ANTIMICROBIAL ACTIVITY, TOXICITY PROFILE AND PHYTOCHEMICAL COMPOSITION OF *Fagaropsis hildebrandtii* (ENGL.) MILNE-REDH. ROOT EXTRACTS (RUTACEAE JUSS.)

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A thesis submitted in partial fulfilment of the requirements for the Degree of Master of Science in Pharmacology and Toxicology of the University of Nairobi

Department of Public Health, Pharmacology and Toxicology

2019

DECLARATION

I hereby declare that this thesis is my original work and it has not been presented in this or any other university for the award of this or any other degree. The various books, journals and other sources consulted for information have been duly acknowledged.

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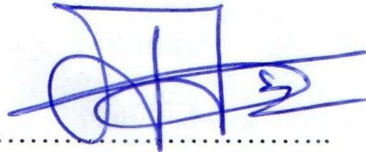 21/08/2019

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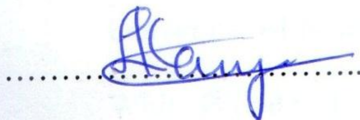 22/08/2019

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ACRONYMS AND ABBREVIATIONS

AIDS	–	Acquired Immune Deficiency Syndrome
ALT	–	Alanine Amino Transferase
AST	–	Aspartate amino Transferase
BWT	–	Bodyweight
DL	–	Decilitres
Hb	–	Haemoglobin
LD50	_	Median lethal dose
MCH	–	Mean Corpuscular Haemoglobin
MCV	–	Mean Corpuscular Volume
MG	–	Micrograms
ML	–	Millilitres
NCCLS	_	National Committee for Clinical Laboratory Standards
NSAIDs	_	Nonsteroidal Anti-inflammatory Drugs
OECD	_	Organisation of Economic Cooperation and Development
PCV	–	Packed Cell Volume
PG	–	Pico litres
RBCs	–	Red Blood Cells
UoN	_	University of Nairobi
WBCs	–	White Blood Cells
WHO	–	World Health Organization

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DEDICATION

To my loving family: my loving husband - Kola and my children - Timela and Tawali.

ABSTRACT

Alternative and herbal medicine has contributed greatly in treatment, management and control of diseases in both developed and developing countries. According to (WHO 2008) approximately 75% - 80% of world's population especially in developing countries use complementary medicine for basic health care. Herbal medicines are preferred because they are cheap, readily available, are accepted easily in various cultures, and are efficacious. However, due to limited research, their safety is questionable and there are limited reports of severe adverse effects following their use. *Fagaropsis hildebrandtii* is a plant commonly used in the treatment of chronic pain, pneumonia, arthritis, ulcers, stomach pains, malaria epilepsy and women's infertility. The ethnomedical claims and safety for use have not been proved despite the continued use. The study will form a basis for continued use of the plant and further validation. This study was carried out on the crude extracts of the *Fagaropsis hildebrandtii* roots for their phytochemical composition, toxicity profile and antimicrobial activity. Oral acute toxicity studies were done and its effects on the vital organs were determined macroscopically and their weights recorded.

Dose levels of 300mg/Kg and 2000mg/Kg body weights of aqueous and organic root extracts. In the toxicity study, female albino mice were used according to the Organization for the Economic Co-operation and Development (OECD) guidelines 423 which recommends the use of one sex animals preferably females which are more sensitive to drug reactions/effects. The control group for the organic extract was administered with extra virgin oil and for the aqueous extract was administered with distilled water. A 28-day sub-acute study of oral administration of both aqueous and organic extracts was done on six groups of both male and female albino mice at doses of 250mg/Kg, 500mg/Kg and 1000mg/Kg body weights. The phytochemical analysis procedures showed the presence of alkaloids,

terpenoids, flavonoids, steroids, tannins, saponins, cardiac glycosides and phenolics. The median lethal dose of *Fagaropsis hildebrandtii* extracts was estimated to be greater than 2000mg/Kg body weight. The vital body function parameters were observed keenly for any changes after 30 minutes, 4 hours, 24 hours, 48 hours, 1 week and 2 weeks. After the sub-acute studies, haematological parameters and biochemical parameters were determined as shown in the results. The major organs were harvested and any changes determined histopathologically. The 1000mg/kg aqueous and hexane extracts doses used caused deaths of 6 females and 2 males. After physical and histopathological studies, the liver, kidney, lungs and spleen were congested. Their biochemical and haematological parameters were increased compared to the controls, P-value <0.05. The body weights in mice on 1000mg/Kg Bwt doses on both aqueous and hexane extracts decreased significantly. For those on 250mg/Kg and 500mg/Kg Bwt, their body weight increased gradually in both the treated and control groups. There was no significant difference in the mean body weight in the treated and control groups of these two doses with P values >0.05. The antimicrobial activity of both aqueous and hexane extracts was determined using *Staphylococcus aureus* (Gram-positive), *Salmonella typhimurium* (Gram-negative) and *Candida albicans* (fungal). The most susceptible organism was *Staphylococcus aureus* and the most resistant was *Candida albicans*. The MIC and MBC for *Staphylococcus aureus* for both aqueous and hexane extracts were 100 mg/ml and 200 mg/ml. The MIC and MBC for the *Salmonella typhimurium* for both aqueous and hexane extracts was 200 mg/ml and 400 mg/ml; 100mg/ml and 200mg/ml.

The study revealed its safety in a single dose (less than 2000mg/kg Bwt) oral administration. However, if administered at doses above 500mg/kg Bwt for more than 28 days can result in deleterious dose-dependent effects especially on the kidneys, the liver and alters biochemical and haematological parameters on prolonged administration.

Isolation, characterization and identification of the phytochemicals are recommended. AST, ALT, urea, creatinine and total proteins levels, WBCs, RBCs and platelets should be closely monitored in prolonged use.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The use of alternate and herbal therapy has been practised for a long time (Sanjoy and Yogeshwer, 2017). Herbal medicine has been in use at a greater extent than conventional medicine by two to three folds (Evans, 1994). The number of people securing complementary medicines is increasing exponentially (80-95% of the world population) mainly in developing countries for primary health care (Robinson and Zhang, 2011). Medicine is the source of therapeutic experiences of many generations practising professionals of indigenous systems of medicine for over many years. Herbal medicine is gaining popularity in the developing world because the medicines are affordable, readily available, easily culturally accepted, and efficacious. They are also believed to have minimal side effects but studies have shown that their safety may be questionable due to reporting of severe drug reactions and adverse effects and consequences after some of their use (Sanjoy and Yogeshwer, 2017; WHO, 2009).

Most herbal products are self-medicated or have very limited medical advice hence the potential risk to human health. In addition, the use of a herb for many years doesn't guarantee its safety hence need for safety and toxicity studies (Joanne *et al.*, 2007). Chemical constituents of plants with medicinal value include glycosides, alkaloids, tannins, flavonoids, terpenes and phenolics (Joanne *et al.,* 2007). According to current statistics, there is a lack of significant research on herbal medicine (WHO, 2009). This is because of the diversity of medicinal plants and herbal medicines.

In China, traditional medicine is still in common use since almost half of the population uses it regularly with high prevalence in rural areas. About 5000 traditional remedies are available in its market accounting to one-fifth of the entire Chinese pharmaceutical market (Li, 2000).

In Japan, most of its herbal remedies find its way from China. It is in this country where herbs were classified in the first pharmacopoeia of Japan traditional medicine in the ninth century (Saito, 2000). In India traditional medicine has been practised in a medical system called Ayurveda for nearly 5000 years. It includes the use of diet and herbal medicines for disease prevention and treatment (Morgan, 2002). In the USA, Europe and other developed countries herbal products have been incorporated into alternative/ complementing or integrative medical systems. In these countries, they have been used for health promotion and disease control strategies (Tyler, 2000).

The World Health Organization (WHO) estimates that 80% of the population of some Asian and African countries (Kenya included) use herbal medicine for primary health care (WHO, 2007). This is due to the fact that pharmaceuticals are prohibitively expensive for most of the world's population. In contrast, herbal medicines can be grown from seed or gathered from nature at little or no cost (WHO, 2007).

Natural products are believed to be a good source of food and medicines for both humans and animals use. They are more affordable, accessible and believed to be safer than conventional modern medicine. Like any other drug, these herbal medicines can cause adverse reactions or acute or chronic side effects in case they are misused or even in their allowed daily dosages. There are also limited information /research/ findings done and documented for reference and precaution purposes. These calls for the need for more information to be researched, documented for their effective and safe use. There is, therefore, need to document knowledge from herbalist and use research finding with credible evidence for the claimed pharmacological effects and efficacy (Sofowora, 1993).

Natural products are believed to be a good source of food and medicines for both humans and animals use. They are more affordable, accessible and believed to be safer than conventional

modern medicine. Like any other drugs, these herbal medicines can cause adverse reactions to normal dosages and/or chronic use. Additionally, there is limited research done and documented for reference and precaution purposes. This calls for the need for more information to be researched and documented for their effective and safe use.

Most herbal products in the market have limited information published about them or have not been subjected to chemical trials to establish their safety and effectiveness (Sanjoy and Yogeshwer, 2017). Toxicological studies by scientists that will help in investigation, interpretation and documentation of nature of these herbal extracts are still wanting and it will be a basis for protection measures (Schwarze *et al*., 2006). *Fagaropsis hildebrandtii* has been used for morning sickness, women's infertility malaria, asthma, ulcers, stomach pains, arthritis, internal abscess, pneumonia, chronic joint pains and epilepsy (Winfred *et al*., 2004; Stanley *et al*., 2011). In the area of study, it has been used for chest pain, stomach pain and chronic joint pains as an aqueous preparation. Many medicinal plants in the area have been used for a long time giving the expected pharmacological outcome.

Candida albicans, Salmonella typhimurium and Staphylococcus aureus are some of the most common human pathogens in low and middle-income countries (Tong *et al.,* 2015). The development of antimicrobial resistance to these pathogens is an ongoing problem and is of public health relevance. Thus the need for alternatives to the present drugs indicated for infections by these microorganisms has never been more important (Kariuki *et al.,* 2015)

Very little or no data has been documented on their use and support for their safety. The herbal products are just boiled or soaked in water and a dose of one glass to be taken three times a day for three days. Most of the herbs are used but the identities of the extracts side effects and adverse effects are not known. They are not purified before use hence the quality is not assured. Therefore the study will give data on the safe dosages of the plant extract and

any expected side effects (Acute and Sub-acute) when used. The obtained data will form a basis for continued use of the plant and further validation.

1.2 Null Hypothesis

Fagaropsis hildebrandtii root bark extracts are toxic, do not have any phytochemical constituents and do not have any antimicrobial activity.

1.3 Research Objectives

1.3.1 General Objective

The general objective of the study was to investigate the phytochemical composition, antimicrobial activity and safety profile of root extract from *Fagaropsis hildebrandtii* (Rutaceae).

1.3.2 Specific Objectives

1. To screen for the phytochemical constituents of *Fagaropsis hildebrandtii* root extracts.
2. To determine acute and sub-acute toxic effects of *Fagaropsis hildebrandtii* root extracts.
3. To evaluate the antimicrobial activity of *Fagaropsis hildebrandtii* root extracts against selected bacteria and fungi strains.

CHAPTER TWO

LITERATURE REVIEW

2.1 Herbal Medicine

It involves the use of plants for intended medicinal purposes or for supplementary diet or nutritional value. Its scope has been extended to include minerals, shells, fungal and bee products and some animal parts. However, it remains an alternative medicine since it lacks enough gathered scientific-based information. Herbal medicine knowledge is passed from generation to generation verbally which can result in lost or distorted information hence need for documentation of gems of knowledge (WHO, 2008; Kokwaro, 2009).

2.2 Prevalence of Herbal Medicine Use

The World Health Organization (WHO) gives an estimate of 80% of the world population using herbal medicine based on plants and animals (WHO, 2007) because pharmaceuticals are expensive for most of the people. In Kenya, herbal medicine has been practised even before the European explorers came (Kokwaro, 2009). The demand and popularity of herbal medicine are increasing exponentially because they are believed to have fewer side- effects, enhanced tolerance, increased accessibility, low cost and high efficiency and potency. It forms the oldest form of healthcare known to man. However, they are limited to not being able to cure emergency cases and accidents, increased risk with self –dosing and complexity in standardizations (Kalyani and Ratna, 2013).

In most plants used in herbal medicine, roots are the most utilized plant parts (38%), then leaves (29%), stem/bark (26%), then other parts like fruits, seeds or the whole plant (5%) (Winfred *et al*., 2014).

2.3 Adverse Effects and Interactions

All medicinal plants have the potential to cause unexpected effects (toxicity and interactions). The pharmacokinetic and pharmacodynamic principles of drugs and herbs can result in drug-drug interactions and therefore herbal products should be considered as medicine and have the potential to have interactions. The risk of adverse effects may be influenced by gender, diet, genetics, and age, interaction with other drugs, underlying diseases, and nutrition status among others. It is therefore important to be aware of the substances which can cause toxicities and can interact with other medications. Adverse reactions mostly involve the skin, gastrointestinal tract, liver, heart and respiratory system. For example, hepatotoxic effects were reported when Kavakava or Echinacea was taken with other hepatotoxic drugs (Sarah, 2011).

2.3.1 Pharmacokinetic Interactions

This refers to interactions affecting absorption, distribution, metabolism and excretion. Interactions affecting absorption will result to either a reduction or increase in the concentration of the other drug for example St. John's Wort causes induction of the P-glycoprotein which results to the reduction of the amount of digoxin absorbed hence reduced drug effect (Williamson, 2003).

In distribution, a drug like warfarin and carbamazepine which are highly plasma protein-bound in a small distribution volume may be displaced by a herb. Competing for the same binding sites hence increased serum drug levels leading to increased therapeutic effects (Scolt and Elmer 2002, Williamson 2003).

In metabolism, a herbal product can either cause enzyme induction or inhibition. For example St. John's Wort causes induction of p450 which are responsible for the metabolism of many drugs. This can result in decreased activity of the drug efficacy. It can reduce the effects of

warfarin, digoxin and theophylline. Liquorice decreases the metabolism of corticosteroids through enzyme inhibition (Williamson, 2003).

Changes in excretion processes affect the serum levels of a given drug for example chronic intake of Liquorice may cause hypokalaemia and water retention which may affect various medications for hypertension and arrhythmias (Scolt and Elmar, 2002).

2.3.2 Pharmacodynamic Interactions

This can result in either additive or antagonistic interactions. Additive interactions result in a case where the herbal drug has the same pharmacological effects as the other drug being taken together. The antagonistic effect may occur if the herb produces an effect which is contrary to the effect desired for the drug hence reducing the drug efficacy (Williamson 2003; Scolt and Elmer 2002).

2.4 Therapeutic Activity of Herbal Drugs

2.4.1 Anticancer Activity

Herbal products with anticancer activity are continuously being subjected to extensive research in drug development. They include plants like *Ficus racemosa, Ocimum basilicum, Embeli ribes, Ficus glomerata, Catharantus roses, Alangium lamarki, Acalypha fruticosa, Terminalia chebula, Tylophora indica, Wringtia tinctoria.* The extracts mostly used for the treatment of pancreatic cancer include *Terminalia belleric, Nigella sativa and Emblica officinali* (Feng *et al.*, 2013; Rodeiro *et al.*, 2008). The extracts used for the treatment of breast cancer includes *Buthus martensi, Squama manitis, Tubercurcumae, Scolopendra subspinipes, Radix paeoniae, Fructus lyciii, Radix angelicae, Colla cornu, Herba epimeda* (Kalyani and Ratna, 2013).

2.4.2 Analgesic Activity

Some of the analgesic agents used are *Zingiber Zerumbet, Toona ciliate, Zataria multiflora, Bougainvilla spetabilis, Ficus glomerata, Nepata italic, Dalbergia lanceolaria, Gilaucium grandiflorum, Chelidonium majus, Stylosanthes fruticosa and Sida acuta* (Feng *et al*., 2013).

2.4.3 Antipsoriasis Activity

Different herbal remedies which have been used for symptomatic relief of psoriasis, for example, turmeric, shark cartilage extract, milk thistle, curcumin, oregano oil. Some of the antimicrobial agents which have been used in the management of psoriasis include *Cassia tora, Azadiracht indica, Wrightia tinctoria* and *Calendula officinalis* (Ben *et al*., 2004).

2.4.4 Antidiabetic Activity

There is a variety of herbal plants with known antidiabetic activity. These include *Annona squamosa, Punica granatum, Salvia officinalis, Zea mays, Scoparia dulcis, Xanthium strumarium, Taraxacum officinale, Urtica dioica, Tecoma stans, Turnera diffusa, Triflolium alexandrinum, Acacia modesta, Polygala senega, Acacia nilotica, Nigella sativa, Ginseng panax, Apium graveolens, Allium sativum, Aloe barbadensis, Embilica officinalis* (Shang, 2000).

2.4.5 Antifertility Activity

Some herbal products have been used as a source of fertility regulating agents because of their little side effects. For example extracts from *Taxus baccata, Stemona japonica, Thuja occidentalis, Raphanus sativus, Pisum sativum, Lonicera ciliosa, Fatsia horrid, Carica papaya, Barberis vulgaris, Amaranthus retroflexus, and Podophyllu peltatum* have been used for this purpose (Nadakishore *et al*., 2007; Kyalani and Ratna, 2013).

2.4.6 Hepatoprotective Activity

Almost 160 phytoconstituents from different plants have been believed to have liver protective activity. Good examples include *Wedelia calendulacea, Annona squamosa, Coccinia grandis, Solanum nigrum, Chamomile capitula, Silybum marianum, Flacourtia indica* (Kamboj, 2000; Kalyani and Ratna, 2013).

2.4.7 Antidepressive Activity

A known number of nutritional and herbal dietary supplements have shown some antidepressive activity; these include *Hypericum perforatum, Rhodiola rosea, Bacopa monniera, Piper methysticum, Valeriana officinalis* and *Panax quinquefolius* (Jeyaprakash, 2007).

2.4.8 Herbals for Dental care

Plants which have been used in the management of dental problems are *Salvia officials, Gardenia gummifera, Eucalyptus globules, Barleria prionitis, Althea officials, Anacyclus* pyrethrum*, Acacia arabica, Holarrhenia antidysenterica* (Akhtar *et al*., 2005).

2.5 *Fagaropsis hildebrandtii*

It is a deciduous shrub/tree which grows up to 24 meters in height. It is usually branched from the base. It has green nice smelling fruits when unripe and turns red when they ripen. It is found in Ethiopia, Somalia and Kenya.

The tree is harvested for local use as herbal medicine. Its local name in the Kamba language is “muvindavindi”. It is used for the treatment of pneumonia, chest pain, arthritis, stomach pains, ulcers, malaria, internal abscesses, epilepsy and women’s infertility (Winfred *et al*., 2014). *Fagaropsis angolensis* is a plant found in the same species whose stem bark is used in for treatment of malaria. The roots are chewed as an expectorant and the powdered root is taken in drinks to treat male sterility (Ken, 2014).

2.6 Toxicity Studies

In most cases, 25% of toxic effects observed in toxicological tests in either rats or dogs are likely to occur in man while 80% of toxic effects observed in toxicological tests in both rats and dogs will likely occur in man when the same substance is used. Dose-time dependent reactions are predictable and can be proved with animal experiments and this forms the fundamental of toxicological studies of new drugs and herbal products (Bhardwaj *et al*., 2012; Frank and Banle, 2008).

These studies are required for the establishment of safety profiles of products which are already in use or new in the market (can be done for pharmaceuticals, food additives, herbal extracts, pesticides, industrial chemicals). Inadequately tested substances have resulted in severe adverse effects to both animals and humans who use the substances or even resulting in death. For instance, in use of sulphanilamides (one of the first antibiotics), use of arsenic to treat syphilis, deaths from ethylene glycol which is solvent, deformities in children born from mother's using thalidomide (anti-nausea drug) (Bhardwaj *et al*., 2012; Frank and Banle, 2008).

The tests performed in the laboratory for toxicity include acute toxicity test (single dose), sub-acute toxicity test (daily dose) and chronic toxicity test (daily dose).

2.6.1 Acute toxicity studies

Acute toxicity testing is one of the most important toxicological investigation procedures which is a key requirement for regulatory purposes for human safety. These results are used to characterize how hazardous a substance is upon exposure to it (David, 2009). A single dose is used for each animal once and gross behaviour is observed and LD_{50} is determined. This is the dose required to kill 50% of the test animals. The tests carried out are for oral, dermal and inhalation routes administration. Most preferred species are rats between the ages

of 6-8 weeks. The least number of animals per dose level is three (ACT method in OECD). The recommended dose levels are 3 with a single dose exposure or fractioned doses up to 24 hours' period for oral and dermal studies. A four-hour exposure is recommended for inhalation studies.

The observation period is 14 days. Data collected from acute studies may help in; the basis of classification and labelling, determining the mode of toxic action of a substance, giving a dose for a new compound, dose determination in animal studies and determination of LD_{50} (Bhardwaj *et al*., 2012). It, therefore, helps to determine the therapeutic index of a substance which is the ratio between the lethal dose and the pharmacologically effective dose.

2.6.2 Sub-chronic toxicity studies

They help to determine toxicity from repeated exposures of several weeks or months. Clinical observations and pathology examinations are conducted. The species used are usually rats and dogs of young age. At least 10 animals of each sex for rodents and four of each sex for non-rodents are used per dose level. Three dose levels including a control group and the no adverse effects level dose are used for a 28- 90 days' study period (Bhardwaj *et al*., 2012).

2.6.3 Chronic toxicity tests

They help to determine the effects of exposure to substance/drug for life. The tests are similar to sub-acute studies but extend for a long period of time and involve a large number of animals. They test for carcinogenicity, mutagenicity, teratogenicity and reproductive toxicity and neurotoxicity effects (Bhardwaj *et al*., 2012).

2.7 Phytochemicals

They are bioactive chemicals which are naturally occurring compounds in plants. For over decades they have been used for health benefits like in disease-preventing functions like detoxifying agents, anticancer agents, dietary fibre and supplements, antioxidants,

pharmacological agents and immune-boosting agents. Most of the phytochemicals have more than one function in the body (Mamta *et al.,* 2013).

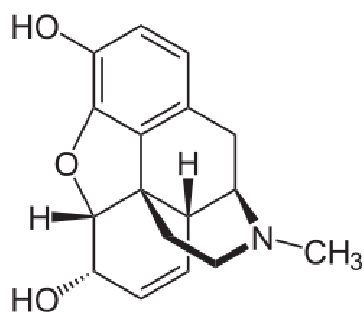
They also protect the plant from damage and diseases by environmental hazards like stress, drought, pollution, UV exposure and pathogens. They also contribute to the plant's flavour, aroma and colour (Mathai, 2000). In study findings, phytochemicals have been found to decrease the risk of coronary heart disease by preventing the oxidation of low-density lipoprotein (LDL) cholesterol, normalizing clotting process and blood pressure in addition to increasing arterial elasticity (Mathai, 2000). Phytochemicals also help to prevent cancer by neutralizing free radicals, inhibition of enzymes which activate carcinogens and activation of enzymes which detoxify carcinogens (Mamta *et al.,* 2013, Meagher and Thomson, 1999).

