sub> of 141, 195 and 231 mg/kg, respectively being significantly different from *A. indica* (ED<sub>50</sub> 319 mg/kg) and *C. citratus* (ED<sub>50</sub> 346 mg/kg) with *V. amygdalina* as the most potent extract among the five plant leaves.

**Conclusion:** The observed activities of the five plants have therefore supported their folkloric uses as antimalarial remedies by the Ugandan traditional medicine practitioners with obvious potentials for drug development.

**Keywords:** Malaria; parasitaemia; chemosuppression; hematological effects.

## 1. INTRODUCTION

Malaria remains a burden in developing countries despite several efforts put in place to eradicate it. Recent reports showed an estimated morbidity of 228 million from which 93% came from African regions and global record of 435,000 mortalities with 92% from Africa [1]. According to the World Health Organization, 85% of the global malaria burden was reported to come from sub-Saharan Africa and India with Uganda being listed among the six countries with high malaria morbidity of 5% [1].

Due to high cost, non-availability and accessibility of conventional antimalarial drugs, people of developing countries use medicinal plants as alternative natural sources for malaria. They are either used in their crude forms, singly as mono-component or in combination as multi-component herbal preparations. Medicinal plants have played important roles as sources of antimalarial drugs, beginning with the discovery of quinine from *Cinchona succirubra* Pav. ex Klotzsch (Rubiaceae) stem-bark [2], which eventually served as starting material for the chemical synthesis of chloroquine, amodiaquine, etc. in the treatment of malaria. Later, artemisinin, isolated from *Artemisia annua* L. (Asteraceae) has served as a skeleton for the

synthesis of artemether, artesunate, dihydroartemisinin, etc. used for the current treatment of chloroquine-resistant *Plasmodium falciparum* malaria [3].

In Uganda, several medicinal plants have been reported through different ethnobotanical surveys on herbal malaria management [4,5], five of which, namely, *Azadirachta indica* A. Juss, *Cymbopogon citratus* Stapf., *Moringa oleifera* Lam., *Tithonia diversifolia* (Hemsl) A. Grey. and *Vernonia amygdalina* Del. have been studied in the present investigation for antimalarial activities.

*Azadirachta indica* A. Juss, (Meliaceae) is an evergreen tree commonly known as neem tree. This plant is indigenous to South Asia and abundant in the tropical belt [6]. The plant has been reported to have anti-hyperglycaemic, antibacterial [7], antifungal, anthelmintic [8], antioxidant, anti-inflammatory and antimalarial [9] activities. The plant has been reported to contain diterpenoids, triterpenoids, alkaloids, flavonoids and phenolic compounds[10].

*Cymbopogon citratus* Stapf. (Poaceae) is a perennial tropical grass that is originated from Indochina, Malaysia and SriLanka. It has been reported to contain anti-inflammatory [11],

analgesic [12], antioxidant, antimicrobial [13], gastroprotective [14], anti-leishmania [15], insecticidal, hypoglycemic and hypolipidemic [16] activities. It contained saponins, alkaloids, flavonoids, tannins, esters, glycosides and essential oils [17]. *In vitro* test of its essential oils was reported to have antiplasmodial activity while its whole plant has been reported for antimalarial activity [18].

*Moringa oleifera* Lam. (Moringaceae) is commonly known as moringa or drumstick. It has antihypertensive, antidiabetic [19], antibacterial [20], antinociceptive, larvicidal, antifungal, anti-inflammatory, analgesic, antimalarial [21,22] and antisickling [23] activities. The plant has been reported to contain phenols (glucosinolates and isothiocyanates), flavonoids, alkaloids, glycosides, amino acids, carotenoids, vitamins and sterols [24].

*Tithonia diversifolia* (Hemsl) A. Grey. (Asteraceae), commonly known as Mexican sunflower is a perennial shrub widely distributed in Africa [25]. The plant has been reported to possess antimalarial [26,27], antiplasmodial [28,29], antibacterial [30], antiulcer [31], antifungal, anti-inflammatory and analgesic [32] activities. The chemical constituents included alkaloids, flavonoids, phlobatannins, tannins, terpenes, saponins and reducing sugars [33]. Nephro-toxicity and hepato-toxicity of the plant have been reported at the dose of 400 mg/kg [34].

*Vernonia amygdalina* Del. (Asteraceae), commonly known as bitter leaf is a small shrub, an edible rainforest, growing in tropical Africa where it is used for the treatment of malaria and other ailments. It has been reported to have antimalarial [35], antimicrobial, antitumoral [36], antihypertensive [37], antinociceptive, antioxidant, hepatoprotective, antidiabetic, anti-inflammatory and anticancer [38,39] properties. The ethanol extract of the leaf showed *in vitro* antiplasmodial activity on *P. falciparum* with IC<sub>50</sub> of 9.82 µg/mL [40]. Steroidal saponins, terpenes, coumarins, tannins, alkaloids, flavonoids and some novel stigmastane-type steroidal glycosides, vernoniosides and diterpenoids have been reported [41,42].

