

*Full Length Research Paper*

# Antioxidant and anti-plasmodial activity of extracts from three Ugandan medicinal plants

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**Extracts from three plants; *Hallea rubrostipulata*, *Vernonia adoensis* and *Zanthoxylum chalybeum*, were tested for antioxidant activity using three assays 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Ability of Plasma (FRAP) and total phenol content) and anti-plasmodial activity using an Enzyme-Linked Immunosorbent Assay (ELISA) on *Plasmodium falciparum* chloroquine sensitive strain MRA-285 line. The objective of the study was to find candidates for making anti-malarial phytomedicines. The water extract of *H. rubrostipulata* showed very high anti-plasmodial activity (IC<sub>50</sub>= 1.95 µg/ml) and high antioxidant activity as well. Thirteen other extracts had high anti-plasmodial activity ranging from 2.14 to 3.63 µg/ml (chloroquine IC<sub>50</sub>= 8 µg/ml). We found high correlation between the different antioxidant assays.**

**Key words:** *Hallea rubrostipulata*, *Vernonia adoensis*, *Zanthoxylum chalybeum*, 2,2-Diphenyl-1-picrylhydrazyl, fluorescence recovery after photobleaching, total phenolic compounds.

## INTRODUCTION

Malaria is the most lethal parasitic disease in the tropical areas. In 2008 there was an estimated 243 million cases of malaria worldwide, that lead to approximately 863 000 deaths. Most of them were young children in Sub Saharan Africa (WHO, 2009). There are four species of *Plasmodium* parasites causing the disease in human: *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium vivax*. *P. falciparum* can cause cerebral malaria and is the most malignant. The parasites are usually transferred to humans by *Anopheles gambiae* female mosquitoes. The parasites multiply in the liver and later in the erythrocytes in the blood. Malaria has been very difficult to fight in Africa due to the efficient mosquito, *A. gambiae*, transferring

the parasites, a high prevalence of *P. falciparum*, the most deadly species of the parasite vectors, weak infrastructure, high costs to address the disease, and favourable climatic conditions to the vector (Center for Disease Control and Prevention, 2009). The problem has been aggravated by development of resistance by *P. falciparum* towards the most familiar and affordable medicine used for malaria, that is chloroquine.

Anaemia is an important cause of morbidity and mortality in children with acute *P. falciparum* malaria. *Plasmodium*-infected erythrocytes are under constant oxidative stress (Griffiths et al., 2001). In a study of blood from Kenyan children with complicated malaria and malarial anaemia it was found that mean membrane  $\alpha$ -tocopherol was significantly reduced and there was a positive correlation between membrane  $\alpha$ -tocopherol and haemoglobin concentrations in malarial subjects compared to control. However, no significant difference in plasma  $\alpha$ -tocopherol was found. This suggests that in

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severe malaria the red blood cell membrane is exposed to a local increase in oxidative stress, and the erythrocyte's resistance to destruction is linked to the erythrocyte membrane  $\alpha$ -tocopherol reserve. It is suggested that compounds exhibiting both anti-plasmodial and antioxidant activities could be very interesting as leads for new anti-malarial drugs (Griffiths et al., 2001; Nguela et al., 2006). In a study of *Plasmodium yoelii* infection in mice oxidative stress and apoptosis (programmed cell death) of liver was demonstrated (Guha et al., 2006) and OH was shown to be the causative agent. Since application of radical scavengers protects the liver from oxidative stress Guha et al. (2006) recommend a combination therapy consisting of antioxidants against the OH and an antimalarial drug. On the other hand some currently used drugs like artemisinin and methylene blue are believed to act by increasing oxidative stress in the parasites (Becker et al., 2004).

Medicinal plants are still widely used in Uganda to treat malaria (Katura et al., 2007; Ssegawa and Kasenene, 2007; Tabuti, 2008). Some of the plants have been tested for anti-plasmodial activity (Waako et al., 2005; Katuura et al., 2007; Waako et al., 2007). In the West African country Mali an Improved Traditional Medicine (ITM) to treat malaria consisting of three indigenous medicinal plants have been developed (Diallo et al., 2004).

