Antiplasmodial, anti-trypanosomal, anti-leishmanial and cytotoxicity activity of selected Tanzanian medicinal plants

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Abstract: The antiplasmodial, anti-trypanosomal and anti-leishmanial activity of 25 plant extracts obtained from seven Tanzanian medicinal plants: *Annickia* (*Enantia*) *kummeriae* (Annonaceae), *Artemisia annua* (Asteraceae), *Pseudospondias microcarpa* (Anacardiaceae), *Drypetes natalensis* (Euphorbiaceae), *Acridocarpus chloropterus* (Malpighiaceae), *Maytenus senegalensis* (Celastraceae) and *Neurautanenia mitis* (Papilonaceae), were evaluated *in vitro* against *Plasmodium falciparum* K1, *Trypanosoma brucei rhodesiense* STIB 900 and axenic *Leishmania donovani* MHOM-ET-67/82. Out of the 25 extracts tested, 17 showed good antiplasmodial activity (IC_{50} 0.04-5.0 μg/ml), 7 exhibited moderate anti-trypanosomal activity (IC_{50} 2.3- 2.8 μg/ml), while 5 displayed mild anti-leishmanial activity (IC_{50} 8.8-9.79 μg/ml). *A. kummeriae, A. annua*, *P. microcarpa*, *D. natalensis*, *M. senegalensis* and *N. mitis* extracts had good antiplasmodial activity (IC_{50} 0.04-2.1 μg/ml) and selectivity indices (29.2-2,250 μg/ml). The high antiplasmodial, moderate anti-trypanosomal and mild anti-leishmanial activity make these plants good candidates for bioassay-guided isolation of anti-protozoal compounds which could serve as new lead structures for drug development.

Keywords*:* Malaria, trypanosomiasis, leishmaniasis, medicinal plants, Tanzania

Introduction

Malaria, trypanosomiasis and leishmaniasis are among the most important public health problems in developing regions (WHO, 2002). Due to their prevalence, virulence and drug resistance status; they are the most serious and widespread parasitic diseases in the tropics (Bryceson,1996;Marsden,1996;Olliaro&Cattani, 1996; WHO, 2002). In view of the complications in dealing with malaria, trypanosomiasis and leishmaniasis, chemotherapy remains a dependable cornerstone in the control of these diseases. In such efforts, plants are considered to be important sources for lead compounds owing to their successful use in the treatment of various ailments since antiquity (Sandberg & Cronlund, 1977).

Several medicinal plants are traditionally used for the treatment of malaria and other protozoal infections in East, Central and West African countries (Akendengue & Louis, 1994; Gessler *et al*, 1995; Dhawahan *et al*, 1997; Nkeh *et al*, 2001, 2003). In 1993, *Artemisa annua* was introduced in Tanzania and is now widely grown in the highlands where it has become a popular remedy for malaria in the form of herbal teas and infusions (Swiss Invest Forum, 2007). Although several phytochemical and pharmacological investigations have been done, *A. annua* was included to assess the efficacy of the local herbal extracts for the treatment of malaria and other protozoal diseases taking into consideration that, it is an exotic plant species growing in different soil and weather conditions. *A. annua* is reported to contain terpenes and flavonoids, but the endoperoxide sesquiterpene, artemisinin is the most pharmacologically useful compound reported to date.

Ethnomedical information reveals that several *Annickia* (formerly *Enantia*) species are widely used for the treatment of malaria and other ailments (Betti, 2002). For instance *Enantia chlorantha* is used for the treament of malaria in West Africa (Wafo *et al*., 1999). *Enantia chlorantha* exhibited *in vivo* activity against *Plasmodium yoelii* in experimentally infected mice (Agbaje & Onabanjo, 1991). Similarly, *E. polycarpa* is used for the treatment of malaria in West Africa (Bouquet & Debray, 1974). The stem-bark extract of *E. polycarpa* showed high *in vitro* antiplasmodial activity against *P. falciparum* K1 strain (IC_{50} 0.126 μg/ml) and high selectivity (SI 616) (Atindehou *et al*., 2004). The chemistry of *E. chlorantha* and *E. polycarpa* has been extensively

