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Evaluation of the cytotoxic activity of extracts from medicinal plants used for the treatment of malaria in Kagera and Lindi regions, Tanzania

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ABSTRACT

A number of medicinal plants used for treatment of malaria in Tanzania have been documented, but information on their safety and efficacy is still based on traditional knowledge accumulated over years and not on pre-clinical and clinical evaluation. The present study aimed to assess the cytotoxic activity of extracts of selected plant species used for treatment of malaria in Tanzania. Ethanol extracts were evaluated for cytoxicity by using MTT assay on LLC-MK2 cells and by brine shrimp lethality assay. Forty five (93.75%) out of 48 crude extracts assessed using LLC-MK2 cells were non-cytotoxic while three extracts (6.25%) were cytotoxic with CC_{50} <30 µg/mL (cut-off point). In the brine shrimp assay 30 (65.2%) out of 46 extracts tested were non-toxic while 16 extracts (34.8%) were toxic (LC_{50} <100 µg/mL). *Antiaris toxicaria* stem bark extract was the most cytotoxic to mammalian cells. This study demonstrates that, most of the antimalarial plants tested were non-toxic. These observations corroborate with traditional healers' claims that the herbal medicines used in their areas are safe. However, further studies using different toxicity models are suggested to further confirm their claims.

INTRODUCTION

The use of plants as source of medicines for treatment of infectious and non-infectious diseases is an old human tradition (Petrovska, 2012), and the practice is now increasing due to increased global health challenges (WHO, 2002). Malaria is one of diseases treated by herbal medicines originating from different plant parts such as roots, stem bark, leaves, flowers and fruits. It is an old life threatening parasitic disease caused by parasites of the genus *Plasmodium*. The parasites infect and destroy red blood cells, leading to fever, severe anaemia, cerebral malaria and death may occur if the patient is not treated properly and on time (Fidock *et al*., 2004; NIAID, 2007).

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Exploration of the accumulated indigenous knowledge on the treatment of malaria using medicinal plants enabled the isolation of two important and currently used antimalarial drugs; artemisinin from *Artemisia annua* and quinine from the bark of *Cinchona spp* (Wells, 2011). Despite plants being a rich source of useful chemical compounds of various structures and with different pharmacological properties on biological systems (Butler, 2004; Moshi *et al*., 2009), some of them may be toxic to humans. For example, some of the toxicities associated with the use of medicinal plants include allergic reactions, irritation of the gastrointestinal tract, destruction of red blood cells, and damage of body organs such as the heart and kidney and carcinogenicity (Westendorf, 1999; IARC, 2012). Several medicinal plants have previously been reported to be toxic. Some of the examples include *Symphytum officinale L.* used for wound healing which contains hepatotoxic pyrrolizidine alkaloids and *Valerian officinalis* used as a sedative for treatment of insomnia and anxiety which causes hepatitis

(Abdualmjid and Segi, 2013). *Aristolochia spp* contain aristolochic acid I and II that cause renal failure **(**Debelle *et al*., 2008); *Drimia sanguinea* and *Bowiea volubilis* which are traditionally used for headache, oedema, infertility and bladder problems contain cardiotoxic bufadienolides (Van der Bijl Jr. and Van der Bijl Sen., 2012). Although the use of herbal medicines is controlled in many countries, information about their efficacy and safety is based on traditional knowledge transmitted through generations over years and not on pre-clinical and clinical evaluation (Chalut *et al*., 1999). Tanzania shares the same experience of having a number of traditional healers who use traditional medicines for treatment of different diseases and is endowed with over 12,000 plant species, of which at least 10% have medicinal values (Mahunnah *et al*., 2012). Furthermore, Tanzania is among the six African countries with many reported cases of malaria (WHO, 2012) and because of the long history of the disease, the practice of using medicinal plants to treat malaria is very common (Mahunnah, 1987; Gessler *et al*., 1995 Kinung'hi *et al*., 2010). Although several antimalarial medicinal plants have been documented in Tanzania, their safety has not been well studied. Therefore in this study the toxicity of crude extracts of medicinal plants used for the treatment of malaria in Kagera and Lindi regions, Tanzania, were assessed using the LLC- MK2 monkey kidney epithelial cell line and the brine shrimp larvae (*Artemia salina* L.).