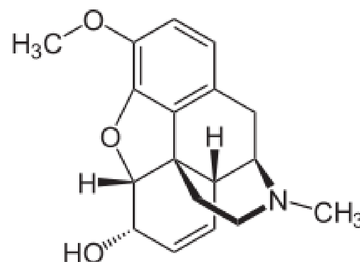
Some of the classes of bioactive phytochemicals in medicinal plants include; detoxifying agents like retinoids, phytosterols, cyanates, carotenoids, flavones, reductive acids, phenols, aromatic isothiocyanates, tocopherols, indoles and coumarins. Others are antioxidants which help in quenching oxygen free radical and inhibition of lipid peroxidation e.g. flavonoids, ascorbic acid, polyphenolic compounds, carotenoids and tocopherols. Another class is the anticancer agents which inhibit tumour cells growth or have anti-metastatic activity and good examples are flavonoids, curcumin, polyphenols and carotenoids. Other classes act as anti-oxidants, some have neuropharmacological activity and are used in cancer chemoprevention like alkaloids, biogenic amines, terpenoids and volatile flavour compounds. Some of the phytochemicals are used as antibacterial and antifungal agents like phenolics, alkaloids and terpenoids. Another important class is the non- starch polysaccharides e.g. lignins, mucilages, gums, pectins, cellulose and hemicellulose which have high water holding capacity, help in binding toxins, bile acids and cause delayed nutrient absorption (Mamta *et al.,*2013).

2.7.1 Phytochemical classes

a) Alkaloids

They form the largest group of phytoconstituents. They are basic in nature and are made of ammonia compounds which are synthesized from amino acids as building blocks (Figure 2.1). Their basicity varies depending on the molecule structure, locations and presence of functional groups (Sanker and Nahar, 2007). Most alkaloids exist in solid form and some as liquids containing Carbon, Hydrogen and Nitrogen. Most of them are soluble in alcohol, sparingly soluble in water and their salts are soluble in water. Their solutions are very bitter. They function as a defence against pathogens and herbivores. They are exploited as pharmaceuticals, narcotics, poisons and stimulants since they are biologically active. (Madziga *et al.,* 2010).





Morphine Codeine

Figure 2.1: Basic structures of examples of some plant alkaloids

In nature, they are found in seeds and roots. More than 12,000 alkaloids are known to exist in about 20% of plant species. Examples are analgesics like codeine and morphine, tubocurarine which is a muscle relaxant, anticancer vinblastine and vincristine, berberine and sanguinafine which are antibiotics, the pupil dilator atropine, the antiarrhythmic ajmaline and scopolamine which is used as a sedative. Others are the stimulants caffeine, nicotine, cocaine, ephedrine and ergotamine. Some methods for detection of alkaloids are listed in Table 1 below (Sanker and Nahar, 2007).

Table 2.1: Methods of screening for alkaloids (Sanker and Nahar, 2007)

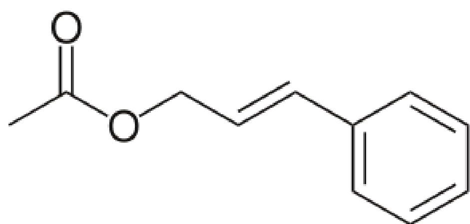
Reagent/Test	Reagent	Result
Meyer's reagent	Potassium Iodide Solution	Cream Precipitate
Wagner's reagent	Iodine in Potassium Iodide	Reddish-brown Precipitate
Tannic Acid	Tannic Acid	Precipitation
Murexide test for Caffeine	The residue is exposed to Ammonia Vapour	Purine alkaloids produce a pink colour
Hager's reagent	A saturated solution of Picric Acid	Yellow Precipitate
Draggendoff's reagent	The solution of Potassium Bismuth Iodide Potassium Chlorate, add hydrochloric acid and evaporate to dryness	Orange or Reddish-brown precipitate

b) Glycosides

These are molecules where sugar is bound as a functional group through its anomeric bond via a glycosidic bond (Figure 2.2). They are stored in plants (cell sap) in their inactive forms which are activated through enzyme hydrolysis that makes the sugar part to be broken making the chemical active. They can be linked by an O- (an O-glycoside), S- (a thioglycoside), N- (a glycosylamine) or C- (a C-glycoside) glycosidic bond. Glycone is the sugar group and aglycone is the non-sugar group which can be alcohol, phenol or glycerol. The glycone can have a single sugar group (monosaccharide) or several sugar groups (oligosaccharide) (Gleadow and Moller, 2014).

Glycosides are colourless and water-soluble phytoconstituents which are neutral in reaction. They are easily hydrolyzed into its building blocks by ferments or acids. They have a bitter taste. Examples are cardiac glycosides (act on the heart), cyanogenic glycosides (flavouring agents), anthracene glycoside (for some diseases and purgatives), amarogentin, chalone

glycosides (anticancer), gentiopicin, polygalin, ailanthone and andrographolide (Sanker and Nahar 2007; Kar, 2007).



Cinnamyl Acetate

Figure 2.2: Basic structure of pharmacologically active plant glycoside

To test for glycosides, the plant extract can be hydrolysed using ferrous chloride/hydrochloric acid and an aqueous base e.g. sodium hydroxide or ammonium hydroxide solutions or using hydrolysed hydrochloric acid/water and aqueous base solution. In both cases, pink or purple colour is formed in the base layer to indicate the presence of glycosides in the plant extract (Sanker and Nahar, 2007). They are classified by the glycone, glycosidic bond and the aglycone. They can also be classified by the type of glycosidic bond depending on whether it is above or below the plane of the cyclic sugar molecule. They include as B-glycosides or α-glycosides. The type of linkages between glycone and aglycone includes C-linkage/glycosidic bond, O- linkage/glycosidic bond, S- linkage/glycosidic bond or N-linkage/glycosidic bond. Glycosides can also be classified according to the type of glycone/sugar present. For example, if it is glucose, the molecule is a glucoside; if it is glucuronic acid, the molecule is glucuronide and fructocide if it is fructose. They can also be classified according to the chemical nature of the aglycone according to the following listed groups; alcoholic glycosides e.g. salicin, anthraquinone glycosides which have a laxative effect e.g. glycosides from species senna, aloe and rhubarb. Others have coumarin glycosides e.g. apterin, chromone glycosides whose aglycone is benzo-gamma-pyrone. Some have cyanogenic glycosides e.g. amygdalin, prunasin, dhurrin, linamarin and lotaustralin,

flavonoid glycoside e.g. hesperidin, naringin, rutin, quercitrin and phenolic glycosides e.g. arbutin, saponins, sapogenin, diosgenin, triterpene. Others have steroidal glycosides or cardiac glycosides, steviol glycosides from stevia rebaudiana; iridoid glycosides e.g. aucubin, geniposidic acid, loganin, catalpol; Thioglycosides e.g. sinigrin, sinalbin. (Gleadow and Moller, 2004)

c) Flavonoids

They are derived from flavans. They are polyphenols which are widely distributed in the plant flora. They are made of more than one benzene ring. They have antioxidant or free radical scavenging property. They are over four thousand known flavonoids. Examples are; quercetin, kaempferol, quercetin, flavones, dihydroflavons, flavonols (Figure 2.3), calchones, anthrocyanidins (Figure 2.4), catechin, proanthocyanidins and leucoanthocyanidins (Kar, 2007; Galcetti *et al.,* 2008).

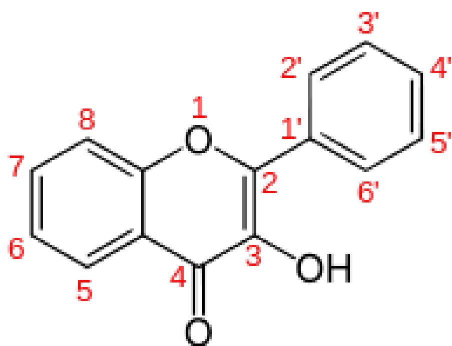


Figure 2.3: Backbone of a flavonol, substituent numbers are indicated

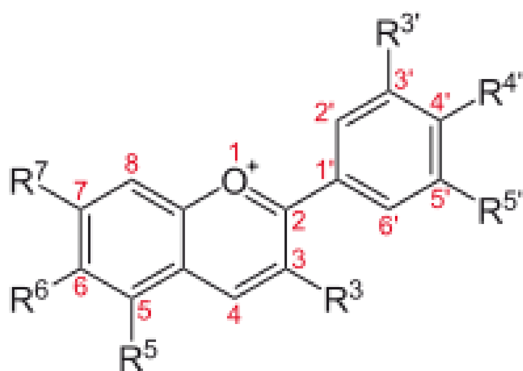


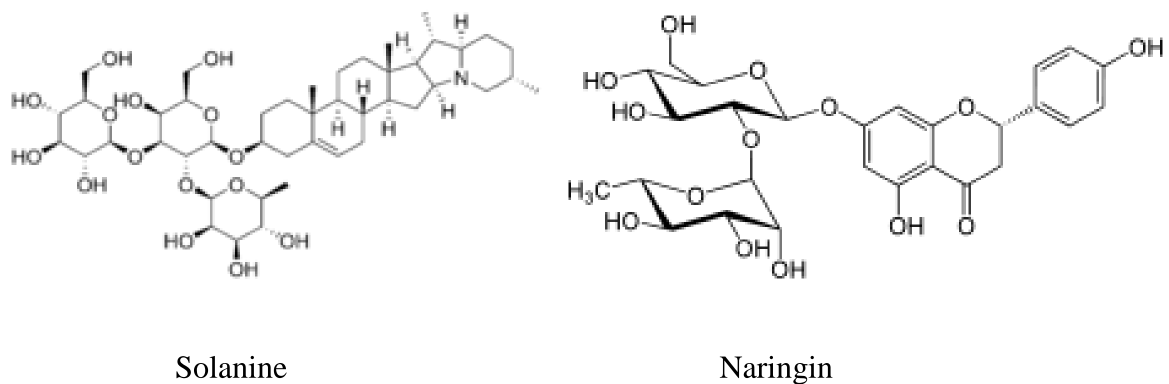
Figure 2.4: Basic structure of an Anthocyanidin ($R^{4'} = -OH$)

Table 2.2: Methods for the screening of plant flavonoids (Cushnic and Lamb, 2011)

Test	Chemicals used	Colour change
Shinoda test	Magnesium fillings added to ethanol, followed by a few drops of concentrated hydrochloric acid	A pink or red colour indicates the presence of flavonoid
Sodium Hydroxide test	About 5mg of the extract is dissolved in water, warmed and filtered. Then 10% sodium hydroxide is added to this solution (Cushnic and Lamb, 2011).	A colour which changes to colourless in addition to hydrochloric acid indicates the presence of flavanoids

d) Saponins

Saponins are high molecular weight compounds in which sugar molecule is combined with triterpene and steroid aglycone (Figure 2.5) which foam in water. On hydrolysis, the aglycone produced is called sapogenin. They are two major groups which include steroid saponins and triterpene saponins. They are soluble in water, alcohol and insoluble in ether. They have a bitter taste irritation on the mucous membranes. They are very poisonous and cause haemolysis of blood and are known to cause cattle poisoning. Pharmacologically they are important in the management of cancer, hyperlipidemia and cardiac arrhythmias. Steroidal saponins (diosgenin and hecogenin) are used for the production of sex hormones. For example, progesterone is made from diosgenin (Sanker and Nahar, 2007; Kar, 2007). Hydrocortisone and cortisone can be synthesized from hecogenin which is found in sisal leaves.



Solanine Naringin

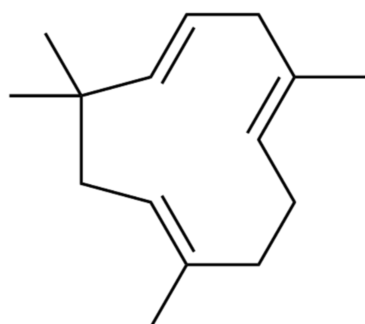
Figure 2.5: Chemical structures of an example of saponins found in plants

e) Terpenes

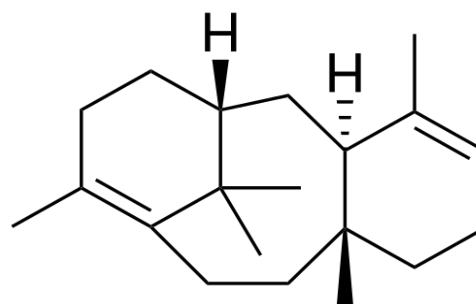
Terpenes are a very widespread and chemically diverse group of natural products. They are produced by a variety of plants e.g. conifers and sore insects. They usually have a strong odour and protect the plants from herbivores, predators and parasites. Terpenes and terpenoids are the basic constituents of essential oils in many plants. Terpenes (Figure 2.6; Table 2.3) are classified according to the number of isoprene units in the molecule (Davis *et al.*, 2000).

Table 2.3: Classification of Terpenes according to the number of Isoprene units (Davis *et al.,* 2000)

Terpene Type	Number of Isoprenes	Examples
Hemiterpenes	One	Prenol, Isovalenic, Acid
Monoterpenes	Two	Geraniol, Terpineol, Limonene, Myrcene, Pinene
Sesquiterpenes	Three	Humulene, Farnesol, Farnesenes
Diterpenes	Four	Taxadiene, Retinol, Phytol, Cafestol, Cambrene
Sesterterpenes	Five	Geranylfarnesol
Triterpenes	Six	Squalene
Sesquiterpenes	Seven	Sasquarterperoids, tetraprenylcurcumene
Tetraterpenes	Eight	Lycopene, Gamma-carotene, Alpha-carotene, Beta-carotene
Polyterpenes	Several	Gutter-percha, Rubber



Humulene

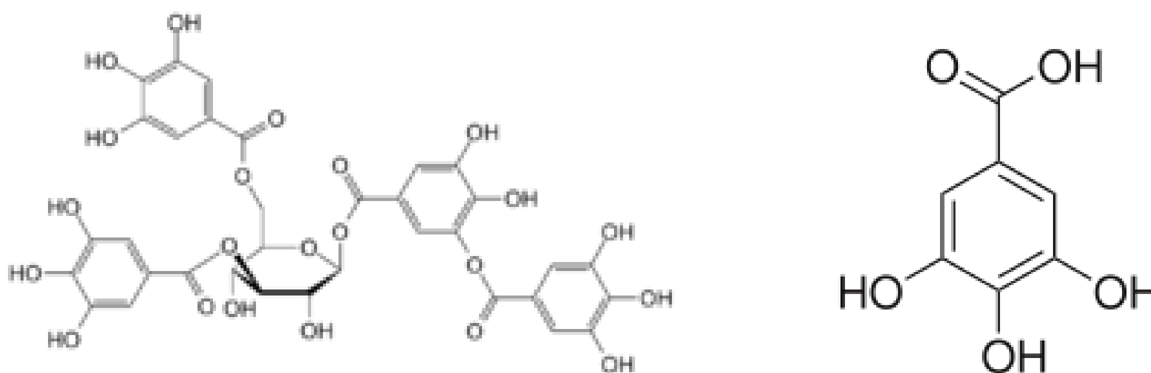


Taxadiene

Figure 2.6: Basic structures of some examples of pharmacologically active Terpenes derived from plants

f) Tannins or Tannoids

They are widely distributed in many plants and are phenolic constituents with high molecular weight. They are water-soluble, insoluble in alcohol, acidic in nature and form complexes with carbohydrates, alkaloids, gelatin and proteins (Figure 2.7) (Kar, 2007; Haslam and Edwin, 2007). They are divided into condensed tannins and hydrolysable tannins which are also called gallotannins or egallitannins. They are used as antiseptics and for treatment of diarrhoea, rhinorrhea and leucorrhoea. Examples of tannins include glycitein, genistein, gallic acid, daidzein and theaflavins. They can be analyzed using precipitation of proteins or alkaloids, reaction with phenolic rings and depolymerization (Haslam and Edwin, 2007; Tao *et al.,* 2014).



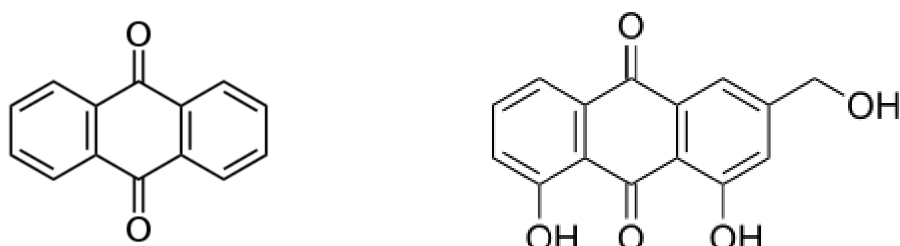
Tannic acid   Gallic acid

Figure 2.7: Examples of plants derived tannins and their structures

g) Anthraquinones

These are naturally occurring phenolic and glycosidic compounds. They are derived from anthracene to give anthrones, anthranols, rhein, salinos, chrysophanol, aloe-emodin and luteolin. They can be used as laxatives e.g. Senna glycoside.

To test Anthraquinones (Figure 2.8), 0.5g of the powdered plant extract is shaken in 10ml of benzene and filtered. Then 5ml of 10% ammonia is added to the filtrate and the mixture is shaken well. The presence of violet, pink or red colour indicates the presence of anthraquinones (Maurga *et al.,* 2008; Akinjojule *et al.,* 2010).



Chemical structure of 9, 10-anthraquinone Aloe-emodin

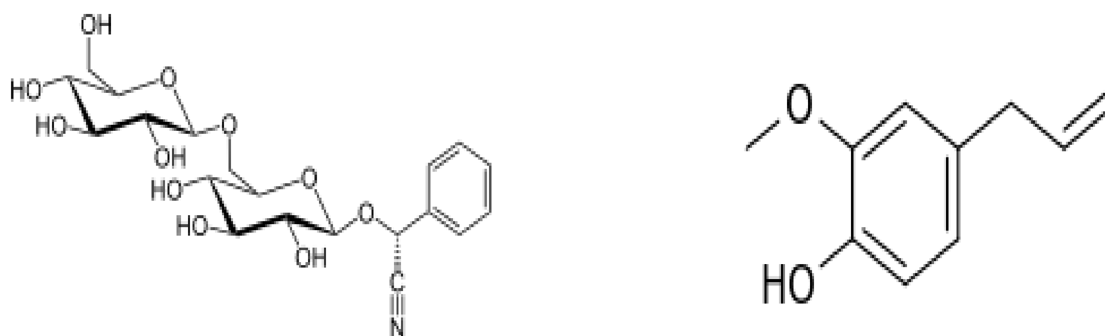
Figure 2.8: Basic structures of some examples of Anthraquinones

h) Essential Oils

These are volatile, odorous substances found in various plants and animals. They are volatile/ethereal oils since they evaporate on exposure to air even at room temperatures. They may be found in the leaves, flowers, roots, rhizomes and leaves. A single volatile oil has more than 200 different chemical compounds (Figure 2.9) which give the characteristic odour and flavour (Matrinez *et al.,* 2008).

The oils are extracted from natural sources through evaporation, distillation, expression, solvent extraction, resin trapping, absolute on extraction, wax embedding or cold pressing. Due to their characteristic fragrance, they are used in cosmetics, perfumes, soaps and other products, for food and drink flavours and for scents in incense and household cleaning products. They are also used in alternative medicine called aromatherapy whose healing effects are attributed to aromatic compounds. In this case, the oils are volatilized, diluted into a carrier oil and can be nebulized, heated over a source of flame or burned as incense or used

for massage. Examples of such oils are lavender, peppermint, tea tree oil, patchouli, eucalyptus, olive oil, thymol, carvacrol, menthol, capsaicin, camphor, anise, juniper, agathosma, turpentine oil, lemongrass and garlic (Lee *et al.*, 2012; Singh *et al.,* 2002).



Amygdalin Eugenol

Figure 2.9: Structures of some examples of pharmacologically important plant-derived essential oils

2.8 Microbiology

Microbiology is from a Greek word meaning 'small'. It is the study of microorganisms some being unicellular (one cell), multicellular (many cells) or acellular (no cells). It entails different sub-disciplines including bacteriology, parasitology, virology and mycology. It is broadly divided into eukaryotic micro-organisms which have membrane-bound cell organelles e.g. fungi, protists and prokaryotic organisms which are conventionally classified as lacking membrane-bound organelles e.g. eubacteria and archaebacteria. Viruses are also classified as micro-organisms because they are simple organisms and very complex molecules. In general, microbiology is classified into pure or applied sciences or divided into phycology, bacteriology and protozoology.

Microbes are known to cause various diseases in humans and other animals but they can also be beneficial in processes like antibiotic production, industrial fermentation (eg the production of vinegar, dairy products, and alcohol) and act as molecular vehicles in

transferring DNA to complex organisms like animals and plants. Micro-organisms have also been used in the production of biotechnologically important enzymes e.g. reporter genes, tag polymerase for use in other genetic systems and novel molecular bio-techniques e.g. yeast two-hybrid system (Nitesh *et al.,* 2011; Keen, 2012).

Bacteria have been used in industrial production of amino acids eg *Corynebacterium glutamicum* which has been used in the production of more than two million tons of amino acids e.g. L-lysine and L-glutamate. Others like streptomyces have been used in the synthesis of antibiotics such as aminoglycoside antibiotics (Keen, 2012; Madigan and Marlinko, 2006).

Some biopolymers like polysaccharides, polyamides and polyester are biotechnologically produced by some micro-organisms and are of suitable high-value medical application such as drug delivery (formulation) and tissue engineering. Microbes have also been used for the biosynthesis of polyhydroxyalkanoates, oligosaccharides, polysaccharides, xanthan, cellulose, levan, hyaluronic acid and organic acids (Keen, 2012). Through symbiosis, microbes benefit human and animal hosts' health in digestion, suppression of pathogenic microbes and production of vitamins and amino acids. The benefits are from eating fermented foods, prebiotics or probiotics (Nitesh *et al.,* 2011).

2.8.1 Microbes and their Classification

A microbe also called a micro-organism can exist in single-celled or multi-celled forms. The existence of these unseen microbial lives was suspected in ancient lives from the Jain scriptures from the 6th Century B.C in India and 1st Century B.C book on agriculture by Marcus Terentius Vamo. Microbes live in almost every habitat (from the poles to the equator, trees, deserts, rocks, geysers and the deep waters). Some adapt to the extreme of environments like very high pressures, high temperatures, low temperatures, high radiations

and can survive for long in vacuums. These micro-organisms are called extremophiles (Schopf *et al;* 2017).

a) Bacteria

It is in the domain of prokaryotes and is single-celled organisms without a nucleus. They have different shapes (spheres, spirals and rods) and are a few micrometres in length. They live in soil, acidic hot springs, water, radioactive wastes and deep parts of the earth's crust. They also have symbiotic and parasitic relationships with animals and plants (Ishige *et al.,* 2005)

Bacteria are found in the environment and in the body. In the body, the largest numbers are found in the gut and a large number on the skin. Most of them are not harmful in the body but offer protection of the immune system. However, some species are pathogenic (Table 4) and cause infectious diseases like cholera, anthrax, syphilis, leprosy and bubonic plague. Respiratory infections are the most fatal bacterial diseases with tuberculosis killing approximately two million people per year mostly in Sub-Saharan Africa (Velimirov, 2001).