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studied (Leboeuf & Cave, 1972; Jössang *et al*., 1977a, b; Wafo *et al*., 1999). The stem-bark and the leaves of *E. chlorantha* and *E. polycarpa* contain many biologically active alkaloids that are closely related. Several quinoline and isoquinoline alkaloids, including quinine and dihydroquinidine, have been isolated from *E. polycarpa* (Buzas *et al*., 1959, 1965). This may explain the extensive use of *E. polycarpa* stem-bark as an anti-malarial remedy in West Africa.

Pseudospondias microcarpa and *P. longifolia* are used widely in African traditonal medicine (Gessler *et al*., 1995; Noumi & Yomi 2001). *Pseudospondias longifolia* is used in Gabon for reducing heart palpitation whereas *P. microcarpa* is used in Tanzania and the Democratic Republic of Congo (DRC) for the treatment of malaria and in Cameroon for constipation (Gessler *et al*., 1995; Noumi & Yomi 2001). Plants in the genus *Drypetes* are used in African traditional medicine for the treatment of various ailments (Akendengue & Louis, 1994; Gessler *et al*., 1995; Nkeh *et al*., 2003). *Drypetes gossweileri* is used for the treatment of rheumatism and filariasis in Gabon (Akendengue & Louis, 1994). *Drypetes molunduana* is used as a pain killer, anti-inflammatory and anti-tumor drug in Cameroon (Nkeh *et al*., 2003). *Drypetes natalensis* is used for the treatment of malaria and other ailments in Tanzania (Gessler *et al*., 1995).

The tuber of *Neorautanenia mitis* is widely used as fish poison in most parts of Africa (Vongtau *et al*., 2000, 2004; Joseph *et al*, 2004). *N. mitis* contains pachyrrhizine, rotenone, 12-hydroxyrotenone, neorotenone, neorautanone, neoduline, nepseudin, 4-methoxyneoduline, rautandiol A and rautandiol B. Several *Maytenus* species are widely used in Africa for the treatment of malaria and other ailments (Gessler *et al*., 1995). *Maytenus senegalensis* is commonly used for the treatment of malaria and bacterial infections in western Tanzania (Gessler *et al*., 1995). The chemistry and pharmacology of *Maytenus senegalensis* are well documented (Hussein *et al*, 1999; Abraham *et al*., 2006).

In our continuing efforts to validate the efficacy and safety of plants most frequently used in the treatment of malaria, we investigated the cytotoxicity and anti-protozoal activity of seven selected Tanzanian medicinal plants. We hereby report the antiplasmodial, anti-trypanosomal, anti-leishmanial and cytotoxicity activity of petroleum ether, dichloromethane and methanol extracts of *Annickia* (*Enantia*) *kummeriae* (Annonaceae), *Artemisia annua* (Asteraceae), *Pseudospondias microcarpa* (Anacardiaceae), *Drypetes natalensis* (Euphorbiaceae), *Acridocarpus chloropterus* (Malpighiaceae), *Maytenus senegalensis* (Celastraceae) and *Neurautanenia mitis*.

Materials and Methods

Collection and processing of plant material

The plants were collected in different parts of Tanzania in 2005 (Table 1). The leaves, seeds, root-bark and stem-bark were dried and powdered using pulveriser. The plant powders (500 g) were sequentially extracted with petroleum ether, dichloromethane and methanol. The extraction was done at room temperature for 48h with intermittent shaking, the solvent extract decanted and filtered off. After filtration, the solvent was removed under reduced pressure and the extracts dried further under a stream of nitrogen for 24h before being weighed and used for biological assays.

