



## RESEARCH ARTICLE

# REVISED Acute and sub-acute toxicity study of the root extracts of *Fagaropsis hildebrandtii* in mice and evaluation of their antimicrobial effects [version 2; peer review: 1 approved, 1 approved with reservations]

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## Abstract

**Background:** Among the Kamba community of Kenya, roots of *Fagaropsis hildebrandtii* (FH) are boiled and used in managing cough, fertility problems, and microbial infections. The safety of this plant in oral administration and the validity of the ethnomedical claims are unverified. This study evaluated the toxicity of the aqueous and hexane root extracts of FH in mice and antimicrobial effects against *Staphylococcus aureus*, *Salmonella typhimurium* and *Candida albicans*.  
**Methods:** Doses (300 and 2000 mg/kg) of the extracts were administered orally to mice for 14 days. The weight, feed, and water consumption, organ weight of mice and gross macroscopy of the liver were used in evaluating acute toxicity. Mice were additionally treated with 250, 500, and 1000 mg/kg body-weight doses of the extracts for 28 days and haematological, biochemical, and histological parameters noted. The minimum inhibitory and minimum bactericidal/fungicidal concentrations (MIC; MBC/MFC) of the extracts against the aforementioned pathogens were determined by broth dilution.  
**Results:** Acute oral toxicity of the extracts was >2000 mg/kg, there were dose dependent changes in haematological and biochemical parameters, all female mice died when treated with doses of 1000mg/kg and doses  $\geq$ 500 mg/kg caused tubular degeneration and haemorrhage of the kidney, cloudy swelling of hepatocytes, and multifocal necrosis and pyknosis in the liver. The MBC/MIC ratio of each of the extracts against *Staph. aureus* and *S. typhimurium* was 2, while *C. albicans* was not sensitive to any of the extracts.  
**Conclusions:** Long term use of FH root extracts was associated with dose-dependent changes in the mice kidney, liver and in biochemical and haematological parameters. Root extracts of FH are bactericidal against *Staph. aureus* and *S. typhimurium* but have no effect on *C.*

## Open Peer Review

Reviewer Status

	Invited Reviewers	
	1	2
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*albicans*. Future work should aim at identifying the metabolites responsible for the observed toxic and bactericidal effects of the FH root extracts.

### Keywords

*Fagaropsis hildebrandtii*, toxicity, antimicrobial activity, mice.



This article is included in the **Antimicrobial Resistance** collection.

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**REVISED Amendments from Version 1**

1. We have amended sections under 'collection and authentication of plant material' to include the season during which the root barks were collected.
2. We have rectified the in-text description of what is presented in Figure 5 and Figure 6
3. We have added a little more information in the last paragraph of the discussion section explaining why we think a dose of 1000 mg/kg of the extracts resulted in the death of all the female mice.

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## Introduction

In the last two decades, the demand for and access to complementary and alternative medicine has grown exponentially<sup>1</sup>. This has been down to a host of reasons including the integration of complementary and alternative forms of medicine into mainstream healthcare, as exemplified in Taiwan<sup>1</sup>, a general lack of access and means to afford therapies from the developed world by people in impoverished areas in Africa and Asia, and cultural attitudes towards health in different parts of the world<sup>2,3</sup>. Notwithstanding, complementary and alternative medicine continues to be sidelined in discussions on the global stage in matters pertaining to public health<sup>4</sup>. This state of affairs means that there is little impetus to unravel the untapped potential that medicinal species used in complementary and alternative medicine hold. The safety profile of some of the medicinal plants used in complementary and alternative therapies are also unknown despite positive ethnomedical reports emanating from various communities around the world. This therefore calls for a thorough scrutiny of not only the ethnomedical claims but also the safety of the medicinal plants used in such therapies.

*Candida albicans*, *Salmonella typhimurium*, and *Staphylococcus aureus* are some of the most common human pathogens in low and middle-income countries<sup>5,6</sup>. The development of antimicrobial resistance to these pathogens is an ongoing problem and is of public health relevance<sup>7,8</sup>. Thus, the need for alternatives to the present drugs indicated for infections by these microorganisms has never been more important.

*Fagaropsis hildebrandtii* (Figure 1) is a deciduous shrub/tree which grows up to a height of 24 meters<sup>9</sup>. It has nice smelling fruits when unripe, which turn red when they ripen. It is native to Ethiopia, Somalia and Kenya<sup>9</sup>. Among the Kamba community of Kenya, it is known as "muvindavindi"<sup>9</sup>. Some ethnomedical claims associated with the plant include treatment of pneumonia, chest pain, arthritis, stomach pains, ulcers, malaria, internal abscesses, epilepsy and resolving infertility in women, as well as chest and respiratory infections<sup>9</sup>. This is administered as follows: the root/bark is soaked in water or boiled and administered as an infusion or decoction at a dose of one glass three times daily<sup>9</sup>. Not only is there a dearth of scientific literature supporting these medicinal claims, but also the safety of this plant for various ethnomedical applications may only be assured after extensive scientific scrutiny. This study therefore aimed to determine the safety of root

extracts of *F. hildebrandtii* on oral administration in mice, as well as to evaluate the antimicrobial effects of the extracts against *Staph. aureus*, *S. typhimurium*, and *C. albicans* which are gram positive, gram negative, and fungal microorganisms, respectively. The null hypothesis for this study was that root extracts of *F. hildebrandtii* are safe on oral administration in mice. Up to 75% of all experiments on toxicity make use of rodents (rats or mice)<sup>10</sup> and it is assumed that the effects detected in rodents are the same as those that would be induced in humans unless there is some specific information of species differences in the test response<sup>10</sup>. Moreover, the response of the test animal is generally considered representative of the average sensitivity of the human population and that for some members of the human population, the risk to health may be much higher<sup>10</sup>.

## Methods

### Chemicals and microorganisms

HPLC grade hexane was purchased from Sigma Aldrich (St. Louis MO, USA). All chemicals used in preparing various reagents for phytochemical screening were analytical grade and high purity. Two bacteria (gram-positive: *Staphylococcus aureus* ATCC 25923; gram-negative: *Salmonella typhimurium* ATCC 722569) and one fungus (*Candida albicans* ATCC 10231) were provided by the Microbiology section of the Department of Public Health, Pharmacology and Toxicology of the University of Nairobi.

### Collection and authentication of plant material

Fresh roots of *F. hildebrandtii* were collected from Kilome constituency, Kitaingo sub location in Makueni County in Kenya (GPS coordinates: 1.7726° S, 37.4498° E) in the month of February, 2017 (Hot, dry season). Identification and authentication of the plants were done at the herbarium, Department of Land Resource Management, University of Nairobi. Identification and authentication involved the determination of the botanical origin



**Figure 1. *Fagaropsis hildebrandtii*. (A) Whole plant and (B) aerial parts. Photos by BM.**

of the plant, determination of the scientific binomial name, determination of the vernacular name, site of collection, habitat and season of collection, altitude and the parts collected<sup>11,12</sup>. The keys available in the book titled 'Kenya trees, shrubs, and lianas'<sup>11</sup> was used to arrive at the said genera and the specific epithet.

### Preparation of extracts

The collected roots were gently washed in tap water to remove dirt. They were then shade dried in the laboratory at room temperature for ~2–3 weeks. After completely drying, plant material was pulverized by use of a mechanical mill and subsequently sieved using a 0.45µm pore Whatman® membrane filter to obtain a powder of suitable consistency. The powdered material was weighed and divided into two portions. One portion (~500 g) was mixed with ~2000 ml of sterile distilled water, allowed to macerate for 72 hours and centrifuged at 3000 rpm for 10 minutes. The supernatant was collected, filtered through a 0.45 µm membrane filter, and the filtrate lyophilized, and appropriately stored in an amber colored bottle (henceforth known as *AQRFH*). To the second portion, 1250 ml of hexane was added and the mixture allowed to macerate for 72 hours, centrifuged at 3000 rpm for 10 minutes, and the supernatant filtered using a 0.45 µm membrane filter. The filtrate was then reduced by use of a rotary evaporator at 40°C to obtain a dried product (henceforth known as *HEXRFH*).

### Qualitative phytochemical screening

*AQRFH* and *HEXRFH* were analyzed for the presence of various phytochemical metabolites, including alkaloids, cardiac glycosides, flavonoids, phenolics, saponins, phytosterols, tannins and triterpenes using standard procedures<sup>13</sup>. See [Table 1](#) for the exact tests carried out.

### Experimental animals and husbandry

Adult healthy male and female albino mice (*Mus musculus*; BALB/c strain; n=66: 42 females, 24 males; age: 8 to 12 weeks; body weight: 20–30 g) were used for this study. The number of animals used were based on the Organization for Economic Corporation and Development (OECD 423) test

guidelines<sup>14</sup> (*Extended data*: [Table S1<sup>15</sup>](#)). Female mice were nulliparous and not pregnant and all animals were sourced from the Animal House of our institution (University of Nairobi). They were housed in polypropylene cages in the laboratory for at least one week to acclimatize to standard conditions (temperature: 25±3°C; relative humidity: 56–60%; 12 hours of light and 12 hours of darkness). Fluorescent room lights were switched on at 0600h–1800h (light cycle) and off at 1800h–0600h (dark cycle). They were fed on standard mice pellets from a commercial feed supplier (Unga Group Plc, Kenya) and water was provided *ad libitum*.

All efforts were made to ameliorate any suffering of the animals by adopting the OECD 423 test guidelines<sup>14</sup> as well as the recommendations of the Biosafety, Animal Use and Ethics Committee (BAUEC) of the University of Nairobi (BAUEC/2018/163) (*Extended data*: [Figure S1<sup>15</sup>](#)).

### Experimental protocols

All treatments on the experimental animals were carried out in rat cages during the daytime/light cycle (0600h to 1800h) at the animal holding unit of the Department of Public Health, Pharmacology, and Toxicology, University of Nairobi.

