Antimalarial activity and *in vivo* toxicity of selected medicinal plants naturalised in Kenya

Authors Names:

A. Caroline Kathambi Murithi[1], (Corresponding Author): E- mail address: carolemurithi@gmail.com or murithiicaro@yahoo.com: Phone +254725876081
B. Dossaji Saifudin Fidahusein [2], E-mail address: saifuddin.dossaji@uonbi.ac.ke; Phone +254721727635
C. Joseph Mwanzia Nguta[3], E-mail address: joseph.nguta@uonbi.ac.ke; Phone +254 735750278
D. Catherine Wanjiru Lukhoba[4], E-mail address: clukhoba@uonbi.ac.ke; Phone +254 20 4449004

Addresses for:

A, B and **D**: School of Biological Sciences, College of Biological and Physical Sciences, University of Nairobi, P.O. Box 30197- 00100, Nairobi, Kenya.

C: Department of Public Health, Pharmacology & Toxicology, College of Agriculture and Veterinary Sciences, University of Nairobi, P.O. Box 29053- 00625, Nairobi, Kenya.

ABSTRACT

Malaria is an epidemic disease in Kenya. With most conventional antimalarial drugs being inaccessible, unaffordable, and reported resistance of *Plasmodium falciparum* to the currently used Artemisinin combined therapy, there is need to explore alternative sources of chemotherapeutic agents and plants are a potential rich source of antimalarial drugs.

The study aimed to investigate the antimalarial activity and acute toxicityof four plants traditionally used to treat malaria in Msambweni District, Kenya.

Results show that the aqueous root extract of *Hoslundia opposita* and the organic leaf extracts of *Flacourtia indica* and *Ocimum gratissimum* suppressed parasitaemia of *Plasmodium berghei* in mice by 90.62%, 87.84% and 88.07% respectively. None of the plant extracts exhibited toxicity on mice at a dose of 2000 mg/Kg body weight.

The antimalarial activity exhibited by extracts of *H. opposita*, *F. indica* and *O. gratissimum* against *P. berghei* suggests that these plants may have active principles against *P. falciparum*.

Key words: Antimalarial, Acute toxicity, Chemosuppression, Crude extracts, *Hoslundia opposita*, *Flacourtia indica, Ocimum gratissimum, Plasmodium berghei*.

1. Introduction

Malaria is the cause of many deaths globally with about 3.3 billion people worldwide at risk of malaria infection (World Health Organization, 2012). The high rate of mortality and morbidity associated with this disease is due to rapid growth and multiplication of *Plasmodium* parasites in the human host blood cells according to Cheng, Kyle and Gatton (2012). Poor and inadequate drainage systems in most towns and villages provide a safe breeding environment for the vectors and this result in a lot of repeated infection on the population as reported by Okokon, Antia, Igboasoiyi, Eissein and Mbagwu (2007).

Decades ago, malaria was effectively treated using antimalarial drugs such as Chloroquine, Fansidar, Mefloquine and Malarone. However, development of resistant strains of *Plasmodium*, particularly *P. falciparum* to these drugs has since led to abandonment of use of the drugs (Nguta, Gakuya, Gathumbi and Kiama (2010); Cheng *et al.*, (2012).

In Africa, about 70% of the population do not have access to affordable conventional medicine according to Were, Kinyanjui, Gicheru, Mwangi and Ozwara (2010). The few and poorly staffed rural health centres (Nguta *et al*., 2010) have made the inhabitants of Msambweni district, Kenya, resort to use of traditional herbal medicine in the treatment of malaria.

Traditional herbal medicines are a potentially rich source of new drugs against malaria and other infectious diseases. A remarkable example is quinine from *Cinchona* bark, which has been used for over 300 years in the treatment of malaria and more recently development of Artemisinin derivatives from *Artemisia annua* (Muthaura, Keriko, Derese, Yenesew and Rukunga, 2011).

Artemisinin based combination Therapy (ACT), is currently used as the first line of treatment of uncomplicated malaria however, Rahmatullah, Hossan, Khatum, Seraj and Jahan (2012) have reported emerging resistance of *P. falciparum* to Artemisinin in the recent past. Malaria is endemic in lowlands particularly in the coastal region and Lake Victoria where there is sufficiently intense transmission.

This study was designed to evaluate the *in vivo* antimalarial activity and acute toxicity of selected medicinal plants commonly used in Msambweni District, South Coast of Kenya for the treatment of malaria.

2. Materials and Methods

2.1 Plant materials

The plant materials *Solanum incanum* L. (Solanaceae) [Mtugudza] leaves, *Flacourtia indica* (Burm.f.) Merr. (Flacourtiaceae) [Mtondombare] leaves, *Ocimum gratissimum* L. (Lamiaceae) [Murihani] leaves and *Hoslundia opposita* Vahl. (Lamiaceae) [Mtserere] roots were collected from Msambweni District of Kenya based on data collected during ethnopharmacological survey on plants used as antimalarial drugs (Nguta *et al*., 2010) and with help from the local community. A written informed consent was obtained from the respondents in this study. Information gathered included their local names (in parentheses) and the parts used in preparation of herbal remedies. Voucher specimens with voucher numbers of the identified plants were deposited at the University

of Nairobi herbarium i.e. *S. incanum* (CM03), *F. indica* (CM02), *O. gratissimum* (CM01) and *H. opposita* (CM04).