Table 1: List of plant species collected

Botanical name	Parts collected	Location of collection
Annickia kummeriae	Leaves, stem and root bark	Amani, Tanga
Acridocarpus chloropterus	Leaves, stem and root bark	Pugu, Dar es Salaam
Pseudospondias microcarpa	Stem and root bark	Kashozi, Kagera
Drypetes natalensis	Stem and root bark	Buyango, Kagera
Maytenus senegalensis	Root bark	Kyamlaile, Kagera
Neurautanenia mitis	Tuber	Ifakara, Morogoro
Artemisia annua	Leaves and seeds	Njombe, Iringa

In vitro assays

Antiplasmodial activity was evaluated against the multi-drug resistant *Plasmodium falciparum* K1 strain (resistant to chloroquine and pyrimethamine), using the methods described by Matile & Pink (1990). The parasites were maintained in a continuous *in vitro* culture and the quantitative assessment of *in vitro* antiplasmodial activity was determined by means of the microculture radioisotope technique. The inhibitory concentration (IC_{50}) represented the concentration that caused 50% inhibition in parasite growth, which was indicated by the uptake of the radio-labeled nucleic acid precursor, [^{3}H]-hypoxanthine, by *P. falciparum* K1 strain maintained on human red blood cells *in vitro*. The definition of the antiplasmodial activity used was: IC_{50} < 0.5 μg/ml - strong activity; 0.5–5.0 μg/ml - moderate activity; 5.0-10 μg/ml - mild activity and IC_{50} > 10 μg/ml - inactive. Chloroquine and artemisinin (IC_{50} 0.063±0.03 μg/ml and 0.002±0.0001 μg/ml, respectively) were used as standards.

The *in vitro* antitrypanosomal activity was evaluated against *Trypanosoma brucei rhodesiense* STIB 900 strain, using the method of Räz *et al*. (1997). Minimum Essential medium (MEM) was supplemented according to Baltz *et al*. (1985) with 2-mercaptoethanol and 15% heat inactivated horse serum which was later added to each well on a 96-well microtiter plate. Drug dilutions together with 10^4 bloodstream forms of *T. b. rhodesiense* STIB 900 in 50ml were added to each well and the plate incubated at 37°C under a 5% CO_2 atmosphere for 72h. Ten microliters of Alamar Blue (12.5mg rezasurin dissolved in one litre of distilled water) were then added to each well and incubation continued for a further four hours. The plate was read using a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) at an excitation wavelength of 536nm and emission wavelength of 588nm (Räz *et al*., 1997). The definition of the antitrypanosomal activity used was: IC_{50} < 1.0 μg/ml – strong activity, 1.0–5.0 μg/ml - moderate activity, 5.0–10 μg/ml mild activity, and IC_{50} > 10 μg/ml – inactive. Melarsoprol (IC_{50} 0.002±0.0001 μg/ml) was used as the standard drug.

The *in vitro* antileishmanial assay was carried out according to the procedure of Kaminsky *et al*. (1996). *Leishmania donovani* MHOM-ET-67/82 strain was maintained in the Syrian golden hamster (*Mesocricetus auratus*). Amastigotes were collected from the spleen of an infected hamster and grown in axenic culture at 37°C in slime mould (*Dictyostelium discoideum*) (SM) medium supplemented with 10% heat inactivated foetal bovine serum (FBS) at pH 5.4 under the atmosphere of 5% CO_2 in air. 100μl of culture medium with 10^5 amastigotes from axenic culture with a serial drug dilution were seeded in 96-well microtitre plates. After 72h of incubation the plates were inspected under inverted microscope to assure growth of the controls and sterile conditions. 10μl of Alamar Blue (12.5 mg rezasurin dissolved in one litre of distilled water) were then added to each well and the plates incubated for another two hours. Miltefosine was used as the standard drug. Then the plates were read with a Spectramax Gemini XS microplate fluorometer using an excitation wavelength of 536nm and an emission wavelength of 588nm. The definition of the antileishmanial activity used was: IC_{50} < 1.0 μg/ml – strong activity; 1.0 - 5.0 μg/ml – moderate activity; 5.0 - 10.0 μg/ml – mild activity; and IC_{50} > 10 μg/ml – inactive. Miltefosine (IC_{50} 0.11±0.001 μg/ml) was used as the standard drug.

