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# The *in vitro* anti-plasmodial and *in vivo* anti-malarial efficacy of combinations of some medicinal plants used traditionally for treatment of malaria by the Meru community in Kenya

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**Abstract**

The use of herbal drugs as combinations has existed for centuries in several cultural systems. However, the safety and efficacy of such combinations have not been validated. In this study, the toxicity, anti-plasmodial and antimalarial efficacy of several herbal drug combinations were investigated. *Lannea schweinfurthii*, *Turraea robusta* and *Sclerocarya birrea*, used by traditional health practitioners in Meru community, were tested for *in vitro* anti-plasmodial and *in vivo* anti-malarial activity singly against *Plasmodium falciparum* and *Plasmodium berghei*, respectively. Methanolic extract of *Turraea robusta* was the most active against *Plasmodium falciparum* D6 strain. Aqueous extracts of *Lannea schweinfurthii* had the highest anti-plamodial activity followed by *Turraea robusta* and *Sclerocarya birrea*. D6 was more sensitive to the plant extracts than W2 strain. *Lannea schweinfurthii* extracts had the highest anti-malarial activity in mice followed by *Turraea robusta* and *Sclerocarya birrea* with the methanol extracts being more active than aqueous ones. Combinations of aqueous extracts of the three plants and two others (*Boscia salicifolia* and *Rhus natalensis*) previously shown to exhibit anti-plasmodial and anti-malarial activity singly were tested in mice. Marked synergy and additive interactions were observed when combinations of the drugs were assayed *in vitro*. Different combinations of *Turraea robusta* and *Lannea schweinfurthii* exhibited good *in vitro* synergistic interactions. Combinations of *Boscia salicifolia* and *Sclerocarya birrea*; *Rhus natalensis* and *Turraea robusta*; *Rhus natalensis* and *Boscia salicifolia*; *Turraea robusta* and *Sclerocarya birrea*; and *Lannea schweinfurthii* and *Boscia salicifolia* exhibited high malaria parasite suppression (chemo-suppression >90%) *in vivo* when tested in mice. The findings are a preliminary demonstration of the usefulness of combining several plants in herbal drugs, as a normal practice of traditional health practitioners.


*Keywords:* Medicinal plants; Anti-plasmodial; Anti-malarial; Combination; Efficacy; Toxicity

## 1. Introduction

Malaria remains a major public health problem in Africa, and is responsible for the death of over 1 million annually (Butler, 1997; Geoffrey, 1998). Drug resistance has been implicated in the enhanced mortality (Trape, 2002), and is a factor in the economic constraints of malaria control (Bloland, 2001). In Kenya, chloroquine (CQ) has been discontinued as the first line treatment for malaria due to overwhelming presence of resistant *Plasmodium falciparum* strains (Dianne et al., 2003). The spread of multi-drug resistant malaria parasite strains has highlighted the urgent need for the development of new, inexpensive, affordable and safe anti-malarial drugs for developing countries where the disease is prevalent (Miller, 1992; Vial, 1996; Wernsdorfer and Trigg, 1988). Due to overwhelming resistance to single

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therapy, combination of drugs has been adopted to combat malaria (Staedke et al., 2001). The use of herbal extracts in combination has existed for decades in cultural systems. For instance, traditional Chinese medical knowledge and practice revealed that *Artemisia annua* was used in combination with other plants in the treatment of fevers (Phillipson and O'Neill, 1987), and this may explain why no resistance was reported for over 2000 years.

Interviews carried out among traditional health practitioners in Meru region, Kenya, revealed the use of many plants, commonly dispensed as combinations against febrifuge and malaria in traditional remedies. The plant extracts were tested singly *in vitro* and *in vivo* and three: *Turrea robusta*, *Sclerocarya birrea*, *Lannea schweinfurthii*, found to exhibit high activity. As a continuation of our efforts to understand traditional medicines of the Meru community, we evaluated the toxicity, anti-plasmodial and anti-malarial activity of herbal drug combinations of the three plants and two others (*Boscia salicifolia* and *Rhus natalensis*) previously shown to exhibit anti-plasmodial and anti-malarial activity (Gathirwa et al., 2007).

## 2. Materials and methods

### 2.1. Collection and processing of plant materials

The plants (Table 1) were collected between February and August 2004, identified, authenticated at the East African Herbarium at National Museum of Kenya (NMK), assigned voucher numbers and the sample specimens deposited for future reference.

The plant materials were air-dried under shade for 14 days, ground using a laboratory mill (Christy & Norris Ltd., Chelmsford, England) and 100 g of powder extracted in 300 ml of sterilized water at 60 °C for 1 h. An equal amount was extracted by soaking in 300 ml of methanol overnight at room temperature. The extracts were filtered and concentrated using a freeze drier or rotary evaporator for water and organic extracts, respectively.