2.2 Preparation of crude extracts

Considering that the people of Msambweni boil the plant parts in preparation of the herbal remedies, decoctions of the aqueous extracts were prepared by heating 50 gms of the ground material in 500cm^3 of distilled water in a water bath at 60^{0}C for one hour and left to stand for twenty-four hours. The extracts were lyophilized by using a freeze drier. Another 50gms of the ground material was extracted by percolation in 500 cm^3 of [Chloroform Methanol (1:1)], for twenty-four hours. The crude organic extracts were concentrated *in vacuo* at 40^{o}C using a rotary evaporator, Harborne (2002).

2.3 Parasites

Chloroquine (CQ) sensitive *P. berghei* (ANKA strain) parasites were used for the *in vivo* antimalarial activity assessment. Cyropreserved parasites stored at -80^{o}C were obtained from Kenya Medical Research Institute (KEMRI). The parasites were revived, stabilized and maintained by serial passage of blood from infected mice to naive mice.

2.4. Preparation of test extracts

Stock solutions of 10,000 µg/ml for the aqueous extracts were prepared by dissolving 0.1 g of each of the extracts in 10 ml of distilled water. The same concentration of organic extracts were prepared by dissolving 0.1 g of each of the organic samples in 0.1ml (0.01%) of DimethylSulphoxide (DMSO) followed by dilution with water to make 10 ml of solution.

2.5 *In vivo* determination of antimalarial activity

This study was conducted with the approval of KEMRI's Animal Care and Use Committee. Swiss albino mice [*Mus musculus* L. (Muridae)] were obtained and housed in the KEMRI animal house and were fed on standard pellets and water. Mice weighing between 18 and 22 g were randomly selected then infected, through intraperitoneal inoculation, at a concentration of 1 x10^7 parasitized erythrocytes.

Ten cages were prepared and five infected mice were randomly assigned to each cage. The treatments were also randomly assigned. Four cages were treated with the aqueous treatments, four with the organic extracts and two were the controls. The aqueous and organic extracts at a concentration of 100 mg/kg/day in a dose of 2 ml were administered. The negative controls received distilled water while the positive controls were treated with 0.2 ml of chloroquine at a concentration of 20 mg/kg of body weight per day. On day 4, thin blood smears were made from a tail cut of each mouse, fixed for 5 minutes using methanol, stained with 10% Geimsa stain in Phosphate buffer, pH7.2 and examined microscopically under oil immersion at x1000 for assessment of parasitaemia. The mean percentage parasitaemia was used to calculate the chemosuppression for each of the

extract. Survival time in days was recorded for all the mice in each group and the mean for each group was calculated.

2.6 Acute toxicity in mice

Forty (40) female Swiss albino mice weighing between 18 and 22 g were randomly divided into 8 groups of five mice in each cage. The mice were fasted for 12 hours, and then weighed prior to administration of 1 ml of the extract at a dosage rate of 2000 mg/kg of body weight as a single dose. The general behaviour of each mouse was observed continuously for the first 30 minutes and then at hourly intervals for 6 hours and then after 24 hours. Food was withheld for one hour after administration of the extract, thereafter, food and water were provided to the mice every day in the morning. The mortality was checked and recorded after every 24 hours. The mice were further observed for 10 days for signs of toxicity. The weights of the mice were taken at 3-day intervals up to the 10^{th} day.

2.7 Data analysis

One way ANOVA was used to analyse chemosuppression means obtained from the 4-day suppressive test and to find out if the chemosuppression caused by one plant extract was significantly different from chemosuppression caused by other plant extracts. Dunnet's test was used to determine whether chemosuppression induced by each of the plant extracts was significantly different from the chemosuppression in the positive control group.

3. Results

3.1 *In vivo* antimalarial activity

All the extracts exhibited varying degrees of chemosuppression. The antimalarial activity of the crude extracts from the four plants against *P. berghei* and the mean survival time of the extracts are summarized in Table 1 below. Values of $p < 0.05$ were considered significant. A therapeutic dose lowering parasitaemia by > 50% was considered high chemosuppression and that inhibiting parasitaemia by < 50% was considered low chemosuppression.

