# ANTIBACTERIAL, ANTIFUNGAL AND SUB-ACUTE TOXICITY PROPERTIES OF *YUSHANIA ALPINA* (AFRICAN MOUNTAIN BAMBOO)

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**A Thesis Submitted in Partial Fulfilment for the Requirements of Masters of Science Degree in Pharmacology and Toxicology of the University of Nairobi**

**2023**

## DECLARATION

This Thesis is my original work and has not been presented for the award of a degree in this University or any other University.

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**Title of the work:** Antibacterial, Antifungal and Sub-Acute Toxicity Study of African Mountain Bamboo (*Yushania alpina*)

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## DEDICATION

I dedicate this research work to my lovely wife and my two sons who have always given me a reason to work hard and without whose support I would not have managed to come this far.

## ACKNOWLEDGEMENTS

I would like to thank my employer Laikipia County for according me a study leave to pursue further studies and the Government of Kenya through the Ministry of Health for awarding me a scholarship to pursue this course. I also acknowledge with a lot of appreciations my University supervisors Prof. T. E Maitho and Prof. James M. Mbaria for their invaluable support and always being available for consultation and guidance throughout my research work. I would also like to acknowledge entire team of staff of Department of Public Health, Pharmacology and Toxicology laboratory for their great support throughout my academic journey in the University of Nairobi.

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## LIST OF ABBREVIATIONS

ALP ……. Alkaline Phosphatase

ALT…… Alanine Transaminase

AST ……. Aspartate Transaminase

GGT ……Gamma-Glutamyl Transpeptidase

IZD……. Inhibition Zone Diameter

MBC……Minimum Bactericidal Concentration

MIC……. Minimum Inhibitory Concentration

MCH ……Mean Corpuscular Haemoglobin

MCHC……. Mean Corpuscular Haemoglobin Concentration

MCV ……Mean Corpuscular Volume

MHA……. Mueller Hinton Agar

MHB ……Mueller Hinton Broth

PCV……. Packed Cell Volume

SPSS ……...Statistical Package for Social Sciences

TSA……. Trypticase Soy Agar

WBC……White Blood Cells

YAS……Yushania Alpina Shoots

YASA……..... Yushania Alpina Shoots Aqueous

## ABSTRACT

In the recent times, chemotherapy against bacterial and fungal infections has faced a huge challenge because of resistance to antibiotics and antifungal agents and also emergence of new microbial infections which have proven difficult to treat. Therefore, traditional medicine forms an alternative therapy for such infections. Most of the local people rely on traditional medicine for the treatment of diseases. Furthermore, traditional medicine is regarded as a cheaper and user-friendly way of treating fungal and bacterial infections. The aim of this study was to investigate the antibacterial, antifungal and sub-acute toxicity properties of *Yushania alpina* shoots extracts which has been used in parts of Central Kenya and Rift Valley and also in Asia for the treatment of wounds and chest infections. For anti-bacterial and antifungal properties, this study applied two methods; Agar well diffusion method where the diameter of zones of inhibition was measured and broth dilution test where growth of microorganism was signified by turbidity in the test tubes once the microorganisms were incubated in relevant media in the presence of Yushania *alpina* shoots extracts. *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Candida albicans* were used as test microorganisms. A 28-day oral subacute toxicity testing was used in this study. A group of 40 healthy Sprague Dawley rats both males and females were selected randomly and allocated to three treatment groups and a control group. The three treatment groups were given orally the plant extracts daily at doses of 1000 mg/kg, 200 mg/kg and 40 mg/kg and control group was given distilled water. Their weights were taken at intervals of 7 days and data on daily food and water consumption was recorded. The animals were monitored daily for any manifestation of treatment related adverse effects in behavioural change, motor function, neurological changes and any other clinical symptoms. After 28 days the animals were sacrificed and examined for any abnormalities related to treatment. Selected organs were harvested in 10% formalin and were taken for histopathology analysis at Department of Veterinary Pathology, Microbiology and