In this study we screened some plants that are commonly used traditionally to treat malaria in Uganda, but where the antioxidant properties of the plants have been poorly investigated. The plants selected for the study included: *Hallea rubrostipulata* (Schum.) J.-F. Leroy (syn: *Mitragyna rubrostipulata*), *Vernonia adoensis* Sch. Bip. ex Walp. (syn: *V. kotschyana* and *Baccharoides adoensis*) and *Zanthoxylum chalybeum* Engl. (syn: *Fagara chalybea*). Flora of Tropical East Africa was used for nomenclature (Beentje, 2000; Bridson and Vercourt, 1988; Kokwaro, 1982). We analyzed the most commonly used plant parts by the respondents in the previous studies (Katura et al., 2007; Ssegawa and Kasenene, 2007; Tabuti, 2008). These included the stem bark of *H. rubrostipulata* and *Z. chalybeum* and the leaves of *V. adoensis*.

Alkaloids have previously been isolated from *H. rubrostipulata* (Seaton et al., 1960; Shellard et al., 1977) and *Z. chalybeum* (Kato et al., 1996). *Z. chalybeum* has been found to exhibit good anti-bacterial (Matu and van Staden, 2003), anti-fungal (Hamza et al., 2006) and anti-plasmodial (Gessler et al., 1994; Rukunga et al., 2009; Muganga et al., 2010; Nguta et al., 2010) activities, while only minimal anti-plasmodial effect was found in *H. rubrostipulata* (Muganga et al., 2010). In several studies on *Z. chalybeum* both root and stem bark were tested and most often the root bark was found to be more active. The stem bark of *Z. chalybeum* is also found to have high antioxidant activity (Kuglerova et al., 2008). The root of *V. adoensis* contains polysaccharides with immunomodulating activity (Nergard et al., 2004) and

steroidal saponins and a glycoside (Sanogo et al., 1998) have been identified. In Mali an ITM to treat gastric ulcer, 'Gastro-sedal', has now been developed from the powdered roots (Nergard et al., 2004). Extracts of leaves were found to have antimicrobial (Deeni and Hussain, 1994), larvicidal and molluscicidal activity (Diallo et al., 2001). In this study we have made five extracts: acetone, dichloromethane, methanol, CMW (chloroform: methanol: water [12:5:3]), and water, from each of the above plant species. The extracts have been tested for radical scavenger effect by the Diphenyl-1-picrylhydrazyl (DPPH) assay, antioxidant activity by the Ferric Reducing Ability of Plasma (FRAP) method and total content of phenolic compounds using the Folin-Ciocalteu reagent. Different Thin Layer Chromatography (TLC) methods to investigate the presence of main secondary compounds (alkaloids, flavonoids, saponins and bitter principles) were carried out, as well as Nuclear Magnetic Resonance (NMR) of the crude extracts. Anti-plasmodium effect was tested using an Enzyme-Linked Immunosorbent Assay (ELISA) that quantifies parasite histidine-rich protein-2 (HRP-2) (40).

Several studies have demonstrated increased oxidative stress in plasmodium infected red blood cells and liver cells. High anti-plasmodial activity has lately been demonstrated in extracts from *Z. chalybeum*. We believe that combination therapies with plant extracts high in antioxidant activity together with a compound that more specifically target and harm the *P. falciparum* parasites would be good candidates for future antimalarial design. In this study we wanted to detect antioxidant activity in the extracts and if there are correlation between the different antioxidant assays; basic properties in the raw extracts, and investigate if we can find better anti-plasmodial activity in some of the extracts.