Although, the five selected Ugandan medicinal plants have been reported as antimalarials, no study has compared and rank-ordered their antimalarial properties. Therefore, the aim of present study was to investigate the antimalarial activities on *P. berghei*-infected mice, using hot

infusion method for extraction, since they are usually prepared through this method by the native communities in Uganda; and also determine their hematological effects, compare their potencies and determine the most active for subsequent development of standardized antimalarials and possible utilization in health care.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

The leaves of *Azadirachta indica* A. Juss, family Meliaceae (AZ), *Cymbopogon citratus* Stapf., family Poaceae (CY), *Moringa oleifera* Lam., family Moringaceae (MO), *Tithonia diversifolia* (Hemsl) A. Grey., family Asteraceae (TD) and *Vernonia amygdalina* Del., family Asteraceae (VA) were collected from Mbarara Municipality, Uganda, in July, 2019 (AZ [S0036'39 E30°39'16], CY [S0°36'22.34 E30°39'42.32], MO [S0°35'59 E30°40'44], TD [S0°35'34.14, E30°38'54.24] and VA [S0°36'57.48 E30°39'25.74]). They were identified and authenticated by the taxonomist, Dr. Eunice Olet, Department of Biology, Faculty of Science, Mbarara University of Science and Technology by comparison with the herbarium specimens of previously collected and preserved samples in the Makerere Herbarium of the Department of Botany, Kampala, Uganda. The leaves were separately air-dried at room temperature and pulverized using a grinder. The reference drugs: chloroquine® and coartem® (artemether-lumefantrine), were purchased from a registered Pharmacy in Mbarara Municipality.

#### 2.1.1 Preparation of infusion

Hot infusion of standard concentration, equivalent to 100 mg/mL of each powdered leaf of AZ, CY, MO, TD and VA, was prepared by separately weighing 5 g of each powdered leaf into a conical flask and pouring 50 mL boiling distilled water into it. The flasks were covered and left for 15 minutes before they were filtered using Sieve no 710 [43,44].

### 2.2 Study Animals and Care

Swiss albino mice of both sexes, weighing 18-20 g, were purchased from the Animal Facility Laboratory of Mbarara University of Science and Technology, Mbarara, Uganda. The animals were housed under a 12 h light /dark cycle with free access to water and were fed with commercial food pellets, purchased from KUYOYO Feeds, Uganda. They were

acclimatized for two weeks prior to the assay. All the animals were kept under standardized environmental conditions and had free access to food and water. The principles of laboratory animal care (NIH publication No. 85-23, revised 1985) [45] were followed.

### 2.3 Malaria Parasite Strain

The parasite was obtained through the United States' BEI Resources, NIAID, NIH: chloroquine-sensitive *Plasmodium berghei*, Strain ANKA, MRA-311, contributed by Thomas F. McCutchan. The vial containing 0.5 mL of *P. berghei* ANKA strain parasite in dried ice, donated by BEI Resources in USA was activated by thawing frozen cryovial in a water bath at 35°C for 2 minutes. This was followed by wiping the outside of the vial with 70% ethanol before opening and 50 µL injected intraperitoneally into each mouse. The growth of parasites was monitored by tail vein bleed sampling and Giemsa-stained thin blood smear microscopy daily, starting on day-3 post-inoculation. The parasites were maintained in continuous blood passage in mice. The parasite was ready for use after the 3<sup>rd</sup> generation of inoculation. A standard inoculum of  $1 \times 10^7$  parasitized erythrocytes was prepared by diluting the blood harvested from a donor mouse (> 30 % parasitaemia) with normal saline and 200 µL injected intraperitoneally to each test mouse.

### 2.4 Early-Malaria Infection (Chemosuppressive) Test

One hundred and thirty-three (133) mice were intraperitoneally inoculated with *P. berghei* ANKA parasite, 2 h prior to drug administration [46]. The mice were randomly divided into 19 groups of 7 mice each; and mice in each of the groups were orally treated with the assigned test leaf infusion at the same doses of 100, 200 and 400 mg/kg [26,47,48], namely: Groups I – III (AZ), Groups IV – VI (CY), Groups VII – IX (MO), Groups X – XII (TD) and XIII – XV (VA). Chloroquine (10 mg/kg) and coartem (4 mg/kg) were administered to Groups XVI and XVII as reference drugs and distilled water (0.2 ml) was given to Group XVIII as negative control, while XIX (uninfected and untreated) represented Normal Group. Drugs were administered once daily, using a metal-feeding cannula for 4 days. On the fifth day ( $D_5$ ), the parasitaemia levels were assessed by examining and counting the parasitized and total red blood cells in the Giemsa-stained (Giemsa in Phosphate

buffer) blood smears. The percentage chemosuppression was determined by recording the number of parasitized red blood cells out of every 500 red blood cells counted in 8 random fields under light microscope at  $\times 1000$  magnification and calculating the average percentage parasitaemia suppression as follows:

**Percentage parasitaemia** =  $100 \{N_p / N_t\}$ , where  $N_p$  is the number of parasitised red blood cells and  $N_t$  is the total number of (parasitised + unparasitised) red blood cells per view of count;

**Percentage chemosuppression** =  $100 \{(A - B) / A\}$ , where  $A$  is the average percentage parasitaemia in the negative control Group and  $B$  is the average parasitaemia in the test Group [49,50].

### 2.5 Haematological Analysis

Three mice from each Group were anaesthetized, its blood collected through cardiac puncture and kept in vacutainer tubes containing anticoagulant (ethylenediamine tetraacetic acid [EDTA]) for haematological analysis. The blood was mixed inside the vacutainer tubes using Blood Mixer (HOSPITEX) RM – 500 and analyzed in an automated Haematology Analyzer; BECKMAN COULTER AC-T 5diff CP, Kraemer Blvd, Brea, USA (WBC Lyse, Fix, Hgb Lyse, Rinse) to determine the Pack Cell Volume (PCV), White Blood Cell (WBC), Red Blood Cell (RBC), Haemoglobin (HGB), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), Platelet, Mean Platelet Volume (MPV), Neutrophils (NE), Lymphocytes (LY), Monocytes (MO), Eosinophils (EO) and Basophils (BA).