Parasitology of the University of Nairobi. Blood samples were also collected and both Haematological and Biochemistry analysis were carried out using automated haematological analyser and automated biochemistry analyser respectively at Mama Lucy Kibaki Hospital in Nairobi in order to check for any signs of toxicity at cellular and enzymes levels. The results from antibacterial activity of *Yushania alpina* shoots extracts demonstrated activity against *S. aureus* from concentrations of 200 mg/ml for the aqueous extracts and 400 mg/ml for the Methanolic extract which showed that aqueous extracts have more potency than the Methanolic extracts against the test organism. Both extracts also resulted in activity against *B. cereous*. However, there was no activity recorded against *Escherichia coli* and *Candida albicans*. Inhibition zone diameter for *Staphylococcus aureus* and *Bacillus cereus* ranged from 8.5 mm to 12 mm which confirmed activity though lower compared with the control where Cefotaxime antibiotic was used. The activity followed a dose dependent response with activity increasing with higher concentrations of *Yushania alpina* shoots extracts. Data from food and water consumption, weight gain was tabulated and analysed using Statistical Package for Social Sciences where their means were compared with those from the control group of rats using independent t-test for significance at 95% confidence interval. The differences were found to be insignificant. Necropsies were done on the sacrificed rats and their organs and there were no abnormalities which were observed. The results from histopathological examination also did not reveal any Histological changes at the cellular level. Collected blood samples were analysed for the following parameters; Blood urea, Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), and Gamma-glutamyl transpeptidase (GGT) Total protein and Albumin levels, Haemoglobin concentration, Red blood cell count (RBC), White blood cell count (WBC), Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC). The results from the parameters which were tested showed values within the normal

ranges. Their means were compared to those of the control group of rats using independent t-test and the results were not significant at 0.05 significance level. The results demonstrate that *Yushania alpina* shoots has significant activity against *Staphylococcus aureus* and against *Bacillus cereous* with more potency observed in the former than the later. The activity observed was dose dependent which gradually changed from bacteriostatic to bactericidal with increase in concentration of the extracts. The *Yushania alpina* shoots extracts have no activity against *E. coli* according to the results. The results obtained indicate that *Yushania alpina* shoots extract has low toxicity in rats at 40mg/ml to 1000mg/ml concentration range since it did not produce significantly different results in the parameters tested for sub-acute toxicity. The plant can therefore be further studied in depth with the aim of having it added in pharmaceutical preparations for the management of infections which are caused by susceptible microorganisms.

# CHAPTER ONE

# INTRODUCTION

## 1.1Background information

Since early times, humans have practised herbalism, the major form of ethnomedicine, to manage various diseases (Ezekwesili *et.al,*.) Medicinal plants are relatively accessible, affordable, considerably efficacious and safer than synthetic drugs, hence their prominence worldwide. Indeed, the World Health Organisation estimates that a higher proportion of the global population (80 %) utilise herbals to meet their essential healthcare requirements, especially in less-developed countries such as Kenya (Theingi *et.al.*,). However, despite the enormous potential of medicinal plants in offering efficacious and safe lead compounds for drug development, due to the diverse array of pharmacologically active phytochemicals they produce, only a few have been investigated empirically (James *et.al.*,).

The lack of standardised methods of preparation, formulation, storage, labelling, marketing, and clear dosage regimens of herbal medicine for various diseases and patients and legal frameworks regulating traditional medicine have elicited safety and credibility concerns .In addition, there is insufficient empirical data on herb-herb and herb-conventional drug interaction and pharmacologic modes of action of various ethnomedicinally important plants, hampering the integration of herbal medicine into conventional healthcare (George *et.al*.,). Thus, it is imperative to empirically validate medicinal plants' pharmacological efficacy and safety to validate their healing claims and valorise promising ones as alternative sources of therapies.

Bamboo belongs to the family Poaceae and is the world’s longest grass. The plant can grow up to a height of 40 meters high and it consists of a hollow stem with nodes in between the segments of the leaves and the stems. The leaves of the plant and the culm stay evergreen throughout the year. In addition, this plant can grow for twenty years and flower only once in

every 7-12 years. This is mostly in Asia and Africa with some species predominating North America and South America. (Shukla *et.al*., 2012). *Oxytenanthera alpina* and *Yushania alpina* are the most predominant species in the African temperate regions (Stapleton, 2013). The two species belong to the tribe *Arundinaria* which is characterized by woody bamboo which lacks the capability to distinguish from indeterminate growth.

