Antimalarial activity and toxicity evaluation of Kenyan *Hugonia castaneifolia* Engl. *Teclea nobilis* Del. and *Turraea mombassana* C.DC

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SUMMARY

The aqueous and methanol extracts of three medicinal plants, *Hugonia castaneifolia, Teclea nobilis* and *Turrae mombassana* were evaluated for *in vitro* antiplasmodial activity against a chloroquine sensitive *Plasmodium falciparum* strain (D6) and i*n vivo*, against a *P. berghei* ANKA strain in mice. The extracts were also assessed for cytotoxicity in Vero cell lines and acute toxicity in mice. The water extracts were moderately active with IC_{50} of 33.07 µg/ml for *T. mombassana* and 23.92 µg/ml for *H. castaneifolia* while the methanol extracts of *T. mombassana, H. castaneifolia and T. nobilis* were highly active *in vitro* with IC_{50} of 6.1 µg/ml, 8.86 µg/ml and 8.61 µg/ml respectively. The methanol extract of *T. mombassana* had the highest chemosupppression followed by the methanol extract of *H. castaneifolia in vivo*. Aqueous extract of *H. casteinofolia* was weakly cytotoxic against Vero cells CC_{50} =22.00 µg/ml. No toxic effect or mortality was observed in mice treated orally with any of the extracts at the highest concentration of 5000mg/kg. These results demonstrate antimalarial potential and safety of the three medicinal plants tested, which are used traditionally for the treatment of malaria in Kenya.

Keywords: Antiplasmodial; Malaria; Toxicity; Meliaceae; Rutaceae; Linaceae

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Introduction

Chemotherapy of malaria worldwide is dependent on nine key drugs: chloroquine, amodiaquine primaquine, mefloquine, quinine, sulfadoxine/pyrimethamine, pyrimethamine/dapsone, halofantrine and artemisinin derivatives [1]. Unfortunately, most of these drugs have been rendered ineffective due to the continued spread of drug resistant *P. falciparum* strains. Sulfadoxine pyrimethamine, a combination antifolate drug, which was an inexpensive antimalarial has had unacceptable levels of therapeutic failure in many countries in Asia, South America and now Africa due to resistance [2]. This is also true for chloroquine, mefloquine and quinine, with several countries abandoning chloroquine as the first-line antimalarial drug. Artemisinins are currently the most effective drug against multi-drug resistant strains of *P. falciparum* [3-5]. However, recent reports of high failure rates associated with artemisinin-based combination therapy, as well as *in vitro* drug-susceptibility data, suggest the possibility of clinical artemisinin resistance along the Thai-Cambodian border [5-7]. Consequently, there is a pressing need for the development of new anti-malarial drugs.

The search for new drugs through the evaluation and validation of traditional medicines offers a good opportunity and a high credible channel for the discovery and development of better medicines. The advantage of such drugs is that their precursors are plants that are widely available in rural areas of Africa. In this study, extracts from three Kenyan medicinal plants, namely *Hugonia castaneifolia, Teclea nobilis* and *Turraea mombassana* were examined for their antimalarial activity and safety, *in vitro* and in mice.

Materials and Methods

Plant collection and preparation

Plants were selected on the basis of their traditional reputation to treat fevers or malaria locally. The plants were collected in November 2008, around the vicinity of the Shimba Hills Game Reserve and adjoining part of Kinango division in Kwale District, Kenya. Permission for a sustainable plant harvesting was obtained from the Kenya Wildlife Services in the forest reserves, and from the local communities outside the forest area. A qualified and experienced taxonomist who was conversant with the flora of the area was part of the collection team. The plant specimens were identified and authenticated and voucher specimens deposited at the East African Herbarium, National Museum of Kenya. The plant parts were air-dried at room temperature under shade for 14 days and pulverized using a laboratory mill (Christy & Norris Ltd., Chelmsford, England) at the Center for Traditional Medicine and Drug Research, Kenya Medical Research Institute. The resulting powders were packed in air tight polythene bags, labeled and stored in the dark until used.

Preparation of plant extracts.

Each plant sample was separately extracted with water and methanol. For the methanol extracts 50 g of the powdered plant material was macerated with 500 ml of methanol at room temperature for 24 h, filtered through Whatman filter paper No. 1. The plant material was re-extracted with 300 ml methanol for the same period and the filtrates pooled and concentrated under vacuum at 40 °C until dry. The concentrate was weighed and transferred to an air tight sample bottle and stored at 4 °C until used.