MATERIALS AND METHODS

Materials

Monkey kidney epithelial cells, LLC-MK2 (ATCC®, USA) were obtained from American Type Culture Collections (USA) and ethanol (Carlo erba®) was purchased from Techno Net Scientific (Dar es Salaam, Tanzania). Foetal Bovine Serum (FBS, BioWhittaker®, Verviers, Belgium), RPMI-1640 medium (Sigma), MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma), sodium bicarbonate (sigma), dimethylsulfoxide (Sigma), cell culture flasks (Corning, NY, USA), 96 well cell culture plates (Costar®, Corning, NY, USA), Centrifuge tubes (Corning, NY, USA), Syringe adapted filters 0.22µm (Corning, NY, USA), Trypsin-EDTA (Sigma) were all purchased from Sigma (Steinheim, Germany). Plant materials were collected from Kagera (November, 2012) and Lindi (July, 2012) regions, Tanzania. Identification of the plants was done by Mr. Haji. O. Selemani, a Botanist at the Department of Botany, University of Dar es Salaam, and the voucher specimens are deposited at the University of Dar es Salaam and at Muhimbili University of Health and Allied Sciences Herbaria, Tanzania.

Extraction of crude extracts

The powdered plant materials were macerated in 80% ethanol at room temperature for 24h and then filtered through cotton wool. The solid plant materials were macerated again in the same solvent for another 24h and the extracts obtained from the first and the second extractions were pooled and concentrated under *vacuo* using a Heldolph® rotary evaporator (Heldolph instruments GmbH, Schwabach, Germany) to obtain viscous extracts which were further dried using a freeze drier (Edwards High Vacuum International, Crawley Sussex, England). The dry extracts were stored at -20°C until use.

Preparation of stock solutions

Stock solutions were prepared by dissolving 4 mg of crude extracts in 100 µL dimethyl sulfoxide and then diluted with RPMI-1640 cell culture medium to make 400 µg/mL. All solutions were sterilized by passing through 0.22 µm syringe-adapted filters and stored at -20°C until use.

Determination of cytotoxic activity on LLC-MK2 cells

Cytotoxicity of the crude extracts was evaluated on LLC-MK2 monkey kidney epithelial cells. Cells were grown in RPMI-1640 culture medium with L-glutamine and 25 mM HEPES (Steinheim, Germany). The medium was supplemented with 2 mg/mL $NaHCO_3$ (sigma), 10 µg/mL hypoxanthine (Sigma), 11.1 mM glucose (sigma), 10% FBS (BioWhittaker®, Verviers, Belgium) and 5µg/mL gentamicin. The cells were incubated at 5% O_2, 5% CO_2, and 90% N_2 in humidified incubator (SHEL LAB™, Sheldon Mfg Inc, OR, USA) at 37°C until confluent before used for cytotoxicity assay. Trypsinated cells were distributed in 96 well plates at 10,000 cells in 100 µL per well and incubated for 48 h to allow them to attach before adding the extract. After 48 h the medium was removed completely from each well, and 100 µL of fresh culture medium was then added. Thereafter 100 µL of crude extracts (400 µg/mL) were added in row H and then serially diluted to row B to give concentrations ranging from 200 – 3.125 µg/mL. Cells in row A served as controls without drug (100% growth). The cells with or without extracts were incubated at 37°C for 72 h before determining their viability. Each concentration level was tested in triplicate.

MTT Assay

Cell viability was determined using MTT assay (Niles *et al*., 2008; 2009). After 72 h of incubation, the culture medium in each well with or without extract was removed completely from the assay plates and replaced by 100 µL of fresh culture medium. Then 10µL of 5 mg/mL Thiazolyl Blue Tetrazolium Bromide, MTT (Sigma) were added into each well to achieve a final concentration of 0.45mg/mL before incubated for 3 h at 37°C. After 3 h, the culture medium with MTT was carefully removed followed by addition of 100µL dimethylsulfoxide to dissolve formazan crystals and then incubated for 1 h before recording the optical density (Emax-Molecular Devices Corporation, California, USA) at 595 nm.

Data analysis

The percentage viability and percentage mortality were calculated from the OD values using Microsoft Excel 2010. The mean results of the percentage mortality were plotted against the logarithms of concentrations using the Fig P computer program Ver 4.189/07 (Biosoft Inc, USA). Regression equations obtained

from the graphs were used to calculate the fifty percent cytotoxic concentration (CC_{50}), which is the concentration killing fifty percent of the cells. An extract with CC_{50}>30 µg/mL is considered non-toxic (Fadeyi *et al*., 2013).