### 2.6 Data Analysis

The percentage parasitaemia reduction was expressed as mean  $\pm$  SEM while the effective doses were determined using Microsoft Excel (2016) and the variation in a set of data was analyzed through the one-way analysis of variance. The difference among the means was considered at 95% confidence level using the post-hoc method of Tukey's Multiple Comparison Test through Graph Pad Prism8.4.2 software 2020 version.

### 3. RESULTS AND DISCUSSION

All the mice treated with AZ, CY, MO, TD and VA showed varying percentage parasitaemia, relative to negative control mice (25.2%), followed by their corresponding percentage chemosuppression values, respectively (Table 1). For example, VA gave 13.5 to 6.3% parasitaemia at 100 to 400 mg/kg which were significantly different from negative control (25.2%). Percentage parasitaemia at 400 mg/kg (6.3 %) was not significantly different ( $q=4.22$ ;  $p=0.057$ ) from that of CQ at 10 mg/kg (1.41%). The corresponding % chemosuppression at 100 (46.4%), 200 (63.9%) and 400 (75.1%) mg/kg were significantly different from one another.

TD gave 15.8 to 8.6% parasitaemia at 100 to 400 mg/kg which were significantly different from negative control (25.5%). Correspondingly, TD gave chemosuppression of 37.3% at 100 mg/kg that was significantly different from chemosuppression of 64.6 and 65.7 % at 200 and 400 mg/kg, respectively (Table 1). Percentage chemosuppression exhibited at 200 and 400 mg/kg were not significantly different from each other ( $q = 0.59$ ;  $P = .99$ ).

MO showed 14.8 to 9.9% parasitaemia at 100 to 400 mg/kg, respectively (Table 1) which were significantly different from negative control (25.5%) as shown in Table 1. MO gave a corresponding chemosuppression of 41.2% at 100 mg/kg that was significantly different from chemosuppression of 54.3 and 60.6% at 200 and 400 mg/kg, respectively. Whereas, chemosuppression at 200 (54.3%) and 400 (60.6%) mg/kg were not significantly different ( $q = 2.66$ ;  $P = .36$ ) from each other (Table 1).

AZ showed 18.13 % parasitaemia at 100 mg/kg (not significantly different from negative control), 14.37 % at 200 mg/kg and 11.37% at 400 mg/kg which were significantly different from the negative control (25.2%), respectively. Correspondingly, AZ gave chemosuppression of 43.0% and 54.9% at 200 and 400 mg/kg, respectively (Table 1) but were not significantly different from each other ( $q = 3.24$ ;  $P = .18$ ) when compared with the lowest dose.

Also, CY showed 19.7% parasitaemia at 100 mg/kg (not significantly different from negative control), 14.15% at 200 mg/kg and 13.2% at 400 mg/kg (Table 1) which were significantly different from the negative control (25.2%), respectively.

CY gave corresponding chemosuppression of 43.9 and 47.7% at 200 and 400 mg/kg, respectively which were not significantly different from each other ( $q = 1.54$ ;  $P = .81$ ).

Thus, the chemosuppressive activities of AZ, CY and TD at 100 mg/kg were not significantly different from one another and similarly at that dose, the activities of MO and VA were not significantly different from each other. At 200 mg/kg, MO, TD and VA extracts gave chemosuppressive activities that were comparable to one another while MO gave a comparable activity with those of AZ and CY. And at 400 mg/kg, VA gave chemosuppressive activity of 75.1 % which was comparable ( $q = 3.60$ ;  $P = 0.18$ ) to that of MO with 65.8 % (Table 1). The  $ED_{50}$  (effective doses) for MO, TD and VA with 231, 195, and 141mg/kg, respectively, were not significantly different from one another but VA ( $ED_{50}$  141 mg/kg) was significantly different from AZ ( $ED_{50}$  319 mg/kg) and CY ( $ED_{50}$  346 mg/kg). The  $ED_{90}$  values of 455 and 454 mg/kg for TD and VA, respectively showed no significant difference (Graph. 1). The negative control group gave a survival time of only 7 days while 25 % of mice, treated with AZ and CY extracts survived for up to day 10 days. However, 50 % of mice treated with either VA or MO gave survival time of 14 days while 50 % mice with TD survived for up to 18 days. The reference standards (CQ and ACT), with 100 % live animals, gave a survival time of 28 days.

The results of the haematological studies showed that the malarial infection, as demonstrated in the untreated but infected negative control mice, produced significantly reduced Red Blood Cell, Haemoglobin, Haematocrit, White Blood Cell, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, Mean Corpuscular Haemoglobin Concentration, Red Cell Distribution Width, Platelet, Mean Platelet Volume, Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils. The reduction in the normal haematological indices, indicating malarial infection, were reversed following the treatments with MO, TD and VA, not significantly different from the uninfected Normal Group but were significantly different from the effects in the untreated infected negative control animals. AZ at the medium dose of 200 mg/kg and CY the highest dose of 400 mg/kg were significantly different from the uninfected Normal Group mice as they did not significantly reverse the haematological defects observed in the untreated but infected negative control Group.