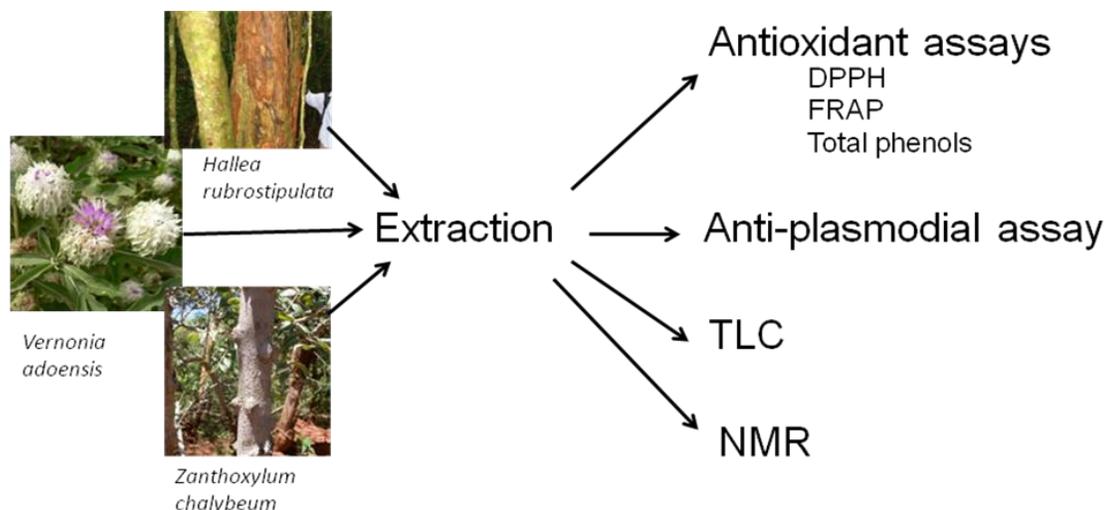
## MATERIALS AND METHODS

### Plant materials

The bark of *H. rubrostipulata* was collected from Sango Bay in Rakai district, leaf material of *V. adoensis* from Mbarara District, while the bark of *Z. chalybeum* was collected in Nakosongola District north of Kampala, all in Uganda. The plant samples for laboratory analysis were air dried in shade to constant weight. Voucher specimen is deposited in the Herbarium of Makerere University (MHU) and at the Norwegian University of Life Sciences (NLH). The plant parts were ground on a laboratory mill, and sieved through a fine meshed copper sieve (0.250 microns mesh width).

### Extraction of plant materials

For each plant 1 g finely ground plant material was extracted with 10 ml solvent in a centrifuge tube. The extract was decanted after centrifugation at 1800 x g for 5 min at 25°C basically as described by Elloff (1998). The process was repeated 3 times. The extractants used were technical grade acetone, Dichloromethane (DCM), methanol (MeOH), a mixture of methanol, chloroform and water



**Figure 1.** Picture of the three plants analyzed in this study. The arrows show the extraction process and different assays conducted.

(MCW; 12:5:3) and water. The extracts were concentrated to dryness using a rotavapor with temperature 40°C, and the dried extracts were stored at -20°C until use. They were re-dissolved in water or methanol to prepare solutions of different concentrations for further studies (Figure 1).

#### Free radical scavenger and antioxidant bioassays DPPH radical scavenging

For testing radical scavenging activity, reaction with the DPPH radical was carried out as previously described (Wangensteen et al., 2004). Extracts were dissolved in methanol, and quercetin (Sigma-Aldrich) was used as a positive control. A Biochrom Libra S32 spectrophotometer (Biochrom, Cambridge, England) was used for measurements of ultraviolet (UV) absorbance at 517 nm.

#### Antioxidant activity using the ferric reducing ability of plasma method

Antioxidant activity in the samples was measured using the Ferric Reducing Ability of Plasma (FRAP) assay (Benzie and Strain, 1996), using some modification (Halvorsen et al., 2002; Stangeland et al., 2009) where the samples were diluted in methanol instead of water. The FRAP-method measures the absorption change that appears when the TPTZ-Fe<sup>3+</sup> complex is reduced to TPTZ-Fe<sup>2+</sup> form in the presence of antioxidants. An intense blue colour with absorption maximum at 593 nm develops. Our measurements were performed at 595 nm with incubation time of 10 minutes at 37 °C by using the KoneLab 30i spectrophotometer (Kone Instruments Corp., Espoo, Finland). The antioxidant activity in the samples was calculated as μmol Fe<sup>2+</sup> per gram (μmol/g) extract.