The aqueous root extract of *H. opposita* and the organic leaf extracts of *F. indica* and *O. gratissimum* induced chemosuppressions that were not significantly different ($p < 0.05$) from that induced by the positive control, chloroquine (Figure1). The organic root extract of *H. opposita* induced a moderate chemosuppression while both extracts of *S. incanum* and the aqueous extract of *O. gratissimum* exhibited relatively low chemosuppressions compared to chloroquine ($P > 0.05$). The lowest chemosuppression was observed in the group treated with the aqueous extract of *F. indica.*

The groups treated with the extracts survived for different periods. Those treated with Chloroquine, the test drug survived for the ten days while those treated with distilled water survived for approximately 5 days. There was no significant difference in the mean survival time of mice treated with the aqueous extract of *F. indica* and those given distilled water. There was no significant difference between the survival time of mice treated with aqueous extracts of *S. incanum* and *H.*

opposita and the organic extracts of *F. indica, O. gratissimum* and *H. opposita* and that of chloroquine (Figure 2).

3.2 Acute toxicity of crude extracts

None of the treatments produced mortality in mice within 24 hours or within the 10-day observation period. No signs of toxicity were observed in the mice treated with a dose of 2000 mg/kg body weight of any of the crude extracts tested for the first 24 hours. Weight observed in all the groups showed steady increase in the 10-day observation period except in the group treated with the organic extract of *F. indica* where there was a decrease in weight from day 3 (Figures 3 and 4).

4. Discussion

The highest parasitaemia of 41.9% was recorded in the group treated with the negative control (distilled water) while the lowest parasitaemia of 1.66 % was recorded in the group treated with the positive control, chloroquine. Except in the group treated with the aqueous extract of *F. indica*, the parasitaemia in all the other groups was less than in the group treated with the negative control. This showed that the treatments had an effect on the multiplication and erythrocyte infectivity of *P. berghei* parasites in mice. Parasitaemia increased gradually in all the groups and by day 5-post infection, all the mice treated with the negative control had died while those treated with Chloroquine survived the ten-day post infection observation period.

Chemosuppression is an important measure of the efficacy of a given crude extract or antimalarial compound. A good antimalarial drug given in the correct dose leads to rapid suppression of the parasite density. In this study, the highest chemosuppression of 95.97% was induced by Chloroquine. The aqueous root extract of *H. opposita* and the organic leaf extracts of *F. indica* and *O. gratissimum* exhibited chemosuppressions that were not significantly different from that induced by Chloroquine ($p < 0.05$).

The aqueous root extract of *H. opposita* suppressed parasitaemia by 90.62%, the highest chemosuppression recorded from all the crude extracts evaluated in the current study. The chemosuppression and mean survival time of the mice were not significantly different from those treated with chloroquine. Earlier ethnobotanical studies have showed that *H. opposita* roots, leaves or the entire aerial parts of the plant are used in the treatment of malaria in Kilifi District (Gathirwa *et al*., 2011). The local community uses *H. opposita* root extracts in Msambweni District to treat malaria (Nguta *et al*., 2010). These findings are in agreement with a similar study by Asase, Hesse and Simmonds (2012), who reported that the leaves of *H. Opposita* are traditionally boiled in combination with those of *O. gratissimum* and *Cymbopogon citratus* and the decoction taken three times a day in Ghana, and this decoction is effective in treating malaria.

The observations in this study on the root extract of *H. opposita* are consistent with *in vivo* antimalarial studies of methanolic extracts of leaves and aerial parts of *H. opposita* that revealed high chemosuppression of 79.67 % for the leaf extract and a medium chemosuppression of 55.05% for the aerial parts (Gathirwa *et al*., 2011). Further, *in vitro* studies of the root bark extract of *H.opposita* revealed good *in vitro* antimalarial activity against *P. falciparum* with an IC_{50} value of

56 µg/ml (Schwikkard and Herdeen, 2002). *In vitro* studies of the methanol root extract by Gathirwa *et al*., 2011, also showed t that the root extract had an IC_{50} value of 79.38 µg/ml and 64.21 µg/ml against CQ sensitive (D6) and CQ resistant (W2) *P. falciparum* strains respectively.

The organic leaf crude extract of *O. gratissimum* revealed a chemosuppression of 88.07% and a mean survival time of 9.6 days post infection. Traditionally in Msambweni district, a leaf decoction of *O. gratissimum* is taken thrice a day for 3-5 days as a remedy for malaria. The *in vivo* antimalarial activity of the organic leaf extract of *O. gratissimum* was reported for the first time in this study. This correlates with earlier *in vitro* studies of ethanol extracts of *O. sanctum, O. basilicum and O. canum*, members of the *Ocimum* species, which exhibited good antiplasmodial activity as reported by Inbaneson, Sundaram and Suganthi (2011). At a dose of 2000 mg/kg body weight, no signs of toxicity were observed and this agrees with an earlier study by Mequanint, Makonnen and Urga (2011),that revealed that both the aqueous and ethanolic extracts and fractions, (Petroleum, Ether, Chloroform and water) of *O. gratissimum* had an $LD_{50} > 8$ g/kg of body weight.