Table 1. Antimalarial activities of the five plant extracts on *P. berghei* ANKA at varying doses

Dose/ Test Drugs	100 mg/kg		200 mg/kg		400 mg/kg	
	% Parasitaemia	% Chemosuppression	% Parasitaemia	% Chemosuppression	% Parasitaemia	% Chemosuppression
AZ	18.13 ± 1.22 (q = 3.77; P = .07)	28.12 ± 4.83 <sup>a,t,g</sup>	14.37 ± 1.25 <sup>***</sup> (q = 6.23; P = .002)	43.04 ± 4.97 <sup>a,b,i</sup>	11.37 ± 1.27 <sup>***</sup> (q = 7.36; P = .0003)	54.91 ± 5.03 <sup>b,k,i</sup>
CY	19.72 ± 0.99 (q = 2.73; P = .26)	21.81 ± 3.90 <sup>l</sup>	14.15 ± 1.00 <sup>***</sup> (q = 6.85; P = .0009)	43.89 ± 3.98 <sup>c,i</sup>	13.20 ± 0.50 <sup>***</sup> (q = 6.89; P = .0009)	47.68 ± 1.98 <sup>c,j</sup>
MO	14.84 ± 1.51 (q = 5.80; P = .004)	41.18 ± 5.97 <sup>g,h</sup>	11.52 ± 0.74 <sup>***</sup> (q = 8.76; P < .0001)	54.31 ± 2.94 <sup>d,i,j</sup>	9.93 ± 10.64 <sup>***</sup> (q = 9.06; P < .0001)	60.63 ± 1.02 <sup>d,k</sup>
TD	15.81 ± 0.50 <sup>***</sup> (q = 5.77; P = .004)	37.31 ± 1.99 <sup>t,h</sup>	9.43 ± 0.48 <sup>***</sup> (q = 10.12; P < .0001)	64.63 ± 1.35 <sup>e,j</sup>	8.63 ± 1.07 <sup>***</sup> (q = 10.18; P < .0001)	65.78 ± 4.26 <sup>e,k,m</sup>
VA	13.51 ± 0.68 <sup>***</sup> (q = 8.95; P < .0001)	46.42 ± 2.70 <sup>h</sup>	9.10 ± 1.17 <sup>***</sup> (q = 12.87; P < .0001)	63.92 ± 4.64 <sup>l</sup>	6.27 ± 0.71 <sup>***</sup> (q = 15.13; P < .0001)	75.13 ± 2.80 <sup>m</sup>
CQ (10 mg/kg)	1.41 ± 0.12 <sup>***</sup> (q = 19.66; P < .0001)	94.41 ± 0.46 <sup>n</sup>	1.41 ± 0.12 <sup>***</sup> (q = 19.66; P < .0001)	94.41 ± 0.46 <sup>n</sup>	1.41 ± 0.12 <sup>***</sup> (q = 19.66; P < .0001)	94.41 ± 0.46 <sup>n</sup>
ACT (4 mg/kg)	0.75 ± 0.09 <sup>***</sup> (q = 20.20; P < 0.0001)	97.04 ± 0.36 <sup>n</sup>	0.75 ± 0.09 <sup>***</sup> (q = 20.20; P < .0001)	97.04 ± 0.36 <sup>n</sup>	0.75 ± 0.09 <sup>***</sup> (q = 20.20; P < .0001)	97.04 ± 0.36 <sup>n</sup>
DW (200 µL)	25.22 ± 3.09	0.00	25.22 ± 3.09	0.00	25.22 ± 3.09	0.00

Data are expressed as Mean ± SEM, \*\*\*P < .001, \*\*P < .01, \*P < .05 compared to negative control (DW); same superscripted letter means P = .05

AZ=Azadirachta indica, CY=Cymbopogon citratus, MO=Moringa oleifera, TD=Titonia diversifolia, VA=Vernonia amygdalina, CQ=Chloroquine, ACT=Artemisinin-based Combination Therapy (Artemether–Lumefantrine)