**Acute toxicity study.** The acute toxic class (OECD 423) test guidelines on acute oral toxicity were used<sup>16</sup>. In total 18, 8–12-week-old female albino mice of 18–20 g were labelled from 1–6 in such a way that there were three animals bearing each number<sup>17</sup>. The number assigned to each animal was then written on a piece of paper (n=18), folded and placed on a receptacle which was then shaken<sup>17</sup>. A paper was withdrawn at random and the animal that bore the number was assigned into the respective cages labelled 1 (distilled water control group), 2 (extra virgin oil control group), 3 (300 mg/kg *AQRFH* extract group), 4 (2000 mg/kg *AQRFH* group), 5 (300 mg/kg *HEXRFH* group) and 6 (2000 mg/kg *HEXRFH* group)<sup>17</sup>. Each animal was individually weighed at day 0, 7, and 14 and the observation of the weights noted (*Extended data*: [Table S2<sup>15</sup>](#)). The mice were then fasted for 4 hours before dosing.

**Table 1. Preliminary phytochemical screening of root extracts of *Fagaropsis hildebrandtii*.**

Plant metabolite	Test	Aqueous extract	Hexane extract
Alkaloids	Dragendorff's test	+	+
	Mayer's test	+	+
Anthraquinones	Borntragger's test	-	-
Cardiac glycosides	Keller-killiani test	-	+
Flavonoids	Alkaline reagent test	+	-
Phenolics	Ferric chloride test	+	+
Saponins	Foam test	+	-
Steroids	Liebermann-Burchard's test	+	+
Tannins	Ferric chloride test	+	+
Terpenoids	Salkowski's test	+	+

+: Present, -: absent

Two doses (300 and 2000 mg/kg of body weight) of either *AQRFH* or *HEXRFH* were prepared by accurately weighing the extracts into 250 ml volumetric flasks, mixing well in distilled water or extra virgin oil respectively, and making up to the mark with either distilled water or extra virgin oil. Dosing of mice followed the OECD 423 test guidelines (i.e. 1ml per 100 g body weight of mice)<sup>14</sup>. Therefore, using the weights of mice as a guide, suitable amounts (in ml, and corresponding to the appropriate doses) of either *AQRFH* or *HEXRFH* suspended in distilled water and extra virgin oil, respectively, were drawn into 2ml syringes attached to a gastric gavage and administered to mice. Two groups (Groups 1 and 2) served as controls, and mice in these groups received by gastric gavage a daily dose of distilled water and extra virgin oil. Group 3 mice received a 300 mg/kg dose of *AQRFH*, Group 4 mice received a 2000 mg/kg dose of *AQRFH*, Group 5 mice received a 300 mg/kg dose of *HEXRFH*, and Group 6 mice received a 2000 mg/kg dose of *HEXRFH*.

Food and water consumption of the mice per treatment was evaluated at 0, 7 and 14 days after treatment (*Extended data*: Table S3<sup>15</sup>). At the end of the 14 days, the mice were sacrificed and organs such as the liver, kidney, lungs and spleen were weighed and used to calculate the relative mean organ weight as below:

$$\text{Relative organ weight} = \frac{\text{absolute organ weight}}{\text{weight of mice at sacrificing}} \times 100$$

The data on relative mean organ weight of mice in the acute toxicity protocol is summarized in *Extended data*: Table S4<sup>15</sup>.

**Sub-acute toxicity studies.** This study was carried out using 24 male and 24 female albino mice (20–30 g). They were grouped by randomized complete block design into 6 treatment groups and 2 control groups (n=6 mice/group). Control group mice received an oral daily dose of 0.6 ml distilled water (aqueous extract control) and 0.3 ml of extra virgin oil (hexane extract control) for 28 days. Treatment group mice received graded doses (250, 500 and 1000 mg/kg body weight) of *AQRFH* and *HEXRFH* which were administered once daily for 28 days. The body weights of the mice were noted at day 0, 7, 14, 21, and 28 and the doses adjusted accordingly (*Extended data*: Table S5<sup>15</sup>).

**Clinical observations.** Changes in fur and skin colour, mucous and eye membranes, respiratory, circulatory autonomic and central nervous systems were noted<sup>16</sup>. Other observations to be noted included tremors, convulsions, confusion, salivation, diarrhoea, coma and death. Observations were done at intervals of 30 minutes, 4 hours, 24 hours, 48 hours, 7 days, 14 days, 21 days and 28 days<sup>16</sup>.

**Body weight.** Individual body weights of mice in the sub-acute toxicity protocol were recorded on day 0, 7, 14 and 28 (*Extended data*: Table S5<sup>15</sup>). Body weight was also recorded prior to the first dosing on day 0 and terminally (after fasting) prior to necropsy (*Extended data*: Table S5<sup>15</sup>). The food and water consumption in the sub-acute toxicity protocol are summarized in *Extended data*: Table S6<sup>15</sup>.

**Necropsy and organ weight.** All animals were fasted overnight prior to necropsy. The animals were euthanized in

0.5% v/v halothane (Piramal Enterprises, India; Batch number K37L17B) in a bell jar and vital organs (stomach, kidney, lung, spleen, heart and liver) were excised from both male and female mice, washed gently and observed macroscopically for lesions or any abnormal signs. Organ weights (absolute and relative) were determined for the excised organs by placing them in absorbent paper for a few minutes and weighed. The relative organ weight of each mice was then determined as below:

$$\text{Relative organ weight} = \frac{\text{absolute organ weight}}{\text{weight of mice at sacrificing}} \times 100$$

The relative mean organ weight of mice in the sub-acute toxicity protocol are summarized in *Extended data*: Table S7<sup>15</sup>.

**Clinical pathology.** At the end of dosing, mice were fasted overnight prior to scheduled necropsy. On day 29, mice were anaesthetized using halothane inhalation in a bell jar, blood samples were collected by use of cardiac puncture into vacutainers containing anticoagulant (EDTA for hematological parameters) and without anticoagulant (plain tubes for biochemical parameters). About 0.5 ml of the blood in plain tubes was centrifuged at 3000 rpm for 10 minutes to obtain serum, which was then stored at -20 °C awaiting further use in biochemical assay of aspartate amino transferase (AST), alanine amino transferase (ALT), total protein (TP), urea and creatinine using specific kits (Catalyst AST Aspartate amino transferase-98-11067-01, Catalyst ALT/GPT Alanine amino transferase-98-11069-01, Catalyst TP Total Protein-98-11085-01, Catalyst BUN Urea-98-11070-01, and Catalyst CREA Creatinine-98-11074-01: Magnum Veterinaria, Laagri Arimaja, Vae 16, Estonia). In addition, ~1.3ml of the blood collected in the anticoagulant containing vacutainers was used to evaluate levels of hematocrit (HCT), total white blood cell (WBC) count, total red blood cell (RBC) count, hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) using IDEXX kits (One Idexx drive, Westbrook, Maine, 04092, United States).

**Histopathological studies.** Histopathological studies were carried out on organ samples of liver and kidney of the mice. These organs were surgically removed from the mice and fixed in 5% buffered formalin. Tissue samples were then dehydrated in graded doses of ethanol (70–99.9%; Fischer Scientific Ltd.; Batch number 0556512, washed in toluene (Fischer Scientific: Lot no: 193377, and enclosed in paraffin (Honeywell Fluka™: 18634H). Thin tissue sections of 5µm were obtained on a rotary microtome and stained with hematoxylin-eosin dye and analyzed microscopically for any pathological alterations. The photomicrographs obtained were labelled for identification.

**Antimicrobial activity.** *Staph. aureus* (gram positive bacteria), *S. typhimurium* (gram negative bacteria) and *C. albicans* (fungus) were cultured on blood agar (Oxoid Ltd.; Lot number 1623360) and incubated overnight at 37°C in an incubator (Mettler, Germany). The cultures were suspended in 5 ml sterile physiological buffered saline and turbidity adjusted to 0.5 McFarland to give a concentration of approximately  $1.5 \times 10^8$  colony forming units per ml (CFU/ml). In total, 800 mg of *AQRFH* and *HEXRFH* were dissolved in 3ml of

sterile distilled water and virgin oil, respectively. Serial dilutions ranging from 3.125–400 mg/ml of the extracts were then prepared using sterile peptone water (Oxoid Ltd; Lot number 1721983). Subsequently, using a sterile 1ml pipette, the adjusted individual micro-organisms (*Staph. aureus*, *S. typhimurium* and *C. albicans*) were inoculated into every tube of the diluted plant extracts to give a final concentration of  $5 \times 10^6$  CFU/ml. The tubes were then incubated at 37°C for 24 hours. Serial dilutions of the plant extracts devoid of micro-organisms were used as negative control. Positive control tubes had the respective microorganisms without serial dilutions of the plant extracts. Peptone water was used to blank the spectrophotometer. Flucloxacillin (10mg/ml; Dawa Pharmaceuticals Ltd-Kenya; Batch number MB18085 and fluconazole (10mg/ml); Universal Corporation Ltd-Kenya; Batch number 5805073) were used as positive reference compounds of antimicrobial activity.

Baseline optical density at a wavelength of 450 nm readings for each of the tubes were taken immediately after all tubes had been prepared using a spectrophotometer (Spectronic 21D, Milton Roy, USA). Optical density readings were also taken after 24 hours. A comparison between baseline optical density readings and optical density readings after 24 hours of inoculation of the tubes was made. This was done to determine whether there was growth or not. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the extracts that inhibited any visible growth of bacteria as determined by the absence of change in the optical density readings between baseline values and those obtained after incubation of the tubes. For the determination of the minimum bactericidal/fungicidal concentration (MBC/MFC), 100µl of broth was taken from all the MIC tubes after 24 hours of incubation at 37°C and sub-cultured aseptically to Plate Count Agar (Oxoid Ltd.; Lot number 1626187). The plates (WOM-CHINA; Lot number RT20171103) were incubated at 37°C for 24 hours and checked for bacterial growth. The lowest concentration of the plant extracts that showed no bacterial growth was considered the MBC/MFC, defined as the lowest concentration of the extract which is responsible for killing greater than 99.9% of the initial bacteria inoculums.