In the industrial sector bacteria is used in sewage treatment, breakdown of oil spills, fermentation process in the production of cheese and yoghurt, biotechnology, recovery of copper, palladium, gold, as well as manufacturing antibiotics and other chemicals (Ishige *et al.,* 2005). Antibiotics are the drugs used in the treatment of bacterial infections.

Bacterial cells have diversity in cell shapes and sizes. The different cell morphologies include coccus, diplococci, diplococci encapsulated, staphylococci, tetrad, sarcina, streptococci, bacillus, coccobacillus, diplobacilli, palisades, streptobacilli, vibrio, helical form, spirochete, filamentous, club rod, enlarged rod, commas form, hypha and stalk. The cocci are spherical in shape, the rod-shaped are bacilli and the vibrio are slightly curved rods or comma-shaped. The spirals shaped are called spirilla and the tightly coiled are the spirochaetes. The cell

shapes are determined by the cell wall and cytoskeleton and it influences their ability to acquire nutrients, swim through liquids, escape predators and adhere to surfaces (Fredrickson *et al.,* 2004; Ishige *et al.,* 2005; Pyang *et al.,* 2016)

Many bacterial species exist as single cells but they form characteristic patterns, for example, Neisseria form diploids, staphylococcus group occur together in clusters and streptococcus form chains. Some group coalesce together to form multicellular structures for example actino-bacteria form elongated filaments, aggregates of myxobacteria and streptomyces form complex hyphae (Rappe and Giavannoni, 2003). Some display complex arrangement of cells and extracellular components like those which form biofilms or microbial mats (Cabeen and Jacobs, 2005; Ishige *et al.,* 2005).

Table 2.4: Common infections in humans and the causative bacteria

Bacterial Infections	Main Species Involved	Prophylactic/therapeutic agents
Bacterial Mengitis	*Streptococcus pneumoniae* *Neisseria meningitis* *Haemophilus influenzae* *Leisteria monocytogenes*	Cefriaxone Vancomycin Ampicillin Cefixime Ceftaxime Flucloxacillin Ampicillin/Cloxacillin NSAIDS
Otitis Media	*Streptococcus Pneumoniae*	Flucloxacillin Ampicillin/Cloxacillin NSAIDS Amocillin/Clavulonic acid
Pneumonia	Community Acquired: *Streptococcus pneumoniae* *Haemophilus influenzae* *Staphylococcus aureus* Atypical: *Mycoplasma pneumoniae* *Chylamydia pneumoniae* *Legionella pneumoniae*	Cefixime Levofloxacin Ciprofloxacin Ceftriaxone Erythromycin Azithromycin
Skin Infections	*Staphylococcus aureus* *Pseudomonas aeruginosa* *Streptococcus pyogenes*	Cephalexin Flucloxacillin Clindamycin Dicloxacillin
Eye Infections	*Chlamydia trachomatis* *Neisseria gonorrhoea* *Staphyloccocus aureus*	Levofloxacin Ofloxacin Ceftriaxone Doxycycline Azithromycin Ciprofloxacin
Sinusitis	*Haemophilus Influenzae* *Streptococcus aureus*	Amoxycillin Azithromycin Antihistamines Trimethoprim/Sulfamethoxazole
Food Poisoning	*Salmonella* *Clostridium* *Escherichia Coli* *Staphylococcus aureus* *Shigella* *Campylobacter jejuni*	Metrnidazole Hydration(normal saline & 5%Dextrose) Secnidazole Doxycyclin Amoxicillin

Gastritis	*Helicobacter Pylori*	Clarithromycin Amoxicillin Metronidazole
Urinary Tract Infections	*Pseudomonas Aeruginosa* *Escherichia coli* *Stapylococcus saprophyticus* *Other Enterobacteriaceae*	Amoxicillin/Clavulonic acid Nitrofuratoin Cefriaxone Erythromycin Azithromycin
Upper Respiratory Tract Infections	*Haemophilus influenzae* *Streptococcus pyogenes*	Levofloxacin Erythromycin Azithromycin Erythromycin Cefixime Ceftriaxone
Sexually Transmitted Diseases	*Chlamydia trachomatis* *Haemophilus ducreyi* *Neisseria gonorrhoeae* *Treponema pallidum* *Ureaplasma urealyticum*	Penicillin G Doxycycline Ceftriaxone Azithromycin Vibramycin Ceftazidime Amikacin

b) Viruses

These are small infectious agents who only replicate inside the living cells of other organisms (plants, animals, micro-organisms, bacteria and archaea). They do not eat, do not produce waste nor do what many other living things do. They only reproduce. They are classified according to their phenotypic characteristics such as nucleic acid types, host organisms, morphology, mode of replication and the disease type they cause. The main classification is a type of nucleic acid is DNA or RNA viruses. Viruses have different morphologies (Koonin *et al.,* 2006).

A virion is a complete virus particle which is made of nucleic acid surrounded by capsid which is a protective coat. The capsid is made of proteins encoded by the viral genome which serves as the basis of morphological distinction with the different shapes as discussed below (Tsagris *et al.,* 2008).

i) Icosahedral

It means nearly spherical and they form a closed shell from identical subunits. A minimum of three triangular identical capsomeres is required and 60 for icosahedrons. Most of the viruses have more than 60 capsomeres and appear spherical. These capsomeres are surrounded by five other capsomeres at the apices to form pentons, for example, rotavirus (Tsagris *et al.,* 2008)**.**

ii) Envelope

Some viruses envelop themselves in viral envelopes which are formed from a modified form of cell membranes of the infected host cell or internal membranes like endoplasmic reticulum or nuclear membrane. The membrane is studded with proteins coded for the host and viral genome, for example, the Influenza and HIV Virus (Tsagris *et al.,* 2008).

iii) Prolate

This is a form of icosahedron elongated along the fivefold axis and its common arrangement of bacteriophages. It has a cylinder with a cap on either end (Koonin *et al,* 2006).

iv) Helical

These viruses have a single capsomere around a central axis to form a helical structure with a central tube or cavity. They are usually filamentous or rod-shaped for example Mosaic virus (Creager and Morgan, 2008).

v) Complex

These viruses have a capsid which is neither purely icosahedral nor helical and have extra structures like protein tails or a complex outer wall e.g. enterobacteria phage T 4, pox-viruses, mimiviruses and pandoravirus (Rossman *et al.,* 2004; Arslan *et al.,* 2011).

Viruses can also be classified according to the genetic material within the virus. They include DNA and RNA viruses.

vi) DNA and RNA Viruses

The genome replications of most of them take place in the cell nucleus and are entirely dependent on the host cells' DNA and RNA synthesizing machinery and RNA processing machinery e.g. Caulimoviridae, Hepandaviridae, Adenoviruses, Parvoviruses (Suzan *et al.,* 2006).

Table 2.5: Overview of the types of human viral infections and the most notable species involved (Hagerstwon *et al.,* 2007)

Disease	Virus	**Prophylactic/therapeutic agents**
Common Cold	Rhinovirus Parainfluenza Virus Respiratory Syncytial Virus	Antihistamines Oral decongestants Topical anticholinergics Corticosteroids NSAIDs Cough suppressants
Eye Infections	Herpes Simplex Virus Adenovirus Cytomegalovirus	Acyclovir Gancyclovir
Encephalitis/Meningitis	Measles Arbovirus Rabies	Measles vaccine Yellow/dengue fever vaccine Antirabies vaccine
Gingivostomatitis	Herpes Simplex Type 1	Acyclovir Gancyclovir
Pharyngitis	Adenovirus Epstein-Barr Virus Cytomegalovirus	Adenovirus vaccine Osetalmivir Zinamivir Peramivir

Parotiditis	Mumps Virus	Measles, Mumps and Rubella vaccine NSAIDs Analgesics
Pneumonia	Influenza Virus Type A and B Parainfluenza Virus Respiratory Syncytial Virus Adenovirus	Flu vaccine Adenovirus vaccine
Myelitis	Poliovirus	Polio vaccine Methylprednisolone
Pancreatitis	Coxsackie B Virus	Analgesics Antipyretics
Hepatitis	Hepatitis Virus	Hepatitis A and B vaccine Entecavir Tenofovir Lamivudine Adefovir Telbivudine
Sexually Transmitted Diseases	Herpes Simplex Type 2 Human Papilloma Virus HIV	Acyclovir Gancyclovir Anti-retrovirals
Skin Infections	Smallpox Rubella Coxsackie A Virus Varicella-Zoster Virus Measles Human Herpes Virus 6	Measles, Mumps and Rubella vaccine Analgesics Antipyretics Acyclovir Gancyclovir

vii) **RNA Viruses**

The genome replication takes place in the cytoplasm. They use their own RNA replicase enzymes to make copies of their genomes eg Metaviridae, Pseudoviridae, Orchomyxoviruses, Picornaviruses (Suzan *et al.,* 2006; Arslan *et al.,* 2011).

2.9 Future of Herbal Medicine

Almost three-quarters of the herbal drugs used worldwide were discovered from leads in local medicine. About 25% of modern medicines are discovered from plants which were first used traditionally, others are synthetic analogues from plant compounds like in India where 70% of modern medicines are made from natural products. Good utilization of these natural resources in bio-prospecting will lead to the discovery of novel lead molecules through drug discovery processes and other disciplines. World Health Organization has great interest in recording herbal medicine information which is verbally passed from generation to generation especially from communities where herbal preparation is used for treatment of gastric disorders for example ulcers, swellings, ageing, eczema, venereal diseases, snakebites, scabies, diabetes, jaundice, asthma, cancer, skin infection, mental illness, among others. In addition, there are intensified efforts in documenting ethnomedical data on medicinal plants in many third world countries. Once these research on herbal medicines have been efficiently carried out and disseminated, it will be a reliable source of information on efficacious drug treatment, expected drug reactions/ side effects and improved health status (Farnsworth and Bingel, 1997; Kyalyani and Ratna, 2013).

The development of an authentic method of analysis for profiling phytochemicals and quantitative analysis of bioactive compounds and other major constituents will sort out a major challenge to scientists. This is because these herbal medicines have been extensively used in the management of chronic diseases like diabetes, arthritis, cancer and AIDS among others for mental comfort and general management (Raina, 2003)

Table 2.6 below shows some of the examples of the plants/herbs used by different communities with medicinal values.

Table 2.6: Plants/herbs used by different communities and their medicinal values

Plant/ herb	Condition Managed
Fagaropsis hildebrandtii	Ulcers, chronic pain, pneumonia, malaria, morning sickness, asthma, (Winfred *et al.,*2007)
Erythrina Abyssinia	Cancerous wound, stomach pain, (Winfred *et al.*, 2007)
Aloe secundiflora	Malaria, diarrhoea, typhoid, oedema(Winfred *et al.,*2007)
Harrisonia abyssinica	Stomach pain(Winfred *et al*., 2007)
Fuerstia Africana	Malaria, pneumonia, ulcers, infertility(Winfred *et al.*, 2007)
Pappea capensis	Post-partum haemorrhage (Catherine *et al.*, 2011)
Solanum incunum	Delayed labour, prevention of abortion, vomiting, (Catherine *et al.,* 2011)
Aspillia mossambicensis	Vomiting, post-partum haemorrhage, abortion prevention(Catherine *et al.*, 2011)
Catharunthus roseus	Treatment of cancers (Ruth and Armin, 2005)
Rauwolfia vomitiria	Treatment of snake bites (Ruth and Armin, 2005)
Physostigma venenosum	Myasthenia, stubborn constipation, postoperative intestinal or bladder atony. (Ruth and Armin, 2005)
Raphionacme hirsute	Tumours (Ruth and Armin, 2005)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The source of the herb was Kithandi forest, Kasikeu ward, Kilome constituency in Makueni County, Kenya shown in Figure 3.1 (Latitude: -1° 48' 14.72" S Longitude: 37° 37' 13.22" E).The climatology of the region is generally dry and exhibiting semi-arid conditions. It exhibits two rainy seasons annually (long and short). The average annual rainfall, evaporation and temperatures are 600mm, 2000mm and 23 degrees Celsius respectively. It is occupied by small scale farmers who carry out agricultural activities including maize and beans farming, cattle and goat keeping and mango farming. (Nyangito *et al.,* 2008) It is an area with many herbs which are extensively used in primary health care management. Examples include *Acacia nilotica, Aloe secundiflora, Fuerstia Africana, Lannea schweinfurthii, Pappea capensis, Lantana camara, Steganotemia araliacea* and *Ximenia americana.* The health facilities are not evenly distributed and the people have to walk for long to seek medical care hence increased use of herbal-medicine.

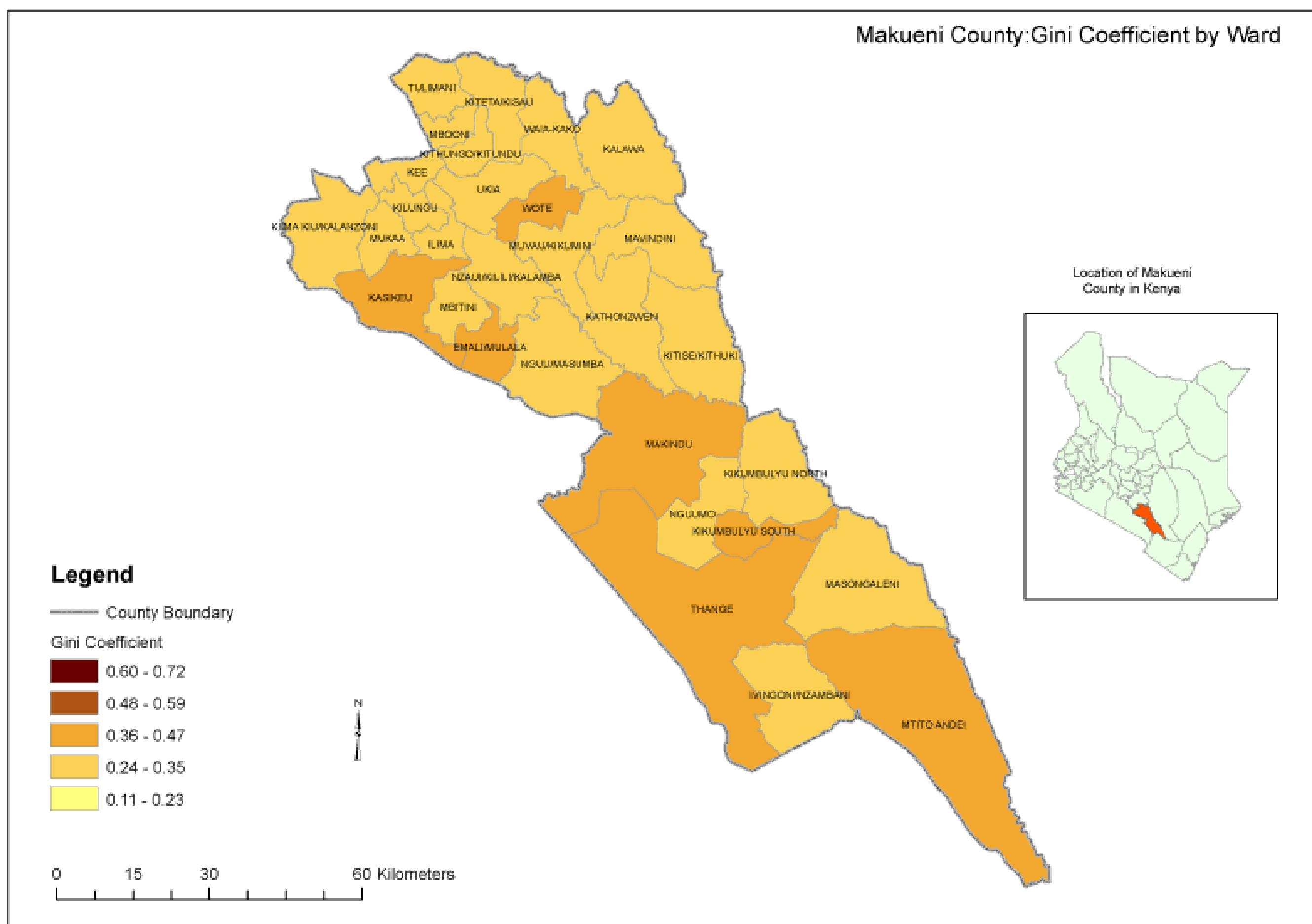


Figure 3.1: Location of Makueni County (shaded red) in Kenya and the location of the study site (Kasikeu ward) in Makueni County

3.2.1 Collection and Identification of the Plant

Fresh roots of a ten-year-old plant of *Fagaropsis hildebrandtii* (Figure 3.2) was collected in February 2017 in Makueni County in the location shown on the map (Figure 3.1) in Kenya. Identification and authentication of the plants were done at the herbarium, Department of Land Resource Management, University of Nairobi (LARMAT). The study of the plant on toxicity, phytochemical constituents and antimicrobial activity was carried out in the University of Nairobi Pharmacology and Toxicology Laboratory and Clinical Studies Laboratory.

Fagaropsis hildebrandtii whole plant

Aerial parts of *Fagaropsis hildebrandtii*

Figure 3.2: *Fagaropsis hildebrandtii* whole plant and aerial parts respectively.

3.3 Preparation of Plant Extracts

3.3.1 Extraction of Aqueous Extracts

The plant roots materials were first rinsed with tap water, then distilled water and air-dried at room temperature (22-26 degrees Celsius) to a constant weight. They were then ground to a fine powder using an electric mill. Then, 500g of the powder was soaked in 2000ml of sterile distilled water and macerated for 72 hours with thorough shaking in the mornings and evenings for increased efficiency in extraction. The mixture was centrifuged at 3000rpm for 10 minutes. The supernatant was removed and filtered using a 0.45um pore size filter paper (Saravanan *et al.,* 2010). The filtrate was freeze-dried for five days to form a dark brown powdered extract of 45 grams yield and packed in an amber coloured bottle. It was kept in a refrigerator at 4°C. The percentage yield was 9%w/v (Okumu *et al.,* 2017).

3.3.2 Extraction of Organic Extracts

The plant materials were first rinsed with tap water then distilled water and air-dried at room temperature (22 - 26 degrees Celsius) to a constant weight. They were then ground to a fine powder using an electric mill and 750grams of the powder was soaked in 1250 millilitres of hexane and macerated for 72 hours with thorough shaking in the mornings and evenings to ensure increased efficiency in the extraction process. The hexane mixture was centrifuged at 3000rpm for 10 minutes. The supernatant was carefully transferred to a beaker and filtered using a 0.45 um pore size filter paper (Saravanan *et al., 2010).* The filtrate was put in a round-bottomed flask and evaporated using a rotary evaporator at 40℃ to remove the excess hexane solvent. The remaining content was put in an amber coloured bottle and placed in a hot sand bath to give a constant content weight. This evaporation process took a period of five days. The procedure was repeated for chloroform solvent but gave a very low yield.

The hexane extract was therefore preferred for the study. The procedure was repeated to give enough yields for the study. This gave a yield of 20.63 grams which is equivalent to 20.74%w/v percentage yield (Okumu *et al.,* 2017).

3.4 Experimental Animals

A total of 66 albino mice (42 females and 24 males) (8-12 weeks) weighing 20-30 grams were used for the study. They were obtained from the animal house in PHPT department, University of Nairobi. The temperatures in the research room were the animals were kept were maintained between 22-25 degrees Celsius by a vanned heater. The room was well ventilated and maintained in light for 12 hours and 12 hours of darkness. The mice were provided with clean water and standard mice pallets food ad-lib. The initial weights for the mice were taken and marked on their tails using a permanent marker. Both males and females were used to give adequate justification.

The use of animals was approved by the "Animal Care and Use Committee (ACUC)" of the facility of veterinary medicine University of Nairobi.

3.5 Ethical Considerations

The use of animals was approved by the "Biosafety, Animal Use and Ethics Committee (BAUEC)" of the Faculty of Veterinary Medicine, University of Nairobi. Reference number FVM BAUEC/2018/163 was assigned.

Any distressed animals as a result of treatment were removed from the study and euthanized using halothane inhalation and disposed of through incineration. All the surviving animals at the end of sub-acute studies were aseptically bled from a cardiac puncture on a dissecting board after euthanasia using halothane and blood collected. The mice carcasses and other biological wastes were buried in a pot to rot or incinerated by staff in the department of Clinical Faculty of Veterinary Medicine, University of Nairobi.

To avoid accidents like extracts spilling on the personnel or pricking oneself when administering medication during the study, good laboratory practices were observed. These included the use of protective gear like lab coats, gloves, masks, use of restrictive boards when injecting the animals and proper disposal of wastes. The staff handling the animals were injected with antirabies as a precaution measure against rabies infection.

3.6 Phytochemical Screening

This was done as described by (Harbone, 1998; Usman *et al*., 2009; Evans and Trease, 2002; Visweswari *et al.,* 2018).

3.6.1 Test for Terpenoids

Two millilitres of chloroform was added to 0.5g of the sample and concentrated sulphuric acid (3ml) carefully added to the mixture to form a layer. A reddish-brown colouration of the interface indicated the presence of terpenoids (Usman *et al*., 2009; Visweswari *et al*., 2018).

3.6.2 Test for Flavonoids

A portion of aqueous filtrate of the extract was added to dilute ammonia (5ml). Then concentrated sulphuric acid (1ml) was added to the mixture. A yellow colouration which disappeared on standing indicated the presence of flavonoids. Also took another portion of the extract and added a few drops of 10% aluminium. A yellow colouration confirmed the presence of flavonoids (Harbone, 1998; Usman *et al*., 2009; Visweswari *et al.,* 2018).

3.6.3 Test for Anthraquinones

Took 0.5 g of the aqueous sample extract and boiled with 10ml of sulphuric acid and was filtered while hot. Then the filtrate was shaken in 5ml chloroform and the chloroform layer was pipetted into another test tube and added 1ml dilute ammonia. Then observations for colour change were being made. The yellow colour indicated the presence of anthraquinones (Trease and Evans, 2002; Visweswari *et al.,* 2018).

3.6.4 Test for Steroids

Added 2ml of chloroform to 0.5g of the sample and carefully added concentrated sulphuric acid (3ml) to the mixture to form a layer. A reddish-brown colouration of the interface indicated the presence of steroids (Usman *et al*., 2009; Visweswari *et al.,* 2018).

3.6.5 Test for Tannins

Approximately 0.5g of the extract was boiled in 10ml of water in a test tube and then filtered. Then a few drops of ferric chloride were added and a brownish-green or blue-black colouration indicated the presence of tannins (Trease and Evans, 2002; Visweswari *et al*., 2018).

3.6.6 Test for Saponins

Approximately 0.5g of the extract was added to 5ml of distilled water in a test tube. Then the solution was shaken and observed for stable persistent froth. The froth was mixed with 3-4

drops of olive oil and shaken vigorously. It was observed for emulsion formation which indicated the presence of saponins (Harbone, 1998; Usman *et al*., 2009; Visweswari *et al.*, 2018).

3.6.7 Test for Alkaloids

Acid alcohol (10ml) was used to dilute 0.5g of the extract and then boiled and filtered. Then 2ml of dilute ammonia was added to 5ml of the filtrate. Added 5ml of chloroform and shaken to extract the alkaloidal base. Acetic acid (10ml) was used to extract the chloroform layer and was divided into two portions. Mayer's reagent was added to one of the portions and Draggendoff's reagent to the other portion. For the positive alkaloids test, it formed a cream precipitate with Mayer's reagent and a reddish-brown precipitate with Draggendoff's reagent (Trease and Evans, 2002; Rahman *et al*., 2017; Visweswari *et al.,* 2018).

