Antimalarial activity and acute toxicity of four plants traditionally used in treatment of malaria in Msambweni District of Kenya

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ABSTRACT

Malaria is a major and constant public health problem in the world. It kills more than one million people per year especially in developing countries where opportunities for treatment are minimal. Since plants have been previously used as a source of antimalarial drugs, they can provide alternatives in the development of drugs against multidrug-resistant strains of Plasmodium falciparum malaria causing parasite. This study aimed to investigate in vivo antimalarial activity and acute toxicity of selected plants used in traditional medicine for treatment of malaria. Results show that organic root extracts of Securidaca longepedunculata Fres. (Polygalaceae) exhibited the highest chemosuppression of parasitaemia (91.03%) among all the crude extracts tested. All the extracts of Commiphora schimperi (Berg.) Engl. (Burseraceae), Ricinus communis L. (Euphorbiaceae) and Grewia hexaminta Burret had chemosuppression levels below 25%. All extracts of C. schimperi, R. communis and G. hexaminta were safe to mice at 2000 mg/kg, while S. longepedunculata was considered non toxic at 300 mg/kg. These results showed that crude extracts of S. longepedunculata demonstrated promising antimalarial activity and there is a potential of isolation of active lead compounds from its extracts.

Keywords: Antimalarial, Acute toxicity, Chemosuppression, *C. schimperi, R. communis, G. hexaminta, S. longepedunculata,* crude extracts, herbal remedies, *P.berghei*, Msambweni district.

1. Introduction

Malaria causes more than 1.1 million deaths per year globally and affects 300-500 million people per year worldwide, with more than 90% of these cases in Africa, Nguta *et al*., (2013); Joanne *et al*., (2009). Due to increased chloroquine resistant *Plasmodium falciparum* and other multidrug resistant parasite strains of malaria, there is need to search for new, active and safe antimalarial drugs, Nguta *et al*., (2011); Bickii *et al*., (2007). Antimalarial plants are a potential source of antimalarial agents with the success of artemisinin isolated from the *Artemisia annua* L. (Asteracea) and the continuous clinical use of quinine isolated from *Cinchona* species (Rubiaceae), Gaurav *et al.,* (2011). However, the safety and efficacy of majority of traditionally used plants is not established scientifically, Nguta *et al.,* (2012), and therefore one is likely to overdose patients in traditional medicine, Fidlock *et al.,* (2004).

Antimalarial and toxicity studies are therefore important to validate anecdotal efficacy and safety reported by communities using these plants. The current study was therefore designed to evaluate *in vivo* antimalarial activity and acute toxicity of selected medicinal plants collected from Msambweni district, South Coast Kenya where they are used traditionally for the treatment of malaria.

2. Materials and methods

2.1 Plant materials

The four pants used in this study were collected from Msambweni district of Kenya between May and November, 2009. They were selected based on their ethnopharmacological use as antimalarial drugs through interviews with local communities, Nguta *et al.,* (2010a,b). The plant material was collected depending on the part used in traditional treatment of malaria and voucher numbers (in parentheses) were given following identification by a taxonomist at the University of Nairobi. The collected species were as follows: *C. schimperi* (OD03) roots, *S. longepedunculata* (OD01) roots, *G. hexaminta* (OD02) leaves and *R. communis* (OD04) leaves. Information gathered included their local names 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.

2.2 Preparation of crude extracts

Aqueous crude extracts were obtained by mixing 50 g of powdered plant material in 500 ml of distilled water in a water bath at 60^{o}C for 1h. Aqueous filtrates were lyophilized using a freeze drier to form dry powder. For organic extracts, 50 g of the powdered plant material was mixed using 500 mls mixture of chloroform and methanol (1:1 v/v) and percolated over 24 hrs for four times to ensure that all phytoconstituents were extracted. Dry crude organic extracts were obtained by concentrating filtrates from organic solvents *in vacuo* at 40^{o}C using a rotary evaporator, and further dried in an oven at 40 ^{0}C. The yields of the dry aqueous extracts ranged between 6.06%and 21.24%, while those of the organic extracts ranged between 4.78% and 25.98%. The dry solid extracts were stored at -20^{0}C in air tight containers until used.

2.2 Preparation of test extracts

Aqueous stock solutions (10,000µg/ml) were prepared by dissolving 0.1 g of each extract in 10ml of distilled water. The organic extracts were dissolved in a lower concentration of Dimethyl sulphoxide (DMSO), < 1% to avoid carry over solvent effect, Musila *et al.,* (2013). Appropriate amounts of test extracts were transferred into 10 mls vials.