The organic leaf extract of *F. indica* induced a chemosuppression of 87.84% that was not significantly different from that induced by chloroquine ($p < 0.05$). The antimalarial activity reported in this study agrees with previous studies of the antimalarial activity of the ethyl acetate aerial shoot extracts of *F. indicia*, which reported strong antiplasmodial activity with an IC_{50} value of 7.4 µM compared to doxycyline, an antimalarial drug that had an IC_{50} value of 6.5 µM (Kaou *et al.*, 2010). Despite being a pure compound, doxycyline had an antimalarial activity lower than that of the crude extracts of *F. indica*, suggesting the presence of potential antimalarial phytoconstituents from the fractions of *F. indica* crude extract (Kaou *et al*., 2010)

At a dose of 2000 mg/kg body weight, the organic *F. indica* extract did not reveal any signs of toxicity on mice and no mortality was produced within 24 hours. It however caused a weight decrease over the ten-day study period, which could have been occasioned by the crude extract effects on absorption of food constituents, calling for further pharmacokinetic studies. In addition, detailed *in vivo* toxicity studies should be carried out.

The aqueous and organic extracts of *S. incanum*, the aqueous extract of *O. gratissimum* and the organic extract of *H. opposita* had chemosuppressions that were significantly different from that of chloroquine. However, there was a decline in the parasite density compared to the negative control. The Msambweni community usually use plant species in combination as therapeutic remedies against malaria, and this could explain the low chemosuppression observed with single species, since there was no synergism as the various species were evaluated alone. The extracts did not exhibit observable signs of toxicity to the mice at a dose of 2000 mg/kg body weight, rendering further support to the ethnopharmacological utilization of the tested species in malaria chemotherapy. The current observation further supports the anecdotal claims of safety of the tested plant species. The current study was designed to validate the traditional use of the selected medicinal plants in the treatment of malaria. The mean survival time of the mice treated with these extracts were higher than that of the negative control group an indication they possessed antimalarial activity, hence cannot wish away the ethnopharmacological reports from the study community. Bioactive compounds do not necessarily have direct activity against the parasites but

they may have other pharmacological properties such as analgesic, antipyretic or immunostimulatory as reported by Cocquyt, Herdewijin, Maes, Van den Steen and Laekeman (2011), properties that could be present in the tested extracts and responsible for the observed chemosuppression. Whereas a good antimalarial activity was recorded for the organic extract of *F. indica*, the aqueous extract had the lowest activity suppressing the parasite by a mere 0.21%. This indicates that the active principles could be present in large amounts in the non-polar component of the crude extract, paving the way for further bioactivity guided isolation of the active principles from the organic crude extract.

Conclusions

The aqueous root extract of *H. opposita* and the organic leaf extracts of *F. indica* and *O. gratissimum* had good antimalarial activities in a *P. berghei* mice model that were not significantly different from that induced by the positive control, chloroquine ($p < 0.05$). This validates the use of these plants by the people of Msambweni in the treatment of malaria.

Although significantly different from that of chloroquine, the chemosuppressions induced by the other extracts ($p > 0.05$) except aqueous extract of *F. indica*, their parasitaemia were lower than that of the negative control (distilled water). This implies that they could still have better antimalarial activity perhaps if combined with other antimalarial species and taken as a decoction to take advantage of synergistic effects. The extracts of the four plants were safe to mice at a dose of 2000 mg/Kg body weight, however; further toxicity studies should be carried out to ascertain their safety in humans following long-term consumption. Isolation and identification of the compounds responsible for the observed antimalarial activities of the selected medicinal plants with interesting antimalarial activity in a *P. berghei* mice model should be carried out.

Acknowledgements

We thank the University of Nairobi and Kenya Medical Research Institute for allowing us to use their facilities to carry out this study.

Tables and Figures

Table 1: Mean (x±S.D) parasite density, chemosuppression and survival time of *P. berghei* infected mice treated orally with the extracts at a dose of 100mg/Kg body weight once for four days

Plant species	Extract	Mean (X± S.D) Parasite density		Mean (X± S.D.) Chemosuppression (%)		Mean (X± S.D.) survival time (days)	
		Aq	Org	Aq	Org	Aq	Org
S. incanum	Leaf	34.45±3.73	28.30± 5.32	14.78±8.63	31.23± 12.87	8.8±1.30	8±1.22
F. indica	Leaf	41.10±2.25	5.02± 1.06	0.21±5.47	87.84± 2.53	7±1	8.6±0.55

O. gratissimum	Leaf	33.79± 1.09	4.91± 0.43	17.95± 2.66	88.07± 1.05	8.2±0.84	9.6±0.55
H. opposita	Root	3.86± 0.18	23.90± 0.89	90.62± 0.43	41.97± 2.10	9.6±0.55	9.2±0.83
Chloroquine	N/A	1.66±0.58		95.97±1.40		10±0	
Distilled water	N/A	41.19±1.55		N/A		5.8±1.30	

Data expressed as ± standard deviation for five determinants per group.