Brine shrimp toxicity assay

The brine shrimp lethality assay is a non-specific toxicity assay that is used in natural products research to detect the presence of pharmacologically active chemical constituents. It uses *Artemia salina* L. (Artemiidae) larvae (Meyer *et al*., 1982). Solutions of plant extracts were made in dimethylsulfoxide. The brine shrimp toxicity assay was conducted and data analyzed as previously reported (Nondo *et al*., 2011). An LC_{50} (concentration killing fifty percent of the brine shrimp larvae) value greater than 100 µg/mL is considered to represent a non-toxic compound or extract (Moshi *et al*., 2010). Each extract was tested in duplicate and the concentrations of dimethylsulfoxide were restricted to a maximum of 0.6% in the final volume.

RESULTS AND DISCUSSION

Forty eight extracts from 38 medicinal plants distributed into 19 different plant families were evaluated for cytotoxic activity on mammalian cells (LLC-MK2 cells) as presented in Table 1. The results revealed that 45 (93.75%) out of 48 extracts tested were non-cytotoxic and exhibited CC_{50} values above the cut-off point which is 30 µg/mL. Of these, 33 extracts (73.3%) from 24 plant species were found to have CC_{50} values above 200 µg/mL, the highest concentration tested. Only three (6.25%) out 48 extracts were found to be cytotoxic with CC_{50} < 30 µg/mL (cut-off point). The extracts were from *Aspilia natalensis* aerial parts (18.57 ± 1.04 µg/mL), *Antiaris toxicaria* leaves (12.51 ± 0.65 µg/mL) and *Antiaris toxicaria* stem bark (1.44 ± 0.48 µg/mL) (Table 1).

The brine shrimp toxicity assay showed that thirty extracts (65.2%) out of the 46 extracts tested had LC_{50} values greater than 100 µg/mL; the cut-off point. Among these, 8 extracts had LC_{50} values greater than 1000 µg/mL, while the remaining had LC_{50} values between 100 and 800 µg/mL. Only Sixteen extracts (34.8%) showed LC_{50} <100 µg/mL, and therefore classified as toxic. *Maesa lanceolata* leaf extract was the most toxic with LC_{50} = 1.55 µg/mL, followed by the extracts from *Dalbergia malangensis* leaves (16.47 µg/mL), *Aspilia natalensis* aerial parts (34.93 µg/mL), *Desmodium salicifolium* stem (36.87 µg/mL), and *Dalbergia malangensis* stem extract (47.59 µg/mL) (Table 2). The high toxicity of *M. lanceolata* leaf extract on brine shrimp larvae may be due to the effect of saponins. Previous studies revealed that the leaves of *M. lanceolata* are rich in triterpenoidal saponins and these compounds were reported to have high molluscicidal and hemolytic activities (Sindambiwe *et al*., 1998; Apers *et al*., 2001).
According to the American National Cancer Institute (NCI), a crude plant extract is considered to be cytotoxic if its CC_{50} value on mammalian cells is <30 µg/mL (Fadeyi *et al*., 2013). On the other hand the cut-off point to consider a crude plant extract non-toxic in the brine shrimp toxicity assay is LC_{50}>100 µg/mL (Moshi *et al*., 2010). Based on the results obtained in the two bioassays, three extracts were found to be toxic against LLC-MK2 cells and 16 extracts were found to be toxic on brine shrimp larvae. Of these only one extract from *A. natalensis* aerial parts was found to be toxic in both assays, which may be an indicator of consensus for cytotoxicity.

The extract from *A. toxicaria* stem bark was ranked the most toxic on the mammalian cells (LLC-MK2 cells) but it was ranked as exceptionally non-toxic using the brine shrimp toxicity assay (with LC_{50} >1,000 µg/mL). On the other hand *M. lanceolata* leaf extract was ranked as the most toxic on brine shrimp assay with LC_{50} = 1.55 µg/mL but ranked as non-toxic on LLC-MK2 cells test (Table 1 and 2). These observations suggest that the two models used in this study complement each other for the detection of toxic compounds that may be attributed to different mechanisms of toxicity; although the brine shrimp bioassay was found to be more sensitive in detecting toxic extracts than LLC-MK2 cells. The difference may be explained partly by the non-specificity of the brine shrimp assay in detecting toxic compounds (Meyer *et al*., 1982) and the differences in the criteria set to define a toxic substance, although in some studies brine shrimp assay has been reported to demonstrate some correlation with cell line results for detecting cytotoxic compounds/extracts (Meyer *et al*., 1982; Carballo *et al*., 2002).