2.4 Parasites

Cryopreserved chloroquine sensitive *Plasmodium berghei* (ANKA) parasites used for *in vivo* antimalarial testing were obtained from KEMRI (Kenya Medical Research Institute). A group of three naive mice was used to revive and stabilize the parasites in the mice host and maintain the parasites through continuous reinjection of *P. berghei* ANKA to new mice. One mouse was randomly selected as a donor and blood was collected via cardiac puncture into bottles with heparin to make an inoculum for new naive mice infection. The donor mouse was sacrificed by cervical dislocation. Passages were done when parasitaemia level reached 20-30%. Passages were used to stabilize cryopreserved parasites in the mouse host. This was done after every four days since by the fourth day the parasitaemia levels reached 20-30% from the day parasites were introduced into the naive mice.

2.5 *In vivo* determination of antimalarial activity

This study was conducted with the approval of KEMRI's Animal Care and Use Committee. Male and female Swiss Albino mice [*Mus musculus* L. (Muridae)] of about 8 to 10 weeks old and weighing 18 to 22 g were used in this study. They were maintained under specific pathogen free conditions and given enough water and food *ad libitum* at KEMRI animal house. Three donor mice were injected with chloroquine sensitive *P. berghei* (ANKA) and parasitaemia was allowed to revive and stabilize for three days before they were infected to experimental mice. Blood smears were prepared only from mice whose parasitaemia levels reached 20-30%. After anesthetizing using chloroform, blood was collected via cardiac puncture into bottles with heparin to make inoculum for infecting all experimental mice in a main chamber before grouping them into cages of five mice each. Experimental mice were infected with 0.2 ml of blood suspension (about 1% parasitaemia) containing about 1×10^7 parasitized erythrocytes through intraperitoneal injection, Waako *et al.,* (2005).

The Peter's 4-day suppressive test was used to determine *in vivo* antimalarial activity, Waako *et al.,* (2005). A completely randomized design was employed in the experiment. From the main chamber, mice were grouped into three groups, with five mice in each group. Each group of five mice was housed in a separate cage. Treatments were assigned randomly in the three separate cages. Extracts were administered orally to the mice at a dosage of 100 mg/kg/day of each crude extract once daily at the same time for 4 consecutive days (days 0-3). The remaining 2 groups were used as controls with one serving as a positive control and another as a negative control. Chloroquine was used as the positive control at a dosage rate of 20 mg/kg/day and distilled water as the negative control at a dose of 0.2 mls/mouse/day. On the 1st day (day 0) drug administration started 2 hours after *P. berghei* parasite infection.

Blood smears from each mouse were prepared from all cages on Day 4 by making a tail cut. The blood smears were fixed with methanol for 5 minutes and then stained with 10% Giemsa. A photographic compound microscope was used to observe the slides under oil immersion at x1000 to determine the number of parasitized cells per given magnification field (MF). Four magnification fields were observed for each blood smear of a given mouse. The number of parasitized cells and the total number of cells in each magnification field was determined. The data obtained was used to determine percentage parasitaemia and parasite growth inhibition (chemosuppression) in each mouse. Mortality of the experimental mice was recorded every day from the start for the next 10 days.

2.6 Acute toxicity

Acute toxicity was used to determine the immediate toxic effects and lethality order of all extracts using the OECD (Organization for Economic Co-operation and Development) Guideline number 423 (2001). Healthy female Swiss albino mice aged 8-10 weeks and weighing 18-22g were randomly selected (25 mice), grouped

in five different cages of 5 each and labeled for individual recognition then fasted overnight, Badkhshan *et al.,* (2011). Food and water were given 1 h after dosing. Dose levels of 50, 300 and 2000 mg/kg of extracts were orally administered using a suitable intubation canula by starting with levels likely to cause mortality (2000 mg/kg). Distilled water was used for the control group. Individual mice were observed after dosing for physical changes, gross behavioral change and mortality with special attention for the first 4 hours and then daily thereafter for the next 10 days.