#### Total phenolic compounds

The total amount of phenolic compounds (TP) of the different extracts was determined using the KoneLab 30i spectrophotometer. The procedure was based on using the Folin-Ciocalteu Reagent

(FCR) (Singleton et al., 1999). In brief, 20 μl of extract were added to 100 μl FCR (diluted 1:10 with dist. water), mixed and incubated at 37°C for 60 s, 80 μl of 7.5 % (w/v) sodium bicarbonate solution was added, the samples were again mixed and incubated at 37°C for 15 min prior to absorbance reading at 765 nm. TP was assessed against a calibration curve of gallic acid, and the results presented as mg Gallic Acid Equivalents (GAE) per g extract.

#### Thin layer chromatography

The extracts were tested in different TLC systems and spray reagents to show the presence of alkaloids, flavonoids, saponins and bitter principles following standard procedures as described by Wagner and Blatt (1996). Components were visualised by UV irradiation (254 and 365 nm) or visual light.

#### Nuclear magnetic resonance

<sup>1</sup>H- and <sup>13</sup>C-NMR spectra were recorded using a Bruker DPX 300 instrument (Bruker Biospin GmbH, Rheinstetten, Germany). Samples were dissolved in CDCl<sub>3</sub>, CD<sub>3</sub>OD or DMSO d-6, and TMS was used as reference.

#### In vitro anti-plasmodial assay

##### Blood stage assays

To test anti-plasmodial activity of the plant extracts the susceptibility micro assay technique was used. *P. falciparum* chloroquine sensitive strain MRA-285 line was maintained in a continuous culture by the method of Trager and Jensen (1976) and used in these assays. The vial of the frozen strain was removed from liquid nitrogen; thawed at 37°C in a water bath for 2 min without shaking. The contents of the vial were transferred into a 50 ml centrifuge tube. 0.1 ml of 12% NaCl solution was added drop-wise to the pellet at a rate of 1 drop per second with constant agitation. 10 ml of 1.6% NaCl was then added drop wise to the pellet at a rate 1 drop per

second with constant agitation to achieve complete mixing. The contents were centrifuged at 2000 RPM for 5 min and the supernatant discarded. 10 ml of pre-warmed complete culture medium was added and the mixture transferred to the culture flask. Giemsa-stained thin blood smears were prepared and examined, parasitemia was determined and contents put in a candle jar and incubated at 37°C for 24 h. After incubation the parasitemia of the surviving trophozoites was determined. The contents were spun at 2000 RPM for 5 min at RT, supernatant removed and discarded. 10 ml of pre-warmed complete culture medium was added and fresh RBC to 2% hematocrit and parasitemia adjusted to less than 1%. 10 ml RPMI 1640 medium supplemented with 25 mM HEPES, 0.2% NaHCO<sub>3</sub>, 0.1 mM hypoxanthine, 100 µg/ml gentamicin, and 0.5% Albumax II serum substitute to produce a packed cell volume of ~2% was used.

### Measurement of *in vitro* drug sensitivity

Sensitivities were measured for chloroquine diphosphate (CQ) (Sigma-Aldrich) as the positive control, no drug as negative control and the plant crude extracts as test samples. Since chloroform is toxic to the parasites, chloride water mixed with methanol was used instead for the CMW extracts. The measurements were performed as described by Nsohya et al. (2010), except that crude plant extracts instead of drugs were tested. All procedures were performed with a vortex in the hood and the surface was wiped with 70% alcohol in order to obtain aseptic conditions.

### Statistical analysis

All assays were analysed in triplicate. Inhibitory concentrations (IC<sub>50</sub>s) for antimalarials were calculated using a polynomial regression model and HN-NonLin software, which is available at <http://malaria.farcH.net>. Correlations between drugs were evaluated by Pearson correlation. Associations between drug sensitivities and specific parasite polymorphisms were evaluated using the Fisher exact test. For all statistical tests, the significance level was set at  $P < 0.05$ . Correlations between the different antioxidant and radical scavenger assays were run in Minitab and the Pearson correlation was calculated.