Cytotoxicity and drug selectivity index

The *in vitro* cytotoxicity assay was carried out according to the procedure described by Kaminsky *et al*. (1996). Rat skeletal myoblast (L-6) cells were maintained as stock culture in RPMI 1640 medium + 10% FBS + 1% *L*-glutamine (200mM). 100μl of cell suspension consisting of 4x10^4 cells/ml were added into each well of columns 1 and 2, 4 and 5, 7 and 8, and 10 and 11 of a 96-well micro-titre plate (Costar™, Corning Inc.). Cells were allowed to attach for 24h, the medium removed completely the next morning, and 100μl of fresh medium added to all wells except in row H. Four drugs were tested on one plate (drug 1 column 1-3, drug 2 column 4-6, drug 3 column 7-9 and drug 4 column 10-12). A serial dilution factor of 1:3 was used to give concentrations in the range 270-0.37μg/ml. Wells in row A served as controls wells without any drugs. The plates were incubated for 72h at 37°C in 5% CO_2 in air. Podophyllotoxin was used as the standard drug. The fluorescent dye, Alamar blue (10 μl) (Trek Diagnostic Systems, East Grinstead, UK), was added to each well and the plates incubated for another two hours. The plates were read using a fluorescence plate reader at excitation and emission wavelengths of 536 and 588 nm, respectively. The definition of the cytotoxicity used was: CC_{50} < 1.0 μg/ml - high cytotoxicity; CC_{50} 1.0–10.0 μg/ml - moderate; CC_{50} 10.0-30.0 μg/ml – mild; and CC_{50} > 30 μg/ml – non

cytotoxic. Podophyllotoxin (CC_{50} 0.009±0.00003 μg/ml) was used as standard cytotoxin.

Data analysis

The antitrypanosomal assay data was transferred into a graphic programme (Softmax Pro, Molecular Devices Corporation, Sunnyvale, CA, USA) to generate sigmoidal inhibition curves from which IC_{50} value for each drug was calculated. The antileishmanial assay data was transferred into a graphic programme to generate sigmoidal inhibition curves from which IC_{50} value for each drug was calculated. The cytotoxicity data was analysed using the plate reader software (Softmax, Molecular Devices Corporation, Sunnyvale, CA, USA) and the CC_{50} value of each drug calculated.

Results

Out of 25 plant extracts, 17 showed good activity against *P. falciparum* K1 strain ($IC_{50} \leq 5.1$ μg/ml), 3 were moderately active (IC_{50} 5.2-10 μg/ml) while the remaining 5 were inactive ($IC_{50} > 10$ μg/ml) (Table 2). Ten extracts were of particular interest since they had IC_{50} values of ≤1 μg/ml: the *n*-hexane extract of *A. annua* leaf (IC_{50} 0.04 μg/ml, SI > 2,250.0), the dichloromethane and methanol extracts of *A. kummeriae* stem-bark (IC_{50} 0.31 μg/ml, SI 187.74 and 173.55 respectively), methanol and dichloromethane of *A. kummeriae* root-bark (IC_{50} 0.35, 0.36 μg/ml and SI 175.43, 29.17, respectively), the ethanol extract of *A. annua* seeds (IC_{50} 0.65 μg/ml, >138.5), the ethanol extract of *D. natalensis* root-bark (IC_{50}1.06 μg/ml, SI 17.92), and ethanol extract of *P. microcarpa* root-bark (IC_{50} 1.13 μg/ml, SI 79.65). Others with good activity (≤ 2 μg/ml) include the ethanol extract of *D. natalensis* stem-bark (IC_{50} 1.42 μg/ml, SI 62.61), the ethanol extract of *N. mitis* tuber (IC_{50} 1.52 μg/ml, SI 14.40), petroleum ether extract of *A. kummeriae* stem-bark (IC_{50}1.85 μg/ml, SI 11.89), and the ethanol extract of *M. senegalensis* (IC_{50} 2.05 μg/ml, SI >43.9). The IC_{50} of the crude extracts were compared with that of the standard drugs chloroquine (IC_{50} = 0.063 μg/ml) and artemisinin (IC_{50} = 0.002 μg/ml). The most promising extract in this assay was the *n*-hexane extract of the leaves of *A. annua* which exhibited 0.6 fold and 20- fold activity higher than that of chloroquine and lower than that of artemisinin, respectively whereas, the methanolic leaves extract of *A. kummeriae* showed 1.9 fold and 60.0 fold activity closer to that of chloroquine and artemisinin, respectively. The stem and roots dichloromethane and methanolic extracts of *A. kummeriae* were also promising showing between 4.9 and 5.7 fold and 155 and 180 fold activity closer to that of chloroquine and artemisinin, respectively. The rest were comparatively far less active as compared to the standard drugs (Table 3).