The cytotoxicity of *A. natalensis* aerial parts extract was predicted by both assays; but it exhibited higher toxicity to mammalian cells than to brine shrimp larvae. The cytotoxic activity of *A. natalensis* (CC_{50} = 18.57 ± 1.04 µg/mL) on LLC-MK2 cells was comparable to that of the standard cytotoxic drug used in this study (Imatinib, Gleevec) which had CC_{50} of 18.61 µg/mL. A previous study revealed that an infusion and paste prepared from leaves of *A. natalensis* are used topically in South Africa to treat skin diseases (Mabona *et al*., 2013), but information regarding its toxicity was limited. Information from the traditional healers who reported these plants indicated that the decoctions of *A. natalensis* leaves and *A. toxicaria* leaves and stem bark are used orally for malaria associated with high fever (‘‘*Malaria kali*’’). They, however, emphasized that the decoction of *A. natalensis* should be consumed in small quantity because if taken in large quantities it causes stomach pain. These results may support the safety concern raised by traditional healers regarding oral administration of extracts from this plant.

Antiaris toxicaria is a known poisonous plant used in arrow poisoning associated with the presence of a number of cardiac glycosides which are inhibitors of Na^+/K^+ -ATPase pump (Kopp *et al*., 1992; Shi *et al*., 2010). In addition, the cardiac glycosides and coumarins isolated from *A. toxicaria* were reported to have cytotoxic activity on various cancer cell lines (Dai *et al*., 2009; Liu *et al*., 2013; Shi *et al*., 2014). In this study we found that ethanolic extracts of the leaves and stem bark of *A. toxicaria* were very toxic to non-cancer cells (LLC-MK2). However, these results do not support the questionnaire-based toxicity information collected from traditional healers.

Table 1: Cytotoxic activity of crude extracts on LLC-MK2 cells (CC_{50} ± SD in µg/mL).