Aq: Aqueous Org: Organic

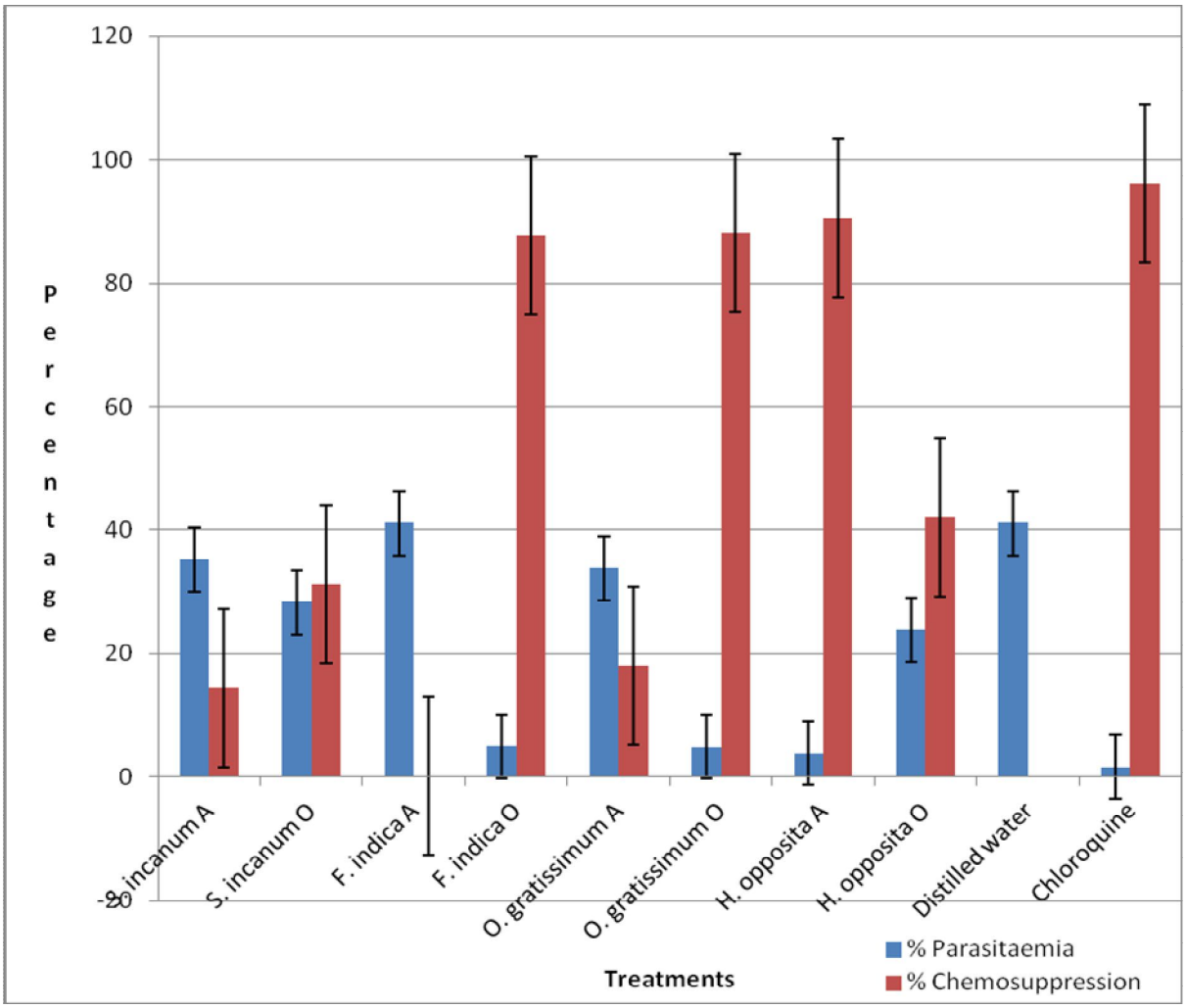

Figure 1: Mean plots for the parasitaemia and chemosuppression for the ten treatments on day 4 following treatments