Another 50 g of the same sample was extracted once with 500 ml of distilled water in a water bath at 60 °C for 1 h, filtered and lyophilized in a freeze dryer. The dry extracts for all the samples similarly treated were weighed into airtight containers and stored at 4 °C until used.

***In vitro* antiplasmodial testing**

The Sierra Leonean Chloroquine sensitive, *Plasmodium falciparum* strain (D6) was used in the study. The parasite cultures were donated by the Malaria Research and Reference Reagent Resource Center (MR4), Manassas, Virginia, USA. Parasite cultivation was carried out using previously described procedures [8,9]. Cultures were maintained in RPMI 1640 culture medium (10.4 g/l) powdered medium without PABA and lactic acid (LA) dissolved in 960 ml of double distilled autoclaved water (DDAW), supplemented with 10% human serum, 25mM (5.94 g/l) HEPES and 25mM NaHCO3. Human O+ red blood cells served as the parasites host cells. Test samples were prepared by dissolving in 100% Dimethyl sulfoxide (DMSO) and diluting in RPMI to lower the concentration of DMSO to ≤1%. Stock solution (1µg/ml) of chloroquine was also prepared for use as the reference drug. Semi-automated micro dilution assay technique that measures the ability of the extracts to inhibit the incorporation of [G-3H] hypoxanthine (Amersham International, Burkinghamshire, UK) into the malaria parasite was used in testing antiplasmodial activity [10]. Aliquots (50µl) of the test solutions were added in the first wells of 96 well flat bottomed micro culture plates (Costar Glass Works, Cambridge, UK) in duplicate and serial diluted down the plate using a Titertek motorized hand diluter (Flow Laboratories, Uxbridge, UK). The last row of wells did not contain the test samples and served as the controls. Two hundred microliters of malaria parasite culture was added into each well. The set plates were incubated at 37 ^{O}C in a gas mixture 3% CO_2, 5% O_2 and 92% N_2 for 48 h, after which each well was pulsed with 25µl of culture medium containing 0.5µCi of [G-3H] hypoxanthine. The plates were incubated for a further 18 hours. The contents of each well were then harvested onto glass fiber filter mats, washed thoroughly with distilled water, dried and the radioactivity in counts per minute (cpm) measured using a beta-counter (Wallac Micro Beta Trilux). The cpms obtained were then used to compute the IC_{50} values as described by Sixsmith et al [11].

***In vivo* antimalarial testing**

Male Balb C mice (6–8 weeks old, weighing 20±2 g) were used in the experiment. The mice were housed in standard macrolon type II cages in air-conditioned rooms at 22 ^{O}C, 50–70% relative humidity, fed with the standard diet and received water *ad libitum*. *Plasmodium berghei* strain ANKA was maintained by serial passage of infected blood through interperitonial injection (ip). The test protocol was based on the 4-day suppressive test [12]. Briefly, *P. berghei* infected blood was obtained by heart puncture, mixed with 1% (w/v) heparin in phosphate buffered saline (PBS) (1:1) and the test animals infected by ip injection with 0.2 ml (1×10^7 parasitized erythrocytes). Infected mice were randomly selected into groups of five for one test sample and the experimental groups treated with a single dose of 800 mg/kg of the test sample in 0.2 ml by oral adminstration from day 0 (immediately after infection) to day 3 [13]. The mice in the positive control group were given chloroquine diphoshate (dissolved in 10% tween 80) at 5 mg/kg/day orally.

Those in the negative control group received the placebo (10% tween 80) at 0.2 ml/kg/day. Parasitaemia was determined on day 4 (24 h after the last treatment) by microscopic examination by counting parasites in 4 fields of ≈100 erythrocytes per view of thin blood film sampled from the tail of the experimental mouse and stained in 10% giemsa solution. The difference between the mean number of parasites per view in the negative control group (100%) and those of the experimental groups was calculated and expressed as percent parasitaemia suppression (chemo suppression) according to the formula: PS = [(A–B)/A]×100 [14]. Where, A is the mean parasitaemia in the negative control on day 4, and B the corresponding parasitaemia in the test group. The standard deviations for the mean values was calculated as described by Armitage and Berry [15]. All *in vivo* experiments were repeated three times. In cases where the standard deviation (SD) was large, the experiment was repeated yet again and in some instances, outliers were not considered while computing the SD. Percentage parasitaemia was described as number of parasitized erythrocytes per 100 erythrocytes while percentage chemo suppression was taken as inhibition of parasite growth / multiplication relative to control expressed in percentage. Parasites in the negative control group were assumed to have experienced 0% chemo–suppression. Chemo suppression is thus the potency of the drug to inhibit parasite growth/multiplication.