Plant family	Plant species	Vernacular name	Plant part	CC_{50} ± SD (µg/mL) 80% EtOH crude extract
Acanthaceae	*Acanthus pubescens* (Oliv.) Vatke	Amatoju	R	>200
Apocynaceae	*Funtumia africana* (Benth) Staff	Mwezamaino/omwelamaino	SB	>200
	Funtumia africana (Benth) Staff	Mwezamaino/omwelamaino	L	>200
	Holarrhena pubescens (Huch-Ham)	Nalupande	R	>200
	Holarrhena pubescens (Huch-Ham)	Nalupande	R	>200[b]
Burseraceae	*Canarium schweinfurthii* Engl.	Omubafu wa kike/muubani wa kike	SB	>200
Celastraceae	*Salacia lovetii* N. Halle & B. Mathew	Omzindabikaka	L	>200
Compositae	*Aspilia mosambecensis* (Oliv.) Wild	Eshurwa rusharila/Esisa	AP	>200
	Aspilia natalensis (Sond) Wild	Kanyamoisa	AP	18.57 ± 1.04
	Guizotia scabra (Vis.) Chiov	Echihongosheija	WP	>200
	Vernonia glabra (Steetz) Vatke	Msangusangu	L	100.75 ± 16.69
Convolvulaceae	*Ipomoea rubens* choisy	Kataba	L	>200
Euphorbiaceae	*Bridelia micrantha* (Hochst.) Bail	Omushamako	SB	156.80 ± 0.44
	Phyllanthus nummulariifolius Poir	Karungi	WP	>200
	Phyllanthus nummulariifolius Poir	Karungi	WP	>200[a]
Fabaceae	*Cassia singueana*	Mlewelewe	R	>200[b]
	Cassia singueana	Mlewelewe	R	>200
	Dalbergia malangensis E.P. Sousa	Omugorora	L	107.29 ± 11.04
	Dalbergia malangensis E.P. Sousa	Omugorora	S	>200
	Desmodium salicifolium (Poir) DC	Batengeliange/Omukongoranwa	AP	>200
	Erythrina sacleuxii Hua	Mlindimila/mnungunungu	SB	>200
	Erythrina schliebenii Harms	Mlindimila	SB	>200
	Erythrina schliebenii Harms	Mlindimila	SB	>200[a]
	Erythrina schliebenii Harms	Mlindimila	R	>200
	Macrotyloma axillare (E. Mey) Verdc	Akaihabukuru	AP	>200
Labiatae	*Leonotis nepaetifolia* (L.) R. Br	Ekitatelante	FL	130.04 ± 0.23
	Leonotis nepaetifolia (L.) R. Br	Ekitatelante	L	137.80 ± 2.29
	Leonotis nepaetifolia (L.) R. Br	Ekitatelante	AP	124.13 ± 11.86
Logamiaceae	*Anthocleista grandiflora* Gilg	Omubagaigana/mbagaigana	SB	>200
Melastomataceae	*Dissotis brazzae* Cogn	Bulitulo	AP	134.47 ± 2.7
	Dissotis melleri Hook. f.	Ekituntun/Etuntun	AP	83.33 ± 3.31
	Dissotis rotundifolia (Sm) Triana	Obwehehe/Obwee	AP	125.90 ± 1.86
	Melastomatrum capitatum (Vahl) A.& R. Fern)	Katuntun/akatuntun	AP	>200
Melianthaceae	*Bersama abyssinica*	Omujalya	SB	>200
Moraceae	*Antiaris toxicaria* (Pers) Lesch	Omujuju	SB	1.44 ± 0.48
	Antiaris toxicaria (Pers) Lesch	Omujuju	L	12. 51 ± 0.65
Myristicaceae	*Pycnanthus angolensis* (Welw.) Warb	Omunonoba	F	136.32 ± 3.09
	Pycnanthus angolensis (Welw.) Warb	Omunonoba	SB	>200
	Pycnanthus angolensis (Welw.) Warb	Omunonoba	L	>200
Myrsinaceae	*Maesa lanceolata* Forsk	Omuzilanyama/omuhanga	L	141.86 ± 2.02
Myrtaceae	*Syzygium cordatum* Krause	Omugege	SB	>200
Rosaceae	*Eriobotrya japonica* (Thunb.) Lindl	Musharazi/Omusharazi	L	>200
Rubiaceae	*Pentas bussei* (K. Krause)	Rusharila kibira	AP	>200
	Hallea rubrostipulata (K. Schum) J.F.Leny	Mchunguchungu	R	141.69 ± 0.61
	Hallea rubrostipulata (K. Schum) J.F.Leny	Mchunguchungu	SB	>200
	Oxyanthus speciosus DC	Omwanikibira	L	>200
	Rhytignia obscura Robyns	Omulokola/lulokola	L	>200
Rutaceae	*Teclea amaniensis*	-	R	>200
Standard drug: Gleevec (Imatinib)				18.61 ± 1.30

a= aqueous extract, b= methanol extract. All other extracts are extracted by 80% ethanol. CC_{50}= cytotoxic concentration fifty percent (mean ± SD, n =3). R = root, S = stem, SB = stem bark, L = leaves, AP = aerial parts (stem + leaves), F = fruits, FL = flowers, WP = whole plant

During our ethnobotanical survey, traditional healers reported that decoctions of leaves and stem bark were non-toxic when taken orally for treatment of malaria. This information from the reporting traditional healers is supported by animal studies. Kang *et al.*, (2008) reported that aqueous and ethanolic leaf extracts of *A. toxicaria* were not toxic to mice even at high doses when given orally. But toxicity was observed when these extracts were administered by intra-peritoneal route. This may suggest that the bioavailability of the cardiac glycosides present in leaves and stem bark is low when given orally compared to when given through other routes. Apart from the toxicity evaluation reported in this study, the plant extracts reported in this study were previously evaluated for *in vitro* antimalarial activity against *P. falciparum* Dd2 strains. At a single concentration of 100 µg/mL, ethanolic extracts from *A. toxicaria* stem bark, *M. lanceolata* leaves, *A. natalensis* and *D. salicifolium* aerial parts inhibited the growth of malaria parasites *in vitro* (Nondo *et al*., 2015**)**. Since the LLC-MK2 cells are normal mammalian cells, toxicity against these cells most likely predicts lack of selectivity and thus it will be toxic to mammalian cells, and therefore the traditional healers and patients should be informed on the risk of toxicity that might arise following use of extracts from these plants.

Table 2: Brine shrimp toxicity results.