Key: A – Aqueous, O- Organic

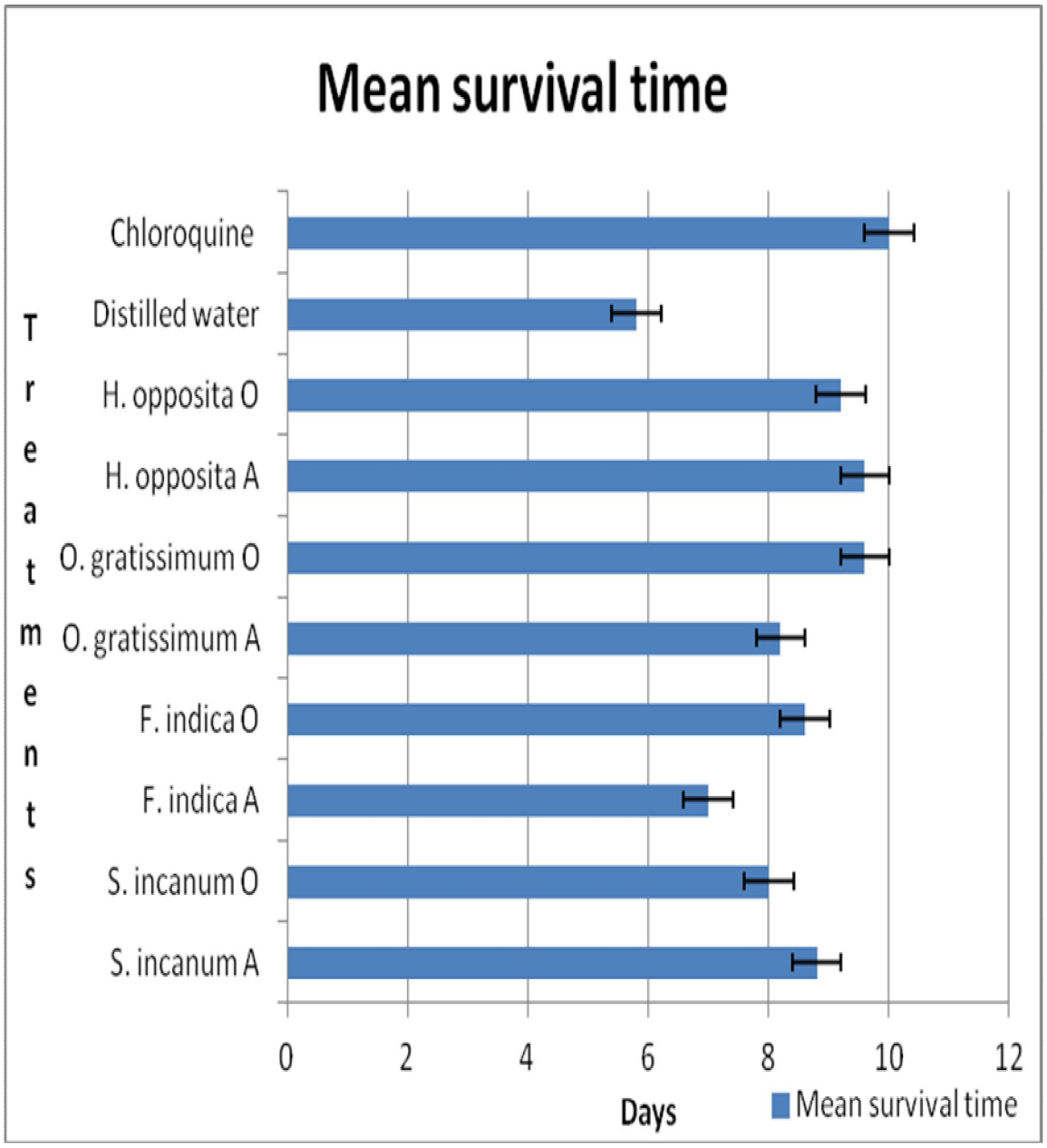

Figure 2: Mean survival time of mice using all extracts

Key: A – Aqueous, O- Organic