### Statistical analysis

Results of hematological and biochemical tests were expressed as mean  $\pm$  of standard deviation of three independent observations and analyzed using ONE-WAY ANOVA on Statistical Package for the Social Sciences (SPSS, version 21.0). Tukey's test was used as post hoc.  $p < 0.05$  was considered significant.

## Results

### Preliminary phytochemical screening

*AQRFH* and *HEXRFH* yielded about 9% and 2.74% of extract, respectively. Preliminary phytochemical screening of *AQRFH* revealed the presence of alkaloids, flavonoids, phenolics, saponins, steroids, tannins and terpenoids while the screening of *HEXRFH* revealed the presence of alkaloids, cardiac glycosides, phenolics, saponins, steroids, tannins and terpenoids (Table 1).

### Acute toxicity study

Acute toxicity studies were carried out for 14 days as per the OECD 423 guidelines<sup>14</sup>. No mortality of mice was observed within the first 4 hours of continuous observation, nor after 24 hours. There was also no lethal effect observed after the administration of the extracts for the experimental period of 14 days. Morphological characteristics such as the fur, and skin colour appeared normal. There was no salivation, diarrhoea, lethargy, unusual behaviour or altered respiration. Mean body weight (*Extended data*: Table S2<sup>15</sup>), food and water intake (*Extended data*: Table S3<sup>15</sup>), and relative mean organ weight (*Extended data*: Table S4<sup>15</sup>) of treatment group animals were not significantly different from the control group animals. Figure 2 illustrates the changes in the mean body weight of mice receiving different treatments over the 14-day study period for acute oral toxicity. Figure 3 illustrates the feed and water consumption of the experimental mice over the 14-day study period.

### Gross macroscopy

On gross macroscopic examination, there was no necrosis, inflammation or change in size of the lungs, liver, spleen, kidney or heart in mice (Figure 4).

### Sub-acute toxicity studies

#### *Mean body weight of mice that received AQFRH over 28 days.*

There was no significant difference in the mean weight of control mice relative to the mice treated with 250 or 500 mg/kg doses of *AQFRH*. However, there was a significant difference in the mean weight of control mice relative to the mice (female) treated with a 1000 mg/kg dose of *AQFRH* after 28 days (*Extended data*: Table S5<sup>15</sup>).

*Feed and water consumption in mice treated with graded doses of AQFRH and HEXFRH.* Feeding mice on graded doses of the root extracts of *F. hildebrandtii* over a 28-day period had no significant effect on the feed and water consumption of mice in treatment groups relative to the control (*Extended data*: Table S6<sup>15</sup>).

*Relative mean weight of mice treated with graded doses of AQFRH and HEXFRH.* Doses of 250 mg/kg and 500 mg/kg of *AQFRH* did not produce any significant effect on the mean weight of mice relative to the controls (*Extended data*: Table S7<sup>15</sup>).

None of the doses of *HEXFRH* had any significant effect on the mean weight of the treatment mice relative to the controls (*Extended data*: Table S8<sup>15</sup>).

*Relative-organ weight ratios of mice treated with AQFRH and HEXFRH.* There was no significant change in the mean relative weight of the stomach, left and right kidneys, lung, spleen, heart and liver in mice treated with a 28-day oral dose of 250 mg/kg and 500 mg/kg of *AQRFH*, respectively (*Extended data*: Table S9<sup>15</sup>). A 1000 mg/kg dose of *AQRFH* significantly lowered the mean relative weight of the spleen in both male and female mice (*Extended data*: Table S9<sup>15</sup>). This dose also

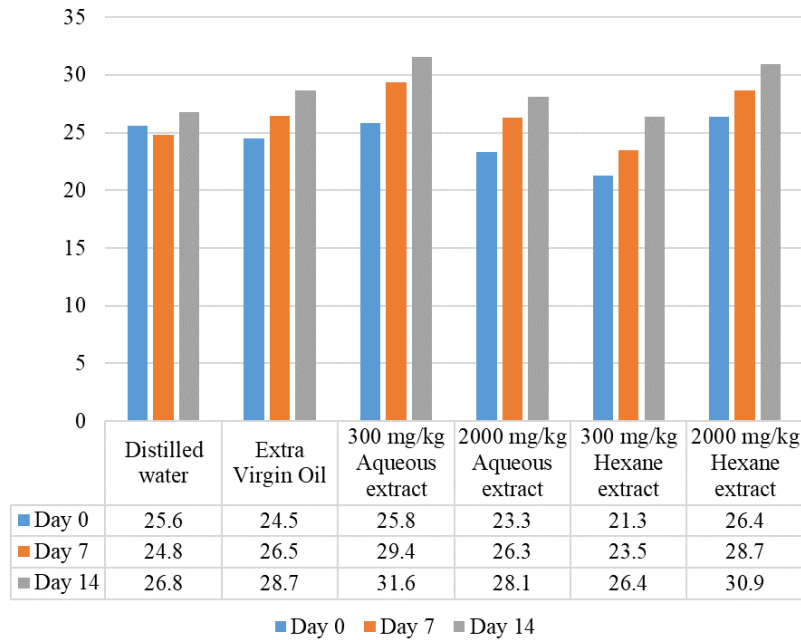


Figure 2. Changes in the mean body weight of mice treated with the root extracts of *F. hildebrandtii* over the 14-day period.

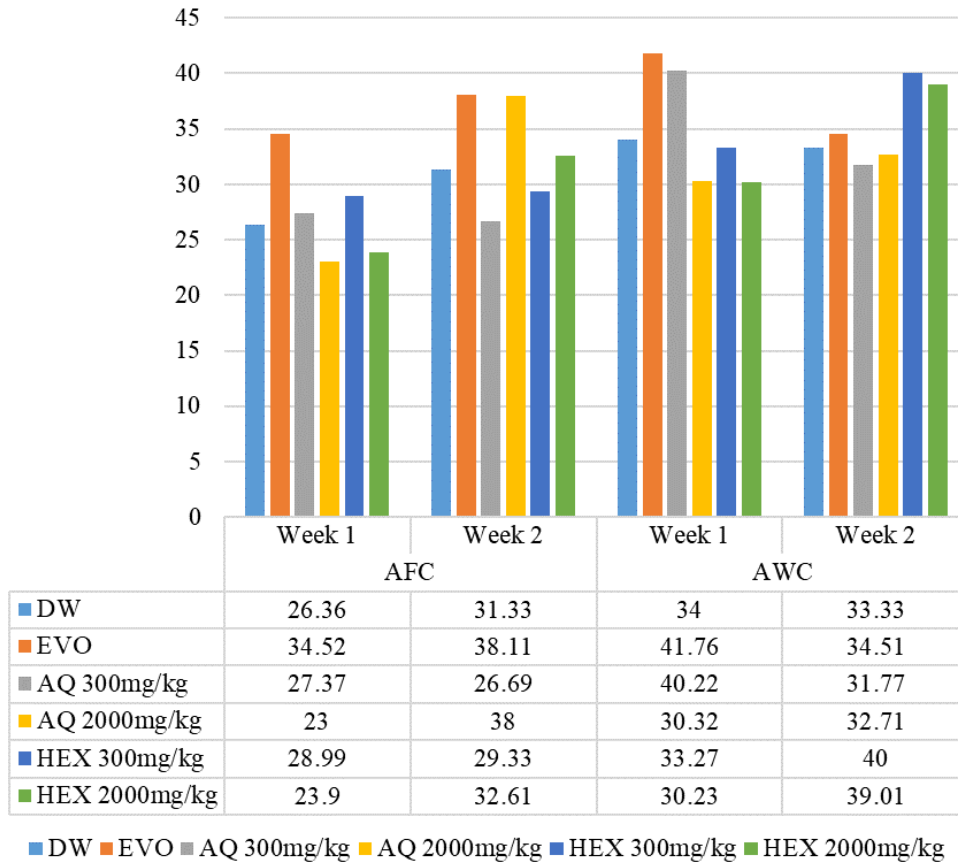
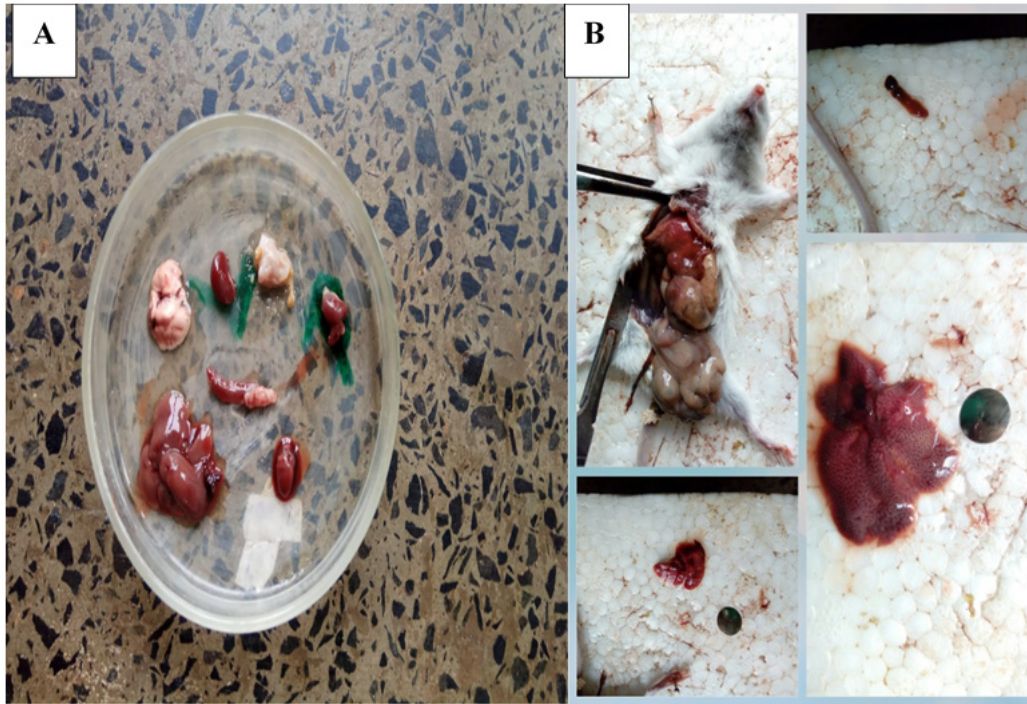


Figure 3. Feed and water consumption in mice receiving various treatments over the 14-day study period. DW: distilled water, EVO: Extra virgin oil, AQ: aqueous, HEX: Hexane, AFC: average food consumption, AWC: average water consumption.