### 2.2. In vitro anti-plasmodial assay

Two strains of *Plasmodium falciparum*: the Sierra Leonean CQ-sensitive D6 and the Indochinese CQ-resistant W2 were used in the study. Parasite cultivation was carried out using previously described procedures (Trager and Jensen, 1976; Schlichtherle et al., 2000). Test samples were prepared by dissolving aqueous extracts in distilled sterilized water while the methanolic extracts were dissolved in 100% DMSO (Sigma Chemical Co., St Louis, MO, USA) and diluted to lower the concentration of DMSO to ≤1%. Stock solutions (1 μg/ml) of CQ and MQ were prepared for use as reference drugs. The semi-automated micro-dilution technique was adapted in assessing *in vitro* anti-plasmodial activity (Desjardins et al., 1979; Le Bras and Deloron, 1983). Briefly, 96-well flat-bottom micro-culture plates were pre-coated with test solutions in duplicate. Wells of the first row (A) contained test solutions of the drug at the highest concentration (125 μg/ml). Serial dilution was carried out under sterile conditions in laminar flow hood (Bellco Glass Inc., USA) using a Titertek motorized hand diluter (Flow Laboratories, Uxbridge, UK) from the first (A) to the second last well (G) achieving a 64-fold dilution while last wells (H) served as control. Parasite cultures at ≥80% ring-stage, ≥4% percentage parasitaemia (%P) and ≥3% growth rate were used in anti-plasmodial assays. Growth media was used to adjust the culture parasitaemia to 0.4% (P) and 1.5% haematocrit from which 200 μl was dispensed into each well of the drug pre-coated micro-culture plate. Plates containing parasitized and non-parasitized erythrocytes were incubated at 37 °C in a gas mixture (3% $CO_2$, 5% $O_2$ and 92% $N_2$) for 48 h, 25 μl of 0.5 μCi [G-$^3$H]hypoxanthine (Amersham International, Buckinghamshire, UK) in culture medium added to each well and incubated for another 18 h. At the end of the incubation period, the radio labelled cultures were harvested onto glass fibre filters and [G-$^3$H]hypoxanthine uptake determined using liquid scintillation and luminescence counter (Wallac MicroBeta TriLux). Drug concentration capable of inhibiting 50% of the *Plasmodium falciparum* ($IC_{50}$) was determined as previously described (Sixsmith et al., 1984).

### 2.3. Cytotoxicity studies

Actively dividing sub-confluent Vero cell growth-inhibition assay was done (Kurokawa et al., 1995). Briefly, Vero cells were grown in Eagle's minimum essential medium (MEM) (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) in 25 ml cell culture flasks incubated at 37 °C in 5% $CO_2$. Upon attainment of confluence, the cells were seeded with $5 \times 10^4$ cells/well in 24-well plates and incubated at 37 °C for 2 days. The culture medium was replaced by fresh MEM containing test extracts at different concentrations, and incubated further for another 2 days. Cells for each sample were detached by trypsinization in triplicate wells, and the number of viable cells determined by a tryphan blue exclusion test. A haemocy-

Table 1
Collected anti-plasmodial plants

| Botanical name | Family | Specimen number | Part used | Extracts yield | |
|---|---|---|---|---|---|
| | | | | Water (%) | Methanol (%) |
| *Turraea robusta* | Meliaceae | (K4) CM036 | Root bark | 4.10 (8.2) | 0.85 (1.7) |
| *Lannea schweinfurthii* | Anacardiaceae | (K4) CM038 | Stem bark | 6.65 (13.3) | 1.15 (2.3) |
| *Sclerocarya birrea* | Anacardiaceae | (K4) CM042 | Stem bark | 6.30 (12.6) | 1.30 (2.6) |

tometer was used to aid in counting viable cells. Inhibition data was plotted as dose-response curves, from which $CC_{50}$ (concentration required to cause visible alterations in 50% of intact cells) was estimated. Selectivity index (SI) was used as parameter of clinical significance of the test samples by comparing general toxins and selective inhibitory effect on *Plasmodium falciparum* calculated as (Wright and Phillipson, 1990):

$$\mathrm{SI} = \frac{\mathrm{CC_{50}\,(Vero)}}{\mathrm{IC_{50}\,(\textit{Plasmodium falciparum})}}.$$

### 2.4. Acute toxicity

*In vivo* acute toxicity was determined in mice using standard procedures (Lorke, 1983; Tona and Mesia, 2001). Groups of five starved animals were given increasing dosages of test sample (250, 500, 750, 1000, 1500 and 5000 mg/kg body weight) orally and the number of deaths occurring within 24–48 h noted. Probit-log treatment was used for data analysis and $LD_{50}$ values determined (Finney, 1964).

### 2.5. In vitro drug interaction experiments

The method described by Canfield et al. (1995) was adopted. Briefly solutions of initial concentrations 20–50 times the estimated $IC_{50}$ values were combined in different ratios of the various drugs. Thus fixed ratios of pre-determined concentrations needed to inhibit parasite growth by 50% ($IC_{50}$) was used to determine the interaction of two drugs. Single and combined drug solutions were dispensed into the 96-well micro-titre plates to give nine combinations in ratios of 90:10 to 10:90 (extract A:extract B) (Fivelman et al., 1999). Incubation and subsequent procedures were followed as previously described for *in vitro* anti-plasmodial assay (Desjardins et al., 1979; Le Bras and Deloron, 1983). Corresponding $IC_{50}$ values were determined for each drug alone and in combination (Sixsmith et al., 1984). The degree of synergy was evaluated according to Berenbaum (1978). Briefly, sum of fractional inhibition concentration (SFIC) ($K$) was calculated using the formula:

$$\frac{A_c}{A_e} + \frac{B_c}{B_e} = K,$$

$A_c$ and $B_c$ are the equally effective concentrations ($IC_{50}$) when used in combination, and $A_e$ and $B_e$ are the equally effective concentrations when used alone. In this system: SFIC < 1 denotes synergism, 1 ≤ SFIC < 2 denotes additive interaction, while SFIC ≥ 2 denotes antagonism (Gupta et al., 2002).