Out of 25 plant extracts, nine were inactive against *T. b. rhodesiense*, 9 others showed weak anti-trypanosomal activity with IC_{50} values ranging between 25 and 10.60 μg/ml while seven had moderate activity ($IC_{50} \leq 7.40$ μg/ml) (Table 2). Six extracts were of particular interest since the anti-trypanosomal activity was ≤ 3 μg/ml. The methanol extract of the leaves and the dichloromethane, chloroform and ethanol extracts of stem-bark and root-bark of *A. kummeriae* had the best activity with IC_{50} values of 2.3- 2.8 μg/ml and SI 3.75-26.70, followed by the methanol extract of root bark of *P. microcarpa* (IC_{50} 5.40 μg/ml, SI 7.26) and the petroleum ether extract of *A. kummeriae* stem-bark (IC_{50} 7.4. μg/ml, SI 2.97). The rest of the extracts were inactive. The IC_{50} values of crude extracts were compared with that of the standard drug melarsoprol (IC_{50} = 0.002 μg/ml). The IC_{50} values indicate that, extracts were relatively far less active as compared to the standard drug (Table 3).

In the antileishmanial assay, 20 out of 25 plant extracts were inactive against *L. donovani* amastigotes ($IC_{50} > 10$ μg/ml) while 5 exhibited mild anti-leishmanial activity (Table 2). The *n*-hexane extract of the leaves of *A. annua* was the most active extract of all with an IC_{50} value of 6.40 μg/ml and SI >14.06, followed by others but with narrow selectivity, these are the: ethanol extract of *N. mitis* tuber (IC_{50} 8.8 μg/ml, SI 2.59), the methanol, petroleum ether and dichloromethane extracts of *A. kummeriae* leaves (IC_{50} 9.25 μg/ml, SI 3.2), stem-bark (IC_{50} 9.74 μg/ml, SI 2.26), and root-bark (IC_{50} 9.79 μg/ml, SI 1.07), respectively. Comparisons of the IC_{50} of the crude extracts with the IC_{50} of the standard drug miltefosine (IC_{50} = 0.11 μg/ml) indicated moderate activities. The IC_{50} values of crude extracts were between 58.2 and >272.7 fold lower compared to that of the standard drug. Thus, *n*-hexane extract of *A. annua* leaves (58.2 fold), ethanol extract of *N. mitis* tuber (80.0 fold), the methanol, petroleum ether and dichloromethane extracts of *A. kummeriae* leaves (84.1->272.7 fold), stem-bark (88.5-176.4 fold), and root-bark (89.0-132.3 fold); and the dichloromethane extract of *A. chloropterus* leaves (106 fold), the remaining crude extracts were comparatively far less active as compared to the standard drug (Table 3).