2.7 Data analysis

Statistical Package for Social Scientists (SPSS) Version 16.0 was used to analyze parasite growth inhibition. Chemosuppression means obtained from the fourth day suppressive assay were analyzed using one way Analysis of Variance (ANOVA) to determine whether chemosuppression caused by one plant extract was different from chemosuppression caused by other plant extracts, Morgan *et al.,* (2004). Dunnett test was used for multiple comparisons when means were found to be different from each another to determine whether chemosuppressions from various treatments were different from the chemosuppression by the positive control (chloroquine). The significance level used in the analysis was 0.05 (Alpha level < 0.05).

3. Results

3.1 *In vivo* antimalarial activity

On day 4 of the suppressive test, thin blood smears were prepared and observed under a photographic compound microscope using oil immersion. Mean parasite and growth inhibition in each mouse was determined by counting total number of parasitized cells and total number of cells in four magnification fields (MFs) in each microscope slide. Percentage parasitaemia and percentage parasite growth inhibition (chemosuppression) of the drugs was calculated according to the method described by Hilou *et al.,* (2006) and summarized in Table 1.

3.2 Acute toxicity

Mortality is critical within 24 hours after drug administration in acute toxicity tests. Dose levels of 50, 300 and 2,000 mg/kg were used. All mice were given the highest dose of 2,000mg/kg body weight that is likely to cause mortality. For both organic and aqueous extracts of *C. schimperi, G. hexamita* and *R. communis,* all mice were alive during the ten experimental days and did not show any signs of toxicity. Extracts of *S. longepedunculata* did not exhibit any signs of toxicity. In its organic form, 30 min to 2 hrs after drug administration, no signs of toxicity were observed. Two mice had signs of toxicity such as convulsions, fur raised and sleepiness at 6 hrs after drug administration, before death. Two mice died on day 4 and day 5 of drug administration. With aqueous extracts, no signs of toxicity were observed for the first four hours. After the 6^{th} hour, one mouse had signs of toxicity such as raised fur, convulsions and lethargy. The mouse died within the first 24 hours after drug administration while others exhibited signs of toxicity. The results are summarized in Figure 1. Dose levels were lowered to 300 mg/kg for both extracts of *S. longepedunculata* and no mortality or signs of toxicity were observed.

4. Discussion

The current study aimed at investigating the antimalarial activity and *in vivo* acute toxicity of selected medicinal plants commonly used in Msambweni ethnomedicine to treat malaria. More than 80% of the global population use medicinal plants to treat different ailments, since they are available, cheap and perceived to be efficacious and safe at the traditionally used doses, Nguta *et al*., (2010a,b). Usage of effective antimalarial drugs in correct dosage causes a decrease in parasitaemia levels that is essential for

recovery. Most antimalarial compounds target the asexual stage in man by acting on the parasite in various ways such as reducing its nutrient uptake and interfering with the parasite metabolic path way. For example, Chloroquine (CQ) acts by affecting haem detoxification where it binds to heam moieties formed from proteolytically processed haemoglobin inside infected red blood cells (RBCs), Fidlock *et al.,* (2004). This leads to decreased parasitaemia and subsequent recovery of symptomatic malaria. Plant extracts exhibiting high chemosuppressive values also reduced parasitaemia levels enabling mice to survive up to the 10^{th} day post infection. Plant extracts in this study which had high levels of chemosuppression showed low parasitaemia levels in *P. berghei* infected mice since chemosuppression is inversely related to parasitaemia.

The positive control group treated with chloroquine had the highest chemosuppression level of 97.95%. This was not surprising since chloroquine is a pure antimalarial compound. Among the plant extracts tested in the current study, organic crude root extracts derived from *S. longepedunculata* showed the highest chemosuppression value of 91.03%, which was not significantly different ($p<0.05$) from that induced by chloroquine. This observation was quite interesting since the extract was only a crude preparation, validating the ethnopharmacological utilization of the plant species in treating patients with malaria associated symptoms. The current observation also gives credit to the information provided by the traditional herbal practitioners who provided information on plants traditionally used to treat malaria in Msambweni district, south coast of Kenya, and hence they can be trusted with ethnobotanical and ethnopharmacological information in future studies. The negative control which was treated with distilled water had the highest parasitaemia with a value of 32.66%. Chloroquine had the least parasitaemia of 0.67% while organic root extracts of *S. longepedunculata* had the lowest parasitaemia with a value of 2.93% among plant extracts investigated in the current study. Chemosuppression level of 55.05% and parasitaemia value of 14.68% associated with aqueous root extracts of *S. longepedunculata* were observed to be significantly different from those associated with the antimalarial drug, chloroquine at $p<0.05$. These results indicate high possibility of antimalarial activity in organic root extracts of this plant. These results are in agreement with those of Haruna *et al.,* (2013) where antimalarial activity was reported in methanol root extracts of *S. logepedunculata* against *P. berghei* (ANKA) in Swiss mice. The observed chemosuppression was 82.6%, 62.7% and 58.4% at doses of 0.56 mg/kg, 0.28 mg/kg, and 0.14 mg/kg respectively, whereas chloroquine had 93.8% and artemether 84.4% at10 mg/kg and 1.6 mg/kg, respectively. The results are also in agreement with reports by Bah *et al.,* (2006) who observed significant antiplasmodial activity from dichloromethane (CH_2Cl_2) leaf extracts of *S. longepedunculata* with an IC_{50} value of 7µg/ml.Various parts of *S. longepedunculata* have been used against malaria in traditional medicine but roots are the most used part against various diseases, Alitonou *et al.,* (2012).