### 2.6. In vivo anti-plasmodial activity

The test extracts were prepared by dissolving the aqueous extracts in sterilized water while the methanol extracts were dissolved in 10% (w/v) tween 80. All the samples were then filter-sterilized by passing through 0.22 μm syringe adaptable filters. Male Balb C mice (6–8 weeks old, weighing 20 ± 2 g) were used as the subjects. The mice were bred in standard macrolon type II cages in air-conditioned rooms at 22 °C, 50–70% relative humidity, fed with the standard diet and water *ad libitum*. *Plasmodium berghei* strain ANKA maintained by serial passage of infected blood through interperitonial injection (i.p.) was used in the study. The test protocol was based on the 4-day suppressive test (Peters et al., 1975). Briefly, *Plasmodium 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 i.p. injection with 0.2 ml ($2 \times 10^7$ parasitized erythrocytes). Infected mice were randomly divided into two groups of five for every test sample such that one group would be treated orally while the other via i.p. route. This being a preliminary study, only one dosage (100 mg/kg) of the plant extracts was used. The experimental groups were treated with 100 mg/kg of the test sample in 0.2 ml by oral administration and i.p. injection 2–4 h post-infection (Gessler et al., 1995). After 1, 2 and 3 days (24, 48 and 72 h post-infection), the experimental groups were treated again with the same dose. Three groups (five mice per group) served as negative and positive controls. The negative group received a placebo (PBS) while the two positive groups were treated with 5 mg/kg day of the reference drug (CQ) one through oral administration and the other by i.p. injection. Parasitaemia was determined daily (24 h interval) by microscopic examination and counting the parasites in 4 fields of ~100 erythrocytes per view of thin blood film sampled from the tail of the experimental mice and stained in 10% Giemsa solution. The difference between the mean number of parasites per view in the control group (100%) and those of the experimental groups was calculated and expressed as percent parasitaemia suppression (PS) according to the formula (Tona and Mesia, 2001):

$$\mathrm{PS} = \left[\frac{A - B}{A}\right] \times 100.$$

$A$ is the mean parasitaemia in the negative control group on day 4 and $B$ the corresponding parasitaemia in the test group.

The standard deviations for the mean values were calculated as described by Armitage and Berry (1991). The mean survival time (days) for all groups was determined. Plant extracts combinations that exhibited strong additive or synergistic interactions *in vitro* were selected for *in vivo* interaction studies. The best performing combinations were mixed from stock solutions and the *in vivo* experiments carried out as described above. Chemosuppression (%) of the blends and individual plant extracts were compared.

## 3. Results and discussion

### 3.1. In vitro anti-plasmodial assays

Results of *in vitro* anti-plasmodial assay of methanolic and aqueous extracts of the three selected medicinal plants against *Plasmodium falciparum* D6 and W2 strains are summarized in Table 2.

Anti-plasmodial activity was classified as follows: high at $IC_{50} \leq 10$ μg/ml, moderate at 10–50 μg/ml, low at 50–100 μg/ml and inactive at >100 μg/ml. The $IC_{50}$ values for W2

Table 2
*In vitro* anti-plasmodial activity ($IC_{50} \pm$ S.D.) of extracts of selected medicinal plants

| Plant | $IC_{50} \pm$ S.D. (μg/ml) | | | |
|---|---|---|---|---|
| | Aqueous | | Methanol | |
| | D6 | W2 | D6 | W2 |
| *Turraea robusta* | 25.32 ± 0.25 | 42.41 ± 2.41 | 2.09 ± 0.13 | 10.32 ± 4.63 |
| *Lannea schweinfurthii* | 10.55 ± 0.62 | 75.90 ± 9.52 | 11.38 ± 0.65 | 36.26 ± 8.52 |
| *Sclerocarya birrea* | 18.96 ± 5.32 | 71.74 ± 4.36 | 5.91 ± 0.36 | 24.96 ± 3.62 |

$IC_{50}$: CQ 3.11 ± 1.21 and 57.34 ± 2.95 ng/ml; MQ 16.08 ± 2.65 and 2.55 ± 0.03 ng/ml for D6 and W2, respectively.

strain were generally slightly higher than those for D6. This is probably due to the resistant nature of the parasite strain used. Methanolic extract of *Turraea robusta* and *Sclerocarya birrea* ($IC_{50}$ 2.09 ± 0.13 and 5.91 ± 0.36, respectively) were the most active against D6 strain while that of *Lannea schweinfurthii* was moderately active on the same. All methanolic extracts were moderately active on W2. Water extracts of all the plants exhibited moderate activity against D6 but were less effective on W2. All the extracts tested show significantly lower activity against resistant clones than in CQ-sensitive isolates, suggesting that they have similar modes of action to CQ. However, being blends of several compounds, resistance development against these extracts may be delayed.