3.6.8 Test for Cardiac Glycosides

To about 0.5g of the sample extract, 5ml of distilled water and 2ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was then underplayed with 1ml of concentrated sulphuric acid. Formation of a brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. In some cases, a violet ring appeared below the brown ring and in the acetic layer, a greenish ring was formed just above the brown ring and spread through the other layers gradually indicating the presence of cardiac glycosides (Harbone, 1998; Usman *et al*., 2009; Visweswari *et al.,* 2018).

3.6.9 Test for Phenolics

Approximately 0.5g of the extract was boiled in 10ml of water in a test tube and then filtered. Then a few drops of ferric chloride were added and a brownish-green or blue-black colouration indicated the presence of phenolics (Trease and Evans, 2002).

3.7 Determination of Antimicrobial Activity

3.7.1 Bacteria and Fungi Cultures

A Gram-positive bacterial strain (*Staphylococcus aureus* ATCC 25923)*,* a Gram-negative (*Salmonella typhimurium* ATCC 7222569*)* and fungi *(Candida albicans* ATCC 10231*)* were used. They were obtained from Bacteriology Laboratory, Department of Public Health, Pharmacology and Toxicology, University of Nairobi.

3.7.2 Broth Dilution Method

Three species of standard microbes, one Gram-positive (*Staphylococcus aureus),* one Gram-negative (*Salmonella typhimurium)* and one fungi *(Candida albicans)* were cultured on blood agar overnight at 37°c in an incubator. The cultures were then suspended in 5mls sterile physiological saline to give a concentration of 2.2 $\times$ 10^{8}cfu/ml. This concentration was confirmed using Mac-Faraday tubes as Mac-faraday 1 (NCCLS, 1997). Plant powders at 800mg for both aqueous and hexane plant extracts were dissolved in 2ml of sterile distilled water and virgin oil respectively. Eight, two-fold serial dilutions of plant extracts at 400, 200, 100, 50, 25, 12.5, 6.25, 3.125mg/ml concentrations (both aqueous and hexane) was done using sterile peptone water to determine any activity against the above microorganisms. Thereafter, the culture tubes were arranged on a rack and clearly labelled.

Using a sterile 1ml pipette, 0.1ml of the individual microorganism was inoculated into every tube of the diluted plant extracts at 37°c for 24 hours. A negative control tube was set by having plant extracts without micro-organisms. A positive control tube had the micro-organisms without the plant extracts while peptone water was used as the blank for the spectrophotometer. Convectional drugs were used for quality control. Flucloxacillin 0.25µg/ml (for both *Staphylococcus aureus* and *Salmonella typhimurium*) and fluconazole 10µg/ml for *Candida albicans* were used. The injectables were used due to the assured sterility in their manufacturing and packaging processes. The optical densities of all the tubes

were read immediately after setting them to obtain the baseline readings and after 24 hours inoculation. The baseline readings were compared to that obtained after 24 hours to determine whether there was growth or not. The MIC (minimum inhibitory concentration) was the lowest concentration of the extract which inhibited any visible bacterial growth determined by no change in optical density between the baseline and incubated tubes (Njue *et al.,* 2014)

In the determination of MBC (Minimum Bactericidal Concentration) 100µl of broth was taken from all the tubes after 24 hours of incubation at 37°c and sub-cultured aseptically to Plate Count Agar. The plates were incubated at 37°c for 24 hours and checked for bacteria growth. The lowest concentration of the plant extracts which showed no bacteria growth was the MBC which is the lowest concentration at which greater than 99.9% of the initial bacteria inoculums are killed (Pavithra *et al*., 2010; Shahidi, 2004; Ronald, 2014; Ronald *et al*., 2013).

a) Preparation of Plate Count Agar

It is a microbiological growth medium mostly used to assess and monitors all viable bacterial growth of a sample. Approximately 17.5g of the powder was weighed and dissolved in one litre of water using a vortex machine. The solution was sterilized by autoclaving at 121℃ for 15 minutes (Atlas, 2004; Eaton *et al.,* 2005).

b) Preparation of Peptone Water

It is a solution composed of peptic digest of animal tissue and sodium chloride used for microbial growth. One litre of purified water was used to dissolve 15 grams of the powder. Then it was warmed slightly with frequent agitation until it completely formed a solution. It was then autoclaved at 121℃ for 15 minutes. The final P.H was 7.2 ±0.2 (Wehr and Frank, 2014).

3.8 Toxicity Studies

The use of animals was approved by the "Biosafety, Animal Use and Ethics Committee (BAUEC)" of the Faculty of Veterinary Medicine, University of Nairobi. Reference number FVM BAUEC/2018/163 was assigned.

Any distressed or dead animals as a result of treatment were removed from the study and euthanized using halothane inhalation and disposed of through incineration. All the surviving animals at the end of sub-acute studies were aseptically bled from a cardiac puncture on a dissecting board after euthanasia using ethyl ether for anaesthesia for blood collection. The mice carcasses and other biological wastes were buried in a pot to rot or incinerated by staff in the department of surgery and clinical studies, school of veterinary medicine, University of Nairobi.

To avoid accidents like extracts spilling on the personnel pricking oneself when administering medication during the study good laboratory practices were observed (use of protective gear like lab coats, gloves, masks, use of restrictive boards when injecting the animals and proper disposal of wastes. The staffs handling the animals were injected with anti-rabies as a precaution measure against rabies infection.

3.8.1 Oral Acute Toxicity Testing

This was adapted from OECD 423 guidelines on oral acute toxicity testing. The animals were divided into 6 groups of 3 female albino mice each which were nulliparous and non-pregnant. Females were used because literature surveys of convectional LD50 tests show that they are more sensitive to toxicity than males (OECD 423). They were randomly selected and kept in different cages in the laboratory for 5 days to allow for acclimatization at a temperature of 22- 25 degrees Celsius and a humidity of 30-70% was maintained. Lighting was artificial

with 12hours light and 12 hours of darkness. Water and food were provided ad-lib. The animals were of the age of 8-12 weeks and a mean weight of 20 -30grams.

Two concentrations of the aqueous and hexane extracts (300 and 2000mg/kg of body weight) were prepared. Then a single dose of 300mg/kg of body weight was used as the start dose administered by gavages using intubation cannula. The weight of the animals was taken before administration of the extracts. They also were fasted prior to dosing for 3-4 hours and food withheld for 1-2 hours after extract administration. Dosing of test animals was done according to the acute oral Toxic class method (OECD 423).

The initial doses were calculated using 300mg/kg (OECD, 2008) according to their body weights and a volume of less than one millilitre was used for each group. A starting dose of 300mg/kg body weight was used because there was no information on the toxicity of *Fagaropsis hildebrandtii* (OECD 423) (Figure 3.3). The next dose level was 2000mg/kg. For the controls, extra virgin oil was used for the hexane extract and distilled water for the aqueous extract. The dosages were as shown in the tables below:

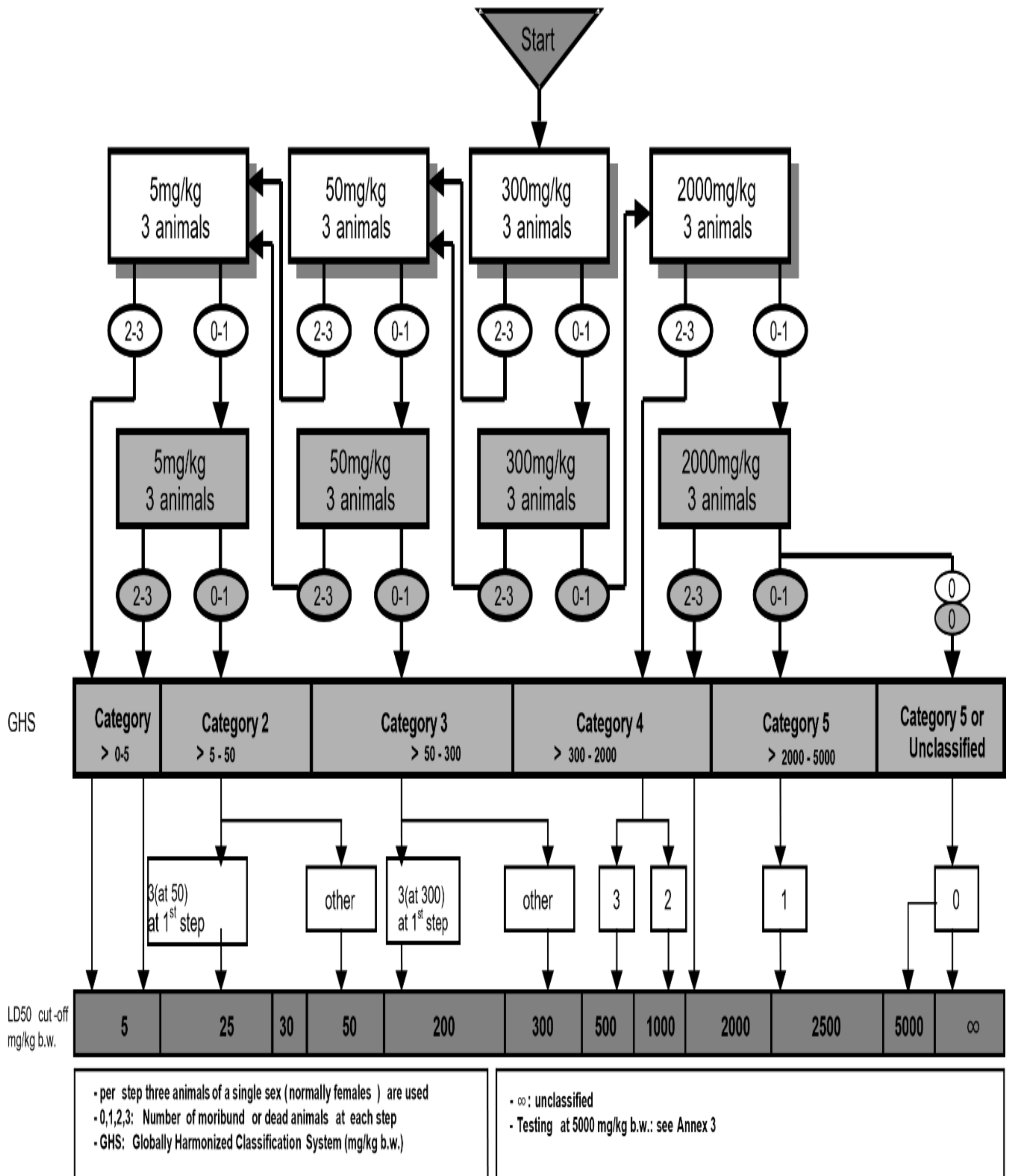


Figure 3.3: Test procedure of acute oral toxicity testing with a starting dose of 300 mg/kg body weight

The mice fasted for three hours before the administration of the extracts using the doses in Table 3.1 below. The initial doses were calculated using 300mg/kg Bwt followed by 2000mg/kg Bwt (OECD, 2008). For the controls, extra virgin oil was used for the hexane extract and distilled water was used for the aqueous extract. For the mice were given 300mg/kg Bwt of the aqueous extract AA1, AA2 and AA3 were given doses of 6.93mg/0.8ml,7.44mg/0.9ml and 8.81mg/1ml in distilled water respectively.

Table 3.1: Dosages of *Fagaropsis hildebrandtii* Extracts used in Acute Oral Toxicity Studies

Group	Mice No.	Extract dose used (Milligrams)	Bodyweight (grams)
Aqueous extract 300mg/kg used (milligrams)	AA1	6.93	23.09
	AA2	7.44	24.80
	AA3	8.81	29.37
Aqueous extract 2000mg/kg used (milligrams)	AB1	50.58	25.29
	AB2	43.42	21.71
	AB3	45.94	22.97
Organic extract 300mg/kg used (milligrams)	OC1	6.17	20.57
	OC2	6.93	23.09
	OC3	6.1	20.34
Organic extract 2000mg/kg used (milligrams)	OD1	44.88	22.44
	OD2	58.18	29.09
	OD3	55.44	27.72
Distilled water(aqueous control)	Ac1	0.6	21.18
	Ac2	0.6	21.83
	Ac3	0.6	24.74
Virgin olive oil (organic control)	Oc1	0.3	26.82
	Oc2	0.3	25.67
	Oc3	0.3	20.89

Those given 2000mg/kg of the aqueous extract AB1, AB2 and AB3 were given 50.58mg/1.2ml, 43.42mg/1.1ml and 45.94mg/1.2ml in distilled water respectively.

The first dose of 300mg/kg Bwt of the hexane extract was used in mice OC1, OC2 and OC3 at 6.17mg/0.93ml, 6.93mg/1ml and 6.1mg/0.9ml doses respectively. Those given 2000mg/kg

Bwt of the organic extract OD1, OD2 and OD3 were given 44.88mg/0.9ml, 58.18mg/1.2ml and 55.44mg/1.1ml of extra virgin oil. Ac1, Ac2 and Ac3 were used as the controls for the aqueous extract and were administered with 0.6ml of distilled water each. Oc1, Oc2 and Oc3 were used as the controls for the organic extract dosages and were administered with 0.3ml of extra virgin oil.

Observations were done for changes in skin and fur colour, mucus and eye membrane, respiratory system, circulatory system, autonomic and central nervous system and somatomotor activity. Effects like tremors, convulsions, confusion, salivations, diarrhoea, coma and death were noted if they occurred. Recordings were done at intervals of 30 minutes, 4 hours, 24 hours, 48 hours, 1 week and 2 weeks. Animal weights were taken weekly up to 14days. LD_{50} value was then determined statistically (OECD, 2008) at given confidence interval if any death occurred.

All the mice were euthanized in a chamber using halothane inhalation. Then they were all mounted on a dissecting board and the liver, spleen, kidneys, lung, heart were carefully removed. They were washed in physiological saline and were macroscopically examined for any notable changes. Their weights and the relative organ weights of the animal were taken during euthanasia.

3.8.2 Experimental Design in Sub-acute Toxicity Studies of *Fagaropsis hildebrandtii*

The study was done using 24 male and 24 female albino mice (20-30g). They were grouped by randomized complete block design to form 8 groups with 6 mice each including the control groups. Each sex in every group of mice was housed in their own cage. The control groups were administered with 0.6ml of distilled water and 0.3ml of extra virgin oil orally once daily, orally for 28days for aqueous and hexane extracts respectively. The other six groups were given the *Fagaropsis hildebrandtii* extract doses (250, 500 and 1000mg/kg body

weight) orally once daily, for 28 days, three groups for the aqueous extract and the other three for the hexane extract. The body weights were taken weekly using an electric balance and recording done. Any abnormal changes noted were to be recorded (Roy *et al.,* 2016).

3.8.3 Blood Collection and Subsequent Blood Parameter Measurements

After 28 days of the study, the animals were weighed, sacrificed after anaesthesia through halothane inhalation (Figure 3.4) and blood was collected using 2ml hyper dermic syringes and needles from cardiac puncture of the animal. A blood aliquot (1.3ml) was put in EDTA tube for haematological assay and 0.5ml of the remaining blood in plain tubes for biochemical assay.

The blood was centrifuged at 3000 rpm for 10 minutes and serum was obtained for biochemical assay and stored at −20 degrees Celsius for AST, ALT, total protein, urea and creatinine measurements (Singh and Rana, 2007). The automatic haematology analyser was used to measure haematocrit, WBC, RBC, Haemoglobin, Thrombocytes, MCH, and MCV.

The animals were then mounted on a dissecting board and the organs (liver, spleen, lung, kidneys, heart, and stomach) carefully removed (Figure 3.5). They were washed using physiological saline, their weights were taken and preserved in 5% buffered formalin solution for histopathology procedure. The relative organ weights were calculated using the formula below.

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight} \times 100}{\text{Weight of the animal at sacrificing}}$$

Figure 3.4: Euthanizing the mice in a glass jar of halothane before harvesting their blood.

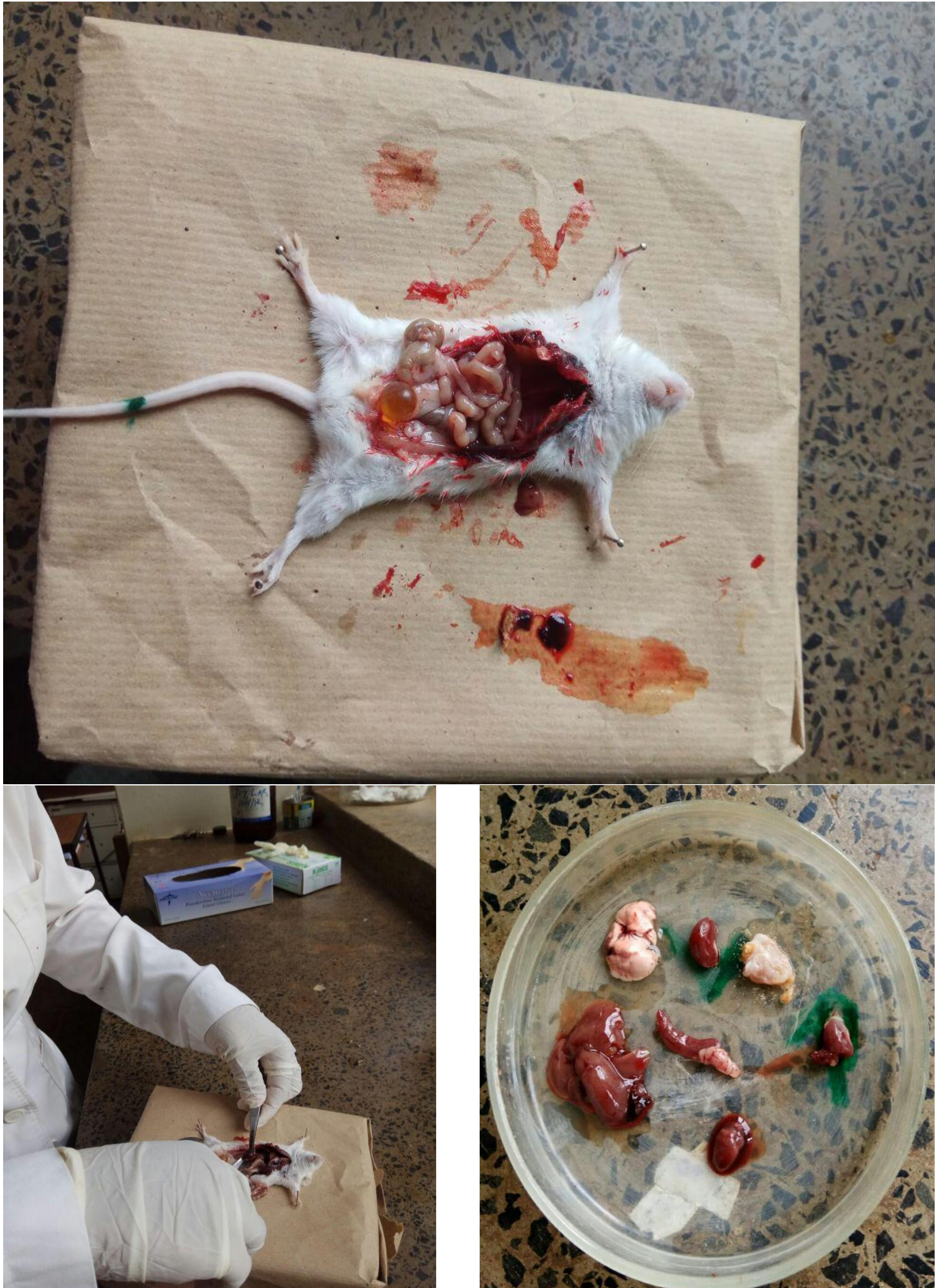


Figure 3.5: Organ identification and harvesting

3.9 Statistical Analysis

The haematological and biochemical test results were expressed as a mean ± of the standard deviation of the mean. Post hoc Tukey test was used to further evaluate the two means of the two groups (control and test group) for all the parameters tested. Differences were considered statistically significant at $P < 0.05$. The data on LD_{50} was presented in tables and determined statistically using OECD 2008 (Acute oral toxicity guideline 423). Minimum inhibitory concentration and minimum bactericidal concentration of different plant extracts results were expressed as the mean ±of standard errors of the mean. Statistical analysis was performed with SPSS V20 (Windows 2010). The P values < 0.05 were considered significant.

CHAPTER 4

RESULTS

4.1 Phytochemical Results

4.1.1 Extracts' Yield

The given quantity of *Fagaropsis hildebrandtii* extract was found to yield the amounts shown in Table 4.1.

Table 4.1: Yields of *Fagaropsis hildebrandtii* extracts

Plant extract	Weight of powder	Amount after extraction (gm)	Percentage yield (%w/w)
Aqueous extract	500 gm in 2000ml of distilled water	45	9
Hexane extract	750gm in 1250ml of hexane	20.63	2.74

4.1.2 Phytochemical Constituents

Table 4.2: Phytochemical results for *Fagaropsis hildebrandtii* hexane and aqueous extracts

Test for:		Hexane extract	Aqueous extract
Alkaloids	**a) Draggendoff's test**	Positive	positive
	b) Mayer's test	Positive	Positive
Terpenoids		Positive	Positive
Flavonoids		Negative	positive
Steroids		Positive	Positive
Anthraquinones		Negative	Negative
Tannins		Negative	Positive
Saponins		Positive	Positive
Cardiac glycosides		Positive	Negative
Phenolics		Positive	Positive

Key: **Positive** – the presence of phytochemical; **Negative** – the absence of phytochemical

Table 4.2 shows some of the medically important bioactive phytochemical constituents present in both hexane and aqueous crude extracts of *F. hildebrandtii.*

4.2 Toxicological Studies

4.2.1 Oral Acute Toxicity Testing

For a period of two weeks, the animals were observed for any changes in the skin, fur, salivation, mucous membrane, skin colour, respiratory system, circulatory system, automatic and central nervous system and somatomotor activity. Effects like tremors, convulsions, confusion, salivation, diarrhoea, coma and death were keenly observed for recording purposes. After the study, there were no significant changes observed for the mice treated with 300mglkg Bwt and 2000mg/kg Bwt of both hexane and aqueous *Fagaropsis hildebrandtii* extract compared with the control group (OECD, 423). The general behaviour of the mice did not change throughout the study.

All the mice were euthanized using halothane. They were placed in a glass chamber with a piece of cotton wool soaked in halothane. Death was confirmed by adjunctive physical method, corneal reflex, respiration and lack of heartbeat. Then post-mortem was done on all the animals as they did not die before the study was over. The organs (kidney, lung, spleen, liver, heart) were weighed and macroscopically examined but there were no abnormalities noted compared with the controls.

a) Mean Weight Change in the Acute Toxicity Study

Administration of different dose extracts of *Fagaropsis hildebrandtii* had an effect on the mean weights of mice in all the groups at day 0, day 7 and day 14 as shown in Table 4.3 and Figure 4.1.