In vitro determination of cell cytotoxicity

The Cytotoxic concentration causing 50% cell lysis and death (CC_{50}) was determined for the extracts by a method described by Mosmann [16]. Vero E6 cells were seeded at a concentration of 2.5×10^4 cells/well in a 24 well plates and grown under 5% CO_2 at 37 ^{O}C in Eagle's Minimum Essential Medium (MEM) supplemented with 5% fetal bovine serum (FBS) for 48 hours. The culture medium was replaced by fresh medium containing a plant extract at various concentrations, and cells further grown for 24 hours. The cells were then treated with trypsin and the number of viable cells determined by the tryphan blue exclusion method. The concentration of herbal extract reducing cell viability by 50% (CC_{50}) was determined from a curve relating percent cell viability to the concentration of extract. Selectivity index (SI = CC_{50}/IC_{50}) was used as a parameter of clinical significance of the test samples by comparing general toxins and selective inhibitory effect on *Plasmodium falciparum* [17].

Determination of acute toxicity

In vivo acute toxicity was determined in mice using standard procedures previously described [18, 19]. Male Swiss mice with a mean body weight of 20±2 g bred at the KEMRI experimental animal care facility in Nairobi, Kenya were used. They were randomly divided into groups of 5 mice, one group for each extract, plus one control group that was given Tween 80. Each mouse was starved overnight prior to treatment. Increasing doses of the test extract (1000, 2000, 3000, 4000, 5000 mg/kg/day) dissolved in 10% Tween 80 was administered orally, 30 minutes later the mice were allowed to take food. The general behavior of mice was observed continuously for 1 hour after the treatment, after 4 hours and thereafter over a period of 24 hours [20]. The mice were further observed daily for up to 14 days following treatment for any signs of toxicity.

Results

Table 1 summarizes the plant species, voucher number, parts used and dry weights of the plants tested. The water extracts had a higher yield compared to their methanol counterparts.

Table 1: Plant species, parts collected and yield of water and methanol extracts

Botanical name	Family	Voucher number	Part used	Dry weight of extract%	
				Water	methanol
Turraea mombassana C.DC	Meliaceae	Cm162	Leaves	6.5	5
Teclea nobilis Del.	Rutaceae	Cm153	Stem bark	5.9	0.9
Hugonia castaneifolia Engl.	Linaceae	Cm164	Twigs	4.3	2.2

In vitro anti-plasmodial assays

Results of *in vitro* antiplasmodial and cytotoxicity assays are summarized in Table 2. Antiplasmodial activity was classified as follows: high at IC_{50} ≤10 µg/ml, moderate at 10–50 µg/ml, low at 50–100 µg/ml and inactive at >100 µg/ml [21]. The methanol extracts were generally more active than those of the aqueous extracts. Methanol extracts of the three test plants *T. mombassana, T. nobilis* and *H. castaneifolia* were the most active against *P. falciparum* D6 strain *in vitro* with IC_{50} of ≤10 µg/ml. The aqueous extracts, of *T. mombassana* and *H. casteinofolia* exhibited moderate activity, while that of *T. nobilis was* inactive.

Table 2: *In vitro* anti-plasmodial activity (IC50 ±S.D.) of extracts of selected medicinal plants

Plant	IC_{50} ± S.D. (µg/ml)	
	Water	Methanol
T. mombassana	33.07±1.42	6.104±0.86
H. castaneifolia	23.92±1.08	8.86±1.87
T. nobilis	130.5±9.4	8.61±1.37
Chloroquine	0.0046 ± 1.8	

***In vivo* antiplasmodial activity**

Results of *in vivo* anti-malarial assay of the plant extracts against *Plasmodium berghei* in mice are summarized in Table 3. Suppression of parasitaemia (chemo-suppression) in mice was used as a measure of efficacy. Chemosuppression of the test samples was categorized as high when it was above 70%, moderate between 50% and 69%, low between 30% and 49%, inactive below 30% [22-25]. The extracts exhibited significant parasite density reduction ($p \leq 0.05$) with chemosuppression ranging from 30.3% to 52.86%. Methanol extracts of *T. mombassana* and *H. castaneiofolia* were the most active with chemo-suppression of 52.86% and 46.76%, respectively.