Extracts of *S. longepedunculata* had high levels of toxicity among the four plants tested. In its organic extracts at a single dose of 2,000 mg/kg, two mice died six hours after drug administration. The other three mice had signs of toxicity such as lethargy, sleepiness, fur raised, tremors and convulsions until the last experimental day. In aqueous extracts at the same single dose as organic extracts, one mouse died within the first 24 hours after drug administration. Two had signs of toxicity; lethargy, sleepiness and fur rose. The remaining two were normal until the last experimental day. These observations indicate levels of toxicity in the plant, calling for dose adjustments in herbal preparations containing *S. longependunculata*. These results agree with those of Auwal *et al.,* (2012) in which aqueous extracts from root bark of *S. longepedunculata* were reported have been shown to be toxic with an LD_{50} value of 771 mg/kg in oral acute toxicity tests in rats. The reported toxicity was attributed to the presence of tannins and saponins in large quantities compared to alkaloids and flavonoids which were also present in the tested crude extract. Phytochemicals such as alkaloids, flavonoids, saponins, tannins, cardiac glycosides, anthraquinones, steroids, balsams and reducing sugars have been reported to be present in chloroform and methanol extracts of *S.*

longepedunculata which are attributed to the antibacterial activity of the plant, Ndamitso *et al.,* (2013), and the same phytoconstituents could have been responsible for the observed antimalarial activity in the current study**.** Methylsalicylate (a phenolic ester) is about 90% of the volatile constituents present in *S. longepedunculata* where its fumigant effect gives the plant insecticidal properties which may be attributed for the acute toxicity of the plant, Okoli *et al.,* (2006). This ester could have partly contributed to the *in vivo* acute toxicity reported in the current study. Bisdesmosidic saponins isolated from the methanol extract of the roots of *S. longepedunculata* have been shown to be deterrent and toxic toward *Sitophilus zeamais* and *Callosobruchus maculatus* which are coleopteran storage pests, Steveson *et al.,* (2009), and could possibly have contributed to the death of mice during the study. Aqueous extracts of *S. longepedunculata* have been shown to have cytotoxic effects (IC_{50} of 67 µg/ml) on cells of Swiss albino mice, Lawal *et al.,* (2012). This report is in agreement and correlates well with the observed *in vivo* toxicity in the current study. Dose levels were lowered to 300 mg/kg for both extracts of *S. longepedunculata* and no signs of toxicity or mortality were observed in any mice during the ten experimental days. This calls for dose adjustments in herbal products containing *S. longepedunculata* to avoid the observed toxicity since the plant species may not be safe in doses above 300 mg/kg bdw.

Organic and aqueous leaf extracts of *G. hexamita* had a chemosuppression of 24.0%. Leaves of *G. hexamita* have been used in traditional medicine for treatment of malaria in Msambweni district, Kenya, Nguta *et al.,* (2010a). This study therefore provides insights into the use of the plant for treatment of malaria in folk medicine. *G. erythraea* Schweinf. (Tiliaceae) has been reported to have considerable antimalarial activity, Al-Musayeib *et al.,* (2012), validating its traditional use in treatment of ailments with malaria like symptoms. Oral acute toxicity of aqueous extracts at a single dose of 2,000 mg/kg was considered safe since all experimental mice were alive and normal for all ten experimental days and no signs of toxicity were observed. These results are supported by those of Ukwani *et al.,* (2012) where hydro-methanolic leaf extracts of *G. crenata* (J.R. Frost.) Schinz & Guillaumin were shown to be toxic during acute and sub-chronic oral toxicity studies in rats, since a single dose of 5,000 mg/kg, did not show mortality or any signs of toxicity in any of the rats studied during the 14 day observation period.