### 3.2. *In vivo assays*

Results of *in vivo* anti-malarial assays of the plant extracts using *Plasmodium berghei* in mice are summarized in Table 3.

Suppression of parasitaemia (chemo-suppression) in mice and mean survival time of the animals were used as measures of efficacy. Test samples were categorized as highly active when chemo-suppression was above 60%. Methanol and water extracts of *Lannea schweinfurthii* and methanol extract of *Turraea robusta* were the most active in i.p. treatment with chemo-suppression of 91.37 ± 9.33, 83.08 ± 9.53 and 78.20 ± 7.59%, respectively. The chemo-suppression of malaria parasites by *Lannea schweinfurthii* extracts was not significantly different ($p < 0.05$) from that of chloroquine. The mean parasite density in animals treated with chloroquine was 0.38 ± 0.03 and 13.27 ± 1.48 for i.p. and oral administration, respectively. However, the mean parasite density in animals treated with PBS (control) was 37.47 ± 6.62 and 35.23 ± 3.61 for i.p. and oral administration, respectively. A similar reduction in parasite density was observed in the animals treated with *Lannea schweinfurthii* methanolic extract (3.17 ± 1.42). There was significant parasite density reduction ($p < 0.05$) in animals treated with most of the herbal test samples compared to the ones treated with PBS (control). The reduced peak parasitaemia on day 4 in all treated groups compared to the negative control group was indicative of anti-malarial potential of the extracts. The survival time of mice that received medicinal plant extracts exhibiting high chemo-suppression lived longer than those that received the ones with low chemo-suppression. The differences in mean survival times for the animals treated with herbal extracts were significant ($p < 0.05$) compared to the ones treated with PBS (control). Since the i.p. treated groups had higher parasite suppression than the ones subjected to oral drug administration, it was expected that the first group would have longer mean survival times. However, this was not true as survival time was similar in the two cases. This may be due to physiological stress or other factors related to i.p. administration of the herbal drugs. These unknown factors could not be addressed in this study and are open to further evaluation. The generated data is interesting and further studies on *Lannea schweinfurthii*, *Turraea robusta* and *Sclerocarya birrea* should be undertaken. Previous *in vivo* anti-malarial data on the extracts of these plants is not available.

Table 3
Effect of selected medicinal plant extracts on *Plasmodium berghei* in mice

| Plant | Extract | Interperitoneal (100 mg/kg) | | | Oral (100mg/kg) | | |
|---|---|---|---|---|---|---|---|
| | | Parasite density | Chemo-suppression (%) | Mean survival time (days) | Parasite density | Chemo-suppression (%) | Mean survival time (days) |
| *Turraea robusta* | $H_2O$ | $13.56 \pm 1.82^{d}$ | $63.81 \pm 5.27^{g}$ | $13.62 \pm 1.33^{k}$ | $18.52 \pm 3.41^{p}$ | $48.83 \pm 3.74^{n}$ | $12.86 \pm 2.56^{k}$ |
| | MeOH | $8.00 \pm 2.35^{c}$ | $78.20 \pm 7.59^{h}$ | $11.81 \pm 5.27^{k}$ | $16.72 \pm 2.87^{p}$ | $52.24 \pm 4.54^{g}$ | $12.97 \pm 3.41^{k}$ |
| *Lannea schweinfurthii* | $H_2O$ | $6.34 \pm 0.83^{c}$ | $83.08 \pm 9.53^{h}$ | $13.27 \pm 5.21^{k}$ | $18.42 \pm 3.36^{p}$ | $48.43 \pm 3.95^{n}$ | $15.26 \pm 4.78^{j}$ |
| | MeOH | $3.17 \pm 1.42^{b}$ | $91.37 \pm 9.33^{i}$ | $12.39 \pm 4.21^{k}$ | $17.12 \pm 3.04^{p}$ | $51.66 \pm 1.85^{g}$ | $14.24 \pm 4.02^{j}$ |
| *Sclerocarya birrea* | $H_2O$ | $12.55 \pm 2.42^{d}$ | 66.51 ± 7.2+ | $15.22 \pm 2.43^{j}$ | $21.06 \pm 2.47^{q}$ | $40.21 \pm 2.78^{n}$ | $15.38 \pm 5.41^{j}$ |
| | MeOH | $13.50 \pm 4.29^{d}$ | $63.49 \pm 8.86^{g}$ | $13.28 \pm 3.54^{k}$ | $20.42 \pm 2.79^{q}$ | $42.04 \pm 2.94^{n}$ | $15.19 \pm 2.84^{j}$ |
| PBS | | $37.47 \pm 6.62^{e}$ | $0.00 \pm 0.00^{f}$ | $7.14 \pm 2.14^{l}$ | $35.23 \pm 3.61^{e}$ | $0.00 \pm 0.00^{f}$ | $8.20 \pm 1.16^{l}$ |
| CQ diphosphate (5 mg/kg) | | $0.38 \pm 0.03^{a}$ | $99.02 \pm 1.26^{i}$ | $18.25 \pm 5.28^{m}$ | $13.27 \pm 1.48^{d}$ | $62.34 \pm 2.96^{g}$ | $17.85 \pm 2.19^{m}$ |

Values with same letters are not significantly different ($p > 0.05$), values with different superscripted letters are significantly different ($p < 0.05$), parasite density and chemo-suppression were calculated on day 4.