## RESULTS AND DISCUSSION

The acetone extract of *H. rubrostipulata* had highest antioxidant activity with IC<sub>50</sub> DPPH 16.6 µg/ml, FRAP 5370.5 µmol/g and total phenols 370.7 mg GAE/g (Table 1, Figure 2). Methanol, Chloroform, Methanol, Water (CMW) and water extract of both *H. rubrostipulata* and *Z. chalybeum* showed quite high activity ranging from 23.9 to 46 µg/ml DPPH, 2478.3 to 4167.5 µmol/g extract FRAP and 187.6 to 333.5 mg GAE/g extract total phenols. The strongest anti-plasmodial activity was found in the water extract of *H. rubrostipulata* (Table 3) (IC<sub>50</sub> = 1.95 µg/ml) which is regarded as very high activity (Gessler et al., 1994). *Vernonia adoensis* has not previously been tested for anti-plasmodial activity. We found that all extracts from *V. adoensis* had very high anti-plasmodial activity ranging from 2.14 to 2.83. Four of the *Z. chalybeum* extracts demonstrated high activity as well (IC<sub>50</sub> ranging from 2.72 – 3.94 µg/ml); only the methanol extract had low activity (IC<sub>50</sub> = 10.92 µg/ml). Chloroquine control (CQ

sensitive strain MRA-285) showed IC<sub>50</sub> = 8 µg/ml. The DPPH radical scavenging activity is due to the hydrogen-donating ability, while the FRAP assay measures the extract's ability to reduce iron (Fe<sup>+++</sup>). The total amount determined to be of phenolic nature by the FCR is based on the chemical reduction of the FCR, a mixture of tungsten and molybdenum oxides, resulting in blue coloured products with absorption maximum 765 nm. The intensity of the absorption is equivalent to the sum of the individual contribution of the different classes of phenols in the mixture (Singleton et al., 1999). Other readily oxidised substances might also react with the reagent. The results show high correlation between the three methods used (Table 1 and Figure 2), and Pearson correlation between the three assays varied between 0.945 and 0.988, all with a P-value of =0.000. In Figure 2 the inverse value of DPPH is used, since in this assay the lowest value show the highest activity.

The DCM extract of all species had the lowest activity, but this solvent also gave low yield (Table 1). Very few studies of antioxidant and radical scavenger effect of extracts from these plants were found. However our results for the DPPH test of methanol extract of *Z. chalybeum* is close to results found by Kuglerova et al. (2008) for ethanol extracts, with IC<sub>50</sub> values of 25.2 and 23 µg/ml, respectively. Diallo et al. (2001) found weak radical scavenger effect in the DCM extract of *V. adoensis* leaves, while no activity in the same extract was found in this study (Table 1). In our study the strongest antioxidant activity when measured with the FRAP assay had H-Ac > Z-Me > Z-CMW > H-CMW > H-W > H-Me > V-CMW. The selected plants were extracted with five different extract systems (Table 1), and the water extract gave highest yield for all plants. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of the water extracts indicated high amounts of sugar, in addition content of compounds with aromatic and olefinic structures in all water extracts was observed. The presence of alkaloids, flavonoids, saponins and bitter principles in the different extracts is shown in Table 2. Alkaloids were found in all three plants as demonstrated earlier (Seaton et al., 1960; Shellard et al., 1977; Kato et al., 1996; Gessler et al., 1994; Deeni and Hussain, 1994), but not in the DCM extract of *H. rubrostipulata* and water extract of *Z. chalybeum*. Flavonoids, saponins and bitter principles were present in most extracts, except for the DCM extracts of *H. rubrostipulata* and *Z. chalybeum* which were nearly deficient of NMR signals in the aromatic region as well. The acetone extract of *V. adoensis* had no saponins or bitter principles.

In a screening of antimalarial activity in 58 plant samples from 43 species of medicinal plants from Tanzania, Gessler et al. (1994) found that extract from *Z. chalybeum* root bark (ethanol and water) were among the four top plant parts with IC<sub>50</sub> values < 1 µg/ml (hypoxanthine assay using the multidrug resistant *P. falciparum* stain K1, and a chloroquine resistant strain NF54). Five other species had IC<sub>50</sub> values = 1- 4.9 µg/ml,

**Table 1.** Yield, DPPH radical scavenging, FRAP antioxidant activity and total phenolics of *H. rubrostipulata*, *Z. chalybeum* and *V. adoensis*.