Plant species	Part	LC_{50} (µg/mL)	95% CI (µg/mL)
Acanthus pubescens (Oliv.) Vatke	R	140.94	113.20 – 175.47
Athocleista grandiflora Gilg	SB	> 1,000	
Antiaris toxicaria (Pers) Lesch	L	154.24	112.18 – 212.08
Antiaris toxicaria (Pers) Lesch	SB	>1,000	
Aspilia mosambecensis (Oliv.) Wild	AP	122.17	93.98 – 158.82
Aspilia natalensis (Sond)	AP	34.93	25.68 – 47.50
Bersama abyssinica	SB	729.14	433.75 – 1,225.68
Bersama abyssinica	R	184.35	126.88 – 267.86
Bridelia micrantha (Hochst.) Bail	SB	>1,000	
Canarium schweinfurthii Engl.	SB	273.51	203.66 – 367.32
Cassia singueana	R	> 1,000	
Cassia singueana	R[b]	332. 36	241.19 – 458.0
Dalbergia malangensis E.P. Sousa	L	16.47	10.78 – 25.17
Dalbergia malangensis E.P. Sousa	Stem	47.59	39.89 – 56.77
Desmodium salicifolium (Poir) DC	AP	36.87	28.96 – 46.94
Dissotis brazzae Cogn	AP	244.39	187.69 – 318.17
Dissotis melleri Hook. f.	AP	116.75	89.26 – 152.71
Dissotis rotundifolia (Sm) Triana	AP	>1,000	
Eriobotrya japonica (Thunb.) Lindl	L	>1,000	
Erythrina schliebenii Harms	SB	729.14	433.75 – 1,225.68
Erythrina schliebenii Harms	R	93.26	74.97 – 116.02
Funtumia africana (Benth) Staff	SB	223.06	175.64 – 283.29
Funtumia africana (Benth) Staff	L	348.56	223.29 – 544.10
Guizotia scabra (Vis.) Chiov	WP	60.14	43.49 – 83.17
Hallea rubrostipulata (K. Schum) J.F.Leny	SB	125.45	99.01 – 158.95
Hallea rubrostipulata (K. Schum) J.F.Leny	R	>1,000	
Holarrhena pubescens (Huch-Ham)	R[a]	291.58	204.76 – 415.21
Holarrhena pubescens (Huch-Ham)	R	63.16	54.77 – 72.82
Holarrhena pubescens (Huch-Ham)	R[b]	135.24	111.40 – 164.19
Ipomoea rubens choisy	AP	97.01	73.05 – 128.83
Leonotis nepaetifolia (L.) R. Br	AP	128.74	90.92 – 182.30
Leonotis nepaetifolia (L.) R. Br	FL	91.75	68.62 – 122.67
Macrotyloma axillare (E. Mey) Verdc	AP	123.91	89.60 – 171.37
Maesa lanceolata Forsk	L	1.55	0.59 – 4.08
Melastomatrum capitatum (Vahl) A.& R. Fern)	AP	390.17	267.79 – 568.48
Oxyanthus speciosus DC	L	229.48	152.28 – 345.83
Pentas bussei (K. Krause)	AP	729.14	433.75 – 1,225.68
Phyllanthus nummulariifolius Poir	WP	86.14	67.83 – 109.40
Phyllanthus nummulariifolius Poir	WP[a]	87.39	68.02 – 110.03
Pycnanthus angolensis (Welw.) Warb	F	81.01	52.74 – 124.43
Pycnanthus angolensis (Welw.) Warb	L	78.55	68.19 – 90.49
Pycnanthus angolensis (Welw.) Warb	SB	93.58	74.98 – 116.79
Rhytignia obscura Robyns	L	489.33	296.20 – 608.37
Syzygium cordatum Krause	SB	99.93	80.33 – 124.31
Vernonia glabra (Steetz) Vatke	L	>1,000	

LC_{50} = lethal concentration fifty, CI = confidence interval, a= aqueous extract, b= methanol extract. All other extracts were extracted by 80% ethanol. R = root, S = stem, SB = stem bark, L = leaves, AP = aerial parts (stem + leaves), F = fruits, FL = flowers, WP = whole plant.

CONCLUSION

Most of the antimalarial medicinal plants tested were non-toxic, and hence support the traditional healers' claims who believe that the herbal medicines they use are safe. However, further studies using different toxicity models are suggested to confirm their claims. Only the extracts of *A. natalensis* and *A. toxicaria* were categorized as toxic to mammalian cells. The evidence of *A. natalensis* toxicity obtained in this study supports the cautionary note that was given by the collaborating traditional healers.

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