**Figure 4.** **A.** Gross macroscopy of the lungs, liver, spleen, kidney and heart excised from a control group mouse, **B.** Gross macroscopy of spleen, lung, and spleen excised from a mouse treated with a 2000 mg/kg dose of *AQRFH*.

significantly lowered the mean relative weight of the liver in male mice, and the stomach and lung in female mice relative to the control group (*Extended data: Table S9*<sup>15</sup>).

There was no significant change in the mean relative organ weight of the stomach, left and right kidneys, lung, spleen, heart and liver in mice treated with a 28-day oral dose of 250 mg/kg and 500 mg/kg of *HEXRFH* (*Extended data: Table S10*<sup>15</sup>). A 1000mg/kg dose of *HEXRFH* significantly increased the mean relative organ weight of the liver in male mice relative to the control. This dose also produced a significant decrease in the mean relative organ weight of the lungs, spleen and heart in male and female mice, and the stomach of female mice relative to the control group (*Extended data: Table S10*<sup>15</sup>).

**Evaluation of hematological parameters.** A 250 mg/kg dose of *AQRFH* significantly lowered the mean RBC levels in male mice, while significantly raising the mean platelet levels in both male and female mice relative to the control group (*Table 2*). This dose also non-significantly increased the mean levels of Hb, HCT, MCV, MCH, and the MCHC in both male and female mice as well as mean levels of WBC in male mice relative to the control (*Table 2*). This dose also non-significantly lowered the mean RBC levels in female mice relative to the controls. A 500 mg/kg dose of *AQRFH* significantly lowered the mean RBC levels in male mice while significantly raising the mean Hb levels in female mice and the mean platelet levels in male mice relative to the control (*Table 2*). This dose also non-significantly increased the mean levels of MCHC, MCH and WBC in both male and female mice, and the mean levels of HCT, platelets and MCV in female mice, as well as

the mean Hb levels in male mice relative to the controls. A 1000 mg/kg dose of *AQRFH* resulted in the death of all female mice tested (n=6). Thus, no hematological parameter was evaluated for these animals. On the other hand, this dose of extract significantly lowered the mean levels of all hematological parameters evaluated in male mice relative to the controls (*Table 2*).

A 250 mg/kg dose of *HEXREF* significantly lowered the mean RBC and platelet levels in male and female mice relative to the controls (*Table 3*). This dose also non-significantly lowered the mean hematocrit, WBC and MCHC levels in both male and female mice, as well as the mean Hb, and MCV levels in female mice relative to the control group. This dose also non-significantly raised the mean Hb and MCV levels in male mice, as well as the MCH levels in both male and female mice relative to the control group. A 500 mg/kg dose of *HEXRFH* significantly lowered the mean RBC levels in both male and female mice, and the mean platelet levels in female mice relative to the control group (*Table 3*). This dose non-significantly raised the mean Hb, MCHC and WBC levels in male and female mice, as well as the mean levels of Hb, MCH and WBC in male mice relative to the control group. This dose also non-significantly lowered the mean Hb, MCHC and WBC levels in both male and female mice, as well as the MCH levels in male mice relative to the control group. A 1000 mg/kg dose of *HEXRFH* resulted in the death of all female mice tested (n=6). Thus, no hematological parameter was evaluated for these animals. On the other hand, this dose of extract significantly lowered the mean levels of all hematological parameters evaluated in male relative to the controls (*Table 3*).



**Table 2. Effect of the aqueous root extract of *F. hildebrandtii* on hematological parameters in male and female mice.**

Parameter	Gender	Control	250 mg/kg	p value	500 mg/kg	p value	1000 mg/kg	p value
<b>RBC</b> ( $\times 10^{12}/L$ )	M	9.91 $\pm$ 0.03	9.00 $\pm$ 0.14	<b>0.00</b>	9.17 $\pm$ 0.22	<b>0.00</b>	5.01 $\pm$ 0.10	<b>0.00</b>
	F	9.19 $\pm$ 0.04	9.15 $\pm$ 0.13	<b>1.00</b>	9.11 $\pm$ 0.09	<b>1.00</b>	-	-
<b>Hb</b> (gm/dL)	M	13.57 $\pm$ 1.00	14.7 $\pm$ 0.98	<b>1.00</b>	14.57 $\pm$ 0.91	<b>1.00</b>	8.85 $\pm$ 0.21	<b>0.02</b>
	F	13.00 $\pm$ 0.87	15.33 $\pm$ 0.21	<b>0.62</b>	17.23 $\pm$ 4.09	<b>0.02</b>	-	-
<b>Hematocrit</b> (L/L)	M	0.48 $\pm$ 0.02	0.51 $\pm$ 0.02	<b>0.93</b>	0.47 $\pm$ 0.02	<b>1.00</b>	0.30 $\pm$ 0.02	<b>0.00</b>
	F	0.48 $\pm$ 0.03	0.50 $\pm$ 0.02	<b>0.97</b>	0.49 $\pm$ 0.02	<b>1.00</b>	-	-
<b>MCV</b> (fL)	M	49.10 $\pm$ 1.05	49.63 $\pm$ 3.72	<b>1.00</b>	48.7 $\pm$ 1.18	<b>1.00</b>	20.65 $\pm$ 0.63	<b>0.00</b>
	F	48.23 $\pm$ 3.56	49.33 $\pm$ 2.90	<b>1.00</b>	48.33 $\pm$ 1.33	<b>1.00</b>	-	-
<b>MCH</b> (pg)	M	13.23 $\pm$ 0.47	14.4 $\pm$ 0.78	<b>0.68</b>	14.73 $\pm$ 0.84	<b>0.32</b>	9.25 $\pm$ 0.64	<b>0.00</b>
	F	13.93 $\pm$ 0.55	14.83 $\pm$ 0.55	<b>0.92</b>	14.63 $\pm$ 0.5	<b>0.99</b>	-	-
<b>MCHC</b> (g/dL)	M	29.13 $\pm$ 0.90	29.7 $\pm$ 0.66	<b>1.00</b>	29.97 $\pm$ 1.20	<b>1.00</b>	19.7 $\pm$ 0.42	<b>0.00</b>
	F	28.27 $\pm$ 0.90	30.53 $\pm$ 0.64	<b>0.37</b>	30.43 $\pm$ 0.76	<b>0.43</b>	-	-
<b>Platelets</b> ( $\times 10^9/L$ )	M	518.33 $\pm$ 2.08	540 $\pm$ 3.06	<b>0.00</b>	546.67 $\pm$ 1.53	<b>0.00</b>	382.00 $\pm$ 0.42	<b>0.00</b>
	F	534.33 $\pm$ 2.52	548.33 $\pm$ 3.51	<b>0.04</b>	540.00 $\pm$ 1.00	<b>0.95</b>	-	-
<b>WBC</b> ( $\times 10^9/L$ )	M	4.38 $\pm$ 0.22	4.90 $\pm$ 0.15	<b>0.9</b>	5.13 $\pm$ 0.04	<b>0.48</b>	2.35 $\pm$ 0.28	<b>0.00</b>
	F	4.64 $\pm$ 0.37	4.26 $\pm$ 0.25	<b>1.00</b>	4.67 $\pm$ 0.34	<b>1.00</b>	-	-

Values expressed as mean  $\pm$  SD (n=3). **Hb**: Hemoglobin, **MCV**: Mean corpuscular volume, **MCH**: Mean corpuscular hemoglobin, **MCHC**: Mean corpuscular hemoglobin concentration, **WBC**: White blood cells

**Table 3. Effect of the hexane root extract of *F. hildebrandtii* on hematological parameters in male and female mice.**