Scientific name Family	Collection	Part	Solvent <sup>c</sup>	Sample	Yield	DPPH	FRAP	Total phenolics
Local name <sup>a</sup>	No.	used <sup>b</sup>		ID	% <sup>d</sup>	IC-50 ± SD	µmol/g extr.±SD	GAE/g extr.±SD
<i>H. rubrostipulata</i>	TS 104	Sb	Aceton	H-Ac	9	16.6 ± 0.5	5370.5 ± 28.1	370.7 ± 0.25
Rubiaceae			DCM	H-DCM	1	>167	253.8 ± 5.4	26.4 ± 0.09
			Methanol	H-Me	11	46 ± 2.1	2562.6 ± 9.6	187.6 ± 0.91
Muziku (GA)			CMW	H-CMW	5	23.9 ± 0.6	3633.1 ± 6.6	269.5 ± 0.48
			Water	H-W	12	28.7 ± 0.9	2863.5 ± 11.0	207.9 ± 0.30
<i>V. adoensis</i>	TS 338	L	Aceton	V-Ac	9	>167	502.0 ± 2.9	32.0 ± 0.11
Asteraceae			DCM	V-DCM	9	>167	193.2 ± 2.1	14.6 ± 0.10
			Methanol	V-Me	9	39.6 ± 3.7	2311.6 ± 5.1	163.1 ± 1.02
Nyakayuma (NYA)			CMW	V-CMW	8	33.3 ± 1.7	2562.3 ± 25.0	186.2 ± 0.25
			Water	V-W	20	53.9 ± 2.5	1474.7 ± 7.9	98.1 ± 0.17
<i>Z. chalybeum</i>	TS 345	Sb	Aceton	Z-Ac	5	80.9 ± 8.7	1663.2 ± 8.8	134.6 ± 0.79
Rutaceae			DCM	Z-DCM	4	>167	184.7 ± 1.6	28.8 ± 0.07
			Methanol	Z-Me	26	25.2 ± 0.7	4167.5 ± 3.0	333.5 ± 1.40
Ntaleyedungu (GA)			CMW	Z-CMW	21	26.7 ± 1.2	3644.4 ± 6.8	286.1 ± 0.28
			Water	Z-W	26	44.4 ± 2.7	2478.3 ± 10.2	233.0 ± 3.32

<sup>a</sup> Local name: GA, Luganda; NYA, Runyankole, <sup>b</sup> Sb, stem bark, L, leaves, <sup>c</sup> DCM, dichloromethane; CMW, chloroform, methanol, water (12:5:3), <sup>d</sup> w/w yield in terms of initial dried material.

**Table 2.** Phytochemical screening of crude extracts.

Sample ID	Alkaloids	Flavonoids	Saponins	Bitter principles
H-Ac	+	+	+	+
H-DCM	-	-	-	-
H-Me	+	+	+	+
H-CMW	+/-	+	+	+/-
H-W	+/-	+	+	+/-
V-Ac	+	+	+/-	-
V-DCM	+	+	+	+/-
V-Me	+/-	+	+	+
V-CMW	+	+	+	+
V-W	+	+	+	+
Z-Ac	+	+	-	+
Z-DCM	+/-	+/-	-	-
Z-Me	+	+	+	+
Z-CMW	+	+	+	+
Z-W	-	+	+	+/-

+/-: indistinct result.