Table 2: Antiplasmodial, anti-trypanosomal, anti-leishmanial and cytotoxicity activity plus selectivity indices of extracts of selected Tanzanian medicinal plants

Extracts	Rat L-6 cells		*P. falciparum* K1		*T.b. rhodesiense*		*L. donovani*	
	CC_{50} (μg/ml)	SI	IC_{50} (μg/ml)	SI	IC_{50} (μg/ml)	SI	IC_{50} (μg/ml)	SI
A. kummeriae								
L (PE)	72.0±3.10	8,000	4.65±0.15	15.48	21.70±3.47	3.32	>30	>2.40
L (DCM)	70.6±2.56	7,844	6.70±1.53	10.54	23.50±1.90	3.00	>30	>2.35
L (MeOH)	30.0±0.74	3,333	0.12±0.01	250.0	2.50±0.19	12.00	9.25±0.54	3.2
ST (PE)	22.0±3.00	2,444	1.85±0.55	11.89	7.40±0.53	2.97	9.74±1.82	2.26
ST (DCM)	58.2±2.80	6,467	0.31±0.05	187.74	2.50±0.24	23.28	18.00±0.42	3.23
ST (MeOH)	53.8±2.40	5,978	0.31±0.04	173.55	2.50±0.10	21.52	19.41±1.66	2.77
RT (PE)	34.1±3.20	3,789	2.51±0.11	13.59	14.10±0.45	2.42	14.55±1.1	2.34
RT (DCM)	10.5±0.62	1,167	0.36±0.06	29.17	2.80±0.23	3.75	9.79±2.5	1.07
RT (MeOH)	61.4±3.97	6,822	0.35±0.04	175.43	2.30±0.43	26.70	12.38±1.12	4.94
A. chloropterus								
L (PE)	>90	>10,000	15.87±1.28	>5.67	47.40±1.51	>1.9	>30	>3.00
L (DCM)	77.3±2.82	8,589	5.50±2.7	14.05	29.40±1.90	2.63	11.66±1.51	6.63
L (MeOH)	80.8±4.03	8,978	15.43±1.42	5.23	42.90±1.30	1.88	28.80±1.21	2.81
ST (PE)	88.1±1.90	9,789	16.68±0.10	5.28	46.40±0.94	1.90	>30	>2.94
ST (DCM)	82.5±4.20	9,167	7.23±1.45	11.41	35.80±1.46	2.30	14.57±1.36	5.66
RT (PE)	>90	>10,000	10.81±0.38	8.33	40.20±2.42	2.24	>30	>3.00
RT (DCM)	64.7±6.90	7,189	5.06±0.92	12.79	28.30±0.80	2.29	>30	>2.16
RT (MeOH)	>90	>10,000	43.83±1.27	2.05	68.20±0.80	1.32	>30	>3.00
P. microcarpa								
ST (EtOH)	>90	>10,000	4.33±1.44	20.79	5.40±0.64	7.26	29.9±4.19	>3.00
RT (EtOH)	>90	>10,000	1.13±0.16	79.65	11.60±1.74	7.38	>30	>5.45
D. natalensis								
ST (EtOH)	88.9±1.10	9,878	1.42±0.54	62.61	10.70±1.99	16.46	19.00±3.27	2.90
RT (EtOH)	19.0±0.38	2,111	1.06±0.20	17.92	12.10±0.38	1.64	29.7±3.52	0.64
M. senegalensis								
RT (EtOH)	>90.00	>10,000	2.05 ± 0.68	>43.9	12.2 ± 1.6	>7.4	16.5 ± 2.32	>5.50
N. mitis								
T (EtOH)	22.8 ± 2.57	2,533	1.58 ± 0.75	14.4	12.4 ± 0.92	1.80	8.8 ± 1.06	2.59
A. annua								
L (*n*-C_6H_{14})	>90.00	>10,000	0.04 ± 0.03	>2,250	15.3 ± 2.66	>5.9	6.4 ± 0.6	>14.06
S (EtOH)	>90.00	>10,000	0.65 ± 0.17	>138.5	27.2 ± 1.9	>3.3	>30.00	>3.00

L – leaves, ST – stem bark, RT – root bark, S – seeds, T – tuber, PE – petroleum ether, DCM – dichloromethane, *n*-C_6H_{14}-n-hexane, MeOH – methanol, CC_{50} 0.009 ± 0.00001 μg/ml for podophyllotoxin, IC_{50} 0.063±0.03 and 0.002 ± 0.0001 μg/ml for chloroquine and artemisinin, respectively, IC_{50} 0.002±0.0001 μg/ml for melarsoprol, IC_{50} 0.11±0.001 μg/ml for Miltefosine.