Table 3: *In vivo* anti-malarial activity of selected medicinal plant extracts on *Plasmodium berghei* in mice

Plant	Extract	Parasite Density	Chemosuppression (%)	Mean Survival Time (Days)
T. mombassana	Methanol	17.88±1.87*	52.86±4.23	10.33±0.58
H. castaneiofolia	Methanol	18.23±4.83*	46.76±2.62	8.6±1.34
	Water	22.83±0.67*	36.6±3.12	9.0±2.61
T. nobilis	Methanol	23.84±0.81*	30.3±5.14	9.7±1.15
	Water	22.61±2.77*	33.9±3.71	9.25±1.25
10% Tween 80		36.15±2.03	0.00±0.00	8.25±0.50
Chloroquine Diphosphate (5mg/kg)		0.34±0.03	98.46±0.12	16.33±5.7

Results are expressed as means ± SD of 5 determinations per experiment.

* = Value statistically significant $p \leq 0.05$ (test verses control by student t- test).

Mammalian toxicity assessment

Results of the *in vitro* toxicity assessment are summarized in table 4. The aqueous extract of *H. castaneifolia* was moderately cytotoxic with CC_{50} of 22 μg/ml. On the other hand the methanol extract of *H. castaneifolia,* aqueous or methanol extracts of *T. mombassana* and *T. nobilis* were not cytotoxic even at 100 μg/ml, the highest concentration tested.

In the acute toxicity assay, no deaths were observed at the highest concentration tested (5000 mg/kg) for

all the extracts. The mice treated with the plant extracts continued to gain weight at a similar rate to that seen in the untreated controls and no external toxic effects or mortality were observed within 14 days.

Table 4: Cytotoxicity (CC_{50}) of selected medicinal plants extracts on VERO cells

Plant	Extract	Cytotoxicity activity CC_{50} (µg/ml)	Antiplasmodial activity IC_{50} (µg/ml)	Selectivity Index (SI)
T. mombassana	Water	≥100.00	33.07±1.42	N.C
	Methanol	≥100.00	6.104±0.86	N.C
H. castaneifolia	Water	22.00	23.92±1.08	0.92
	Methanol	≥100.00	8.86±1.87	N.C
T. nobilis	Water	≥100.00	130.5±9.4	N.C
	Methanol	≥100.00	8.61±1.37	N.C
Control	Chloroquine	24.74	0.0046 ± 1.8	5378.26

SI = CC_{50} / IC_{50}

N.C: Not Cytotoxic at the highest concentration tested.

Discussion

The aqueous extracts had lower antiplasmodial activity *in vitro* compared with their corresponding methanol extracts. A possible explanation for the lower antiplasmodial activity observed in the aqueous extracts is that they were not prepared according to the traditional methods, in the traditional context, its very common for several plant taxa to be administered as mixtures or boiled in meat soup (water plus fat in the meat). In other cases, extraction is traditionally done by adding honey and letting it to ferment [25]. For this reason, it is possible that synergism could exist between the various phytochemicals or the different constituents could help extract and keep active lipophilic compounds in an aqueous solution in the traditional way of preparation [26]. This may explain why the methanolic extracts were more active, since methanol extracts have both polar and non polar compounds.

In a previous study, Gakunju et al reported that the root water extract of *T. mombassana* had antiplasmodial activity with an IC_{50} of 90 µg/ml against chloroquine sensitive *P. falciparum* strain (K67), while the water extract of the leaves of this plant,

investigated in the present study was found to have an IC_{50} of 33.07 µg/ml against the chloroquine sensitive *P. falciparum* strain (D6), affirming antimalarial potential of this plant [27]. The knowledge that the leaves are more active than the roots is encouraging as harvesting of leaves for medicinal purposes is not destructive to the plants. Harvesting of the roots means uprooting the whole plant, hence destroying it. The water extract of *Hugonia castaneifolia* twigs, had good antimalarial activity, with no toxic signs in mice. However, the same extract showed cytotoxic activity on Vero E6 cell line *in vitro*. The isolation of a cytotoxic rosane diterpenoid hugorosenone from *H. castaneifolia* root bark has been reported [28] revealing that a similar compound could also be present in the twigs. *In vitro* cytotoxicity does not inevitably mean an extract cannot be used in humans [28,29] as there is potential for isolation of safe non toxic compound/s. For instance, *Galega officinalis* is a plant that has proved too toxic for widespread agricultural use, with the potential to induce tracheal frothing, pulmonary oedema, hydrothorax, hypotension, paralysis and even death in man and yet Metformin the current gold standard for management of type 2 diabetes was isolated from it. Experimental and clinical evaluations of galegine, a substance produced by the herb *G. officinalis* provided the pharmacological and chemical basis for the subsequent discovery of metformin [30].