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

| Plant | Extract | $CC_{50}$ (μg/ml) | SI[a] (D6) | SI[a] (W2) |
|---|---|---|---|---|
| *Turraea robusta* | MeOH | 24.38 | 11.67 | 2.36 |
| | $H_2O$ | 45.72 | 1.81 | 1.08 |
| *Lannea schweinfurthii* | MeOH | 225.25 | 19.79 | 6.21 |
| | $H_2O$ | 3256.52 | 308.67 | 42.91 |
| *Sclerocarya birrea* | MeOH | 361.24 | 61.12 | 14.47 |
| | $H_2O$ | 3375.22 | 178.02 | 47.05 |

[a] SI-selectivity index calculated as $CC_{50}/IC_{50}$.

### 3.3. In vitro toxicity assays

Results of *in vitro* toxicity assay of the medicinal plant extracts are summarized in Table 4.

*Turraea robusta* exhibited toxicity ($CC_{50}$ 24.38, 45.72 μg/ml for methanol and aqueous extracts, respectively) with a low selectivity index for aqueous extract against D6 (1.81). Its selectivity indices for W2 were also low (2.36 and 1.08 for methanol and aqueous extract, respectively). The methanol extract of *Lannea schweinfurthii* had a slightly low-selectivity index against W2 (6.21) indicative of mild toxicity. In the acute toxicity assay, no deaths were observed at the highest concentration tested. The $LD_{50}$ values for all the plants were above 5000 mg/kg body weight when tested by oral administration. Administration by the i.p. injection was not done due to the viscous nature of the extracts at high dosage (>100 mg/kg). Another pointer to the safety of most of 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 (Jutamaad et al., 1998) suggesting that the therapeutic index is too low. The aqueous extracts were generally less toxic that methanolic ones. Few scientific studies have been undertaken to ascertain the safety of traditional remedies prior to putting them to clinical use despite several scientific reports showing that many plants used as food and traditional medicine are potentially toxic, mutagenic and carcinogenic (Schimmer et al., 1994).

### 3.4. In vitro drug interactions

In our earlier communication (Gathirwa et al., 2007), we observed that the methanolic extract of *Boscia salicifolia* exhibited high anti-plasmodial activity against *Plasmodium falciparum* D6 and W2 strains ($IC_{50}$ $1.03 \pm 0.03$ and $8.86 \pm 0.24$ μg/ml, respectively). The water and methanol extracts also exhibited the highest selective inhibition (SI > 34). Similarly, the methanol extract of *Boscia salicifolia* was very active *in vivo* with chemo-suppression of $86.50 \pm 4.37\%$. Although the aqueous and methanolic extracts of *Rhus natalensis* were the least active (>100 μg/ml), the water extract of *Rhus natalensis* exhibited high anti-malarial activity with chemo-suppression of $83.15 \pm 3.61\%$ and corresponding significant reduction in parasite density in the animals treated with the extract ($6.31 \pm 0.97$). The methanol extract of *Rhus natalensis* was the most toxic ($CC_{50}$ 211.78 μg/ml, SI 2.76 and 2.15 for D6 and W2, respectively). However, the water extract exhibited high selective inhibition ($CC_{50}$ 3958.16 μg/ml; SI 35.47 and 37.7 for D6 and W2, respectively). Consequently, *Boscia salicifolia* and *Rhus natalensis* methanol and aqueous extracts were recommended for *in vitro* and *in vivo* drug combination assays together with *Turraea robusta*, *Lannea schweinfurthii* and *Sclerocarya birrea*.

Results of *in vitro* interaction of the aqueous extracts of selected medicinal plants against *Plasmodium falciparum* W2 (CQ resistant clone) are summarized in Tables 5–8.

Moderate to weak synergism was observed in most combinations of *Turraea robusta* and *Lannea schweinfurthii* except towards equal proportion of each extract (50:50) where moder-

Table 5
Interaction of aqueous extracts of *Turraea robusta* with selected medicinal plants

| | Ratio | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 90:10 | 80:20 | 70:30 | 60:40 | 50:50 | 40:60 | 30:70 | 20:80 | 10:90 |
| *Lannea schweinfurthii* | 0.90[c] | 0.84[c] | 0.76[c] | 0.83[c] | 1.04[b] | 1.65[b] | 0.68[c] | 0.68[c] | 0.56[c] |
| *Sclerocarya birrea* | 1.21[b] | 1.02[b] | 1.02[b] | 1.01[b] | 1.17[b] | 0.78[c] | 1.00[b] | 1.01[b] | 1.16[b] |
| *Boscia salicifolia* | 1.03[b] | 1.95[b] | 1.25[b] | 1.23[b] | 1.50[b] | 1.09[b] | 1.03[b] | 0.92[c] | 0.92[c] |
| *Rhus natalensis* | 1.36[b] | 1.38[b] | 1.42[b] | 1.41[b] | 1.39[b] | 1.33[b] | 1.33[b] | 1.31[b] | 0.98[c] |

The figures in the table are SFIC values, [a]antagonistic; [b]additive; [c]synergistic.