Table 2. Haematological response of mice treated with the five plant extracts

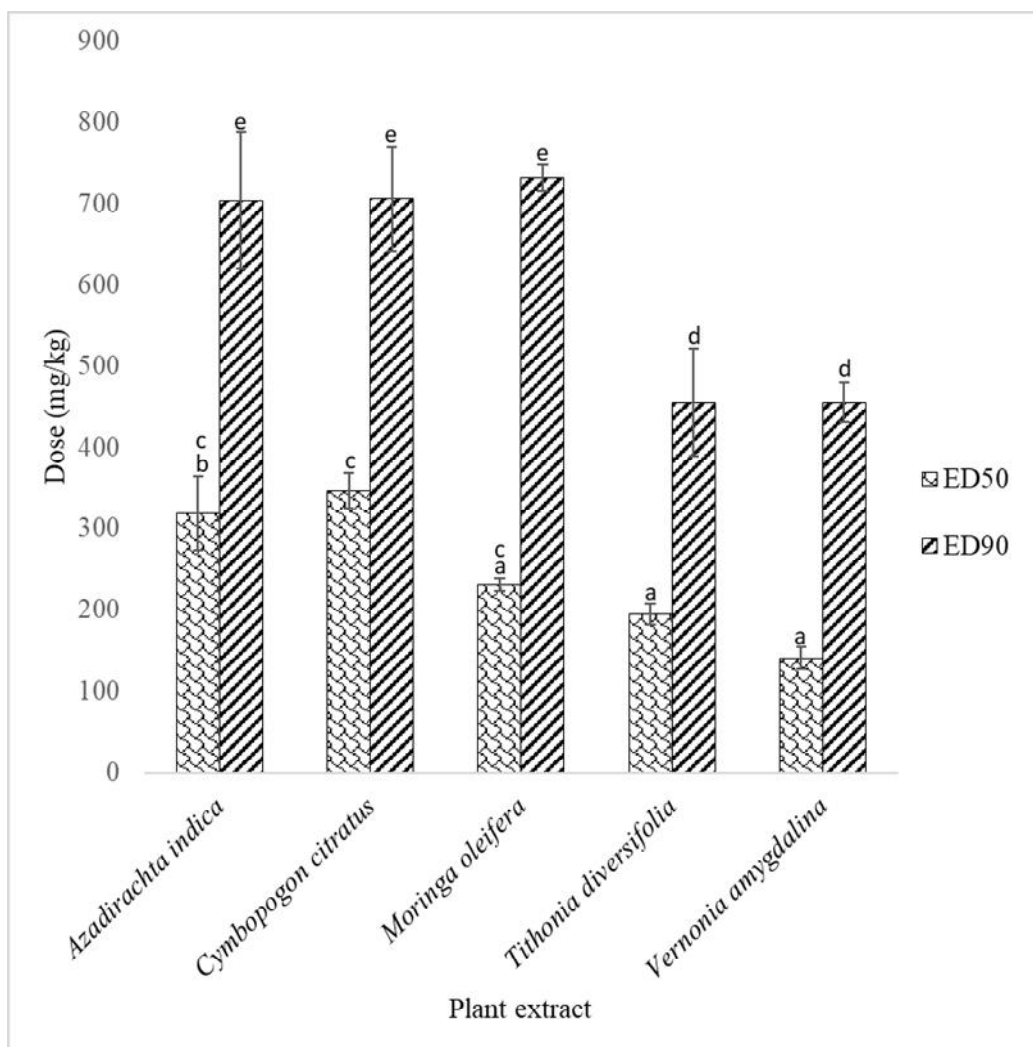
Parameters/ Test drugs (mg/kg)	WBC ( $\times 10^9/L$ )	RBC ( $\times 10^{12}/L$ )	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT	MPV	NE (%)	LY (%)	MO (%)	EO (%)	BA (%)
Normal	8.16 $\pm$ 0.68 <sup>a</sup>	8.94 $\pm$ 0.20 <sup>b</sup>	14.56 $\pm$ 0.24 <sup>c</sup>	43.54 $\pm$ 0.74 <sup>d</sup>	48.86 $\pm$ 1.46 <sup>e</sup>	16.29 $\pm$ 0.22 <sup>f</sup>	33.41 $\pm$ 0.23 <sup>g</sup>	12.73 $\pm$ 0.31 <sup>h</sup>	1035.00 $\pm$ 72.92 <sup>i</sup>	6.00 $\pm$ 0.22 <sup>j</sup>	13.01 $\pm$ 2.94 <sup>k</sup>	72.23 $\pm$ 2.62 <sup>l</sup>	12.63 $\pm$ 1.14 <sup>m</sup>	0.60 $\pm$ 0.18 <sup>n</sup>	1.53 $\pm$ 0.51 <sup>o</sup>
AZ 100	10.93 $\pm$ 0.58 <sup>a</sup>	5.24 $\pm$ 0.89	9.20 $\pm$ 1.19	24.77 $\pm$ 3.44	48.00 $\pm$ 2.00	17.83 $\pm$ 0.88	37.20 $\pm$ 0.35	13.67 $\pm$ 0.29 <sup>h</sup>	349.70 $\pm$ 4.37	7.67 $\pm$ 0.54	6.27 $\pm$ 1.20 <sup>k</sup>	78.00 $\pm$ 1.65 <sup>l</sup>	8.90 $\pm$ 1.86 <sup>m</sup>	0.07 $\pm$ 0.07 <sup>n</sup>	6.77 $\pm$ 0.90
200	10.63 $\pm$ 1.53 <sup>a</sup>	6.29 $\pm$ 0.15 <sup>b</sup>	11.17 $\pm$ 0.55 <sup>c</sup>	31.33 $\pm$ 0.82	50.00 $\pm$ 0.58	17.70 $\pm$ 0.80	35.47 $\pm$ 1.32 <sup>g</sup>	13.50 $\pm$ 0.93 <sup>h</sup>	304.50 $\pm$ 145.50	9.30 $\pm$ 0.60	3.57 $\pm$ 1.42 <sup>k</sup>	81.97 $\pm$ 3.88 <sup>l</sup>	7.23 $\pm$ 2.41 <sup>m</sup>	0.00 <sup>n</sup>	7.23 $\pm$ 0.09
400	13.80 $\pm$ 5.88 <sup>a</sup>	7.11 $\pm$ 1.03 <sup>b</sup>	12.13 $\pm$ 1.95 <sup>c</sup>	35.20 $\pm$ 6.86 <sup>d</sup>	49.00 $\pm$ 2.3	17.00 $\pm$ 0.42	34.97 $\pm$ 1.24 <sup>g</sup>	12.70 $\pm$ 0.46 <sup>h</sup>	530.70 $\pm$ 161.20	7.40 $\pm$ 0.31 <sup>j</sup>	5.03 $\pm$ 1.29 <sup>k</sup>	74.20 $\pm$ 7.47 <sup>l</sup>	14.47 $\pm$ 3.26 <sup>m</sup>	0.13 $\pm$ 0.13 <sup>n</sup>	6.07 $\pm$ 0.94
CY 100	10.30 $\pm$ 1.94 <sup>a</sup>	7.53 $\pm$ 1.73 <sup>b</sup>	12.60 $\pm$ 2.38 <sup>c</sup>	37.50 $\pm$ 8.50 <sup>d</sup>	50.00 $\pm$ 0.58	17.23 $\pm$ 1.15	34.57 $\pm$ 2.44 <sup>g</sup>	13.67 $\pm$ 6.89 <sup>h</sup>	861.00 $\pm$ 93.09 <sup>i</sup>	6.30 $\pm$ 0.35 <sup>j</sup>	6.67 $\pm$ 1.84 <sup>k</sup>	79.07 $\pm$ 2.96 <sup>l</sup>	10.20 $\pm$ 3.07 <sup>m</sup>	0.13 $\pm$ 0.07 <sup>n</sup>	3.93 $\pm$ 1.93
200	6.73 $\pm$ 1.14 <sup>a</sup>	8.62 $\pm$ 0.26 <sup>b</sup>	14.20 $\pm$ 0.27 <sup>c</sup>	43.20 $\pm$ 2.16 <sup>d</sup>	50.00 $\pm$ 1.53	16.50 $\pm$ 0.25	32.90 $\pm$ 1.55 <sup>g</sup>	13.23 $\pm$ 0.19 <sup>h</sup>	724.70 $\pm$ 137.10 <sup>i</sup>	8.30 $\pm$ 0.45	6.77 $\pm$ 1.62 <sup>k</sup>	73.10 $\pm$ 4.81 <sup>l</sup>	13.30 $\pm$ 3.64 <sup>m</sup>	0.20 $\pm$ 0.20 <sup>n</sup>	6.63 $\pm$ 0.87
400	10.77 $\pm$ 0.72 <sup>a</sup>	5.73 $\pm$ 0.81	9.43 $\pm$ 1.14	27.23 $\pm$ 3.61	47.67 $\pm$ 0.33	16.57 $\pm$ 0.52	34.70 $\pm$ 0.81 <sup>g</sup>	14.80 $\pm$ 0.21	182.00 $\pm$ 40.81	7.97 $\pm$ 0.46	6.47 $\pm$ 1.87 <sup>k</sup>	76.10 $\pm$ 6.15 <sup>l</sup>	11.03 $\pm$ 3.46 <sup>m</sup>	0.00 <sup>n</sup>	6.40 $\pm$ 0.95
MO 100	14.10 $\pm$ 0.10 <sup>a</sup>	6.11 $\pm$ 0.11 <sup>b</sup>	10.25 $\pm$ 0.25 <sup>c</sup>	29.45 $\pm$ 0.45 <sup>d</sup>	48.00 $\pm$ 0.00	16.75 $\pm$ 0.05 <sup>f</sup>	35.00 $\pm$ 0.00 <sup>g</sup>	12.75 $\pm$ 0.05 <sup>h</sup>	197.50 $\pm$ 32.50 <sup>i</sup>	8.55 $\pm$ 0.05	8.95 $\pm$ 3.35 <sup>k</sup>	73.85 $\pm$ 2.75 <sup>l</sup>	10.55 $\pm$ 0.45 <sup>m</sup>	0.00 <sup>n</sup>	6.65 $\pm$ 0.15