Table 4.3: Effects of administration of different dose extracts of *Fagaropsis hildebrandtii* on the mean weights of mice

Group M =3	Mice number	Dosage used	Day 0 Mean Weight(g)	Day 7 Mean Weight(g)	Day 14 Mean Weight(g)	P-values
1	AA1 AA2 AA3	Aqueous 300mg/kg btw	25.75±3.25	29.40±2.70	31.60±1.93	0.33
2	AB1 AB2 AB3	Aqueous 2000mg/kg btw	23.32±1.82	26.26±1.27	28.11±0.76	0.99
3	OC1 OC2 OC3	Hexane 300mg/kg btw	21.33±1.52	23.52±1.60	26.39±1.74	0.72
4	OD1 OD2 OD3	Hexane 2000mg/kg btw	26.42±3.51	28.71±2.95	30.86±3.11	0.88
5	Ac1 Ac2 Ac3	0.6ml distilled water	25.58±1.90	24.76±1.75	26.76±1.82	-
6	Oc1 Oc2 Oc3	0.3ml extra virgin oil	24.46±3.14	26.46±3.14	28.68±3.08	-

Note: All values are expressed as mean ± SD of three animals.

SD= Standard deviation M Number= mice

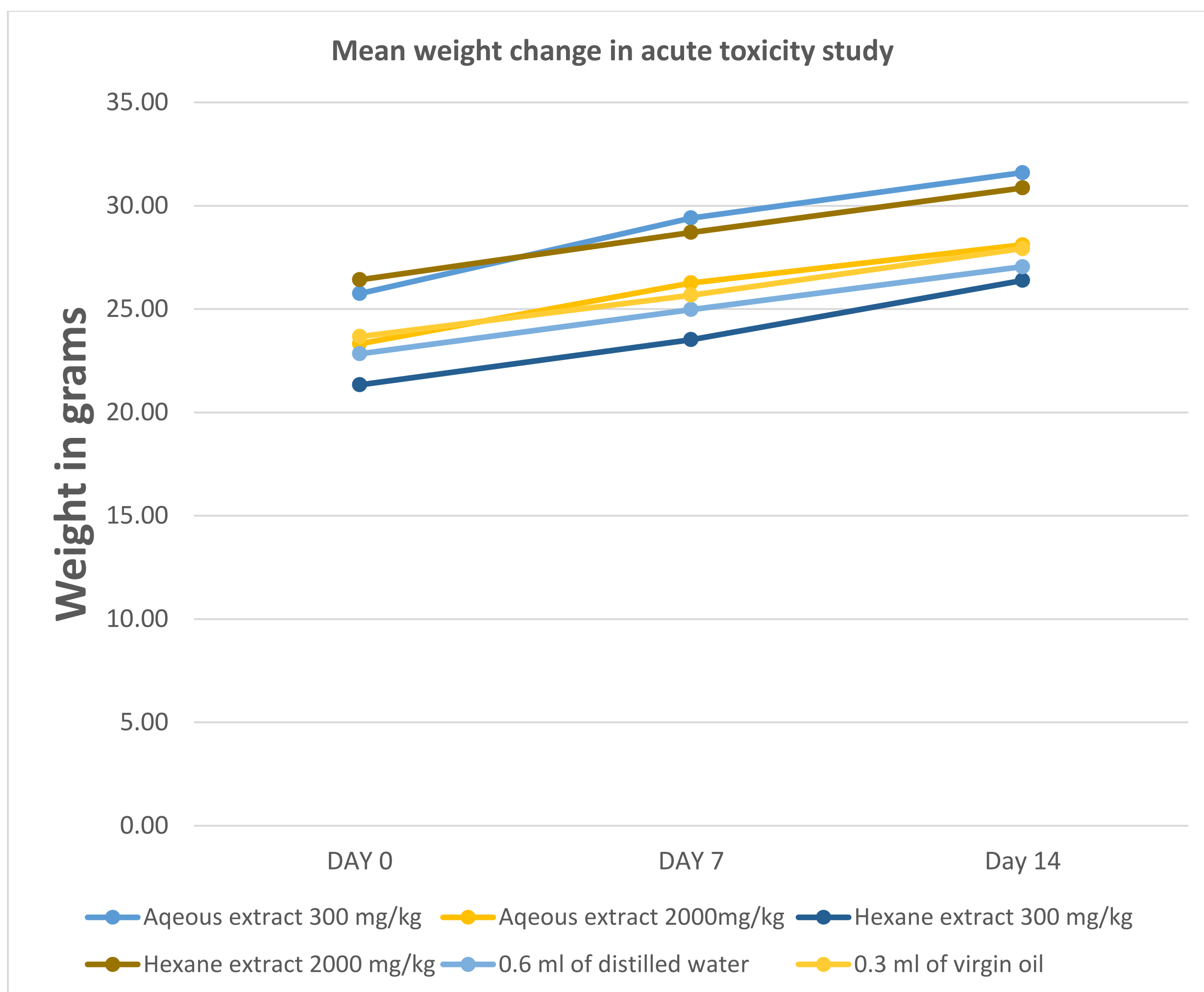


Figure 4.1: Effects of *Fagaropsis hildebrandtii* extracts on mean weight change in acute toxicity studies

There were no significant effects on the weights when the mean weights of all the treated groups and the controls were compared (OECD, 423) as shown in Table 4.3 and Figure 4.1. This was supported by the P-values of 0.33,0.99,0.72 and 0.88 for aqueous extract 300mg/kg Bwt, aqueous extract 2000mg/kg Bwt, hexane extract 300mg/kg Bwt and hexane extract 2000mg/kg Bwt respectively compared with the controls at 95% confidence interval.

b) Feed and water consumption of acute toxicity studies

Table 4.4: Effects of different *F. hildebrandtii* extracts on feed and water consumption

The dose of extracts used/ Bwt	Week 1 AWC (ml)	Week 2 AWC (ml)	P - values	Week 1 AFC (gm)	Week 2 AFC (gm)	P- values
A 300mg/kg	40.22	31.77	0.94	27.37	26.69	1.00
A 2000mg/kg	30.32	32.71	1.00	23.00	38.00	1.00
Distilled water	34.00	33.33	-	26.36	31.33	-
O 300mg/kg	33.27	40.00	1.00	28.99	29.33	0.76
O 2000mg/kg	30.23	39.01	0.96	23.90	32.61	0.67
Extra virgin oil	41.76	34.51	-	34.52	38.11	-

AWC - average water consumption **AFC -** average feed consumption

O - Hexane extract **A -** Aqueous extract

The general water and food consumption rates of all the mice treated with *F. hildebrandtii* aqueous and hexane extracts at 300mg/kg Bwt and 2000mg/kg Bwt dosages didn't change significantly over the two weeks of study as shown in Table 4.4. In addition, they didn't have a significant difference compared with the mean consumption rates of the control groups as the P-values were greater than 0.05 as shown in Table 4.4.

Gross necropsy was done to rule out any pathological changes in the major organs (lungs, liver, spleen, kidneys and heart) for all the *F. hildebrandtii* doses and extracts used. All the organs were normal and didn't show any abnormalities, necrosis, inflammation or changes in size after the macroscopic examination. This was observed with all the dose extracts used.

c) Relative-organ mean weights in acute toxicity studies

The relative organ mean weights of the kidneys, liver, lungs, heart and spleen of the treated groups were compared with the controls and there was no significant difference between them as shown by the p values which were all greater than 0.05 as shown in Table 4.5.

Table 4.5: Effects of *Fagaropsis hildebrandtii* extracts on relative organ mean weights of various organs of the treated animals and controls

Group M=3	The dose used/Bwt	Relative Mean weights(grams) of organs					
		Liver	Left kidney	Right kidney	Heart	Spleen	Lung
1	Aqueous extract 300mg/kg	6.20±0.26 *	0.61±0.02*	0.65±0.00*	0.54±0.06*	0.63±0.02*	1.33±0.14*
2	Aqueous extract 2000mg/kg	6.17±0.29*	0.59±0.01*	0.63±0.02*	0.65±0.11*	0.57±0.04*	1.18±0.18*
3	Hexane extract 300mg/kg	6.17±0.21*	0.55±0.05*	0.58±0.03*	0.66±0.07*	0.59±0.02*	1.05±0.21*
4	Hexane extract 2000mg/kg	6.12±0.16*	0.58±0.10*	0.62±0.05*	0.68±0.07*	0.58±0.09*	1.25±0.22*
5	Distilled water	5.79±0.39*	0.59±0.02*	0.62±0.03*	0.54±0.09*	0.60±0.03*	1.28±0.15*
6	Extra virgin oil	5.88±0.43*	0.55±0.07*	0.58±0.01*	0.60±0.05*	0.60±0.02*	1.27±0.14*

* P> 0.05 ** P< 0.05 M= Mice number

4.2.2 Sub-Acute Toxicity Studies

a) Average Feed and water consumption of sub-acute toxicity studies of *F. hildebrandtii*

Table 4.6 shows the average water and feed consumption of mice administered with the different doses of aqueous and hexane extracts of *F. hildebrandtii* plant and their respective controls over a 28-day study.

The values show no significant effects on the rate of feed and water consumption on the different groups of mice administered with 250mg/kg Bwt, 500mg/kg Bwt and 1000mg/kg Bwt of both hexane and aqueous extracts of *Fagaropsis hildebrandtii*. This is after comparison with their controls which were administered with extra virgin oil and distilled water respectively for 28 days. There were no significant changes observed in the feeding and water consumption behaviour of the treated mice on weekly assessment for 28 days as shown by the average water consumption, average feed consumption and P values which are greater than 0.05 in Tables 4.5 and 4.6 respectively.

Table 4.6: Effects of Different *F. hildebrandtii* Extracts and Dosages on Feed and Water Consumption

The dose given/Bwt	Sex	Week 1		Week 2		Week 3		Week 4	
		AWC (ml)	AFC (gm)	AWC (ml)	AFC (gm)	AWC (ml)	AFC (gm)	AWC (ml)	AFC (gm)
Aqueous 250mg/kg	F	40	32.28	50	33.44	43.33	33.4	41.67	29.32
Aqueous 500mg/kg	F	34.17	25.11	33.33	30.69	33.33	27.6	31.76	21.39
Aqueous 1000mg/kg	F	35	28.29	34.17	32.2	29	35.41	_	_
Distilled Water	F	38.33	40	33.33	32.5	31.7	26.4	26.67	31.81
Hexane 250mg/kg	F	40	32.42	48.3	34.32	43.33	34.29	40	31.07
Hexane 500mg/kg	F	34.67	26.71	33.33	29.81	34	26.81	32.67	22
Hexane 1000mg/kg	F	36.33	27.92	35.33	30.22	26.67	33.45	_	_
Extra virgin Oil	F	35	38	32.33	31.49	33	27.11	33.33	33.72
Aqueous 250mg/kg	M	29	27.9	24	22.4	42.5	21.8	32.5	31.1
Aqueous 500mg/kg	M	34.17	31	33.33	31.5	30	28.22	32	26.63
Aqueous 1000mg/kg	M	40	33.5	43.33	33.24	40	34.91	39	21.80
Distilled Water	M	40	44	40	43	46.7	45.21	43.33	31.84
Hexane 250mg/kg	M	30.67	29.8	24.67	23.09	41.67	22.09	34	28.98
Hexane 500mg/kg	M	34.67	30.1	34.33	30.84	30.67	29.12	33.33	27.24
Hexane 1000mg/kg	M	40.67	32.5	42.33	34	41.33	33.4	28	33.10
Extra virgin oil	M	39.3	40	42.33	40.29	45.33	43	41.67	33.41

Note: All values are expressed as mean ± SD of three animals.

KEY: **AWC** - Average Water Consumption **F**- Females

AFC - Average Feed Consumption **M** -Males

Table 4.7 shows the P-values of the average water and feed consumption of mice administered with the different doses of aqueous and hexane extracts obtained from *F. hildebrandtii* plant and their respective controls over a 28-day study.

Table 4.7: P-values of average water consumption and average feed consumption of both males and female mice used in the sub-acute studies

Dose given/Bwt	**Sex**	**P-values (AWC)**	**P-values (AFC)**
Aqueous 250mg/kg	F	0.84	1.00
Aqueous 500mg/kg	F	1.00	0.85
Aqueous 1000mg/kg	F	1.00	1.00
Distilled Water	F	-	-
Hexane 250mg/kg	F	0.72	1.00
Hexane 500mg/kg	F	1.00	0.87
Hexane 1000mg/kg	F	1.00	0.45
Extra virgin Oil	F	-	-
Aqueous 250mg/kg	M	0.78	1.00
Aqueous 500mg/kg	M	0.98	0.16
Aqueous 1000mg/kg	M	0.99	0.34
Distilled Water	M	-	
Hexane 250mg/kg	M	0.15	0.43
Hexane 500mg/kg	M	0.19	0.53
Hexane 1000mg/kg	M	0.92	0.48
Extra virgin oil	M	-	-

KEY: **AWC** - Average Water Consumption **F-** Female

AFC - Average Feed Consumption **M-** Male

b) Haematological analysis in sub-acute toxicity studies of *F. hildebrandtii*

Effects on Haematological parameters at day 28 in sub-acute toxicity of mice administered with aqueous extracts obtained from *F. hildebrandtii* and distilled water (control) are as shown in Table 4.8 and Figure 4.2.

Table 4. 8: Effects on Haematological parameters at day 28 in Sub-acute Toxicity of mice administered with aqueous extracts obtained from *F. hildebrandtii* and distilled water

	Sex	**Control**	**250mg/kg Bwt**	**P-value**	**500mg/kg Bwt**	**P-value**	**1000mg/kg Bwt**	**P-value**
RBC	M	9.91±0.03	9.00±0.14	0.00	9.17±0.22	0.00	5.01±0.10	0.00
(x 10^{12}/L)	F	9.19±0.04	9.15±0.13	1.00	9.11±0.09	1.00	-	-
Hb	M	13.57±1.00	14.7±0.98	1.00	14.57±0.91	1.00	8.85±0.21	0.02
(gm/dL)	F	13.00±0.87	15.33±0.21	0.62	17.23±4.09	0.02	-	-
Haematocrit	M	0.48±0.02	0.51±0.02	0.93	0.47±0.02	1.00	0.30±0.02	0.00
(L/L)	F	0.48±0.03	0.50±0.02	0.97	0.49±0.02	1.00	-	-
MCV	M	49.10±1.05	49.63±3.72	1.00	48.7±1.18	1.00	20.65±0.63	0.00
(fL)	F	48.23±3.56	49.33±2.90	1.00	48.33±1.33	1.00	-	-
MCH	M	13.23±0.47	14.4±0.78	0.68	14.73±0.84	0.32	9.25±0.64	0.00
(pg)	F	13.93±0.55	14.83±0.55	0.92	14.63±0.5	0.99	-	-
MCHC	M	29.13±0.90	29.7±0.66	1.00	29.97±1.20	1.00	19.7±0.42	0.00
(g/dL)	F	28.27±0.90	30.53±0.64	0.37	30.43±0.76	0.43	-	-
Platelets	M	518.33±2.08	540±3.06	0.00	546.67±1.53	0.00	382.00±0.42	0.00
(x10^9/L)	F	534.33±2.52	548.33±3.51	0.04	540.00±1.0	0.95	-	-
WBC	M	4.38±0.22	4.90±0.15	0.9	5.13±0.04	0.48	2.35±0.28	0.00
(x10^9/L)	F	4.64±0.37	4.26±0.25	1.00	4.67±0.34	1.00	-	-

Note: All values are expressed as mean ± SD of three animals.

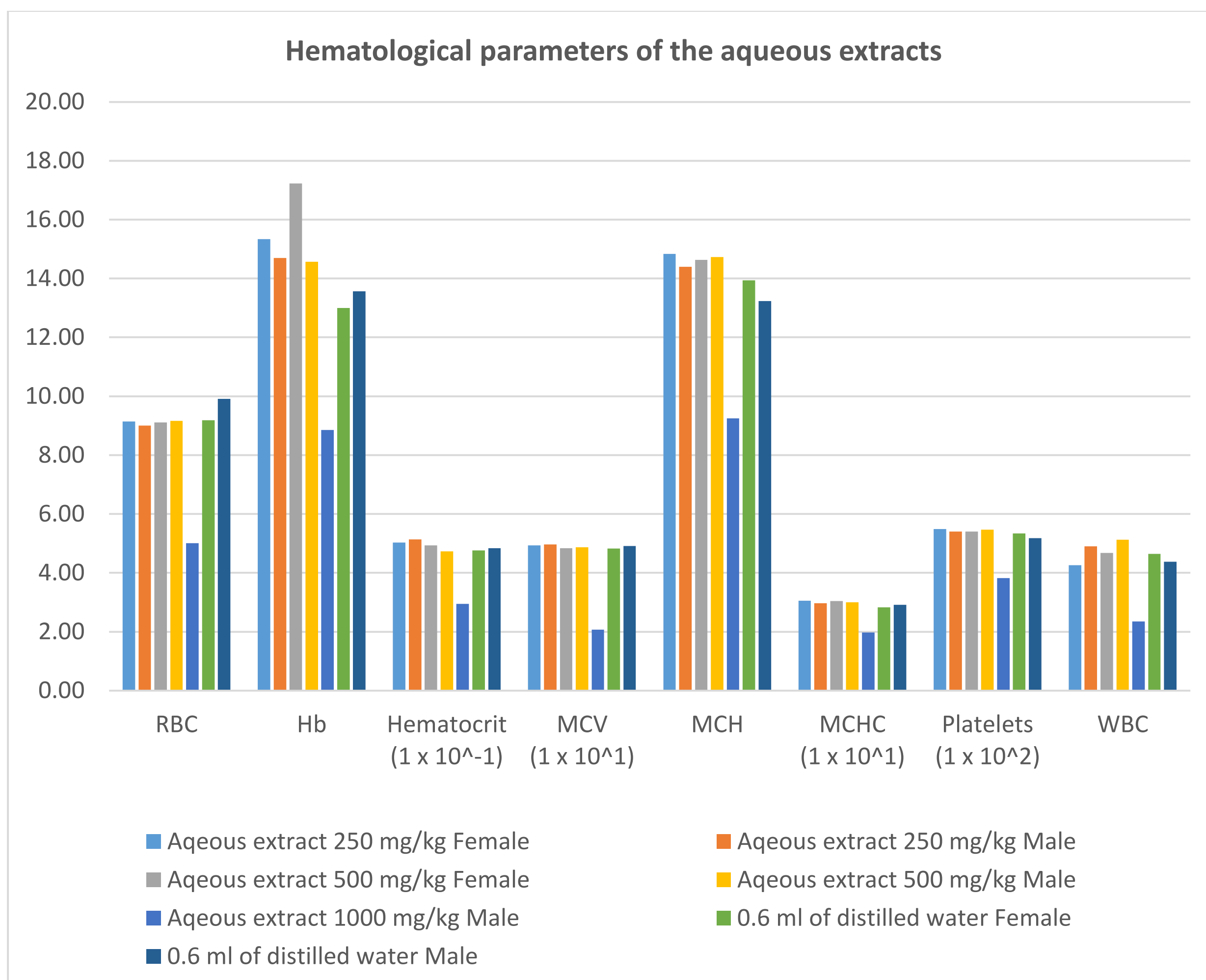


Figure 4.2: Effects of *Fagaropsis hildebrandtii* extracts on Haematological parameters

One-way ANOVA showed a significant difference in the treated groups and controls used in the study with a P-value of 0.00. Further analysis was done using the Post hoc- Tukey test to give the p-value of individual treated groups compared with their respective controls as presented in Table 4.9 and Figure 4.3. For those administered with 1000mg/kg body weight for aqueous extract showed a significant difference with the controls and had a P value of less than 0.05 except for the missing values (of the six females which died before the end of the study) for all the haematological parameters.

There was also a significant difference $P < 0.05$ as shown in the tables above with the controls on RBCs count for the mice administered with aqueous 250mg/kg Bwt and aqueous 500mg/kg Bwt doses for both males and females.

After the study, mean weights of WBCs of the female mice administered with aqueous 500mg/kg Bwt dose had a P value of 0.023 which was significantly different from the control group.

The platelets mean weight showed a significant difference with $P < 0.05$ for the following groups also (males on aqueous 250mg/kg body weight and 500mg/kg body weight.)

Table 4.9: Effects on Haematological parameters at day 28 in sub-acute toxicity of mice administered with Hexane extracts obtained from *F. hildebrandtii* and extra virgin oil (control)

	Sex	Control	250mg/kg Bwt	P value	500mg/kg Bwt	P value	1000mg/kg Bwt	P value
RBC (x 10^{12}/L)	M	9.88±0.12	9.00±0.07	0.00	9.02±0.09	0.00	4.98±0.06	0.00
	F	9.64±0.07	9.13±0.11	0.00	8.96±0.13	0.00	-	-
Hb (gm/dL)	M	15.23±0.64	15.24±0.07	1.00	14.48±0.05	1.00	13.05±0.21	0.83
	F	14.50±0.62	14.1±0.53	1.00	14.16±0.12	1.00	-	-
Haematocrit (L/L)	M	0.5±0.04	0.5±0.02	1.00	0.51±0.03	1.00	0.28±0.01	0.00
	F	0.49±0.01	0.45±0.02	0.67	0.49±0.03	1.00	-	-
MCV (fL)	M	45.33±1.00	49.30±3.64	0.95	48.3±3.21	1.00	25.8±1.13	0.00
	F	49.80±6.36	49.47±0.45	1.00	52.9±4.70	0.99	-	-
MCH (pg)	M	13.6±0.75	13.7±0.78	1.00	13.43±0.85	1.00	11.25±0.22	0.04
	F	13.9±0.56	14.87±0.85	0.87	15.1±0.56	0.64	-	-
MCHC (g/dL)	M	28.91±1.60	26.97±0.29	0.60	27.07±1.76	0.67	21.09±1.15	0.00
	F	29.87±1.11	29.47±1.33	1.00	29.57±0.68	1.00	-	-
Platelets (x10^9/L)	M	500.67±1.53	492.33±4.16	0.00	510.67±1.53	0.72	357.00±2.83	0.00
	F	515.00±2.00	540.33±5.03	0.00	500.33±13.01	0.01	-	-
WBC (x10^9/L)	M	5.87±0.85	4.94±0.22	0.85	5.20±0.36	0.36	2.16±0.08	0.00
	F	4.75±0.55	4.71±0.72	1.00	3.77±0.60	0.14	-	-

Note: All values are expressed as mean ± SD of three animals.