Parameter	Gender	Control	250 mg/kg	p value	500 mg/kg	p value	1000 mg/kg	p value
<b>RBC</b> ( $\times 10^{12}/L$ )	M	9.88 $\pm$ 0.12	9.00 $\pm$ 0.07	<b>0.00</b>	9.02 $\pm$ 0.09	<b>0.00</b>	4.98 $\pm$ 0.06	<b>0.00</b>
	F	9.64 $\pm$ 0.07	9.13 $\pm$ 0.11	<b>0.00</b>	8.96 $\pm$ 0.13	<b>0.00</b>	-	-
<b>Hb</b> (gm/dL)	M	15.23 $\pm$ 0.64	15.24 $\pm$ 0.07	<b>1.00</b>	14.48 $\pm$ 0.05	<b>1.00</b>	13.05 $\pm$ 0.21	<b>0.83</b>
	F	14.50 $\pm$ 0.62	14.1 $\pm$ 0.53	<b>1.00</b>	14.16 $\pm$ 0.12	<b>1.00</b>	-	-
<b>Hematocrit</b> (L/L)	M	0.5 $\pm$ 0.04	0.5 $\pm$ 0.02	<b>1.00</b>	0.51 $\pm$ 0.03	<b>1.00</b>	0.28 $\pm$ 0.01	<b>0.00</b>
	F	0.49 $\pm$ 0.01	0.45 $\pm$ 0.02	<b>0.67</b>	0.49 $\pm$ 0.03	<b>1.00</b>	-	-
<b>MCV</b> (fL)	M	45.33 $\pm$ 1.00	49.30 $\pm$ 3.64	<b>0.95</b>	48.3 $\pm$ 3.21	<b>1.00</b>	25.8 $\pm$ 1.13	<b>0.00</b>
	F	49.80 $\pm$ 6.36	49.47 $\pm$ 0.45	<b>1.00</b>	52.9 $\pm$ 4.70	<b>0.99</b>	-	-
<b>MCH</b> (pg)	M	13.6 $\pm$ 0.75	13.7 $\pm$ 0.78	<b>1.00</b>	13.43 $\pm$ 0.85	<b>1.00</b>	11.25 $\pm$ 0.22	<b>0.04</b>
	F	13.9 $\pm$ 0.56	14.87 $\pm$ 0.85	<b>0.87</b>	15.1 $\pm$ 0.56	<b>0.64</b>	-	-
<b>MCHC</b> (g/dL)	M	28.91 $\pm$ 1.60	26.97 $\pm$ 0.29	<b>0.60</b>	27.07 $\pm$ 1.76	<b>0.67</b>	21.09 $\pm$ 1.15	<b>0.00</b>
	F	29.87 $\pm$ 1.11	29.47 $\pm$ 1.33	<b>1.00</b>	29.57 $\pm$ 0.68	<b>1.00</b>	-	-
<b>Platelets</b> ( $\times 10^9/L$ )	M	500.67 $\pm$ 1.53	492.33 $\pm$ 4.16	<b>0.00</b>	510.67 $\pm$ 1.53	<b>0.72</b>	357.00 $\pm$ 2.83	<b>0.00</b>
	F	515.00 $\pm$ 2.00	540.33 $\pm$ 5.03	<b>0.00</b>	500.33 $\pm$ 13.01	<b>0.01</b>	-	-
<b>WBC</b> ( $\times 10^9/L$ )	M	5.87 $\pm$ 0.85	4.94 $\pm$ 0.22	<b>0.85</b>	5.20 $\pm$ 0.36	<b>0.36</b>	2.16 $\pm$ 0.08	<b>0.00</b>
	F	4.75 $\pm$ 0.55	4.71 $\pm$ 0.72	<b>1.00</b>	3.77 $\pm$ 0.60	<b>0.14</b>	-	-

Values expressed as mean  $\pm$  SD (n=3). **Hb**: Hemoglobin, **MCV**: Mean corpuscular volume, **MCH**: Mean corpuscular hemoglobin, **MCHC**: Mean corpuscular hemoglobin concentration, **WBC**: White blood cells

**Evaluation of biochemical parameters.** A 250 mg/kg dose of *AQRFH* did not produce any significant change in the mean levels of any of the biochemical parameters evaluated (Table 4). This dose produced a non-significant decrease in the mean levels of urea, creatinine and ALT in both male and female mice and AST and TP in male mice relative to the control group. This dose also produced a non-significant increase in the mean levels of AST and TP in female mice relative to the control group. A 500 mg/kg dose of *AQRFH* significantly raised the mean levels of ALT in female mice relative to the controls (Table 4). This dose produced a non-significant decrease in the mean levels of AST in male and female mice, and in mean levels of ALT, urea, creatinine, and TP in male mice only relative to the controls. This dose also produced a non-significant increase in the mean levels of urea, creatinine and TP in female mice only relative to the controls. A 1000 mg/kg dose of the aqueous extract of *F. hildebrandtii* resulted in the death of all female mice tested (n=6) (Table 4). Thus, no biochemical parameter was evaluated for these animals. On the other hand, this dose of extract significantly raised the mean levels of all biochemical parameters evaluated in male mice relative to the controls.

A 250 mg/kg dose of *HEXRFH* produced a significant increase in the mean levels of ALT in female mice (Table 5). This dose produced a non-significant decrease in the mean levels of urea in male and female mice, creatinine in female mice and AST in male mice relative to the controls. This dose also produced a non-significant increase in the mean levels of TP in male and female mice, creatinine and ALT in males, and AST in female mice relative to the controls. A 500 mg/kg dose of *HEXRFH* significantly raised the mean levels of ALT in both male and female mice relative to the controls (Table 5). This dose produced a non-significant decrease in the mean levels of urea and AST in male mice relative to the controls, and in mean levels of ALT, urea, creatinine, and TP in male mice

only relative to the controls. This dose also produced a non-significant increase in the mean levels of urea, creatinine and TP in female mice only relative to the controls. A 1000 mg/kg dose of *HEXRFH* resulted in the death of all female mice tested (n=6) (Table 5). Thus, no biochemical parameters were evaluated for these animals. On the other hand, this dose of extract significantly raised the mean levels of all biochemical parameters evaluated in male mice relative to the controls.

**Histopathological studies on kidney and liver sections of mice receiving AQRFH and HEXRFH over 28 days.** The effect of the extracts on the kidneys of mice used in the sub-acute toxicity protocol is summarized in Figure 5. The kidney section of a mouse treated with a 250 mg/kg dose of *AQRFH* was characterized by regular glomeruli and normal renal tubules with complete smooth epithelia and clear lumen (A) while the kidney section of a mouse treated with a 500 mg/kg dose of *AQRFH* was characterized by degeneration of renal tubules and loss of epithelium (B). On the other hand, the kidney section of a mouse treated with a 1000 mg/kg dose of *AQRFH* was characterized by multifocal tubular degeneration and loss of most of the epithelium (C) while the kidney section of a mouse treated with a 500 mg/kg dose of *HEXRFH* was characterized by mild tubular degeneration (D).

The effect of the extracts on the liver of mice used in the sub-acute toxicity study protocol are summarized in Figure 6. Liver sections of mice treated with distilled water and extra virgin oil respectively were characterized by normal parenchymal architecture with normal hepatic cells that were evenly distributed and separated by hepatic sinusoids (A and B). The liver section of a mouse treated with a 500 mg/kg dose of *AQRFH* was characterized by diffuse cloudy swelling of the hepatocytes (C) while the liver section of a mouse treated with a 1000 mg/kg dose of *AQRFH* was characterized by multifocal hepatocyte necrosis and cloudy swelling with nuclear pyknosis (D). The liver section of

**Table 4. Effect of a 28-day oral administration of graded doses of the aqueous root extract of *F. hildebrandtii* on biochemical parameters in mice.**

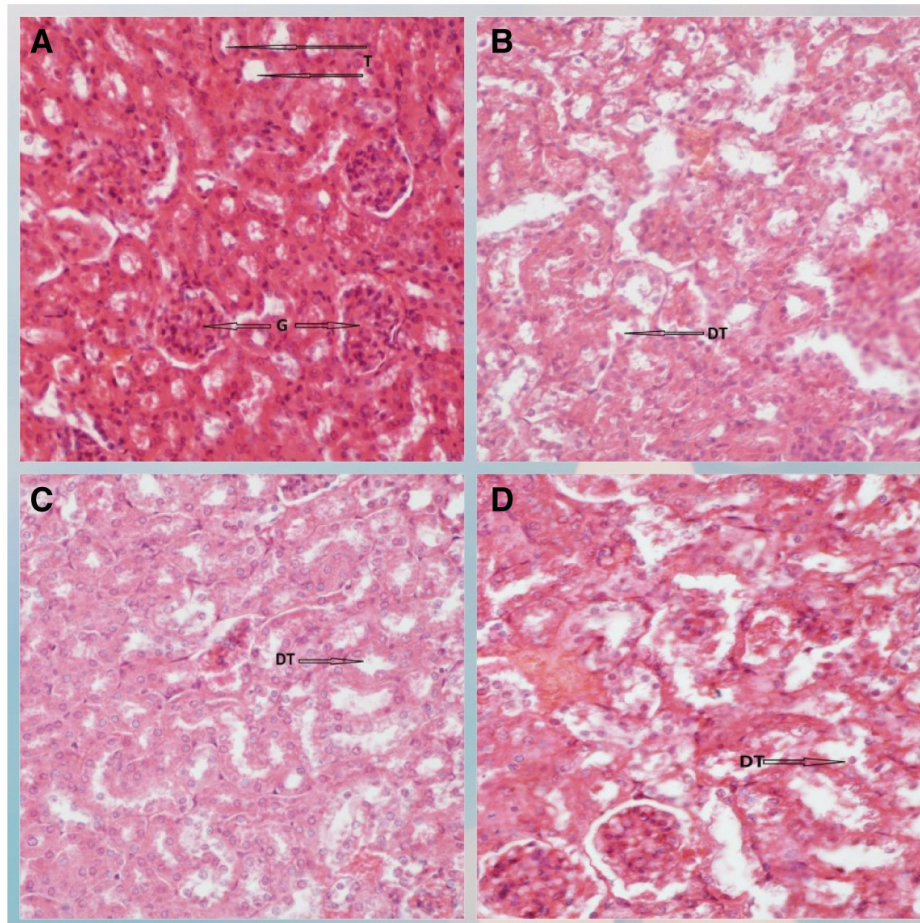
Parameter	Gender	Control	250 mg/kg	p value	500 mg/kg	p value	1000 mg/kg	p value
Urea (mg/dl)	M	7.33±0.67	5.9±0.26	<b>0.66</b>	6.57±0.15	<b>1.00</b>	11.6±2.12	<b>0.00</b>
	F	7.67±0.81	5.45±0.01	<b>0.21</b>	7.80±1.47	<b>1.00</b>	-	-
Creatinine (mg/dl)	M	95.67±5.03	92.33±8.37	<b>1.00</b>	90.00±3.00	<b>0.95</b>	150.5±2.12	<b>0.00</b>
	F	79.00±4.36	89.00±4.24	<b>0.24</b>	91.33±5.51	<b>0.10</b>	-	-
ALT (mg/l)	M	95.33±3.21	92.33±3.06	<b>1.00</b>	93.00±2.65	<b>1.00</b>	140.00±5.66	<b>0.00</b>
	F	81.00±7.55	80.00±5.66	<b>1.00</b>	97.67±2.08	<b>0.01</b>	-	-
AST (ml/l)	M	105.67±1.53	105.00±2.00	<b>1.00</b>	101.23±1.66	<b>0.97</b>	120.5±0.71	<b>0.00</b>
	F	102.67±1.15	104.19±2.70	<b>0.99</b>	101.67±3.06	<b>1.00</b>	-	-
T.P (g/l)	M	68.67±3.06	65.00±3.06	<b>0.89</b>	65.33±2.08	<b>0.94</b>	95.5±2.12	<b>0.00</b>
	F	65.33±3.51	69.00±1.41	<b>0.74</b>	69.33±1.2	<b>0.82</b>	-	-