200	6.07 $\pm$ 1.56 <sup>a</sup>	7.79 $\pm$ 0.67 <sup>b</sup>	12.93 $\pm$ 0.94 <sup>c</sup>	39.13 $\pm$ 3.76 <sup>d</sup>	50.00 $\pm$ 0.58	16.63 $\pm$ 0.29 <sup>f</sup>	33.13 $\pm$ 0.95 <sup>g</sup>	12.50 $\pm$ 0.50 <sup>h</sup>	621.70 $\pm$ 152.90	6.70 $\pm$ 0.60 <sup>j</sup>	13.70 $\pm$ 4.05 <sup>k</sup>	61.03 $\pm$ 6.75 <sup>l</sup>	19.27 $\pm$ 4.46 <sup>m</sup>	0.17 $\pm$ 0.12 <sup>n</sup>	5.83 $\pm$ 1.97
400	7.85 $\pm$ 1.55 <sup>a</sup>	6.22 $\pm$ 2.97 <sup>b</sup>	10.90 $\pm$ 4.80 <sup>c</sup>	31.55 $\pm$ 14.85 <sup>d</sup>	50.50 $\pm$ 0.50	17.90 $\pm$ 0.80	35.20 $\pm$ 1.30 <sup>g</sup>	13.10 $\pm$ 0.60 <sup>h</sup>	409.00 $\pm$ 56.00	7.80 $\pm$ 0.60	7.05 $\pm$ 2.65 <sup>k</sup>	73.20 $\pm$ 12.90 <sup>l</sup>	12.95 $\pm$ 6.25 <sup>m</sup>	0.10 $\pm$ 0.10 <sup>n</sup>	6.70 $\pm$ 3.90
TD 100	11.43 $\pm$ 3.29 <sup>a</sup>	7.47 $\pm$ 0.90 <sup>b</sup>	12.47 $\pm$ 1.05 <sup>c</sup>	35.83 $\pm$ 4.70 <sup>d</sup>	48.00 $\pm$ 0.58	16.80 $\pm$ 0.72 <sup>f</sup>	35.20 $\pm$ 1.72	14.27 $\pm$ 0.22	437.70 $\pm$ 133.70	7.03 $\pm$ 0.54	5.87 $\pm$ 2.43 <sup>k</sup>	79.90 $\pm$ 2.65 <sup>l</sup>	8.10 $\pm$ 2.06 <sup>m</sup>	0.00 <sup>n</sup>	6.13 $\pm$ 2.97
200	8.13 $\pm$ 1.38 <sup>a</sup>	7.78 $\pm$ 0.27 <sup>b</sup>	12.90 $\pm$ 0.23 <sup>c</sup>	39.07 $\pm$ 0.84 <sup>d</sup>	50.33 $\pm$ 0.88	16.63 $\pm$ 0.34 <sup>f</sup>	33.07 $\pm$ 0.13	13.27 $\pm$ 0.39 <sup>h</sup>	407.30 $\pm$ 65.53	8.33 $\pm$ 0.27	5.77 $\pm$ 1.88 <sup>k</sup>	73.87 $\pm$ 3.94 <sup>l</sup>	12.80 $\pm$ 1.82 <sup>m</sup>	0.13 $\pm$ 0.88 <sup>n</sup>	7.43 $\pm$ 0.54
400	11.97 $\pm$ 2.95 <sup>a</sup>	7.37 $\pm$ 1.41 <sup>b</sup>	12.80 $\pm$ 1.71 <sup>c</sup>	36.43 $\pm$ 6.90 <sup>d</sup>	49.33 $\pm$ 0.67	17.83 $\pm$ 1.35	36.07 $\pm$ 2.52	13.87 $\pm$ 0.90 <sup>h</sup>	700.70 $\pm$ 362.2 <sup>i</sup>	9.30 $\pm$ 1.71	8.90 $\pm$ 2.07 <sup>k</sup>	68.17 $\pm$ 6.95 <sup>l</sup>	14.40 $\pm$ 3.86 <sup>m</sup>	0.07 $\pm$ 0.03 <sup>n</sup>	8.47 $\pm$ 1.07
VA 100	12.00 $\pm$ 1.50 <sup>a</sup>	3.86 $\pm$ 0.14	7.25 $\pm$ 0.45	19.00 $\pm$ 0.90	49.50 $\pm$ 0.50	18.75 $\pm$ 0.45	38.00 $\pm$ 0.40	13.05 $\pm$ 0.65 <sup>h</sup>	420.50 $\pm$ 82.50	8.60 $\pm$ 0.20	3.70 $\pm$ 0.60 <sup>k</sup>	84.85 $\pm$ 1.05 <sup>l</sup>	6.40 $\pm$ 0.60 <sup>m</sup>	0.00 <sup>n</sup>	5.05 $\pm$ 0.15
200	10.65 $\pm$ 0.75 <sup>a</sup>	5.77 $\pm$ 0.21 <sup>c</sup>	10.45 $\pm$ 0.25 <sup>c</sup>	28.50 $\pm$ 0.10	49.00 $\pm$ 2.00	18.05 $\pm$ 0.25	36.60 $\pm$ 0.90	13.20 $\pm$ 0.90 <sup>h</sup>	303.50 $\pm$ 47.50	8.45 $\pm$ 1.55	3.00 $\pm$ 0.60 <sup>k</sup>	87.20 $\pm$ 2.00	3.60 $\pm$ 0.80	0.05 $\pm$ 0.05 <sup>n</sup>	6.15 $\pm$ 0.55
400	9.17 $\pm$ 2.52 <sup>a</sup>	7.68 $\pm$ 0.90 <sup>b</sup>	13.17 $\pm$ 1.62 <sup>c</sup>	37.87 $\pm$ 4.94 <sup>d</sup>	49.00 $\pm$ 0.58	17.10 $\pm$ 0.12 <sup>f</sup>	34.83 $\pm$ 0.26 <sup>g</sup>	13.30 $\pm$ 0.64 <sup>h</sup>	460.00 $\pm$ 162.80	7.97 $\pm$ 0.29	7.33 $\pm$ 1.26 <sup>k</sup>	73.80 $\pm$ 3.35 <sup>l</sup>	13.23 $\pm$ 1.65 <sup>m</sup>	0.07 $\pm$ 0.03 <sup>n</sup>	5.57 $\pm$ 0.92