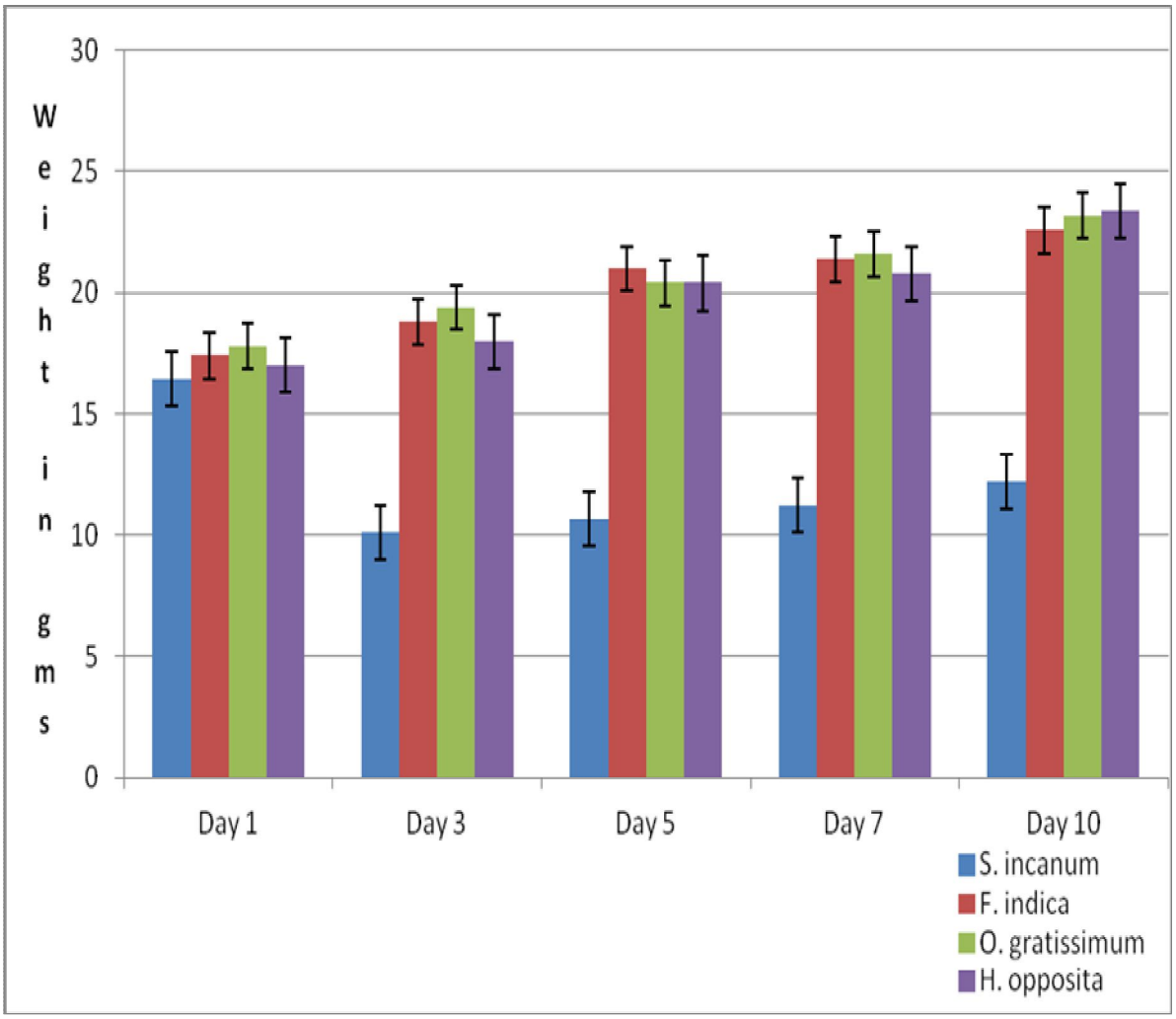

Figure 3: Variation of mean weights of the mice treated with aqueous extracts with time.