Values are expressed as mean ± SD of three animals. **ALT:** Alanine aminotransferase, **AST:** Aspartate amino transferase, **TP:** Total protein

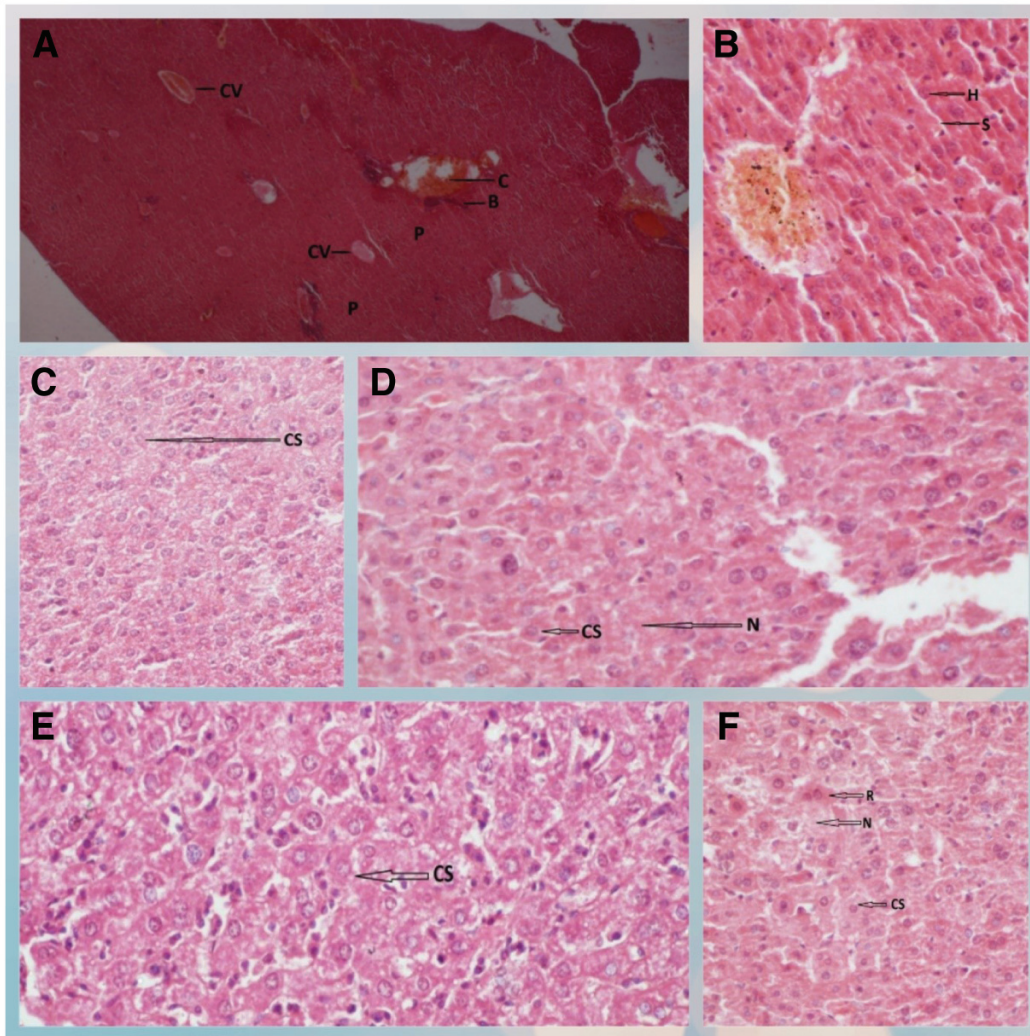
**Table 5.** Effect of a 28-day oral administration of graded doses of the hexane root extract of *F. hildebrandtii* on biochemical parameters in male and female mice.

Parameter	Gender	Control	250 mg/kg	p value	500 mg/kg	p value	1000 mg/kg	p value
Urea (mg/dl)	M	7.13±1.38	6.33±0.21	<b>0.99</b>	6.63±0.15	<b>1.00</b>	13.5±0.07	<b>0.001</b>
	F	6.83±0.75	6.37±1.46	<b>1.00</b>	7.10±0.26	<b>1.00</b>	-	-
Creatinine (mg/dl)	M	81.67±5.51	85.67±5.69	<b>1.00</b>	90.67±4.16	<b>0.47</b>	119.00±0.71	<b>0.00</b>
	F	89.67±1.15	89.33±2.08	<b>90.92</b>	92.00±3.00	<b>0.53</b>	-	-
ALT (mg/l)	M	80.67±6.43	81.33±4.93	<b>1.00</b>	96.00±7.00	<b>0.024</b>	137.5±0.71	<b>0.00</b>
	F	79.33±4.16	94.33±5.86	<b>0.00</b>	93.33±3.51	<b>0.00</b>	-	-
AST (ml/l)	M	104.00±3.00	103.26±5.80	<b>1.00</b>	102.33±2.08	<b>1.00</b>	118.00±1.41	<b>0.00</b>
	F	100.67±1.15	102.33±1.52	<b>1.00</b>	102.67±2.08	<b>1.00</b>	-	-
T.P (g/l)	M	66.67±1.53	72.33±1.53	<b>0.36</b>	68.00±2.00	<b>1.00</b>	93.5±2.12	<b>0.00</b>
	F	65.67±1.00	69.32±3.21	<b>0.45</b>	66.66±1.52	<b>0.99</b>	-	-

Values are expressed as mean ± SD of three animals. **ALT:** Alanine aminotransferase, **AST:** Aspartate amino transferase, **TP:** Total protein



**Figure 5.** **A:** Kidney section of a mouse treated with a 250 mg/kg dose of *AQFRH* (×400); regular glomeruli (G) and normal renal tubules (T) with complete smooth epithelia and clear lumen (×100), **B:** Kidney section of a mouse treated with a 500 mg/kg dose of *AQFRH* (×100); degeneration of renal tubule (DT)/loss of epithelium, **C:** Kidney section of a mouse treated with a 1000 mg/kg dose of *AQFRH* (×100; multifocal tubular degeneration (DT), most of the epithelium is lost, **D:** Kidney section of a mouse treated with a 500 mg/kg dose of *HEXFRH* (×400); mild tubular degeneration (DT).



**Figure 6.** **a.** Liver section of a mouse treated with distilled water ( $\times 40$ ); C: congestion, CV: portal vein, P: portal area, **b.** liver section of a mouse treated with extra virgin oil only ( $\times 100$ ); normal parenchymal architecture with normal hepatic cells (H) that are evenly distributed and separated by hepatic sinusoids (S), **c.** liver section of a mouse treated with a 500 mg/kg dose of AQRFH ( $\times 100$ ); diffuse cloudy swelling (CS) of the hepatocytes (early necrosis), **d.** liver section of a mouse treated with a 1000 mg/kg dose of AQRFH ( $\times 100$ ): multifocal hepatocyte necrosis, CS: cloudy swelling with nuclear pyknosis, N: hepatocyte necrosis, **e.** liver section of a mouse treated with a 250 mg/kg dose of HEXRFH ( $\times 400$ ); focal area of hepatocyte degeneration characterized by cloudy swelling, **f.** liver section of a mouse treated with a 1000 mg/kg dose of HEXRFH ( $\times 100$ ); hepatocyte necrosis and pyknosis, CS: cloudy swelling/pyknosis, R: regenerating hepatocyte.

a mouse treated with a 250 mg/kg dose of HEXRFH was characterized by cloudy swelling (E), and the liver section of a mouse treated with a 1000 mg/kg dose of HEXRFH was characterized by hepatocyte necrosis, pyknosis, and cloudy swelling (F).

**Minimum inhibitory concentration of root extracts of *F. hildebrandtii* against *Staph. aureus*, *Salmonella typhimurium* and *C. albicans*.** *C. albicans* was not sensitive to any of the concentrations of AQRFH and HEXRFH. *Staph. aureus* was sensitive to a concentration of 100 mg/ml, 200 mg/ml and 400 mg/ml of both AQRFH and HEXRFH. *Salmonella typhimurium* was sensitive to concentrations of 200 mg/ml and 400 mg/ml of AQRFH and a concentration of 100mg/ml, 200mg/ml and 400 mg/ml of HEXRFH (Table 6).