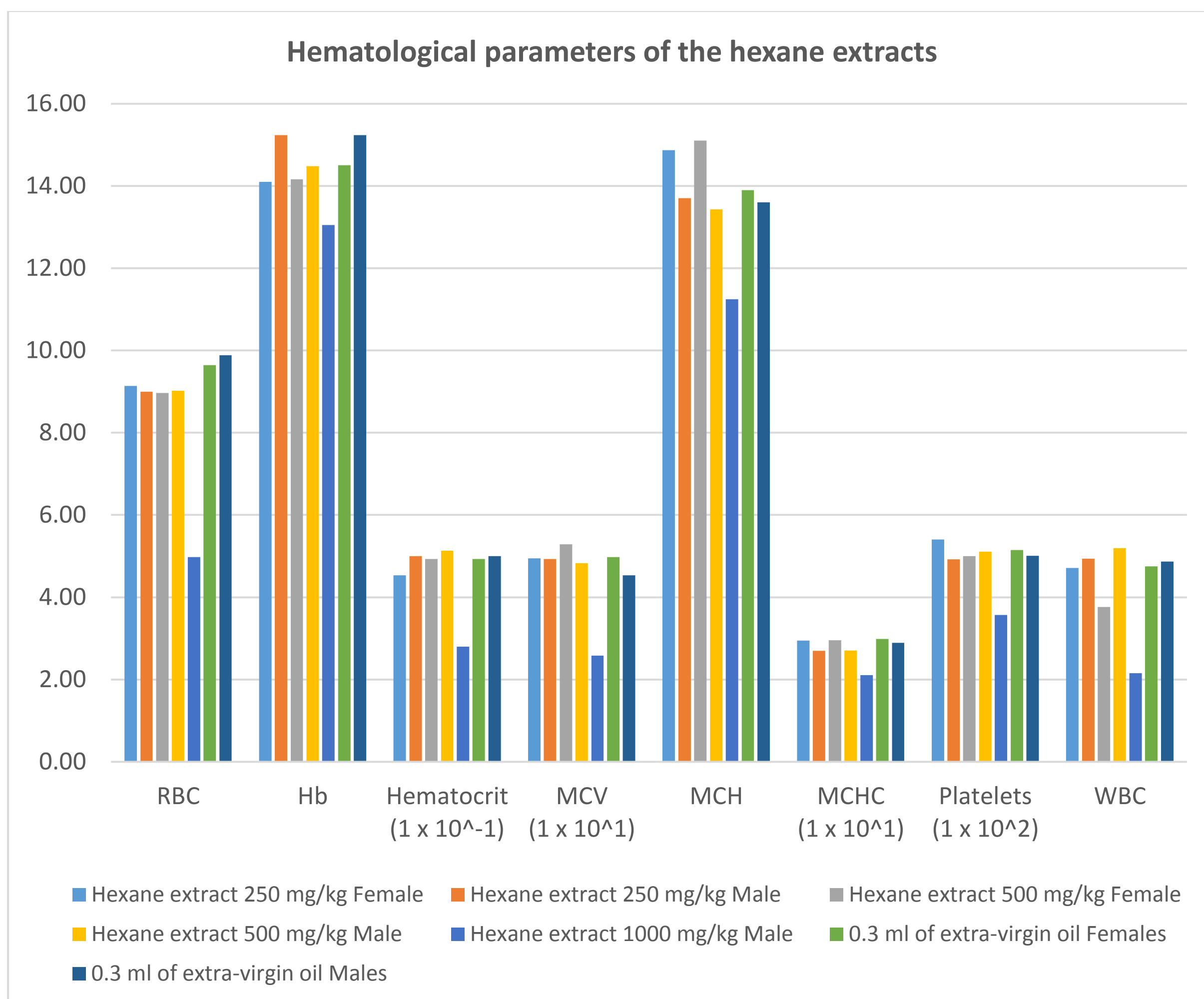


Figure 4.31: Effects of *Fagaropsis hildebrandtii* extracts on Haematological parameters

One-way ANOVA showed a significant difference in the treated groups and controls used in the study with a P-value of 0.00. Further analysis was done using the Post hoc- Tukey test to give the p-value of individual treated groups compared with their respective controls as presented in Table 4.9. For those administered with 1000mg/kg body weight for hexane extract showed a significant difference with the controls and had a P value of less than 0.05 except for the missing values of the six females which died before the end of the study for all the haematological parameters.

There was also a significant difference P <0.05 as shown in the tables above with the controls on RBCs count for the mice administered with hexane 250mg/kg Bwt and hexane 500mg/kg Bwt doses for both males and females.

The platelets mean weight showed a significant difference with P<0.05 for the following groups also (females administered with hexane 250mg/kg body weight and 500mg/kg body weight; males on hexane 250mg/kg body weight.)

c)* Biochemical parameters analysis in sub-acute toxicity studies of *Fagaropsis hildebrandtii

Table 4.10 and Figure 4.3 shows the analysis of the biochemical parameters of both males and females administered with aqueous extracts obtained from *F. hildebrandtii.*

Table 4.10: Biochemical parameters at day 28 in sub-acute toxicity in mice administered with aqueous extracts obtained from *F. hildebrandtii* and distilled water (control)

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

Note: All values are expressed as mean ± SD of three animals.

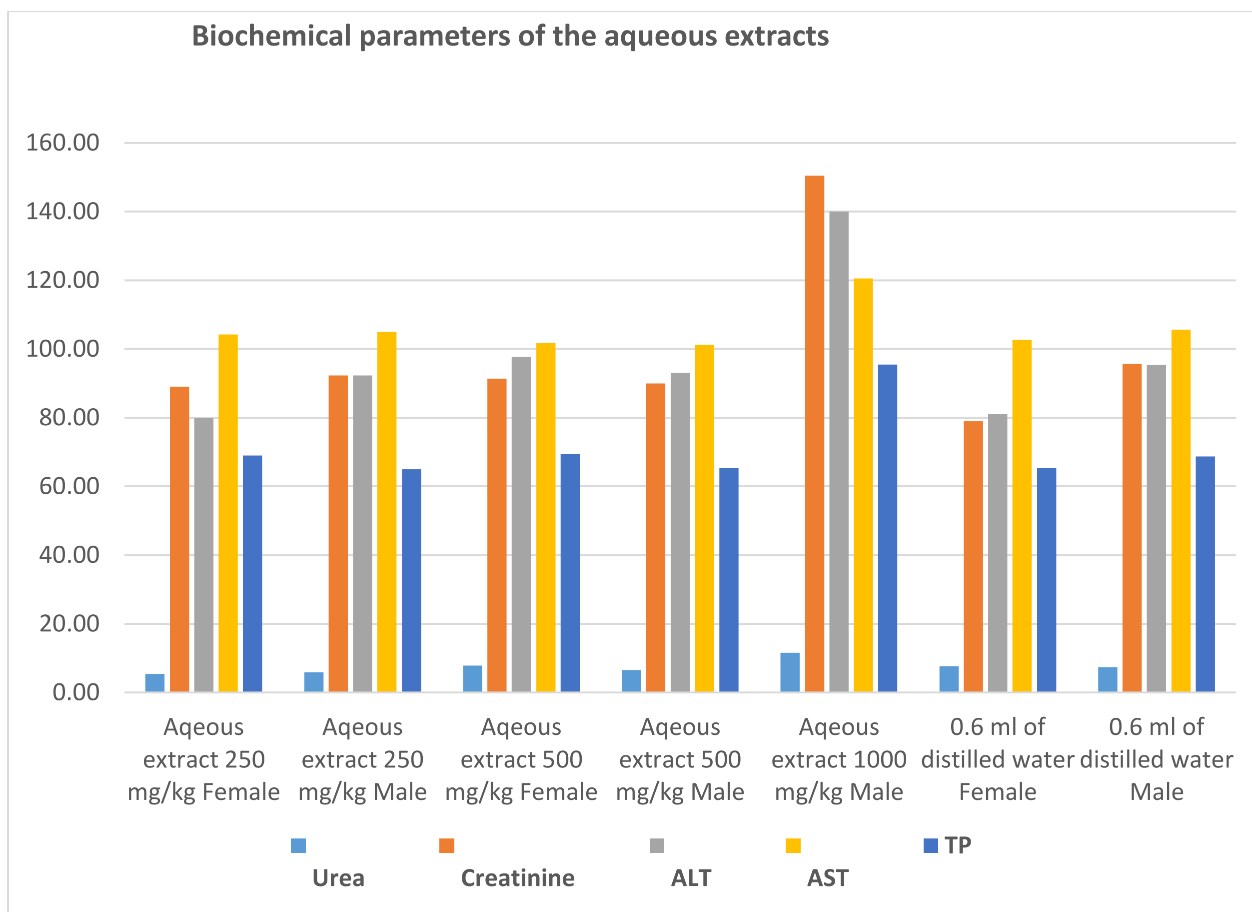


Figure 4.4: Biochemical parameters: in sub-acute toxicity of *F. hildebrandtii* aqueous extract doses

All biochemical parameters were significantly different in 1000mg/ kg Bwt for an aqueous extract from their respective controls P<0.05. These values were not recorded for females administered with 1000mg/kg Bwt dose aqueous extract since they died before day 28.

All biochemical parameters were significantly different in 1000mg/ kg Bwt dose for hexane extract from their respective controls P<0.05 as shown in Table 4.11 and Figure 4.4. These values were not recorded for females administered with 1000mg/kg Bwt dose for hexane extract since they died before day 28.

Table 4.11 shows the analysis of the biochemical parameters of both males and females administered with hexane extracts of *F. hildebrandtii.*

Table 4.11: Effects of *Fagaropsis hildebrandtii* extracts on Biochemical parameters at day 28 in sub-acute toxicity in mice administered with hexane extracts and extra virgin oil (control)

	Sex	**Control**	**250mg/kg Bwt**	**P value**	**500mg/kg Bwt**	**P value**	**1000mg/kg Bwt**	**P value**
Urea mg/dl	M	7.13±1.38	6.33±0.21	0.99	6.63±0.15	1.00	13.5±0.07	0.001
	F	6.83±0.75	6.37±1.46	1.00	7.10±0.26	1.00	-	-
Creatinine Mg/dl	M	81.67±5.51	85.67±5.69	1.00	90.67±4.16	0.47	119.00±0.71	0.00
	F	89.67±1.15	89.33±2.08	0.92	92.00±3.00	0.53	-	-
ALT mg/l	M	80.67±6.43	81.33±4.93	1.00	96.00±7.00	0.024	137.5±0.71	0.00
	F	79.33±4.16	94.33±5.86	0.00	93.33±3.51	0.00	-	-
AST ml/l	M	104.00±3.00	103.26±5.80	1.00	102.33±2.08	1.00	118.00±1.41	0.00
	F	100.67±1.15	102.33±1.52	1.00	102.67±2.08	1.00	-	-
T.P g/l	M	66.67±1.53	72.33±1.53	0.36	68.00±2.00	1.00	93.5±2.12	0.00
	F	65.67±1.00	69.32±3.21	0.45	66.66±1.52	0.99	-	-

Note: All values are expressed as mean ± SD of three animals.

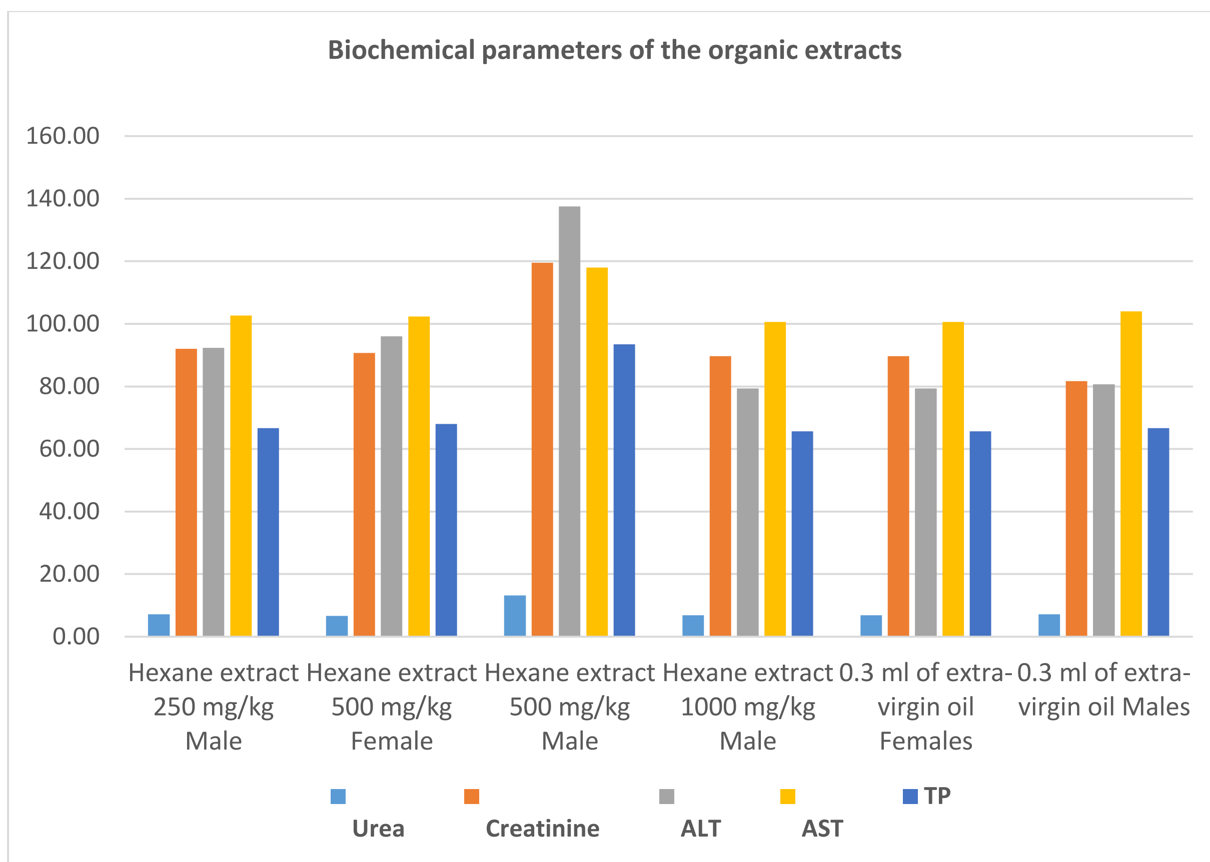


Figure 4.5: Biochemical parameters in sub-acute toxicity of *F. hildebrandtii* hexane extract doses

The ALT mean levels were also significantly different in males administered with hexane 500mg/kg Bwt, females on hexane250mg/kg Bwt and females on hexane 500mg/kg Bwt with P>0.05 compound with the controls.

d) Relative-Organ Weight Ratios

Table 4.12 and Figure 4.6 show the effects of *F. hildebrandtii* aqueous extracts on absolute organ-body weight ratios in both males and females and their controls.

Table 4.12: Effects on Relative organ- body weight ratios in groups treated with aqueous extract obtained from *F. hildebrandtii* and distilled water (control)

Organ	Sex	Control	250mg/kg Bwt	P value	500mg/kg Bwt	P value	1000mg/kg Bwt	P value
Stomach	M	1.36±0.13	0.92±0.13	0.34	1.42±0.16	1.00	0.82±0.08	1.00
	F	1.33±0.11	1.36±0.21	1.00	1.46±0.26	1.00	0.74±0.24	0.05
Left kidney	M	0.66±0.07	0.58±0.03	1.00	0.67±0.15	1.00	0.63±0.03	1.00
	F	0.56±0.08	0.51±0.09	1.00	0.69±0.08	0.90	0.57±0.03	1.00
Right kidney	M	0.68±0.02	0.62±0.09	1.00	0.75±0.12	1.00	0.60±0.03	0.99
	F	0.57±0.09	0.59±0.03	1.00	0.70±0.08	0.71	0.55±0.02	1.00
Lung	M	0.99±0.11	1.28±0.16	0.73	1.36±0.10	0.38	0.58±0.04	0.24
	F	1.20±0.07	1.21±0.35	1.00	1.40±0.11	0.98	0.52±0.00	0.002
Spleen	M	0.68±0.12	0.71±0.05	1.00	0.82±0.05	0.96	0.23±0.03	0.00
	F	0.65±0.05	0.67±0.08	1.00	0.64±0.08	1.00	0.20±0.03	0.00
Heart	M	0.56±0.05	0.49±0.04	1.00	0.55±0.22	1.00	0.34±0.08	0.24
	F	0.59±0.08	0.62±0.12	1.00	0.62±0.10	1.00	0.32±0.02	0.06
Liver	M	5.12±0.08	4.92±0.49	1.00	5.73±0.82	0.97	3.25±0.17	0.004
	F	5.25±0.75	4.74±0.23	0.99	5.58±0.24	1.00	4.01±0.40	0.33

Note: All values are expressed as mean ± SD of three animals.

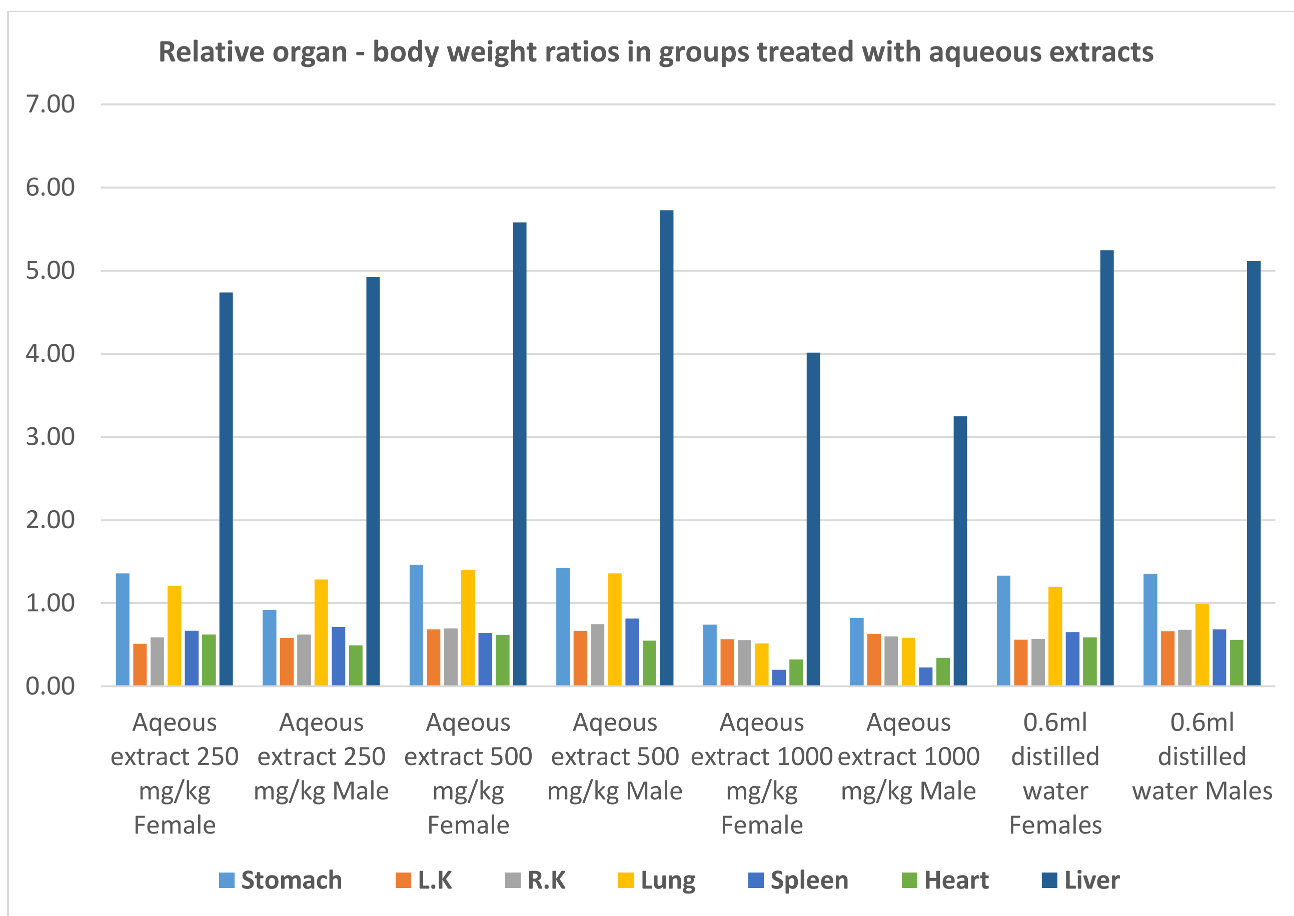


Figure 4.6: Effects of *F. hildebrandtii* aqueous extracts on absolute organ-body weight ratios in both males and females and their controls.

There was no significant difference in relative organ weights in all the doses for the stomach, left the kidney and right kidney since all the P values were more than 0.05. This is as shown in the above results in Table 4.12 and Figure 4.6.

The mean relative weights of the lungs and spleen female mice treated with aqueous 1000mg/kg Bwt dose showed a significant difference in absolute organ ratio compared to those of the control.

The mean spleen relative weights of males group treated with aqueous 1000mg /kg Bwt dose had a significant difference compared with the controls.

The mean relative weights of the liver in males treated with both aqueous 1000mg/kg Bwt dose extracts showed a significant difference in absolute organ –body weight ratio compared to those of control groups.

Table 4.13 shows the effects of *F. hildebrandtii* hexane extracts on relative organ-body weight ratios in both males and females and their controls.

Table 4. 13: Effects on Relative organ- body weight ratios in groups treated with Hexane extract obtained from *F. hildebrandtii* and extra virgin oil

Organ	**Sex**	**Control**	**250mg/kg Bwt**	**P value**	**500mg/kg Bwt**	**P value**	**1000mg/kg Bwt**	**P value**
Stomach	M	1.32±0.07	1.32±0.07	1.00	1.57±0.34	0.96	0.82±0.04	0.17
	F	1.48±0.09	1.26±0.16	0.99	1.31±0.40	1.00	0.87±0.17	0.04
Left kidney	M	0.56±0.04	0.54±0.04	1.00	0.60±0.06	1.00	0.51±0.11	1.00
	F	0.53±0.01	0.49±0.06	1.00	0.61±0.08	1.00	0.48±0.16	1.00
Right kidney	M	0.59±0.05	0.54±0.06	1.00	0.64±0.04	1.00	0.46±0.12	0.72
	F	0.55±0.06	0.52±0.06	1.00	0.66±0.10	0.86	0.52±0.01	1.00
Lung	M	1.23±0.26	1.33±0.05	1.00	1.58±0.06	0.42	0.53±0.06	0.001
	F	1.07±0.32	1.28±0.05	0.97	1.28±0.16	0.96	0.34±0.22	0.001
Spleen	M	0.76±0.04	0.74±0.16	1.00	0.80±0.15	1.00	0.16±0.04	0.00
	F	0.65±0.06	0.69±0.18	1.00	0.81±0.14	0.85	0.24±0.12	0.001
Heart	M	0.61±0.07	0.55±0.07	1.00	0.49±0.12	0.97	0.31±0.001	0.02
	F	0.60±0.10	0.53±0.02	1.00	0.49±0.06	0.97	0.30±0.03	0.022
Liver	M	0.57±0.23	5.35±0.38	1.00	5.75±0.34	1.00	3.31±0.41	0.001
	F	5.63±0.52	5.50±0.06	1.00	5.17±0.09	1.00	4.45±1.11	0.237

Note: All values are expressed as mean ± SD of three animals

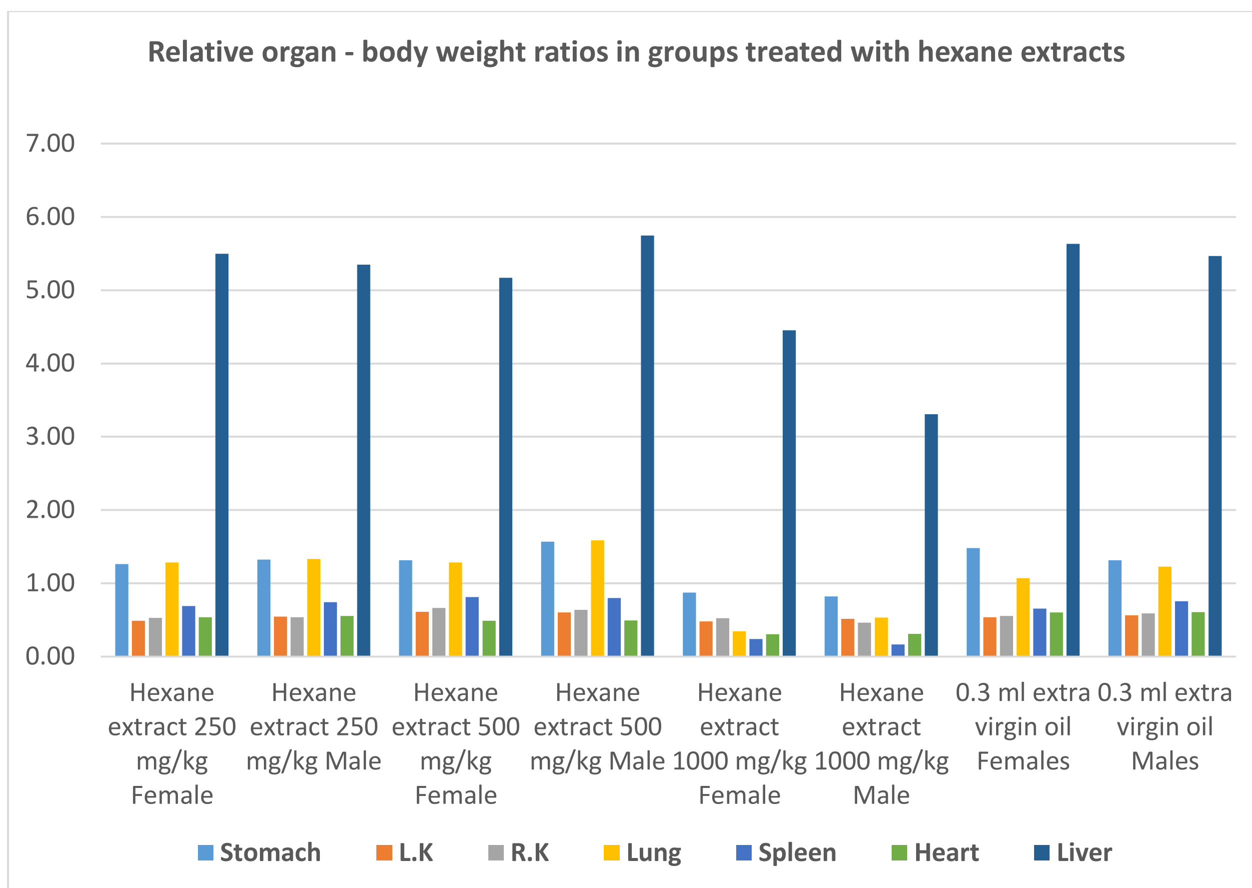


Figure 4.7: Effects of *F. hildebrandtii* hexane extracts on relative organ-body weight ratios in both males and females and their controls.