Parameters/ Test drugs (mg/kg)	WBC ( $\times 10^9/L$ )	RBC ( $\times 10^{12}/L$ )	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT	MPV	NE (%)	LY (%)	MO (%)	EO (%)	BA (%)
DW (200 $\mu$ L)	12.30 $\pm$ 1.00 <sup>a</sup>	6.56 $\pm$ 0.39	11.38 $\pm$ 0.43	30.88 $\pm$ 2.12	46.75 $\pm$ 0.75	17.45 $\pm$ 0.41	37.15 $\pm$ 1.24	14.15 $\pm$ 0.84	575.70 $\pm$ 165.50	7.45 $\pm$ 0.19	7.88 $\pm$ 2.12 <sup>k</sup>	71.98 $\pm$ 5.86 <sup>l</sup>	12.48 $\pm$ 3.59 <sup>m</sup>	0.03 $\pm$ 0.03 <sup>n</sup>	7.65 $\pm$ 0.46
CQ (10)	5.27 $\pm$ 1.11 <sup>a</sup>	8.45 $\pm$ 0.35 <sup>b</sup>	14.20 $\pm$ 0.60 <sup>c</sup>	41.70 $\pm$ 2.43 <sup>d</sup>	49.33 $\pm$ 1.20	16.80 $\pm$ 0.10 <sup>f</sup>	34.10 $\pm$ 0.85 <sup>g</sup>	12.77 $\pm$ 0.44 <sup>h</sup>	848.30 $\pm$ 122.30 <sup>i</sup>	6.17 $\pm$ 0.18 <sup>j</sup>	14.77 $\pm$ $\pm$ 5.29 <sup>k</sup>	70.77 $\pm$ 7.38 <sup>l</sup>	10.97 $\pm$ 2.78 <sup>m</sup>	0.37 $\pm$ 0.13 <sup>n</sup>	3.13 $\pm$ 1.84
ACT (4)	5.60 $\pm$ 1.80 <sup>a</sup>	8.49 $\pm$ 0.29 <sup>b</sup>	13.90 $\pm$ 0.40 <sup>c</sup>	44.70 $\pm$ 3.10 <sup>d</sup>	52.50 $\pm$ 1.50	16.35 $\pm$ 0.50 <sup>f</sup>	31.15 $\pm$ 1.25 <sup>g</sup>	13.15 $\pm$ 0.15 <sup>h</sup>	860.50 $\pm$ 159.50 <sup>i</sup>	5.85 $\pm$ 0.05 <sup>j</sup>	7.85 $\pm$ 1.65 <sup>k</sup>	71.50 $\pm$ 5.90 <sup>l</sup>	17.30 $\pm$ 3.80 <sup>m</sup>	0.30 $\pm$ 0.30 <sup>n</sup>	3.05 $\pm$ 0.75