**Minimum bactericidal/fungicidal concentration (MBC/MFC) of the root extracts of *F. hildebrandtii* against *S. aureus*, *S. typhimurium* and *C. albicans*.** *C. albicans* was not sensitive to any of the concentrations of either AQRFH or HEXRFH. *Staph. aureus* was sensitive to a concentration of 200 mg/ml and 400 mg/ml of both AQRFH or HEXRFH. *Salmonella typhimurium* was sensitive to a concentration of 400 mg/ml only of the AQRFH and a concentration of 200 mg/ml and 400 mg/ml of HEXRFH (Table 7). Based on the findings of MBC and MIC of the extracts against the tested pathogens, the MBC/MIC ratio of the extracts was found to be 400/200=2; for AQRFH against *Salmonella typhi*, 200/100=2; for HEXRFH against *Salmonella typhi*, 200/100=2; for AQRFH against *Staph. aureus*, 200/100=2; for HEXRFH against *Staph. aureus*

**Table 6. Determination of the minimum inhibitory concentration of root extracts of *F. hildebrandtii* against *Staph. aureus*, *S. typhimurium* and *C. albicans*.**

Type of pathogen	Identity of the extract	Extract concentrations tested (mg/ml)										
		3.125	6.25	12.5	25	50	100	200	400	Negative control	Standard drug	
<i>C. albicans</i>	AQ	+	+	+	+	+	+	+	+	+	+	–
	HEX	+	+	+	+	+	+	+	+	+	+	–
<i>S. aureus</i>	AQ	+	+	+	+	+	–	–	–	–	+	–
	HEX	+	+	+	+	+	–	–	–	–	+	–
<i>S. typhimurium</i>	AQ	+	+	+	+	+	+	–	–	–	+	–
	HEX	+	+	+	+	+	–	–	–	–	+	–

**Table 7. Determination of the minimum bactericidal concentration of root extracts of *F. hildebrandtii* against *Staph. aureus*, *S. typhimurium* and *C. albicans*.**

Type of pathogen	Identity of the extract	Extract concentrations tested (mg/ml)										
		3.125	6.25	12.5	25	50	100	200	400	Negative control	Standard drug	
<i>C. albicans</i>	AQ	+	+	+	+	+	+	+	+	+	+	–
	HEX	+	+	+	+	+	+	+	+	+	+	–
<i>S. aureus</i>	AQ	+	+	+	+	+	–	–	–	–	+	–
	HEX	+	+	+	+	+	–	–	–	–	+	–
<i>S. typhimurium</i>	AQ	+	+	+	+	+	+	–	–	–	+	–
	HEX	+	+	+	+	+	–	–	–	–	+	–

## Discussion

Herbal medicine is a popular form of medicine but is limited by safety concerns. We believe that the present study provides the first account of the safety of any part of *F. hildebrandtii* on oral administration in any animal model. Moreover, there has been no report of the secondary metabolites that may be present in the plant. However, it is worth noting that Yiaile and colleagues<sup>18</sup> have previously reported on the preliminary composition of *Fagaropsis angolensis*, a plant of the same genus as our plant of interest. Previous studies have reported that flavonoids, phenolics, tannins, and terpenoids have antimicrobial properties with wide-ranging mechanisms of action<sup>19–22</sup>. In our case, however, the absence of flavonoids in the hexane root extract of *F. hildebrandtii* does not appear to have resulted in the abolishment of the antimicrobial properties of the extract. This observation may suggest that other phytochemicals distinct from flavonoids may be responsible for the observed antimicrobial properties.

Substances that possess antimicrobial properties are considered as bacteriostatic agents when the ratio of MBC to MIC is  $>4$  and bactericidal agents when the same ratio is  $\leq 4$ <sup>23</sup>. Using this criterion, we suggest that both of our extracts were bactericidal in nature. However, caution should be exercised in interpreting these results. This is because according to Martins and colleagues<sup>24</sup>, the higher the MIC values, the more likely that test results evaluated lose clinical relevance. Moreover, other studies have reported much lower MIC values for *Tarenaya spinosa*<sup>24</sup>, *Buchenavia tetraphylla*<sup>25</sup>, *Thymus vulgaris*<sup>26</sup> and *Origanum vulgare*<sup>26</sup> against

*Staph. aureus* and *S. typhi*. We posit that the differences in MIC values observed between our findings and those of the aforementioned studies may have something to do with differences in the abundance of phytochemicals between the plants.

Changes in the mean body weight, relative organ weight, and feed and water consumption may be indicative of acute toxicity<sup>27–29</sup>. In our study, administration of a single dose of aqueous and hexane extracts of *F. hildebrandtii* did not produce any significant changes in these parameters. Moreover, no animals died during the 14-day study period. It may therefore be inferred that these extracts may not have had any untoward effects in mice when administered orally for 14 days. However, sub-acute treatment resulted in an increase in the weight of female mice. Changes (positive or negative) in the body weight of experimental animals may be indicative of alterations in the physiology of the animals and may stem from the variations in the levels of hormones, disorders of major organs, such as the liver or kidney, or decreased absorption of nutrients as a result of treatment with test substances<sup>30</sup>. Moreover, sub-acute treatment of mice with a 1000 mg/kg dose of the root extracts of *F. hildebrandtii* was associated with a significant decrease in the mean relative organ weight of the stomach and heart in female mice, liver in male mice, and spleen in both male and female mice. According to Teo and colleagues<sup>31</sup>, the reduction in body weight of experimental animals may negatively impact the weight of internal organs. Given that the acute and sub-acute treatment of mice with root extracts of *F. hildebrandtii* did not

significantly change the consumption of food and water, we believe that the observed effects on animal weight and internal organs were the result of physiological changes in the model adopted rather than a consequence of lower food consumption.

Physiological/pathological response to toxic insult in man or animals can be gauged by evaluating the hematopoietic system<sup>32</sup>. In this study, the main significant changes were the dose dependent decrease in RBCs in both male and female mice, and significant decreases in the mean levels of Hb, HCT, MCV, MCH, MCHC, platelets and WBCs at 1000 mg/kg in both male and female mice. The observations of these effects following the oral administration of root extracts of *F. hildebrandtii* may be related to the toxicity caused by the extracts. Detoxification is a key function of the liver. Thus, the accumulation of toxic compounds in the liver is bound to be associated with liver damage. This damage is usually assessed by the determination of serum transaminases (ALT and AST) as well as measurement of total proteins<sup>32,33</sup>. Significant increases in the serum levels of these enzymes particularly at a dose of 1000 mg/kg for both aqueous and hexane extracts of *F. hildebrandtii* and the observations of cloudy swelling, necrosis, and pyknosis on histological analysis of the liver further confirms that the oral administration of these extracts in mice may indeed cause liver damage at high doses. The kidney is another organ that is considered a frequent target of toxicity<sup>32,34</sup>. In our study, renal function was evaluated by serum levels of urea and creatinine and by histological analysis. The kidney of mice treated with root extracts of *F. hildebrandtii* at doses of 500 mg/kg and above were characterized by degeneration of renal tubules, loss of epithelium and hemorrhage. Moreover, there were significant increases in the mean levels of urea and creatinine in male mice treated with a 1000 mg/kg dose of the aqueous and hexane root extracts of *F. hildebrandtii*. According to Burtis and Bruns, there is usually an increase in the levels of creatinine when the cortex and/or glomeruli is damaged<sup>35</sup>; therefore such an increase may be a good indicator of chronic kidney disease<sup>36</sup>. The histological evaluation of this organ further confirms the damage particularly for mice treated with doses of root extracts of *F. hildebrandtii* above 500 mg/kg.

Finally, mortality is usually an important index of the safety or toxicity of a xenobiotic. Although 14-day acute oral exposure of mice to root extracts of *F. hildebrandtii* did not result in the death of any of the mice, it is damning that female mice treated with a 1000 mg/kg dose of the root extracts of *F. hildebrandtii* in the sub-acute protocol all died before the end of the experiment, and their male counterparts exhibited significant alterations in biochemical, hematological and pathological indices. According to guidelines by the Organization for Economic Development (OECD), in the event that a xenobiotic causes toxicity in rodent models, females are more susceptible to toxicity than males<sup>16,37</sup>. Therefore, it could be argued that the death of female mice treated with high doses of the extracts may have stemmed from the physiological (biochemical, hematological) and pathological alterations which were more severe in female mice than their male counterparts.

## Conclusion

In conclusion, the short-term use of the root extracts of *F. hildebrandtii* at doses of 300 and 2000 mg/kg may not result in toxic manifestations in mice. However, long term administration of the extracts (beyond 14 days) was associated with dose-dependent histopathological changes in the liver and kidney of mice, as well as alterations in hematological and biochemical parameters. Considering that the roots of this plant have various ethno medicinal indications, including the treatment of pneumonia, arthritis, chest and stomach pain, ulcers, malaria, internal abscess, epilepsy, infertility in women and chest and respiratory infections, our results may be useful as a valuable piece of information on the safety profile of roots of this plant and may be important as a reference material for any future work on *F. hildebrandtii*. Further evaluation on the potential of this plant to induce carcinogenicity, mutagenicity and genotoxicity is warranted in order to paint a better picture on the complete safety profile of this plant. Studies on the identity of the metabolites responsible for the observed toxicity is also warranted.

## Data availability

### Underlying data

Figshare: Underlying data on the study titled 'Acute and sub-acute toxicity study of the root extracts of *Fagaropsis hildebrandtii* in mice and evaluation of their antimicrobial effects', <https://doi.org/10.6084/m9.figshare.9104894.v1><sup>38</sup>; <https://doi.org/10.6084/m9.figshare.9101231.v2><sup>39</sup>; <https://doi.org/10.6084/m9.figshare.9104480.v3><sup>40</sup>

This project contains the following underlying data:

- Data on the mean body weight of mice used in the acute toxicity protocol<sup>39</sup>
- Data on the feed and water consumption in mice in the acute toxicity protocol<sup>39</sup>
- Data on the relative mean organ weight of mice in the acute toxicity protocol<sup>39</sup>
- Data on the mean body weight of mice treated with sub-acute toxicity protocol<sup>40</sup>
- Data on the feed and water consumption in mice in the sub-acute toxicity protocol<sup>40</sup>
- Data on the relative mean organ weight of mice in the sub-acute toxicity protocol<sup>40</sup>
- Data on the haematological parameters in mice in the sub-acute toxicity protocol<sup>38</sup>
- Data on the biochemical parameters in mice in the sub-acute toxicity protocol<sup>38</sup>

Figshare: Photomicrographs of kidney and liver sections of mice treated with distilled water, extra virgin oil, and graded doses of aqueous and hexane root extracts of *Fagaropsis hildebrandtii*, <https://doi.org/10.6084/m9.figshare.9199406.v2><sup>15</sup>

## Extended data

Figshare: Extended data on the study titled ‘Acute and sub-acute toxicity study of the root extracts of *Fagaropsis hildebrandtii* in mice and evaluation of their antimicrobial effects’, <https://doi.org/10.6084/m9.figshare.9083348.v1><sup>41</sup>.