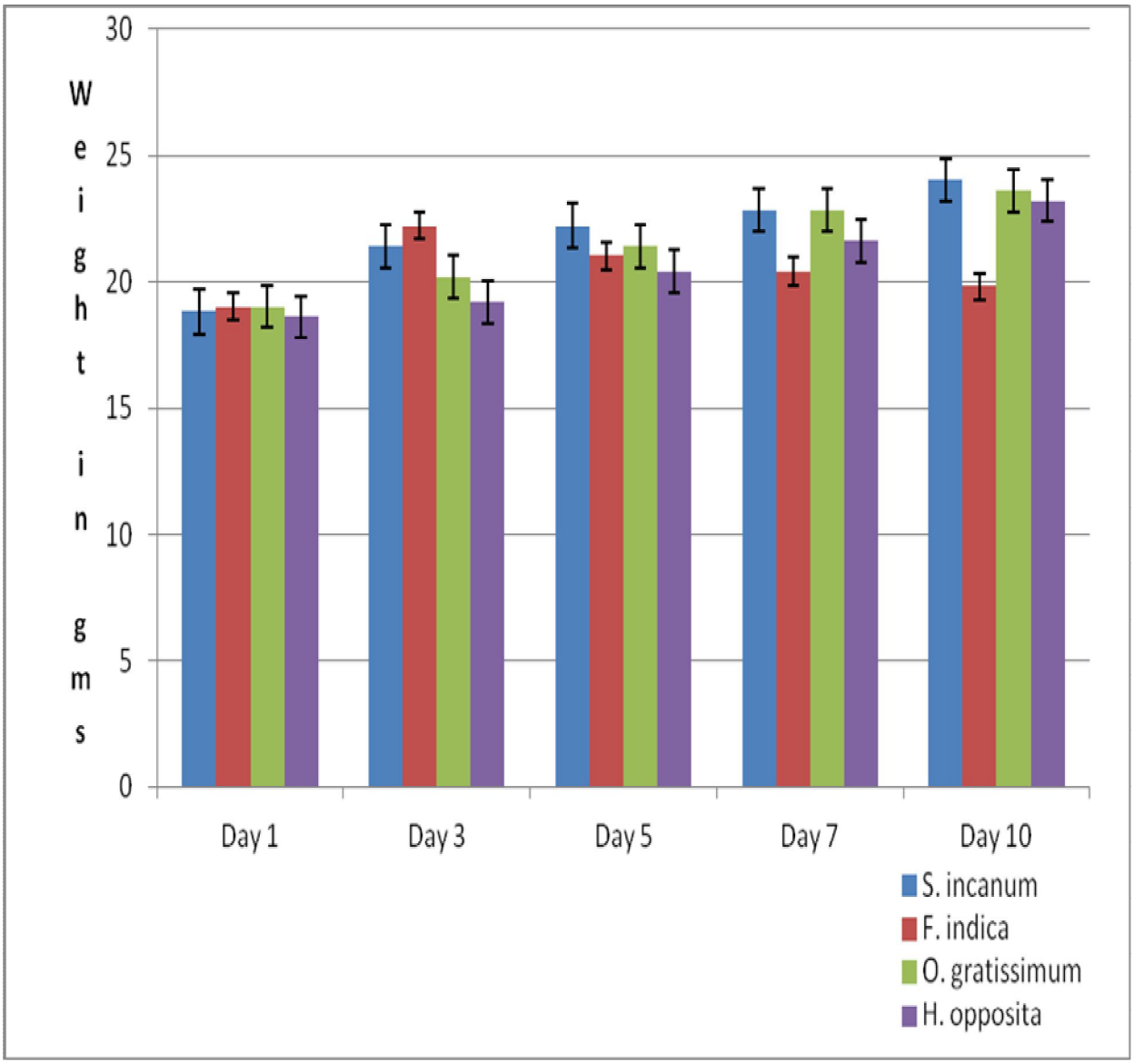

Figure 4: Variation of mean weights in the mice treated with organic extracts with time.

References

Asase, A., Hesse, D.N., Simmonds, M.S., 2012. Uses of multiple plants prescriptions for treatment of malaria by some communities in Southern Ghana. *Journal Ethnopharmacology*, 144 (2), 448-452.

Cheng, Q., Kyle, D.E., Gatton, M.L., 2012. Artemisinin resistance in *Plasmodium falciparum*: A process linked to dormancy. *International Journal for Parasitology, Drugs and Drug Resistance*, 2, 249–255.

Cocquyt, K.,Cos, P., Herdewijin, P., Maes, L., Van den Steen, P.E., Laekeman, G., 2011. *Ajuga remota* Benth: From ethnopharmacology to phytomedical perspective in the treatment of malaria. *Phytomedicine*, 18 (14), 1229–1237.

Gathirwa J.W, Rukunga, G.M., Mwitari, P.G., Mwikabe, N.M., Kimani, C. W., Muthaura, C.N., Kiboi D. M., Nyangacha, R.M., Omar, S. A., 2011. Traditional herbal antimalarial therapy in Kilifi district, Kenya. *Journal of Ethnopharmacology*, 134 (2), 434–442.

Harborne, J.B., 2002. Phytochemical Methods: A guide to modern techniques of plant analysis. Third edition, Champman & Hall, London.

Inbaneson, S.J., Sundaram, R., Suganthi, P., 2012. In vitro antiplasmodial effect of ethanolic extracts of traditional medicinal plant *Ocimum* species against *Plasmodium falciparum*. *Asian Pacific Journal of tropical medicine*, 5 (2), 103–106.

Kaou, A.M., Mahiou- Leddet, V., Canlet, C., Debrauwer, L., Hutter, S., Laget, M., Faure, R., A zos, N., Ollivier, E., 2010. Antimalarial compounds from the aerial parts of *Flacourtia indica* (Flacourtiaceae). *Journal of Ethnopharmacology*, 130 (2), 272-274.

Mequanint, W., Makonnen, E., Urga, K., 2011. *In vivo* anti-inflammatory activities of leaf extracts of *Ocimum lamiifolium* in mice model. *Journal of Ethnopharmacology*, 134(1), 32–36.

Muthaura, C.N., Keriko, J.M., Derese, S.,Yenesew, A., Rukunga, G.M., 2011. Investigation of some medicinal plants traditionally used for treatment of malaria in Kenya as potential sources of antimalarial drugs. *Experimental Parasitology*, 127 (2), 609–626.

Nguta, J.M., Mbaria, J.M., Gakuya, D.W., Gathumbi, P.K., Kiama, S.G., 2010. Antimalarial herbal remedies of Msambweni Kenya. *Journal of Ethnopharmacology*, 128(2), 424-432.

Okokon, J.E., Antia, B.S., Igboasoiyi, A. C., Essien, E.E., Mbagwu, H.O., 2007. Evaluation of antiplasmodial activity of ethanolic seed extract of *Picrali manitida. Journal of Ethnopharmacology*, 111 (3), 464–467.

Rahmatullah, M., Hossan, S., Khatum, A., Seraj, S., Jahan, R., 2012. Medicinal plants used by various tribes of Bangladesh for treatment of malaria. *Malaria Research and treatment*, 2012 (371798).

Schwikkard, S., Heerden, F.R., 2002. Antimalarial activity of plant metabolites. *RSC Publishing*, 19, 675-692.

Were, P.S., Kinyanjui, P., Gicheru, M., Mwangi, M.E., Ozwara, H.S., 2010. Prophylactic and curative activities of extracts from *Warburgia ugandensis* Sprague (Canellaceae) and *Zanthoxylum usambarense* (Engl.) Kokwaro (Rutaceae) against *Plasmodium knowlesi* and *Plasmodium berghei. Journal of Ethnopharmacology*, 130(1), 158–162.

World Health Organization, 2012. Ten facts on Malaria, Retrieved from www.who.org [accessed on 12th September 2013)