Table 3: Comparison of extracts IC_{50} values with those of standard drugs

Extracts	IC_{50} Extract / IC_{50} Chloroquine	IC_{50} Extract / IC_{50} Artemisinin	IC_{50} Extract / IC_{50} Melarsoprol	IC_{50} Extract / IC_{50} Miltefosine
A. kummeriae				
L (PE)	73.8	2,325.0	10,850.0	>272.7
L (DCM)	106.3	3,350.0	11,750.0	>272.7
L (MeOH)	1.9*	60.0*	1,250.0	84.1
ST (PE)	29.4	925.0	3,700.0	88.5
ST (DCM)	4.9*	155.0	1,250.0	163.6
ST (MeOH)	4.9*	155.0	1,250.0	176.4
RT (PE)	39.8	1,255.0	7,050.0	132.3
RT (DCM)	5.7*	180.0	1,400.0	89.0
RT (MeOH)	5.6*	175.0	1,150.0	112.5
A. chloropterus				
L (PE)	251.9	7,935.0	23,700.0	>272.7
L (DCM)	87.3	2,750.0	14,700.0	106.0
L (MeOH)	244.9	7,715.0	21,450.0	261.8
ST (PE)	264.8	8,340.0	23,200.0	>272.7
ST (DCM)	114.8	3,615.0	17,900.0	132.5
RT (PE)	171.6	5,405.0	20,100.0	>272.7
RT (DCM)	80.3	2,530.0	14,150.0	>272.7
RT (MeOH)	695.7	21,915.0	34,100.0	>272.7
P. microcarpa				
ST (EtOH)	68.7	2,165.0	2,700.0	271.8
RT (EtOH)	17.9	565.0	5,800.0	>272.7
D. natalensis				
ST (EtOH)	22.5	710.0	5,350.0	172.7
RT (EtOH)	16.8	530.0	6,050.0	270.0
M. senegalensis				
RT (EtOH)	32.5	1,025.0	6,100.0	150.0
N. mitis				
T (EtOH)	25.1	790.0	6,200.0	80.0
A. annua				
L (*n*-C_6H_{14})	0.6*	20.0*	7,650.0	58.2
S (EtOH)	10.3*	325.0	13,600.0	>272.7

L – leaves, ST – stem bark, RT – root bark, S – seeds, T – tuber, PE – petroleum ether, DCM – dichloromethane, C_6H_{14}- n-hexane; *Strong activity closer to that of standard drug

Discussion

Our results show that, ten extracts presented strong to moderate antiplasmodial activity with IC_{50} in between 0.04 and 2.05 μg/ml. These were the leaves and seed extracts of *Artemisia annua,* the leaves, stem and root bark extracts of *Annickia kummeriae,* root and stem bark extract of *Drypetes natalensis,* root bark extract of *Pseudospondias microcarpa,* tuber extract of *Neurautanenia mitis* and the root bark extract of *Maytenus senegalensis.* All this confirms their traditional use as antimalarial plants. Our results confirmed that the methanol extracts of the leaves, stem-bark, root-bark and dichloromethane extract of the stem-bark of *A. kummeriae* have high *in vitro* antiplasmodial activity against *P. falciparum* K1 strain (IC_{50} 0.12-0.36 μg/ml) and high selectivity (SI 29.17-250.0). In addition to that, the dichloromethane and methanolic extracts of the stem and roots of *A. kummeriae* showed IC_{50} values which were between 4.9 and 5.7 fold and 155 and 180 fold closer antimalarial activity to that of chloroquine and artemisinin, respectively. However, the petroleum ether and other dichloromethane extracts exhibited moderate to mild activity (IC_{50} 1.85-6.70 μg/ml) and low selectivity (SI 10.54-11.89). An exceptional good activity (IC_{50} 0.36 μg/ml) and moderate selectivity (SI 29.17) was found in dichloromethane extract of the root-bark, while a moderate to mild anti-leishmanial and anti-trypanosomal activity of *A. kummeriae* extracts were noted. *Annickia kummeriae* is a rare plant species that has not been reported outside Tanzania and should be conserved. The antiplasmodial principles of *A. kummeriae* are currently under investigation.