In the acute toxicity assay no deaths were observed at the highest concentration tested which was (5000 mg/kg), indicating that the plant extracts were not toxic. Another pointer to the safety of most of the samples tested is that all the animals that received the extracts stayed alive for the entire period of the 4-day suppressive test. Generally, if the test mice die before day 5, then the cause of death is usually attributed to the effect of the test drug rather than the parasites [31] suggesting that the therapeutic index is too low.

Conclusion

The activities observed both *in vitro* and *in vivo* as well as the safety studies for most of the plants selected, validate their use in traditional medicine to treat malaria. Out of the five extracts that had remarkable antiplasmodial activity *in vitro,* only the aqueous extract of *H. castaneifolia* was found to be cytotoxic. Intrestingly, it was not found to be toxic in mice. In view of this, it would be interesting to determine if the compounds responsible for the moderate cytotoxicity in *H. castaneiofolia* are the same or different from those responsible for *in vitro* antimalarial activity.

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References

1. Willcox M, Bodeker G and Rasoanaivo P. (2004). Traditional Medicinal Plants and Malaria. CRC Press,Washington D.C. 2004.

2. Plowe CV. Monitoring antimalarial drug resistance: making the most of the tools at hand. *Journal of Experimental Biology.* 2003; **206**: 3745–3752.

3. Katuura E, Waako P, Tabuti JRS, Bukenya-Ziraba R, and Okeng OJ. Antiplasmodial activity of extracts of selected medicinal plants used by local communities in western Uganda for treatment of malaria. *African Journal of Ecology*. 2007; **45**: 94–98.

4. Dianne J, Jeanne M, Margarette S, Oren S, Aggrey J, Piet A, Altaf A, Bernard L and Feiko O. Treatment history and treatment dose are important determinants of sulfadoxine-pyrimethamine efficacy in children with uncomplicated malaria in Western Kenya. *Journal of Infectious Diseases.* 2003; **187**: 467-468.

5. Wongsrichanalai C and Meshnick RS. Declining Artesunate-Mefloquine efficacy against Falciparum Malaria on the Cambodia–Thailand Border. *Journal of Emerging Infectious Diseases.* 2008; **14**: 716-9.

6. Jambou R, Legrand E and Niang M. Resistance of Plasmodium falciparum field isolates to in-vitro artemether and point mutations of the SERCA-type PfATPase6. *Lancet*. 2005; **366**: 1960-3.

7. Vijaykadga S, Rojanawatsirivej C, Cholpol S, Phoungmanee D, Nakavej A and Wongsrichanalai C. In vivo sensitivity monitoring of mefloquine monotherapy and artesunate-mefloquine combinations for the treatment of uncomplicated falciparum malaria in Thailand in 2003. *Tropical Medicine and International Health*. 2006; **11**: 211-9.

8. Trager W. and Jensen J. B. Human malaria parasites in continuous culture. *Science.* 1976; **193**: 673-675.

9. Schlichtherle, M., Wahlgren, M., Perlmann, H. and Scherf, A. *Methods in Malaria Research. MR4/ATCC,* Manassas, Virginia. 2000; 1-74.

10. Desjardins RE, Canfield C, Haynes J and Chulay J. Quantitative assessment of anti-malarial activity *in vitro* by a semi-automated microdilution technique. *Antimicrobial Agents and Chemotherapy.* 1979; **16**: 710-718.

11. Sixsmith DG, Watkins WM, Chulay JD and Spencer HC. *In vitro* anti-malarial activity of tetrahydrofoliate dehydrogenase inhibitors. *American Journal of Tropical Medicine and Hygiene.* 1984; **33**: 772-776.

12. Peters W, Portus JH and Robinson BL. The chemotherapy of rodent malaria, XXII. The value of drug -resistant strains of *Plasmodium berghei* in screening for blood schizontocidal activity. *Annals of Tropical Medicine and Parasitology* 1975; **69**: 155-171.

13. Gessler M C, Tanner M, Chollet J, Nkunya MHH and Heinrick M. Tanzanian medicinal plants used traditionally for the treatment of malaria*: in vivo* anti-malarial and *in vitro* cytotoxic activities. *Phytotherapy Research.* 1995; **9**: 504–508.