Table 6
Interaction of aqueous extracts of *Rhus natalensis* with selected medicinal plants

| | Ratio | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 90:10 | 80:20 | 70:30 | 60:40 | 50:50 | 40:60 | 30:70 | 20:80 | 10:90 |
| *Sclerocarya birrea* | 1.19[b] | 1.70[b] | 1.79[b] | 2.06[a] | 2.16[a] | 2.06[a] | 1.15[b] | 1.08[b] | 0.54[c] |
| *Lannea schweinfurthii* | 1.33[b] | 1.32[b] | 1.21[b] | 1.00[b] | 1.00[b] | 1.02[b] | 1.34[b] | 1.33[b] | 1.36[b] |
| *Boscia salicifolia* | 0.98[c] | 0.94[c] | 0.88[c] | 0.89[c] | 1.06[b] | 1.12[b] | 1.22[b] | 1.22[b] | 1.28[b] |

The figures in the table are SFIC values, [a]antagonistic; [b]additive; [c]synergistic.

Table 7
Interaction of aqueous extracts of *Lannea schweinfurthii* with selected medicinal plants

| | Ratio | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 90:10 | 80:20 | 70:30 | 60:40 | 50:50 | 40:60 | 30:70 | 20:80 | 10:90 |
| *Boscia salicifolia* | 1.12[b] | 1.11[b] | 0.92[c] | 1.01[b] | 1.03[b] | 1.06[b] | 1.16[b] | 1.24[b] | 1.28[b] |
| *Sclerocarya birrea* | 1.24[b] | 1.16[b] | 1.28[b] | 1.44[b] | 1.45[b] | 1.54[b] | 2.17[a] | 2.22[a] | 2.24[a] |

The figures in the table are SFIC values, [a]antagonistic; [b]additive; [c]synergistic.

ate additive interaction was noted. Only one combination (40:60) of *Turraea robusta* and *Sclerocarya birrea* exhibited moderate synergistic interaction with the rest displaying strong additive behavior. A similar scenario was observed in combinations of *Turraea robusta* and *Boscia salicifolia* with two combinations with more of *Boscia salicifolia* extract exhibiting weak synergism. As the amount of *Boscia salicifolia* extract in the combination increased from 1:1 to 1:9 the interaction changed from moderate addition to weak synergism. As the amount of *Turraea robusta* extract in the combination decreased from 90:10 to 50:50, the interaction changed from strong to moderate additive behavior. The interaction of *Turraea robusta* with *Rhus natalensis* was mainly additive with only the 9:1 ratio exhibiting weak synergy.

Combination of *Rhus natalensis* and *Sclerocarya birrea* extracts gave additive interaction at high concentration of *Rhus natalensis*, antagonism towards equal proportions and synergism at low concentrations of *Rhus natalensis.* The interaction of *Rhus natalensis* and *Lannea schweinfurthii* was additive at all tested ratios. The interaction of *Rhus natalensis* and *Boscia salicifolia* was synergistic at high amounts of *Boscia salicifolia* and additive as the later was reduced.

All combinations of *Lannea schweinfurthii* and *Boscia salicifolia* extracts gave additive interaction except at 70:30 where synergistic behavior was observed. At high amount of *Lannea schweinfurthii* while blended with *Sclerocarya birrea*, additive behavior was observed which changed to antagonism at high amount of the later.

Interaction of *Boscia salicifolia* with *Sclerocarya birrea* was additive at high concentration of *Boscia salicifolia* and antagonistic as its amount was reduced in the blend.

*In vitro* interaction studies carried out indicated synergistic or additive effects of the drug combinations on parasite development. These results highlight the interest in combination of plants extracts for the treatment of malaria. Synergism permits the quantity of each drug to be reduced, enhance efficacy and delay resistance. *In vitro* drug interaction of the plants tested showed promise of better results than when singly used. However, it is often difficult to predict *in vivo* drug interactions in humans on the basis of the *in vitro* results.

Table 8
Interaction of aqueous extracts of *Boscia salicifolia* with *Sclerocarya birrea*

| Ratio | *Sclerocarya birrea* |
|---|---|
| 90:10 | 1.33[b] |
| 80:20 | 1.30[b] |
| 70:30 | 1.27[b] |
| 60:40 | 1.08[b] |
| 50:50 | 1.98[b] |
| 40:60 | 2.22[a] |
| 30:70 | 2.65[a] |
| 20:80 | 2.65[a] |
| 10:90 | 2.84[a] |

The figures in the table are SFIC values, [a]antagonistic; [b]additive; [c]synergistic.

### 3.5. In vivo drug interactions

Combinations of aqueous extracts of medicinal plants giving the optimum anti-plasmodial activity (synergistic or additive effects) *in vitro* were subjected to further investigation for antimalarial potential in mice. Results of *in vivo* interaction of combinations of aqueous extracts of selected medicinal plants are summarized in Table 9.