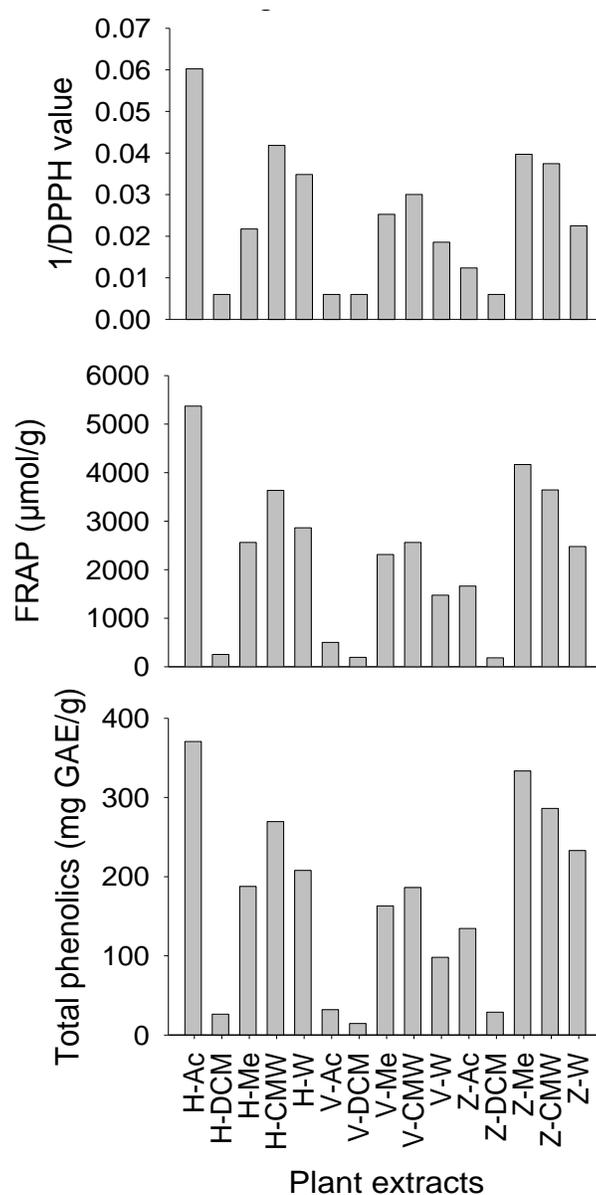
which was regarded as high activity. All of the three plants in this study had extracts with very high or high

activity according to Gessler et al. (1994). In a study of Rwandan medicinal plants (Muganga et al., 2010), both

**Table 3.** Anti-plasmodial activity in extracts from *H. rubrostipulata*, *V. adoensis* and *Z. chalybeum* with chloroquine as positive control on chloroquine sensitive strains.

Species	Ac		DCM		MeOH		CMW		W	
	% Y	IC <sub>50</sub> *	% Y	IC <sub>50</sub> *	% Y	IC <sub>50</sub> *	% Y	IC <sub>50</sub> *	% Y	IC <sub>50</sub> *
<i>H. rubrostipulata</i>	9	2.97	1	2.83	11	3.94	5	2.70	12	1.95
<i>V. adoensis</i>	9	2.54	9	2.83	9	2.57	8	2.67	20	2.14
<i>Z. chalybeum</i>	5	3.05	4	2.85	26	10.92	21	2.72	26	3.63
Chloroquine										8

Ac, Acetone; DCM, Dichloromethane; MeOH, Methanol; CMW, chlorine water and methanol, Y= Yield, W = Water, \*  $\mu\text{g/ml}$ .



**Figure 2.** The inverse value of DPPH IC<sub>50</sub> radical scavenger activity (top), antioxidant activity measured by the FRAP assay (middle) and total phenols measured by the Folin-Ciocalteu assay (bottom) in plant extracts from *H. rubrostipulata*, *V. adoensis* and *Z. chalybeum*. For plant extract sample IDs see Table 1.