Chemosuppression level due to organic extracts of *C. schimperi* (2.88%) was higher than that of aqueous extracts (0.18%). Roots and stem bark of *C. schimperi* has been reported to be used to treat malaria in traditional medicine, Statish and Ranjana, (2013). Organic and aqueous extracts that were tested for their oral acute toxicity at 2,000 mg/kg did not show any signs of toxicity. These observations are in accordance with those of Mekonnen *et al.,* (2003), who did not observe any signs of toxicity while examing crude extracts of *C. myrrha* and *C. guidotti.*

Aqueous and organic leaf extracts of *R.communis* recorded chemosuppression levels of 15.65% and 2.88%, respectively. The observed parasitaemia levels were not significantly different from those of distilled water ($P<0.05$). The plant has been used in traditional medicine for treatment of malaria in Ghana, Asase *et al.*, (2005). This study provides more information on its use for treatment of malaria. Its antimalarial activity could be due to synergism since traditional practitioners prepare decoctions with multiple medicinal plants. In oral acute toxicity studies, both organic and aqueous extracts at a single dose of 2,000 mg/kg, exhibited no mortality or signs of toxicity.

5.0 Conclusions and Recommendations

In the current study, organic extracts of *S. longependuculata* had a chemosuppression of 91.03% and aqueous extracts 55.05%. These chemosuppression levels were high especially for the organic extract which was not significantly different from that of chloroquine [(97.95%) ($P< 0.05$)]. This shows a possibility of

antimalarial phytochemicals in the roots of the plant. Screening for selected phytochemicals in this plant may provide insights into active antimalarial compounds in the plant. Phytochemical analysis of the plant is highly recommended to identify the active compounds associated with the interesting antimalarial activity. Further *in vivo* toxicity studies are also recommended to determine safe dose levels of the plant. Crude extracts of *C. schimperi*, *R. communis* and *G. hexaminta* had lower chemosuppression than chloroquine but they had lower parasitaemia levels when compared with the negative control, distilled water. This validates their ethnopharmacological use as antimalarial herbal remedies by the Msambweni community. *In vitro* antiplasmodial studies, further *in vivo* antimalarial studies, *in vivo* toxicity studies, fractionation, purification and compound identification from the most active crude extracts is recommended. This could yield safe and efficacious compounds that could be used as biomarkers for standardization of antimalarial herbal preparations or serve as templates that could yield novel antimalarial drugs following pharmacophore modulation.

6.0 Acknowledgements

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7.0 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 (Family)	Part of the plant used	Mean (X± S.D) Parasite density		Mean (X± S.D.) Chemosuppression (%)		Mean (X± S.D.) survival time (days)	
		Aqueous	Organic	Aqueous	Organic	Aqueous	Organic
S. longepedunculata Fres. (Polygalaceae)	roots	14.68±0.84$^{(e)\,(f)}$	2.93±0.54$^{(e)}$	55.05±2.21$^{(b)}$	91.03±1.64	8±1.58$^{(c)\,(d)}$	8±2.0 $^{(c)\,(d)}$
R. communis L. (Euphorbiaceae)	leaves	27.55±2.15$^{(e)\,(f)}$	31.72±8.0 $^{(f)}$	15.65±6.53$^{(b)}$	2.89±24.51 $^{(a)}$	7.8±1.30$^{(c)}$	9.4±1.34$^{(d)}$
G. hexamita Burret. (Tiliaceae)	leaves	24.81±5.2 $^{(e)\,(f)}$	24.82±3.16$^{(e)\,(f)}$	24.04±15.93$^{(b)}$	24.13±9.7 $^{(a)}$	8±1.87 $^{(c)}$	7.8±1.48$^{(c)}$
C. schimperi (Berg.) Engl. (Burseracea).	roots	32.57±3.88 $^{(f)}$	29.26±1.8 $^{(e)\,(f)}$	0.19±11.89$^{(b)}$	10.41±5.51 $^{(a)}$	7.6±0.55$^{(c)}$	7.6±0.89$^{(c)}$
Chloroquine	0.67±0.39			97.95±1.2		10±0	
Water	32.66± 13.46			N/A		6.6±0.55	

[a]**P <0.05**, organic extracts compared with chloroquine

[b]**P <0.05,** water extracts compared with chloroquine

[c]**P <0.05,** mean survival time of mice treated with extracts compared with chloroquine

[d]**P <0.05,** mean survival time of mice treated with extracts compared with negative.