There was no significant difference in relative organ weights in all the doses for the stomach, left the kidney and right kidney since all the P values were more than 0.05. This is as shown in the above results in Figure 4.7.

There was also a significant difference in the mean relative weights of lungs and spleen males treated with hexane 1000mg/kg Bwt dose compared with the controls.

The mean relative weights of the liver in males treated with hexane 1000mg/kg Bwt dose extracts showed a significant difference in absolute organ-body weight ratio compared to those of control.

The mean relative weights of the heart of females treated with hexane 100mg/kg Bwt dose extract showed a significant difference P-value 0.022 compared with the controls. The mean

relative weights of the heart of males treated with hexane 1000mg/kg Bwt dose extract showed a significant difference compared with the controls P-value 0.020.

e) Mean Body Weights

Table 4.14 shows the mean weights of both male and female mice treated with aqueous extracts compared with the controls administered with distilled water.

Table 4.14: Mean body weights of mice during sub-acute toxicity after administration of aqueous extract obtained from *F. hildebrandtii* and distilled water (control)

Day	Control	250mg/kg Bwt	P-value	500mg/kg Bwt	P-value	1000mg/kg Bwt	P-value
0	F	23.37±1.71	1.00	23.09±3.19	1.00	23.72±3.25	0.024
	M	24.17±1.16	0.97	24.69±1.73	1.00	23.05±0.41	1.00
7	F	22.80±2.18	1.00	22.48±2.74	1.00	23.42±3.08	0.024
	M	23.42±1.45	0.97	24.65±2.75	1.00	22.41±0.77	1.00
14	F	24.51±1.16	1.00	23.13±2.99	1.00	23.02±2.95	0.024
	M	24.05±1.01	0.97	25.39±2.25	1.00	21.62±0.49	1.00
21	F	25.52±1.38	1.00	24.26±2.52	1.00	22.62±2.49	0.024
	M	25.22±1.19	0.97	26.30±1.84	1.00	21.09±0.81	1.00
28	F	27.54±0.55	1.00	26.79±1.73	1.00	18.28±0.67	0.024
	M	27.35±1.07	0.97	27.94±0.85	1.00	17.12±0.76	1.00

Note: All values are expressed as mean ± SD of three animals.

The mean weights of both the females and males in the groups treated with both 250mg/kg Bwt and 500mg/kg Bwt dose of aqueous extracts of *Fagaropsis hildebrandtii* didn't show a significant difference $P>0.05$ when compared with the controls. The females treated with aqueous 1000mg/kg Bwt extract showed a significant difference $P<0.05$ when compared with the controls. They were weighed after they died in the last week of the study. This is as shown in Table 4.14 and Figure 4.8.

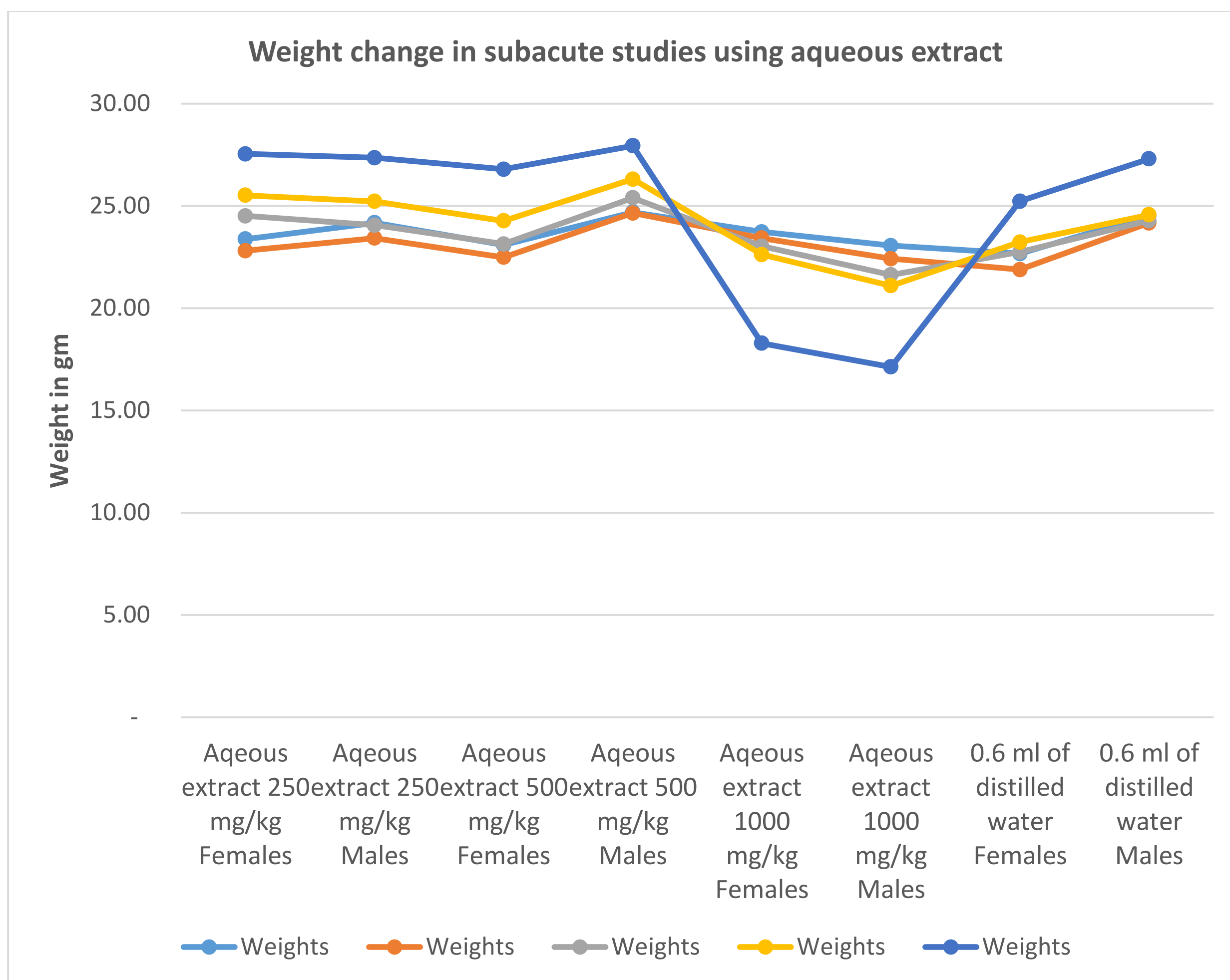


Figure 4.8: weight profile in sub-acute toxicity testing of the aqueous extract obtained from *F. hildebrandtii*

Table 4.15 shows the mean weights of both male and female mice treated with hexane extracts compared with the controls administered with extra virgin oil.

Table 4.15: Mean body weights of mice during sub-acute toxicity after administration of hexane extract obtained from *F. hildebrandtii* and extra virgin oil (control)

Day	**Control**	**250mg/kg Bwt**	**P value**	**500mg/kg Bwt**	**P value**	**1000mg/kg Bwt**	**P value**
0	F	23.49±3.29	1.00	21.69±0.65	1.00	22.67±0.68	0.99
	M	21.53±1.15	0.97	23.00±0.64	1.00	24.08±1.00	1.00
7	F	23.22±2.69	1.00	21.69±0.56	1.00	21.71±0.50	0.99
	M	20.54±1.15	0.97	22.56±0.56	1.00	22.87±1.15	1.00
14	F	23.45±3.05	1.00	22.20±0.67	1.00	22.66±1.16	0.99
	M	21.49±1.37	0.97	23.28±0.67	1.00	22.47±0.05	1.00
21	F	24.99±2.47	1.00	22.93±0.50	1.00	22.17±1.58	0.99
	M	22.62±0.96	0.97	24.03±0.50	1.00	21.43±1.12	1.00
28	F	25.14±2.58	1.00	23.89±0.21	1.00	19.48±0.60	0.99
	M	24.30±1.10	0.97	25.06±0.21	1.00	18.43±1.15	1.00

Note: All values are expressed as mean ± SD of three animals.

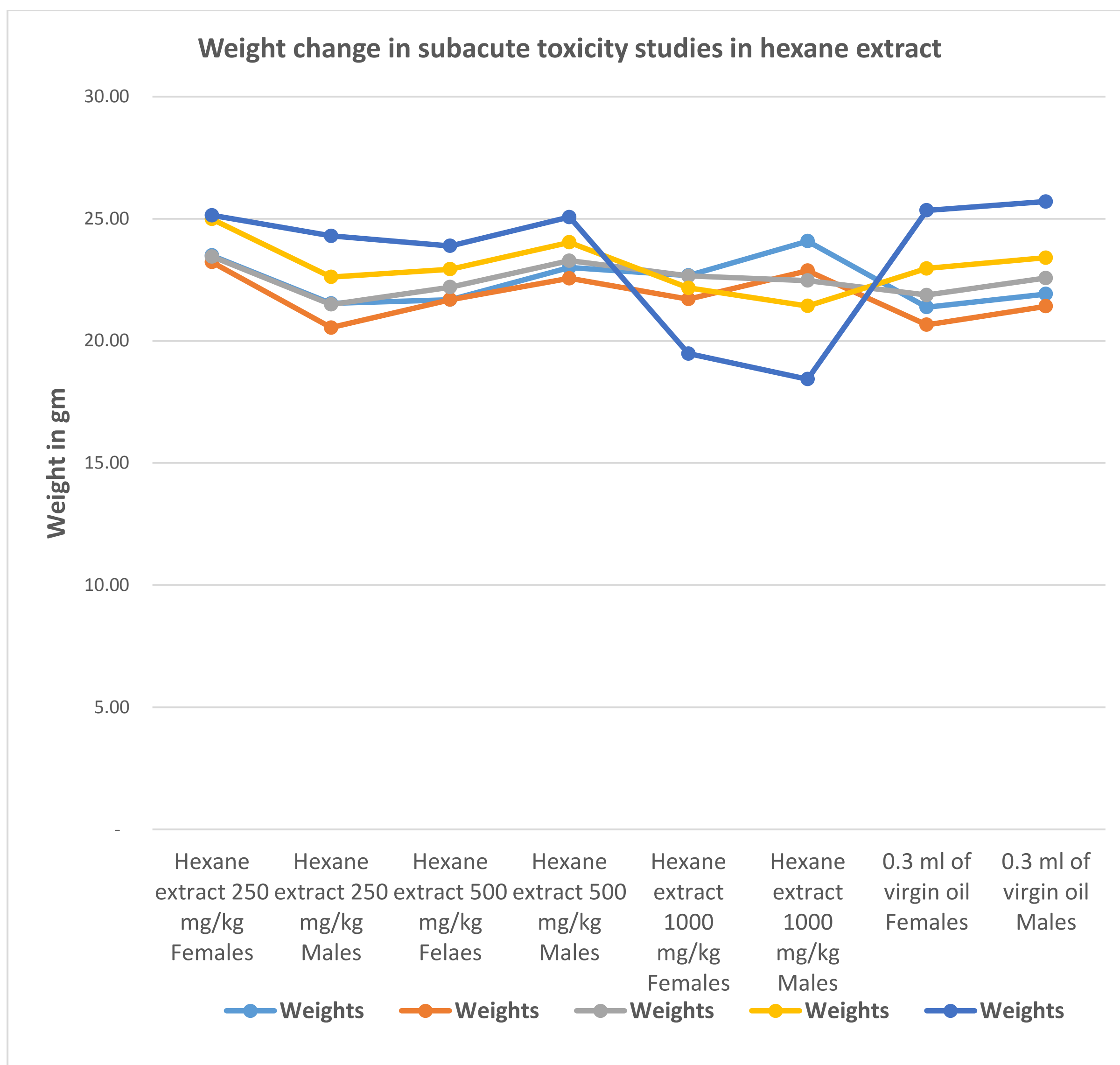


Figure 4.9: Weight profile in sub-acute toxicity testing of hexane extract of *F. hildebrandtii*

The mean weights of both the females and males in all the treated groups with *F. hildebrandtii* hexane extracts didn't show a significant difference P>0.05 when compared with the controls. This is as shown in Table 4.15.

f) **Histopathological studies on kidney and liver sections of mice treated with extracts of *F. hildebrandtii***

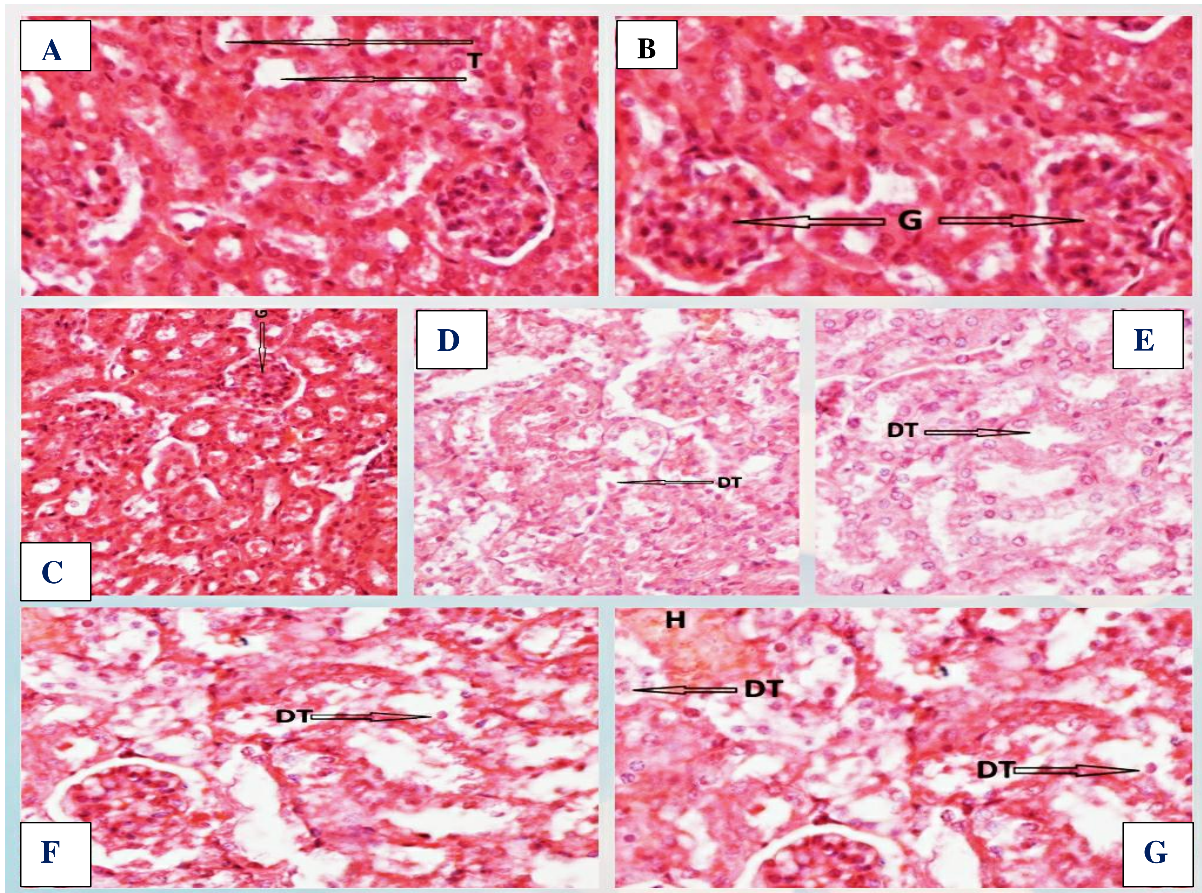


Figure 4.10: Kidney sections from mice receiving different treatments.

A: kidney section from a mouse treated with distilled water/extra virgin oil, **B**: kidney section from a mouse treated with a 250mg/kg dose of aqueous extract of *F. hildebrandtii*, **C**: kidney section from a mouse treated with a 250mg/kg dose of hexane extract of *F. hildebrandtii*, **D**: kidney section from a mouse treated with a 500mg/kg dose of aqueous extract of *F. hildebrandtii*, **E**: kidney section from a mouse treated with a 500mg/kg dose of hexane extract of *F. hildebrandtii*, **F**: kidney section from a mouse treated with a 1000mg/kg dose of aqueous extract of *F. hildebrandtii,* **G**: kidney section from a mouse treated with a 1000mg/kg dose of hexane extract of *F. hildebrandtii*.

Kidney sections A, B, C were characterized by regular glomeruli and normal renal tubules with complete smooth epithelia and clear lumen (X400). Kidney section D was characterized

by degeneration of renal tubules (DT) and loss of epithelium. Kidney section E was characterized by mild tubular degeneration. Kidney section F was characterized by multifocal tubular degeneration (DT) with loss of epithelium. Kidney section G was characterized by degeneration of tubules (DT) and haemorrhage (H).

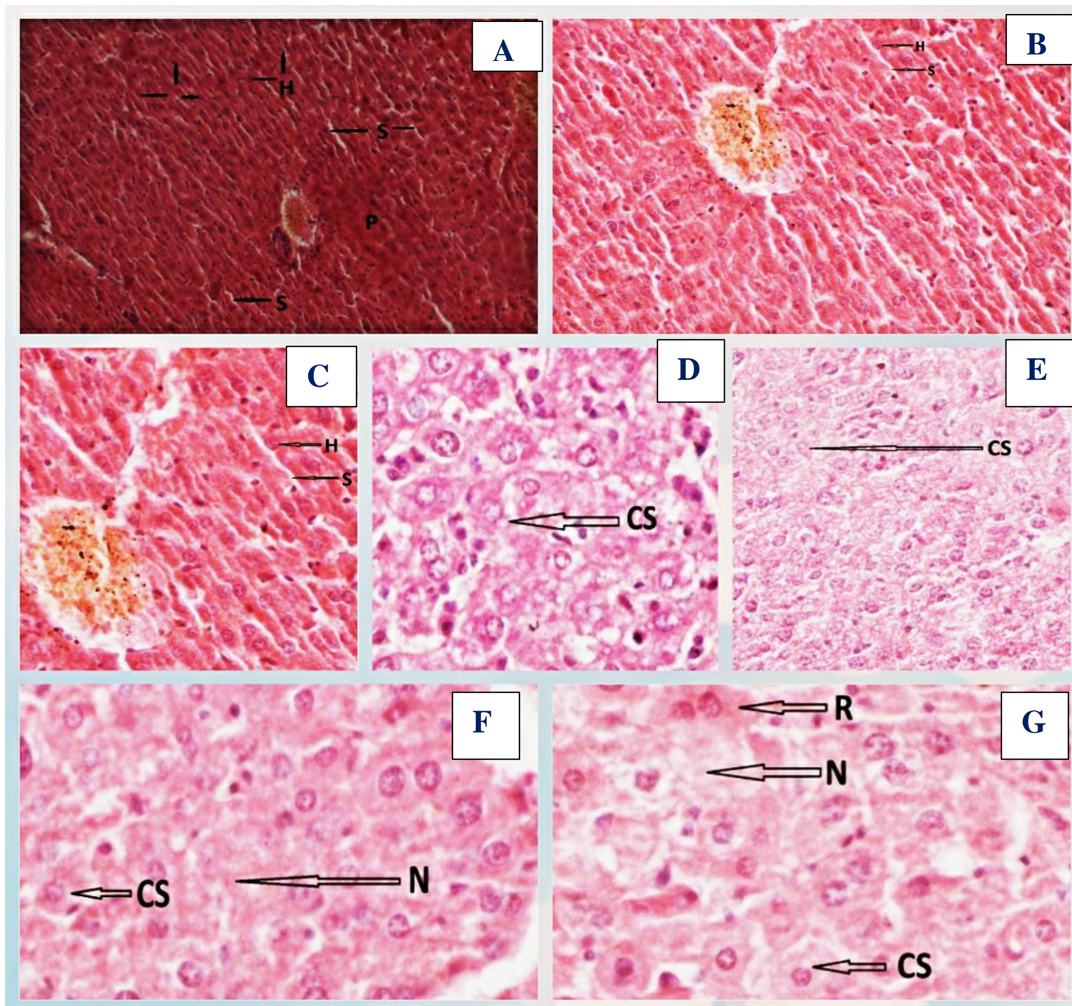


Figure 4.11: Liver sections from mice receiving different treatments.