*H. rubrostipulata* and *Z. chalybeum* were among 13 plants tested for anti-plasmodial activity against a chloroquine-sensitive strain of *P. falciparum* (3D7), and the most active extracts were evaluated against a chloroquine sensitive strain (W2). Root bark of *Z. chalybeum* showed high activity (Me:  $4.2 \pm 2.7$   $\mu\text{g/ml}$ ; DCM:  $6.2 \pm 0.6$   $\mu\text{g/ml}$ ), while methanol extract of *H. rubrostipulata* only showed slight activity ( $39.9 \pm 2.8$   $\mu\text{g/ml}$ ). In this study the limit for activity was defined to  $> 50$   $\mu\text{g/ml}$ . However the activity of DCM extract from stem bark of *H. rubrostipulata* and *Z. chalybeum* was quite similar, as it was in our study as well (our study: 2.83 and 2.85  $\mu\text{g/ml}$ ; Muganga et al. (2010): 39.9 and 41.5  $\mu\text{g/ml}$  for *H. rubrostipulata* and *Z. chalybeum* respectively). In a study of anti-plasmodial activity in plant extracts traditionally used in Kenya the highest activity was found in *Z. chalybeum* root bark water extract with  $\text{IC}_{50}$  value of 3.65  $\mu\text{g/ml}$  (Rukunga et al., 2009). There have lately been several promising studies of the anti-plasmodial activity of *Z. chalybeum*, especially the root bark. We have analyzed the stem bark, and found high activity in several of the extracts. In further work both the root and stem bark should be analysed in more detail. *Z. chalybeum* is one of the species heavily harvested both for medicine and for charcoal production, and it has been found difficult to cultivate. The species has been ranked as one of the 35 most important medicinal species of conservation concern in the world (Hawkins, 2008). Measures to multiply and sustain the species should be intensified.

The stem bark water extract of *H. rubrostipulata* was found to have the highest activity against the *P. falciparum* strain tested. This extract also had relative high score in the antioxidant tests (DPPH  $\text{IC}_{50}$ : 28.7  $\mu\text{g/ml}$ , FRAP: 2863.5  $\mu\text{mol/g}$ , TP: 207.9 mg GAE/g), and should be investigated further for potential development of phytomedicines. *H. rubrostipulata* has been found easy to germinate and grow (Stangeland et al., 2008) as opposed to *Z. chalybeum*, and it will thus be possible to secure raw material for potential phytomedicines.

We found that there is a strong correlation between the different antioxidant assays (Figure 2), and many of the extracts showed high anti-plasmodial activity. Some of the with high anti-plasmodial activity also have a extract Rather high antioxidant activity, like the water extract of all the three species and the methanol extracts of *H. rubrostipulata* and *V. adoensis*. According to Nguela et al. (2006) these extracts could be interesting leads to look for good anti-malarial drugs. Gessler et al. (1994), Muganga et al. (2010), and Rukunga et al. (2010) all found very high *in vitro* anti-plasmodial activity in some of the extracts from *Z. chalybeum* root bark, and moderate activity in the stem bark. This is in line with our findings for activity in stem bark. Muganga et al. (2010) only found moderate anti-plasmodial activity in the *Hallea (Mitragyna) rubrostipulata* stem bark, but they did not test the water extract of *M. rubrostipulata*, where we found the highest activity. According to previous studies and our findings, stem bark and root bark of *Z. chalybeum* and *M. rubrostipulata* are

good candidates for further investigation. *In vitro* assays cannot precisely reproduce *in vivo* situation. Some extracts may not become active without certain metabolic action *in vivo*. In further work the extracts should be tested with *Plasmodium berghei* in mice and toxicological investigations.

## Conclusion

During malaria infection increased reactive oxygen species are generated that may contribute to erythrocytic damage and anaemia. On the other hand some hypothesise that important antimalarials act as pro oxidants in the plasmodium parasite. In any case, oxidative stress seems to play an important role in erythrocytes and parasites during malaria infection. In our bioassays many plant extracts showed high anti-plasmodial as well as high antioxidant activity. We do not know if it is the same or different compounds in the raw extracts that have these effects, and neither do we know at this stage the bioavailability of the active principles. But since the water extracts of these plants have been traditionally used against malaria there is reason to believe that there is some bioavailability. We believe that it may be favourable to select extracts with high anti-plasmodial and antioxidant activity for further investigation. In future work the most promising raw extracts, like the water extract of *H. rubrostipulata* and *V. adoensis*, should be tested both on chloroquine sensitive and chloroquine resistant strains and bioassay guided fractionation for antimalarial effect and toxicity should be performed to see if effective and safe antimalarials can be developed.

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