Data are expressed as Mean  $\pm$  SEM, values carrying different superscripts from the negative control for each parameter are significantly different ( $P < .05$ )

WBC=White Blood Cell; RBC=Red Blood Cell; HGB=Haemoglobin; HCT=Haematocrit; MCV=Mean Corpuscular Volume; MCH=Mean Corpuscular Haemoglobin; MCHC=Mean Corpuscular Haemoglobin Concentration; RDW=Red Cell Distribution Width; PLT=Platelet; MPV=Mean Platelet Volume; NE=Neutrophils; LY=Lymphocytes; MO=Monocytes; EO=Eosinophils; BA=Basophils; Normal=uninfected/untreated control group; DW=infected and treated with distilled water control group; AZ=Azadirachta indica; CY=Cymbopogon citratus; MO=Moringa oleifera; TD=Tithonia diversifolia; VA=Vernonia amygdalina; CQ=Chloroquine; ACT=Artemisinin-based Combination Therapy





**Graph 1. Effective doses (ED<sub>50</sub> and ED<sub>90</sub>) of plant extracts in the chemosuppressive test**

Data are expressed as Mean  $\pm$  SEM, same superscripted letter means  $P = .05$

All the five plant extracts, AZ, CY, MO, TD and VA, exhibited varying dose-dependent antimalarial activities from 100 to 400 mg/kg. VA was the most active, followed by TD and MO while CY exhibited the least antimalarial activity. Although, AZ gave an average antimalarial activity of 54.91% chemosuppression but at the highest dose (400 mg/kg) and also at medium dose (200 mg/kg), it gave 43.04 %, similar to 43.89% by CY (adjudged the least active), hence, AZ was not considered a promising candidate, which has supported the previous report of Farahna et al. [48] on the antimalarial inefficiency of its ethanol extract. The dose-dependent activities of TD ethanol extract was reported by Elufioye and Agbedahunsi [26] at 100 – 400 mg/kg, supporting the results of the

present study which showed TD as potent in dose-dependent manner; for example, the results of 200 mg/kg dose (64.6%) and 400 mg/kg dose (65.8%) were comparable to the activities of VA at same doses with 63.9% and 75.1% activities, respectively. Some compounds, e.g. sesquiterpene lactones, predominantly tagitinin C, isolated from TD, were found to possess antiplasmodial activities [28] and they could be partly responsible for the observed antimalarial actions in the present study. CY gave the lowest activity (47.68%) in the present study at the highest dose of 400mg/kg and 43.89% at the medium dose of 200 mg/kg which correlated with that of Ukpai and Amaechi [51] on the ethanol extract of CY when no noticeable antimalarial activity was found. It is noted however that the

essential oil of CY possessed an *in vitro* antiplasmodial activity [52]. MO (54.3%) at 200 mg/kg in this study, with dose- dependent antimalarial activities, was comparable to VA (63.9 %) and TD (64.6 %) at the same dose but at 400 mg/kg, its activity of 60.6 % was not comparable with VA (75.1%). Then at 400 mg/kg, VA that gave the 75.1 % chemosuppression, was found to be the most promising of the five plant extracts investigated. These results have therefore supported the findings of Abosi and Raseroka [53] on the dose-dependent chemosuppression of VA extract, and that of Njan et al. [53] who reported its curative activities. Furthermore, VA also produced the lowest ED<sub>50</sub> and ED<sub>90</sub> values, thereby supporting its potency over the other four plant materials investigated and hence, the most promising for drug development. Two of the well-known chemical constituents of VA, vernodalol and vernodalin, have been reported to possess antiplasmodial activities [42]. Consequently, the five plants investigated in this study can be rank-ordered in a decreasing order of antimalarial potencies as follows: VA≥TD≥MO>AZ≥CY.

The haematological measurements were necessary to assess the possible harmful effects on the blood parameters and blood constituents, comparatively in both the treated and untreated normal mice [54,55]. These haematological parameters are the dynamic tools for detecting any abnormality in the system of animal or human subjects under study. The results of the haematological experiments showed that the plant extracts at high doses, were able to reverse the blood abnormalities caused by the malarial parasites in the infected and treated animals where their blood parameters fell within the normal ranges. Thus, VA, TD and MO were not only found as the most active extracts but also the best in reversing the haematological eventualities caused by the malaria infection. Further research efforts in our laboratories will be reported later on the development of antimalarial remedies from the three most active plants investigated.

#### 4. CONCLUSION

All the hot infusions of the five medicinal plants studied, following ethnomedical preparation methods by the native users of Uganda, have shown antimalarial activities in varying degrees and thus establishing their folkloric claims by the traditional healers. The hot infusions of MO, TD and VA were found to be the most active plants

which could be developed as antimalarial remedies for Uganda.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

The study protocol was approved by the Mbarara University of Science and Technology Research Ethics Committee (MUREC1/7) and the Uganda National Council for Science and Technology (HS359ES).

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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