The hexane extract of *A. annua* leaves showed the highest *in vitro* antiplasmodial activity (IC_{50} 0.04 μg/ml) and selectivity index (SI > 2,250) confirming its efficacy and safety (Tawfiq *et al.*, 2004). Although the cytotoxicity of hexane leaf and ethanol seed extracts are the same, the activity and selectivity of the latter are lower, suggesting reduced amount of the active principles.

P. microcarpa root-bark extract has high *in vitro* antiplasmodial activity against *P. falciparum* K1 strain (IC_{50} 1.13 μg/ml) and good selectivity (SI 79.65). Unfortunately, *P. microcarpa* showed mild anti-trypanosomal activity (IC_{50} 5.40 μg/ml, SI 7.26). Similar findings have been reported from Congo Brazzaville (Mbatchi *et al*., 2006). Extract of *P. microcarpa* was inactive against *Leishmania donovani* parasites. Stem-bark and root-bark extracts of *Drypetes natalensis* exhibited high *in vitro* antiplasmodial activity against *P. falciparum* K1 strain (IC_{50} 1.42 and 1.06 μg/ml, respectively) and good selectivity (SI 62.61 and 17.92, respectively). *Maytenus senegalensis* showed moderate *in vitro* antiplasmodial activity against *P. falciparum* K1 strain (IC_{50} 2.05 μg/ml) and good selectivity (SI > 43.9). Unfortunately, *M. senegalensis* extracts were inactive against *T. b. rhodesiense* and *L. donovani*, respectively. Other studies have shown that methanol extract of *M. senegalensis* has significant antiplasmodial activity (Gessler *et al.*, 1995; Rukunga *et al.*, 2009). *Neurautanenia mitis* tuber extract exhibited high *in vitro* antiplasmodial activity against *P. falciparum* K1 strain (IC_{50} 1.52 μg/ml) and a moderate selectivity (SI 14.43).

The high percentage (68%) of extracts with good antiplasmodial with moderate anti-trypanosomal (20 %) and mild anti-leishmanial activity (20 %) suggests that these plants may be useful in the traditional management of malaria and other protozoal diseases. In view of the high antiplasmodial activity and good selectivity of *Artemisia annua* extract just 0.6 fold higher activity than chloroquine and 11.8 fold lower activity than artemisinin, and the usually envisaged ten-fold improvement in biological activity of the isolated pure compound, cost benefit analysis discourages the purification downward to the pure artemisinin compound. The crude extract is itself with enough antiplasmodial activity and the required safety to serve as an antimalarial drug. However, quality control of the crude drug/extract must be undertaken and therapeutic indices determined. The *in vitro* anti-protozoal activity and cytotoxicity of *A. kummeriae*, *P. microcarpa*, and *D. natalensis* are being reported here for the first time, and bioassay-guided isolation of bioactive principles should be undertaken to provide new anti-parasitic drugs or lead compounds for anti-malarial anti-trypanosomal and anti-leishmanial drug development. Since *Neurautanenia mitis* is an insecticidal and a potent fish poison with moderate selectivity, this plant poses health risks to users and there is a strong need for further pharmacological investigations to elucidate its toxic potential and principles for possible evaluation and development of new insecticides.

In African communities, medicinal plants have always been used for the treatment of malaria and other ailments. However, without a scientific validation the traditional preparations cannot be integrated in "modern" medicine. This study has highlighted six promising plants for further antiplasmodial investigations, and the determination of their active constituents, with a view to rationalize and optimize their utilization.

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