No activity was exhibited by the combination of *Turraea robusta* and *Boscia salicifolia* (20:80) administered by i.p. route despite the fact that it exhibited weak synergism *in vitro*. This is surprising since aqueous extracts of *Boscia salicifolia* (Gathirwa et al., 2007) and *Turraea robusta* exhibited moderate antimalarial activity *in vivo* when tested singly (63.81 and 43.75% chemo-suppression, respectively). Oral administration of the same also resulted in reduced activity (21.46%) compared to singly tested extracts. It can be concluded that the combination is antagonistic *in vivo.* By definition, a strong antagonism refers to the loss of schizontocidal effect when the drugs are used in combination, requiring higher concentrations of the drugs to produce the same effect as the drugs alone (Berenbaum, 1978). This may have occurred in the case of the 20:80 combination of *Turraea robusta* and *Boscia salicifolia* aqueous extracts where loss of activity was observed *in vivo*. Mice in this cage died at the same time as the ones treated with PBS (control). Combination of aqueous extracts of *Turraea robusta* and *Lannea schweinfurthii* (10:90) also exhibited antagonistic interaction with chemo-suppression of the combination (57.47%) being lower than that of the single extracts (63.81 and 83.08%, respectively). The reduction in activity was observed for both i.p. and oral drug administration. These are cases of *in vivo* antagonism, which raise concern for resistance development to herbal preparations. If the drugs in combination are antagonistic, the efficacies of such regimens might be compromised and the chances of resistance development and spread increase, as less effective drugs may be allowing weakly resistant clones to survive and be transmitted. The observed *in vivo* antagonism should be a subject of further studies to establish exactly what happens under such situations. However, since *Turraea robusta* extract was shown to be weakly cytotoxic, the explanation to loss of activity would be the potentiated toxicity resulting into

Table 9
*In vivo* anti-malarial assay of combinations of aqueous extracts of selected plants extracts

| Combination | Extract ratio | SFIC | Drug administration | Parasite density | Chemo-suppression (%) | Survival time (days) |
|---|---|---|---|---|---|---|
| *Turraea robusta and Sclerocarya birrea* | 40:60 | 0.78 | i.p. | 3.52 ± 2.77$^{b}$ | 93.61 ± 3.74$^{g}$ | 16.43 ± 2.28$^{p}$ |
| | | | Oral | 23.94 ± 1.58$^{e}$ | 54.58 ± 3.25$^{i}$ | 15.12 ± 1.52$^{p}$ |
| *Turraea robusta and Boscia salicifolia* | 20:80 | 0.92 | i.p. | 55.23 ± 6.12$^{f}$ | 0.00$^{j}$ | 8.33 ± 1.95$^{n}$ |
| | | | Oral | 41.38 ± 3.54$^{f}$ | 21.46 ± 2.24$^{k}$ | 9.25 ± 2.78$^{n}$ |
| *Turraea robusta and Lannea schweinfurthii* | 10:90 | 0.56 | i.p. | 23.43 ± 3.81$^{e}$ | 57.47 ± 9.59$^{i}$ | 10.25 ± 1.71$^{q}$ |
| | | | Oral | 38.57 ± 3.33$^{f}$ | 26.78 ± 2.55$^{k}$ | 9.74 ± 2.45$^{n}$ |
| *Rhus natalensis and Lannea schweinfurthii* | 60:40 | 1.00 | i.p. | 15.3 ± 4.21$^{d}$ | 71.87 ± 5.74$^{h}$ | 15.21 ± 3.25$^{p}$ |
| | | | Oral | 26.07 ± 2.85$^{e}$ | 50.66 ± 4.63$^{i}$ | 11.16 ± 1.54$^{q}$ |
| *Rhus natalensis and Sclerocarya birrea* | 10:90 | 0.54 | i.p. | 7.05 ± 3.46$^{c}$ | 87.21 ± 6.29$^{h}$ | 12.82 ± 1.25$^{q}$ |
| | | | Oral | 29.66 ± 2.74$^{e}$ | 43.71 ± 2.58$^{l}$ | 11.33 ± 1.14$^{q}$ |
| *Rhus natalensis and Turraea robusta* | 10:90 | 0.98 | i.p. | 3.1 ± 1.78$^{b}$ | 94.38 ± 3.44$^{g}$ | 8.04 ± 1.53$^{n}$ |
| | | | Oral | 22.94 ± 3.58$^{e}$ | 57.71 ± 2.54$^{i}$ | 14.65 ± 2.74$^{p}$ |
| *Rhus natalensis and Boscia salicifolia* | 70:30 | 0.88 | i.p. | 3.02 ± 0.99$^{b}$ | 94.51 ± 5.27$^{g}$ | 14.80 ± 2.74$^{p}$ |
| | | | Oral | 20.44 ± 1.48$^{e}$ | 61.25 ± 1.75$^{m}$ | 16.48 ± 2.84$^{p}$ |
| *Lannea schweinfurthii and Boscia salicifolia* | 70:30 | 0.92 | i.p. | 5.17 ± 2.67$^{c}$ | 90.62 ± 8.48$^{g}$ | 18.96 ± 1.88$^{r}$ |
| | | | Oral | 20.53 ± 2.35$^{e}$ | 61.03 ± 4.36$^{m}$ | 18.15 ± 2.94$^{r}$ |
| *Lannea schweinfurthii and Sclerocarya birrea* | 80:20 | 1.16 | i.p. | 33.6 ± 3.58$^{e}$ | 39.02 ± 2.96$^{k}$ | 8.75 ± 1.52$^{n}$ |
| | | | Oral | 22.17 ± 1.53$^{e}$ | 57.19 ± 3.34$^{i}$ | 17.74 ± 1.28$^{r}$ |
| *Boscia salicifolia and Sclerocarya birrea* | 60:40 | 1.08 | i.p. | 2.29 ± 1.01$^{b}$ | 95.84 ± 1.99$^{g}$ | 18.45 ± 2.44$^{r}$ |
| | | | Oral | 23.04 ± 2.14$^{e}$ | 56.35 ± 2.85$^{i}$ | 17.96 ± 2.04$^{r}$ |
| CQ diphosphate | – | – | i.p. | 0.20 ± 0.00$^{a}$ | 99.64 ± 0.48$^{g}$ | 19.43 ± 3.52$^{r}$ |
| | | | Oral | 13.27 ± 1.48$^{d}$ | 62.34 ± 2.96$^{m}$ | 17.85 ± 2.19$^{r}$ |
| PBS | – | – | i.p. | 55.10 ± 5.54$^{f}$ | 0.00$^{j}$ | 8.11 ± 2.53$^{n}$ |
| | | | Oral | 52.71 ± 4.32$^{f}$ | 0.00$^{j}$ | 7.62 ± 2.14$^{n}$ |