This project contains the following extended data:

- Figure S1: The biosafety, animal use and ethics committee approval document for the study
- Table S1: A summary of the animals used in the study
- Table S2: Effect of distilled water, extra virgin oil, and the aqueous and hexane root extracts of *F. hildebrandtii* on the mean body weight in mice over a 14-day period
- Table S3: Effect of distilled water, extra virgin oil and the aqueous and hexane root extracts of *F. hildebrandtii* on feed and water consumption in mice over a 14-day period
- Table S4: Effect of distilled water, extra virgin oil and the aqueous and hexane root extracts of *F. hildebrandtii* on the relative mean organ weight in mice over a 14-day period
- Table S5: Effect of graded doses of the aqueous root extract of *F. hildebrandtii* on the mean body weight in mice over a 28-day period
- Table S6: Effect of graded doses of root extracts of *F. hildebrandtii* on water and feed consumption in mice over a 28-day period

- Table S7: Effect of graded doses of the aqueous root extract of *F. hildebrandtii* on the mean body weight of mice over a 28-day period
- Table S8: Effect of graded doses of the hexane root extract of *F. hildebrandtii* on the mean relative organ weight in mice over a 28-day period
- Table S9: Effect of graded doses of the aqueous root extract of *F. hildebrandtii* on the relative mean organ weight in mice over a 28-day period
- Table S10: Effect of graded doses of the hexane root extract of *F. hildebrandtii* on the relative mean organ weight in mice over a 28-day period

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

## Acknowledgements

The authors would like to acknowledge the support accorded to them by Dr. Ojoo Rodi, immediate former director of the biosafety, animal care and use committee of the University of Nairobi, and the team of laboratory technologists, including Ms Lucy Mwangi, Mr Joseph Nderitu, Mr Ken Maloba and Mr James Macharia, for their assistance and guidance in the laboratory work. The authors are also grateful to the staff at the animal house, department of public health, pharmacology and toxicology for their support.

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# Open Peer Review

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Version 2

Reviewer Report 01 June 2020

<https://doi.org/10.5256/f1000research.23451.r63349>

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## Candice van Wyk

Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Sciences, University of Pretoria, Pretoria, South Africa

Some concerns regarding the preparation of the extracts and the starting concentrations for the toxicity study.

'Doses (300 and 2000 mg/kg) of the extracts were administered orally'. What was the starting concentration of the extracts for the toxicity study/MIC? If the MIC was used, this part should move to after the preparation of the plant extracts for clearer understanding.

'QRFH and HEXRFH were dissolved in 3ml of sterile distilled water and virgin oil, respectively. Serial dilutions ranging from 3.125–400 mg/ml of the extracts were then prepared using sterile peptone water'. Why were these used as solvents?

Manufacturers info missing: 'pulverized by use of a mechanical mill' (info).

"They were fed on standard mice pellets from a commercial feed supplier (Unga Group Plc, Kenya) and water was provided *ad libitum*."

"Feeding mice on graded doses of the root extracts of *F. hildebrandtii* over a 28-day period had no significant effect on the feed and water consumption of mice in treatment groups relative to the control (Extended data: Table S615)".

My concern regarding the above: what is the composition of the pellets the mice were fed on and could this not have an effect on the outcome of the toxicity studies? Could the pellets not influence toxic uptake? If not, this needs to be discussed.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**

Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Toxicity, isolation of compounds, antimicrobial.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Reviewer Report 11 February 2020

<https://doi.org/10.5256/f1000research.23451.r59721>

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The authors have well addressed our comments, and accommodated suggested corrections. Accordingly we approve the manuscript .

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Toxicological effects of herbal medicines on hematological and biochemical parameters and histopathology of vital organs including liver and kidneys.

**We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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Version 1

Reviewer Report 07 October 2019

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**Fikre Bayu**

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This is a very good and up to the standard study that comprehensively investigated the acute and subacute toxicity and antimicrobial effects of the root extract of *Fagaropsis hildebrandtii* which is a widely used herbal medicine. It has a significant contribution towards the currently increasing search for safe, affordable and effective drugs of plant origin and we approve it. However, we have the following comments which would have been accommodated/explained. These include:

1. How was the starting doses of the extract (as 300 mg/kg for acute and 250 mg/kg for sub-acute) determined. Why was LD50 not determined?
2. When and during what season was the herb collected and the study conducted?
3. The root of the plant is mentioned used traditionally as boiled. However, the investigation

was carried out on fresh root extract. Why not the boiled ones same as traditionally used? or do the authors believe heat has no effect on the active ingredients of the plant?

4. What could be the cause for the death of the female mice treated with the extract at the highest doses (1000mg/kg body wt)? Why macroscopic and microscopic pathological observations were not done? At what date(s) did the mice die?
5. The description of histopathological observations made on page 10 appears an abridged version of figure legends. There are also statements that do not go along with the figure legends as pointed out below:
  - In Figure 5 micrographs for histopathology of kidney, only 4 pictures are presented. However, in the text, page 10, seven pictures (A-G) are described, and does not go along.
  - In Figure 6 micrographs for histopathology of liver.
  - Picture "A" appears as at a lower magnification as compared to "B-F", but is indicated as x400 same as others, and what B is meant to indicate is not stated.
  - Picture "C" in the figure legend is stated to show diffuse cloudy swelling (CS) of the hepatocytes, but mentioned as normal same as A & B in the text, page 10.

We suggest these should be corrected in the text and better be briefly stated in a manner of reporting the observations.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Toxicological effects of herbal medicines on hematological and biochemical parameters and histopathology of vital organs including liver and kidneys.

**We confirm that we have read this submission and believe that we have an appropriate level**

of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 08 Nov 2019

**Beatrice Muia**, University of Nairobi, Nairobi, Kenya

**We are grateful to the reviewers for taking the time to provide feedback on our manuscript. We wish to respond to each of their queries as below;**

### **1. Selection of the starting doses and determination of LD<sub>50</sub>**

- The choice of 300 mg/kg as a starting dose in evaluating the acute toxicity of the root bark extracts of *F. hildebrandtii* was informed by the Organization for Economic Cooperation and Development (OECD) test guidelines no 423 (1). According to these guidelines, in cases where there is no information on the toxicity of a substance, it is recommended to use a starting dose of 300 mg/kg body weight for animal welfare reasons (1).
- The choice of 250 mg/kg as a starting dose in evaluating the sub-acute toxicity of root bark extracts of *F. hildebrandtii* was informed by OECD test guidelines no 407 (2). According to these guidelines, at least three test groups, and a control group should be used and a limit dose of up to 1000mg/kg is considered (2). Thereafter, a descending sequence of dose levels should be selected to demonstrate any dose-related responses and no-observed-adverse effects at the lowest dose (NOAEL) (2). In view of this, the sequential dose level we selected was 1000mg/kg, 500mg/kg, and 250mg/kg.
- Unfortunately, unlike other methods of LD<sub>50</sub> determination, it is only possible to determine a precise LD<sub>50</sub> using OECD guidelines when at least two of the tested doses result in mortality greater than 0% and lower than 100% (1). In our case, none of the doses tested resulted in the death of any mice. In such a case, the OECD guidelines allow for the determination of defined exposure ranges where lethality may be expected (1). This is what we have provided in reporting the acute oral toxicity of the extracts (i.e. LD<sub>50</sub> >2000mg/kg).

### **2. The season of plant collection and when the study was conducted.**

- Roots of *F. hildebrandtii* were collected during the hot dry season in February 2017.
- Acute and sub-acute studies were conducted between January 2018 and February 2018. Before this period, extraction, phytochemical analysis, and evaluation of the effect of the extracts on some human pathogens had been evaluated (April 2017 to December 2017).

### **3. Why the investigations were carried out on fresh root extracts rather than boiled root extracts.**

- This was done to preserve the integrity of the phytochemicals conferring antimicrobial activity to our plant of interest. According to a 2013 study on the antibacterial activity of selected medicinal plants against some common human pathogens, cold water extracts exhibit better inhibitory effects against *S. aureus*, *B. subtilis*, *P. vulgaris*, *P. aeruginosa*, and *E.coli* than hot water extracts (3).

- Based on the OECD test guidelines, female rodents (rats and mice) are usually more susceptible to toxic compounds than their male counterparts (1). Therefore, it could be argued that the physiological (biochemical, hematological) and pathological alterations we observed were more severe in female mice than their male counterparts. The metabolic roles of the kidney and liver may have exposed these organs to dose-dependent toxic effects.
- The mice died on weeks 3 and 4 (Between February 2<sup>th</sup> 2018, and February 17<sup>th</sup>, 2018)

**4. The discrepancy between the figure legends of liver and kidney sections and the description provided in the text.**

a. Thank you for your observation. We have rectified the in-text description to better reflect the figure legends provided in Figures 5 and 6.

We wish to sincerely thank the reviewers for taking the time to provide feedback on this manuscript.

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**Competing Interests:** The authors declare no competing interests

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