[e]**P < 0.05,** Parasite density versus Distilled water

[f]**P < 0 .05**, Parasite density versus Chloroquine

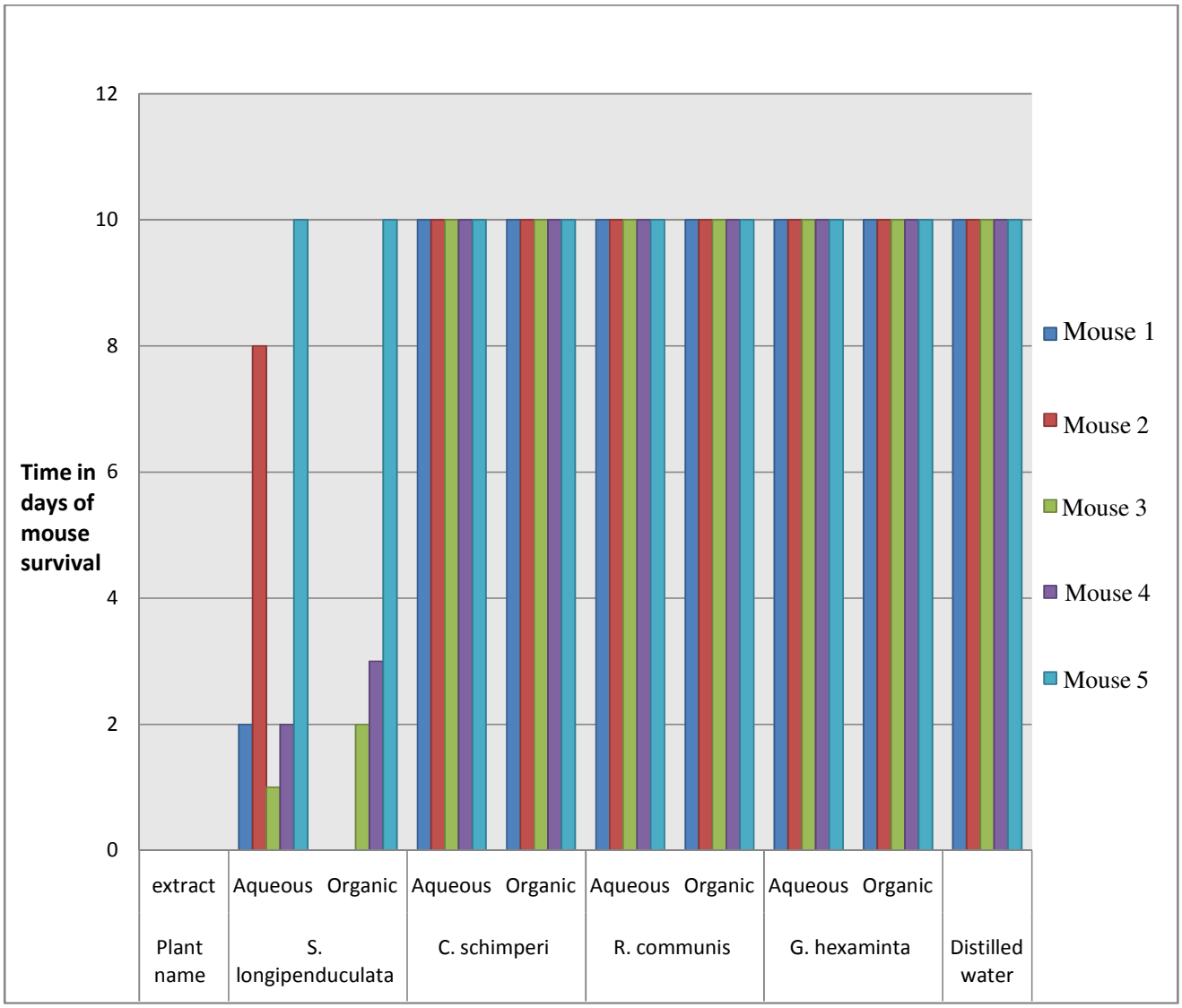

Figure 1: Oral acute toxicity of plant extracts at 2,000mg/kg

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