Values with same letters are not significantly different ($p > 0.05$), values with different superscripted letters are significantly different ($p < 0.05$) parasite density and chemo-suppression were calculated on day 4.

immune-compromised mice thus exposing them to enhanced parasite attack.

The following combinations: *Boscia salicifolia* and *Sclerocarya birrea*, *Turraea robusta* and *Sclerocarya birrea*, *Rhus natalensis* and *Boscia salicifolia*, *Rhus natalensis* and *Turraea robusta* and *Lannea schweinfurthii* and *Boscia salicifolia* exhibited high chemo-supression (95.84, 93.61, 94.51, 94.38 and 90.62, respectively). Testing these combinations *in vivo* demonstrated enhanced anti-malarial activity compared to the single plant extracts with some giving chemo-suppression close to that of CQ. The enhanced anti-malarial activity for the combinations was evident in mice treated by both i.p. and oral administration. The mean survival times of mice treated with blends of *Lannea schweinfurthii* and *Boscia salicifolia* as well as that of *Boscia salicifolia* and *Sclerocarya birrea* were not significantly different ($p < 0.05$) from the control group treated with chloroquine. The test plant extracts were able to suppress parasites significantly thereby alleviating deaths associated with parasitaemia effects. However, it was noted that despite high parasite suppression in mice treated with a combination of *Rhus natalensis* and *Turraea robusta*, the mean survival time was reduced substantially. Combining the two plants may have resulted to enhanced toxicity. Further work, including testing this combination at lower concentration as well as exhaustive toxicological studies, is needed. The fact that mice treated with the same combination through oral drug administration had a longer mean survival time than those subjected to i.p. injection is indicative of higher toxin exposure in the former.

These findings demonstrate that it is useful to combine drugs as is usually done in traditional preparations and encourage testing of other plant combinations. Traditional healers seem to have already taken advantage of enhanced efficacy of combined decoctions. For example, in Mali, *Nauclea latifolia* was associated with *Mitragyna inermis* or *Guiera senegalensis* and *Feretia apodanthera* with *Mitragyna inermis* (Azas et al., 2004). Since total extracts were used, the bioactive compounds should be isolated and their interactions investigated. The mechanism of synergistic and additive interactions of the plant extracts could not be addressed in this study. However, additive effect may be due to the two separate entities binding to the same receptor in the parasite while in synergy different sites on the parasite may be the target points but are open to further debate.

Drug combination is one of the effective means to counter parasite resistance in anti-malarial chemotherapy (White and Olliaro, 1996). Drug combinations also help reduce risk of resistance development (Anne et al., 2001; Olliaro and Taylor,

2003). For instance when artemisinin is used in combination, its derivatives are rapidly eliminated and reduce the parasitaemia considerably within a single life cycle of the parasites, and residual parasites may be eliminated by a second drug with a minimal risk of selecting mutant, resistant parasite populations (White and Olliaro, 1996). Ideally, combination chemotherapy for malaria should take advantage of synergistic interactions, as these would enhance therapeutic efficacy and lower the risk of resistance emergence. The findings from studies with animal models may be more predictive of the drug action in humans (Chawira et al., 1987).

## 4. Conclusions

In this study, a preliminary demonstration of anti-plasmodial potential and safety of the five selected medicinal plants commonly used by the Meru community for the treatment of malaria has been achieved. The enhanced efficacy of certain combinations of herbal extracts remotely explains the practice and use of combined concoctions in the management of malaria by traditional health practitioners. This study revealed that at least two out of the five plants studied had good anti-plasmodial and anti-malarial properties and should be subjected to further chemical and biochemical investigations. Considering that natural products from plants used in traditional medicine, which have potent anti-plasmodial action represent potential sources of new anti-malarial drugs or templates (Wright and Phillipson, 1990; Gasquet et al., 1993), it is envisaged that further studies on these plants may lead to development of new affordable and effective phytomedicines for malaria.

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