A: Liver section from a mouse treated with distilled water/extra virgin oil. **B:** Liver section from a mouse treated with a 250mg/kg dose of aqueous extract of *F. hildebrandtii*. **C:** Liver section from a mouse treated with a 250mg/kg dose of hexane extract of *F. hildebrandtii*. **D:** Liver section from a mouse treated with a 500mg/kg dose of aqueous extract of *F. hildebrandtii*. **E:** Liver section of a mouse treated with a 500mg/kg dose of hexane extract of *F. hildebrandtii*. **F:** Liver section of a mouse treated with a 1000mg/kg dose of aqueous

extract of *F. hildebrandtii*. **G:** Liver section from a mouse treated with a 1000mg/kg dose of hexane extract of *F. hildebrandtii*. Liver sections A, B, C were characterized by normal parenchymal architecture with normal hepatic cells that were evenly distributed and separated by sinusoids. Liver sections D and E were characterized by diffuse cloudy swelling of the hepatocytes (CS) indicative of early necrosis with the pyknotic nucleus. Liver section F was characterized by multifocal hepatocyte necrosis with hepatocytes undergoing cloudy swelling with nuclear pyknosis (N). Liver section G was characterized by hepatocyte necrosis and pyknosis, cloudy swelling, necrotic hepatocyte and regenerating hepatocyte around the necrotic hepatocyte.

4.3 Antimicrobial susceptibility

Table 4.16 shows the antimicrobial activity of aqueous extract obtained from *F. hildebrandtii* at different concentrations. The MBC for *Salmonella typhimurium* and *Staphylococci aureus* was 400mg/ml and 200mg/ml respectively.

Table 4.16: Antimicrobial activity of aqueous extract with controls

	The minimum bactericidal concentration of *Fagaropsis hildebrandtii* aqueous extract									
	400mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml	Quality control	Positive control
Salmonella typhimurium	-	+	+	+	+	+	+	+	-	+
Staphylococci Aureus	-	-	+	+	+	+	+	+	-	+
Candida albicans	+	+	+	+	+	+	+	+	-	+

KEY: **+ - growth** **- - No growth**

Positive control – an organism with no drug

Quality control – an organism with the drug (flucloxacillin for the bacteria and fluconazole for the fungi)

Table 4.17 shows the antimicrobial activity of hexane extract obtained from *F. hildebrandtii* at different concentrations. The MBC for *Salmonella typhimurium* and *Staphylococci aureus* was 200mg/ml.

Table 4. 17: Antimicrobial activity of hexane extract with controls

	The minimum bactericidal concentration of *Fagaropsis hildebrandtii* hexane extract									
	400mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125 mg/ml	Quality control	positive control
Salmonella typhimurium	-	-	+	+	+	+	+	+	-	+
Staphylococci Aureus	-	-	+	+	+	+	+	+	-	+
Candida albicans	+	+	+	+	+	+	+	+	-	+

KEY: + **-growth** - **- No growth**

Positive control – an organism with no drug

Quality control – an organism with the drug (flucloxacillin for the bacteria and fluconazole for the fungi)

Table 4.18*: Optical density readings for* Candida albicans *for determination of MIC*

Aqueous Extract Concentration (mg/ml)	Baseline Optical Density Reading at start	Optical Density reading after 24 hours	Organic Extract Concentration (mg/ml)	Baseline Optical Density Reading at the start	Optical Density reading after 24 hours
400	0.23	0.24	400	0.24	0.25
200	0.22	0.23	200	0.24	0.25
100	0.22	0.23	100	0.23	0.24
50	0.20	0.21	50	0.22	0.23
25	0.17	0.18	25	0.18	0.20
12.5	0.13	0.15	12.5	0.15	0.19
6.25	0.90	0.10	6.25	0.11	0.16
3.125	0.08	0.09	3.125	0.05	0.15
Quality control	0.02	0.01	Quality control	0.02	0.01
Positive control	0.01	0.04	Positive control	0.01	0.05
Blank	0	0	Blank	0	0

KEY: **Quality control** - an organism with the drug (Fluconazole)

Positive control - an organism with no drug

Blank - peptone water

The MIC for *Candida albicans* could not be determined using the concentrations used since none of them showed activity against the organism for both aqueous and hexane extracts.

Table 4. 19: Optical density readings for *S. typhimurium* for determination of MIC

	AQUEOUS			*ORGANIC*	
Aqueous Extract Concentration (mg/ml)	start Optical Density Reading	Optical Density reading after 24 hours	Organic Extract Concentration (mg/ml)	start Optical Density Reading	Optical Density reading after 24 hours
400	0.23	0.20	400	0.24	0.22
200	0.23	0.23	200	0.23	0.21
100	0.23	0.24	100	0.23	0.23
50	0.21	0.22	50	0.22	0.24
25	0.91	0.20	25	0.21	0.24
12.5	0.15	0.17	12.5	0.20	0.22
6.25	0.10	0.20	6.25	0.19	0.21
3.125	0.06	0.09	3.125	0.18	0.20
Quality control	0.03	0.10	Quality control	0.02	0.01
Positive control	0.01	0.04	Positive control	0.01	0.05
Blank	0	0	Blank	0	0

KEY: **Quality control** - an organism with the drug (Flucloxacillin)

Positive control - an organism with no drug

Blank - peptone water

The MIC for *Salmonella typhimurium* was 200mg/kg for the aqueous extract and 100mg/kg for the hexane extract obtained from *F. hildebrandtii*. These are the concentrations were no change in baseline and after 24 hours optical density readings were detected.

Table 4. 20: Optical density readings for Staphylococcus aureus for determination of MIC

AQUEOUS			ORGANIC		
Aqueous Extract Concentration (mg/ml)	Start Optical Density Reading	Optical Density reading after 24 hours	Organic Extract Concentration (mg/ml)	Start Optical Density Reading	Optical Density reading after 24 hours
400	0.23	0.19	400	0.24	0.22
200	0.23	0.21	200	0.24	0.23
100	0.22	0.22	100	0.23	0.23
50	0.19	0.20	50	0.22	0.23
25	0.13	0.15	25	0.20	0.22
12.5	0.07	0.10	12.5	0.19	0.25
6.25	0.04	0.70	6.25	0.15	0.40
3.125	0.02	0.10	3.125	0.10	0.30
Quality control	0.02	0.01	Quality control	0.05	0.01
Positive control	0.02	0.09	Positive control	0.01	0.1
Blank	0	0	Blank	0	0

KEY**: Quality control** – an organism with the drug (flucloxacillin)

Positive control – an organism with no drug

Blank - peptone water

The MIC for *staphylococci aureus* was 100mg/kg for both aqueous and hexane extracts obtained from *F. hildebrandtii* plant root barks. This is the concentration where there was no difference in baseline reading and after 24 hours reading in optical densities.

Table 4.21**: Optical density readings for Negative controls in MIC determination**

AQUEOUS			ORGANIC		
Aqueous Extract Concentration (mg/ml)	Immediate Optical Density Reading	Optical Density reading after 24 hours	Organic Extract Concentration (mg/ml)	Immediate Optical Density Reading	Optical Density reading after 24 hours
400	0.20	0.20	400	0.21	0.21
200	0.20	0.20	200	0.21	0.21
100	0.19	0.19	100	0.19	0.19
50	0.17	0.17	50	0.19	0.19
25	0.14	0.14	25	0.15	0.15
12.5	0.10	0.10	12.5	0.11	0.11
6.25	0.04	0.04	6.25	0.06	0.06
3.125	0.02	0.02		0.01	0.01
Blank	0	0	Blank	0	0

KEY: **Blank** – peptone water

The negative control tubes only contained the plant extracts alone. There was no difference in the readings of the optical densities before and after the experiment.

CHAPTER 5

DISCUSSION

5.1 DISCUSSION

The medicinal value of *Fagaropsis hildebrandtii* can be based on the presence of bioactive phytochemical constituents as proved by results which produce a definite physiological action in the different body systems like other plants where extensive research has been done (Akinmoladun *et al.*, 2007). Alkaloids, saponins, flavonoids, tannins, terpenoids, and essential oils among others are some of the important bioactive compounds found in plants (Edeoga *et al.,* 2005) and they were found in this plant. This, therefore, explains the uses of *F. hildebrandtii* extracts in the treatment and management of various diseases.

F. hildebrandtii contains tannins which are astringent and therefore used in treating intestinal disorders like stomach ache, diarrhoea, typhoid among others (Akinpelu and Onakoya, 2006). Flavonoids were also present in *F. hildebrandtii* and therefore it can be used as an antioxidant (a scavenger of free radicals) (Mamta *et al.,* 2013). The presence of terpenoids can also explain why the plant is used in the management of respiratory tract infections like cough, asthma, chest pains because they help in soothing the irritated tissues and the mucous membrane in the respiratory tract lining (Krishnaiah *et al.,* 2009). Cardiac glycosides were also present in the hexane extract and therefore the plant can also be used in treatment and management of congestive heart failure and cardiac arrhythmias. This is by inhibiting Na +/ K+ pump leading the increased sodium ions in the myocytes and increased calcium ion leads. The calcium ions help to increase heart muscle contraction hence improved cardiac output and reduced heart distention (Krishnaiah *et al.,* 2009). The presence of saponins can also be used to explain why the plant has been used in the management of hypertension and cardiovascular diseases. Saponins are known to reduce the excessive intestinal absorption of

cholesterol which increases the risk of hypertension and cardiovascular diseases onset (Akinpelu and Onakoya, 2006). Saponins are also known to bind cholesterol forming insoluble complexes which are extracted in bile hence their additional use in the management of hypercholesterolemia. Alkaloids were also present in the plant and this supports why it has been used in the management of chronic pain, and hypertension like other plants which contain alkaloids. Alkaloid (morphine) from plants like *Papaver somniferum* have been used as narcotic analgesics. Reserpine from *Rauwolfia serpentina* has been used as an antihypertensive agent and cocaine from *Erythroxylum coca* has been used as an anaesthetic agent and as a central and nervous system stimulant. Colchicine which is also an alkaloid which has been used in gout management (Akinpelu and Onakaya, 2006; Priya, 2014). Alkaloids are also known to have antimicrobial activity in many plants (Karou *et al.,* 2005) like cryptolepine from *Sida acuta.*

The presence of phenolic compounds can explain the use of *F. hildebrandtii* in the management of inflammatory conditions and chronic pains. These compounds are known for their properties against oxidative damage which leads to degenerative diseases like inflammation, cancer, cardiovascular diseases among others (Priya, 2014).

Steroids are known for their use in the treatment of inflammatory conditions like systemic vasculitis, myositis, arthritis, and gout among others hence the use of the plants with steroids in the management of gout, arthritis, chronic pain and respiratory conditions (Bishop *et al.,* 2001).

According to OECD guidelines 423, the results shown by *F. hildebrandtii* aqueous and organic extracts at dosages of 300mg/kg and 2000mg/kg body weight of experimental animals, it is non-toxic since its LD50 is greater than 2000mg/kg according to OECD 423 guidelines. No death occurred during the study and therefore the LD50 of aqueous and

hexane extracts of *F. hildebrandtii* could not be determined. It is above the maximum single dose of 2000mg/kg Bwt administered to the experimental animals. The extracts are therefore safe for human use in single doses lower than the ones used in the experiment. This is because in all the dose levels used in the study, with close monitoring of the wellness parameters as shown in the results section indicate no cases of toxicity and significant behavioural changes on the animals (OECD,2008). Keen observation on the different groups of animals during the study period was done to note any changes in food intake, digestion, and frequency of urination, respiration rate, temperature changes, eye colour, sleep, and cases of sedation, tremors, coma, convulsions and even death. There were no signs of acute toxicity noted. There was no significant difference in mean body weight gain for the different treated groups compared with the controls. In all the experimental groups and the controls, there was a gradual increase in body weight which indicates that the extracts had no effect on the growth of the animals (Klaassen, 2001; Roy *et al.,* 2016).

There were no significant changes in body weight with the different doses administered and the control groups used. This indicates that single dose of *F. hildebrandtii* below 2000mg/kg Bwt has insignificant levels of toxicity/effects on the growth of mice, therefore most likely there were no effects on the metabolism of fat, carbohydrates and proteins. There was no change in the rate of water and food consumption during this study period. It remained within the normal range of 4-7ml water intake per day and 3-6gm feed intake per day (Michael, 2008; Alexander *et al.,* 2002). Necropsy and macroscopic examinations of the liver, kidneys, heart, spleen and lungs showed no abnormalities in size and colour in the treated mice after comparison with the controls. This was also observed by Roy *et al.,* (2016) when another plant *Senna allata* was used in Swiss albino mice. The relative organ weights of the kidney, liver, lungs, heart and spleen were compared between the treated and controlled groups and

the differences were not significant. The P-value was >0.05. The relative weight is a better indicator of toxicity than absolute weight (Demma *et al.,* 2007).

On the 28th day, the animals were sacrificed after diethyl ether inhalation of anaesthesia after which blood for haematological and biochemical tests was collected. Important organs like the liver, kidneys, heart, lungs and stomach were also harvested and preserved for histopathological procedures (Figure 3.5).

The body weights of mice treated with both the hexane and aqueous extracts of *F. hildebrandtii* of both 250mg/kg and 500mg/kg Bwt doses increased gradually throughout the study. Statistically, there was no significant difference in the mean weights of these treated groups compared with the controls. This was also observed in males treated with both hexane and aqueous 1000mg/kg Bwt doses and females on hexane 1000mg/kg Bwt extract. The P values were more than 0.05. This is an indication that these dose levels had negligible effects/toxicity on the growth of animals. There was no disturbance in carbohydrate, fat or protein metabolism (Klaassen, 2001). Females treated with aqueous extract 1000mg/kg Bwt dose showed a significant difference compared with the controls with a P-value of 0.024. This indicates there was an effect on the growth and rate of metabolism of fat carbohydrates and proteins (Klaassen, 2001; Roy *et al.,* 2016).

The liver and the kidneys are important organs in maintaining the major metabolic processes in the body hence their major consideration for the study. The liver detoxifies harmful substances in the body and the kidneys help in maintaining haemostasis through reabsorption of vital substances and waste excretion (Koffi *et al.,* 2014). ALT and AST are significant liver enzyme indicators of liver functioning. Their levels are used to indicate any damage to the liver (Shashi, 2007). As indicated by the results, the AST and ALT levels were elevated in mice treated with the highest dose of both aqueous and hexane *F. hildebrandtii* extracts

compared with the controls. The P-value was less than 0.05 as shown in the results. This may be an indication that this high dose had some toxic effects on the liver to cause tissue injury (Martin, 2006). This calls for caution when the plant extracts are being used for a prolonged period by people with known liver diseases or dysfunction.

The haematological parameters are of much interest because any changes from their normal values are indicative of possible toxic effects in the body and majorly the bone marrow and the spleen (Adebayo et al., 2005). They can also be used to determine any malfunctioning, diseases or histomorphology of major organs in the body (Olson *et al.,* 2000). The mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) are RBC indices used in anaemia classification. Diseases, chemical substances, drugs, toxins or any other substances which can cause damage to the bone marrow, spleen and toxic effects on the blood components can affect the levels of the haematological parameters.

The histopathological studies revealed that both males and females were affected the same by both the aqueous and hexane extracts obtained from *F. hildebrandtii* at different doses. However, the females were more sensitive to the toxic effects since more females died compared to males at the highest dose of 1000mg/kg Bwt. The histological studies did not reveal any abnormalities or severe tissue damage on the liver, spleen, kidneys and lungs in the mice treated with 250mg/kg Bwt aqueous dose in comparison with the controls and normal tissue organs. However, the liver from the mice treated with organic 250mg/kg Bwt dose showed very mild degenerative effects on the hepatocytes characterized by some cloudy swelling and focal hepatocyte necrosis.

The kidneys play a very vital role in the excretion of waste products from the body and reabsorption of minerals and water (osmoregulation). The kidneys from both the treated

groups and controls showed no significant or any pathology like degeneration, inflammation, necrosis, enlargement, congestion on the glomerulus, bowman's capsule, proximal convoluted tubule and the distal convoluted tubule (Thomas,2005).

The liver had a normal architecture for both treated and control mice with no significant signs of damage on the portal triad, sinusoidal vein, central vein and hepatocytes. The normal parenchymal architecture was maintained with normal hepatic cells that were evenly distributed and separated by hepatic sinusoids except in mice treated with 250mg/kg Bwt organic dose, 500mg/kg Bwt and 1000mg/kg Bwt doses for hexane and aqueous preparations.

The histopathological studies indicated that both the aqueous and hexane doses of 250mg/kg Bwt and 500mg/kg Bwt didn't have significant adverse effects on the morphology of tissues of the organs examined after a 28 day period of administration. This is further supported by the normal range of parameter values for these doses in the haematological and biochemical results. However, the 500mg/kg Bwt doses for both hexane and aqueous preparations showed some mild degenerative effects on the renal tubules characterized by a slight loss of epithelium and some early signs of hepatocyte necrosis. The vascular congestion in the liver noted in both control and a study animal on the 250mg/kg Bwt aqueous dose was not an effect of the drug administration.

The histopathological results of the mice which received the highest dose of 1000mg/kg Bwt for both aqueous and hexane extracts (those which died and those which survived) revealed that the kidneys were congested, the liver was mottled, the spleens were pale and congested and the lungs were congested. The kidneys had multifocal tubular degeneration and had lost most of the cell epithelium. The liver showed multifocal hepatocyte necrosis which was indicated by the presence of cloudy swelling with nuclear pyknosis in all the hepatocytes. In addition, for the dead ones the heart was rounded and enlarged. This was a sign of organ and

tissue damage by the 100mg/kg dose of *F. hildebrandtii*. This was further supported by the haematological and biochemical results of the surviving mice on 1000mg/kg Bwt doses which indicated a significant difference in platelets, RBCs and WBCs between the treated groups and controls with P-value <0.05. The levels of urea, creatinine and total proteins were elevated when the treated groups were compared with the controls with P-value <0.05. ALT and AST levels were also elevated in this group of mice. This is an indication that this highest dose was toxic to mice causing adverse effects and damage to the liver, spleen and kidneys. The accumulation of toxic compounds in the liver is bound to be associated with liver damage which is usually assessed by the determination of serum transaminases AST and ALT) as well as the measurement of total proteins (Araujo *et al.,* 2017; Okumu *et al.,* 2017)

Staphylococcus aureus is the most dangerous of all of the many common staphylococcal bacteria. Staphylococcal infections are a significant and common clinical problem in medical practice and most of them are resistant to commonly available antibiotics like penicillin and methicillin hence the use of flucloxacillin for positive control which is effective against both gram-positive and gram-negative bacteria (Rayner and Munkhof, 2005).

Salmonella typhii causes typhoid fever in humans which affects approximately 17 million people in a year causing 600,000 deaths. It is a very common disease in developing countries due to poor sanitary systems and lack of antibiotics (Goldrick and Barbara, 2003; Murray *et al.,* 2009).

Candida albicans is a member of human gut flora and becomes pathogenic in immunocompromised individuals in a variety of conditions. It is the most common fungal species isolated from the human tissue or implanted medical devices. It is also commonly used as a model organism in biology (dimorphic fungus) because it grows as both yeast and filamentous cells. Candida is also found worldwide and ranked as among the most common

groups of organisms which cause nosocomial infections. Candidiasis is the fourth most common hospital-acquired infection in the world (Gow, 2017; Erdogan and Rao, 2015).

All the tubes with the different dilutions of both aqueous and hexane extracts and the bacterial strains showed either increased or decreased optical density values and turbidity after 24 hours of incubation were all cultured in PCA plates for 24 hours and the colonies counted on each plate where growth occurred. This was used to determine the MIC and MBC values.

The MIC and MBC values of both aqueous extract and hexane extract for *Salmonella typhimurium* was 200 mg /ml and 400mg /ml ; 100mg/ml and 200mg/ml respectively. This shows that both aqueous and hexane extracts of *F. hildebrandtii* had inhibitory activity on *Salmonella typhimurium*. The MIC and MBC for *Staphylococci aureus* for both aqueous and hexane extracts were 100mg/ml and 200mg/ml; 100mg/ml and 200mg/ml respectively. It is also proved that they had an inhibitory activity of *Staphylococci aureus.* Gram-positive bacteria were more susceptible than gram-negative bacteria.

All the tubes with the different dilutions of both aqueous and hexane extracts and the fungal strain showed increased optical density values and turbidity after 24 hours of incubation. They were all cultured in PCA plates for 24 hours and the colonies counted on each plate were more than 300. Both extracts had no inhibitory activity and fungicidal effects on *Candida albicans.* The fungi were resistant to both aqueous and hexane extracts of *F. hildebrandtii* extract concentrations of 400mg/ml which is the highest concentration used which could be allowed by the yields using the extraction methods used.

The minimum inhibitory concentration and minimum bactericidal concentration shown for the different plant extracts on different bacterial species at different concentrations supports why the plant has been used for treatment and management of various infections in the body.

It has been used for respiratory tract infections, gastrointestinal infections, joint infections among others (Winfred *et al.,* 2007). This can also be supported further by the presence of phytochemical compounds (alkaloids, phenolic compounds, flavonoids, terpenes, coumarins) with an antibacterial activity which was present in the plant extracts as demonstrated by other studies on other plants (Priya, 2014).

5.2 CONCLUSION

The phytochemical screening showed that aqueous and hexane extracts of his plant contain alkaloids, tannins, saponins, terpenoids, flavonoids, cardiac glycosides, phenolics and steroids which are bioactive plant constituents. The isolation, purification and identification of these active compounds is a lead to drug discovery and development.

F. hildebrandtii aqueous and hexane extracts are non-toxic at a single dose below 2000mg/kg body weight.

The LD50 of *F. hildebrandtii* aqueous and hexane extracts are above 2000mg/kg body weight.

The acute toxicity studies indicate the safety of the oral administration of these root extracts of *F. hildebrandtii* as a single dose.

The research has revealed potentials of the plant being a good source of very important drugs. It has also formed a basis for why *F. hildebrandtii* has been used in the treatment, management and prevention of various diseases and disorders.

5.3 RECOMMENDATIONS

Further isolation, characterization and identification of the phytochemicals in this plant are highly recommended since it forms the basis for drug discovery and development.

For persons on long term use of this plant should have close monitoring of AST, ALT, urea, creatinine and total proteins levels to avoid damage on the kidneys and liver. The haematological parameters (especially WBCs, RBCs and platelets) should also be closely monitored to detect bone marrow and spleen damage.

It is important to do a chronic toxicity study for *F. hildebrandtii* plant to have more data on any captured adverse effects and the safe use of the plant.

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Appendix i: Ethical Approval Form

UNIVERSITY OF NAIROBI
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Dr Beatrice M. Muia
C/o Dept of PHPT

REF:FVM BAUEC/2018/163

17/08/2018

Dear Dr Muia

RE: Approval of Proposal by Biosafety, Animal use and Ethics committee

Studies on phytochemical composition, antimicrobial activity and toxicity profile of *Fagaropsis hildebrantii* root extracts

By Beatrice Mwende Muia : J56/88061/2014

We refer to the above MSc proposal that you submitted to our committee for review and approval. We have now reviewed the proposal and note that you have satisfactorily dealt with the issues that had been raised concerning the numbers of animals to be used, the sexes, animal husbandry and euthanasia.

We hereby approve your work as per the revised proposal that you submitted.

Rodi O. Ojoo BVM M.Sc Ph.D
Chairman,
Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine

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Appendix 2: Plagiarism Screening Report

Appendix 3: Paper Published from the study

Attached herewith is the paper which has been published in the F1000Research Journal on August 16th, 2019.