14. Tona L, Mesia K, Ngimbi NP, Chrimwami B, Cimanga OK, De Bruyne T, Pieters L and Vlietinck AJ. *In-vivo* anti-malarial activity of Cassia occidentalis, Morinda morindoides and phyllanthus niruri. *Annals of Tropical Medicine and Parasitology.* 2001; **95**: 47–57.

15. Armitage P and Berry G. Statistical Methods in Medicinal Research. Blackwell Science Ltd, Oxford, UK, 1991; 90–92.

16. Mossman T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods.* 1983; **65**: 55–63.

17. Phillipson J D and Wright C W. Can ethnopharmacology contribute to the development of anti-malarial agents? *Journal of Ethnopharmacology.* . 1991; **32**: 155–165.

18. Lorke D. A new approach to practical acute toxicity testing. *Archives of Toxicology.* 1983; **54**: 275–287.

19. WHO/EDM/TRM/2000.1. General guidelines for methodologies on research and evaluation of traditional medicines. WHO, Geneva, 2000.

20. Twaij HAA, Kery A and Al Khazraji NK. Some pharmacological, toxicological and phytochemical investigations on Centaurea phyllocephala. *Journal of Ethnopharmacology.* 1983; **9**: 299–314.

21. Gathirwa JW, Rukunga G M, Njagi ENM, Omar SA, Guantai AN, Muthaura CN, Mwitari PG, Kimani CW, Kirira PG, Tolo FM., Ndunda TN and Ndiege IO. *In vitro* anti-plasmodial and *in vivo* antimalarial activity of some plants used in the treatment of malaria by the Meru community in Kenya. *Journal of Natural Medicines.* 2007; **61**: 261–268.

22. Muregi FW, Ishih A, Miyase T, Suzuki T, Kino H, Amano T, Mkoji GM and Terada M. Antimalarial activity of methanolic extracts from plants used in Kenyan ethnomedicine and their interactions with chloroquine (CQ) against a CQ-tolerant rodent parasite, in mice. *Journal of Ethnopharmacology.* 2007; **111**: 90–195.

23. Carvalho LH, Brandão MGL, Santos-Filho D, Lopes JLC and Krettli AU. Antimalarial activity of crude extracts from Brazilian plants studied in vivo in *Plasmodium berghei*-infected mice and in vitro against *Plasmodium falciparum* in culture. *Brazilian Journal of Medical and Biological Research*. 1991; **24**: 1113– 1123.

24. Andrade-Neto VF, Brandão MGL, Stehmann JR, Oliveira LA and Krettli AU. Antimalarial activity of *Cinchona*-like plants used to treat fever and malaria in Brazil. *Journal of Ethnopharmacology.* 2003; **87**: 253–256.

25. Gathirwa JW, Rukunga GM, Mwitari PG, Mwikwabe MN, Muthaura CN, Kimani CW, Kiboi DM, Nyangacha RM and Omar SA. Traditional herbal antimalarial therapy in Kilifi district, Kenya. *Journal of Ethnopharmacology.* 2011; **134**: 434–442.

26. Clarkson C, Maharaj VJ, Crouch NR, Grace OM, Pillay P, Matsabisa MG, Bhagwandhin N, Smith PJ and Folb PI. *In vitro* antiplasmodial

activity of medicinal plants native to or naturalized in South Africa. *Journal of Ethnopharmacology* 2004. **92**: 177-191.

27. Gakunju DMN, Mberu IEK, Dossaji SF, Gray AI, Waigh R D, Waterman PG and Watkins WM. Potent antimalarial activity of the alkaloid nitidine, isolated from a Kenyan herbal remedy. *Antimicrobial Agents and Chemotherapy.* 1995; **39**: 2606-2609.

28. Baraza DL, Cosam C, Joseph CC, Joan JE, Munissi JEJ, Nkunya HHM, Arnold N, Porzel, A and Wessjohann L. Antifungal rosane diterpenes and other constituents of *Hugonia castaneifolia* . *Phytochemistry.* 2008; **69**: 200-205.

29. Kokwaro JO. *Medicinal Plants of East Africa*. Kenya Literature Bureau, Nairobi, 1993.

30. Bailey CJ, Campbell IW, Chan JCN, Davidson JA, Howlett HCS and Ritz P. *Metformin: the Gold Standard. A Scientific handbook*. Chichester, Wiley, 2007.

31. Jutamaad S, Noppamas S, Aimon S and Yodhathai T. Toxicological and anti-malarial activity of eurycomalactone and *Eurycoma longifolia* Jack extract in mice. *Thai Journal of Phytopharmacy.* 1998; **5**: 14-17.