The buds of this species emanate from basal spikelet bracts, three stamens in each of the floret and ebracteate synflorescenceparaclades (Stapleton, 2013). Chusqueinae have been shown to be produced by *Arundinaria* of Asia, Africa, extending further to North, Central, and South America. This production is as a result of the evolution of reluctant inflorescences containing three stamens and minimal branched sheathing. Evolution traits have also been shown to have retransferred the *Guaduinae* and *Arthrostylidiinae* genus (Bamboo Phylogeny Group, 2012). *Arundinaria* genus was initially considered to contain three stamens but is not currently regarded as having three stamens (Zeng et al., 2010). The species within the tribe *Arundinarieae* vary depending on the weight of the characters and generic concept breadth. For instance, the Asian species close to the *Arundinaria* may be placed either into tribe *Arundinaria* and tribe *Bashani* on the basis of polyphyletic interpretation or tribe *Sarocalamus*on the monophyletic basis (Bamboo Phylogeny Group, 2012).Nonetheless, other morphologically distinct species of bamboo may be placed in other genera (Zhang *et.al.*, 2012).*Yushania alpina* (previously known as *Arundinaria alpina*) is indistinguishable from the American and Asian bamboo species, however, has a characteristic open panicle with reduced sheathing at the branching points which renders it ebracteate. Moreover, it has sheaths which resemble small tough bracts and sheath hairs (Stapleton, 2013). In the recent past, rapid diversification within the three stamens of bamboo appears to have materialized (Bamboo Phylogeny Group, 2013). Most of the diversification is inclined to the temperate and montane regions of the Andes Mountains, North East Asia and the Himalayas (Hodkinson et al., 2010).

Additionally, hybridization has been coupled with diversification which is linked to long-time generation (Hodkinson et al., 2010).

Bamboo shoots have low-fat content and high vitamins, potassium, carbohydrates, and dietary fibres (Chongtham *et.al.,* 2011). The shoots also contain glycoside and flavones which have been shown to exhibit antimicrobial properties. In addition, the shoots appear cream yellowish in colour after harvesting and have a good taste and strong smell. Studies have shown that the shoots and leaves are extracted to manufacture antimicrobial capsules and tablets. Banslochan are siliceous concentrations present in bamboo shoots and are used in Indian traditional medicine. Similarly, bamboo manna is another siliceous ingredient with healing properties but has been replaced by synthetic salicylic acid (Stapleton, 2013).

Bamboo shoots contain cyanogens, glycosides, urease, and hetain which are used for the treatment of threadworms, diarrhoea, jaundice, and cough (Chongtham *et.al.,* 2011). Boiled shoots of bamboo have been used traditionally in cleaning wound. Also, they have been known to treat ulcers and maggot infections. Bamboo shoots and leaves have also been utilized widely for the treatment of microbial infections especially in Japan and China (Choudhury *et.al.,* 2012). The plant's shoots are processed in a variety of ways which influence their chemical profile. There are also some accompanying physical changes in the nutritional and medicinal value of bamboo during canning, fermentation, and boiling (Srivastava *et.al*., 2011). For instance, the protein and sugar contents tend to decrease during boiling, nevertheless, fibre content remains stable. Studies reveal that fresh bamboo shoots have a higher nutrient content than canned and fermented bamboo shoots whereas moisture content remains high in canned shoots (Choudhury *et.al*., 2012). As such, the bamboo plant still remains an important plant for use both nutritionally and medicinally and therefore more research should be conducted on the plant.

Modern-day chemotherapy against bacterial and fungal infections has faced a huge blow as a result of resistance to antibiotics and antifungal agents This has resulted in the rise of bacterial and fungal infections with limited treatment options. As such, traditional medicine forms an alternative therapy for such infections. Most of the local people rely on traditional medicine as a source of disease treatment. Though similar research has been conducted elsewhere in the world *Yushania alpina* being a common grass plant in Kenya, very little study has been conducted scientifically on this specific species in regard to its antibacterial and antifungal medicinal uses. The species difference and also differences in climatic conditions and soil constituents under which this indigenous bamboo grows could lead to variations in pharmacologically active constituents in their extract. The current study is aimed at investigating the antibacterial and toxic properties of *Yushania alpina* in the laboratory by conducting both *in vitro* and *in vivo* studies.

## 1.2 Objectives

### 1.2.1 General objective

The overall objective of this study is to determine the antibacterial, antifungal and sub-acute toxicity properties of *Yushania alpina* shoots extracts.

### 1.2.2 Specific objectives

The specific objectives of the study are:

i. To determine the antibacterial properties of *Yushania alpina* shoots against gram positive and gram-negative bacteria.
ii. To determine antifungal properties of *Yushania alpina* shoots against *Candida albican.*
iii. To determine sub-acute toxicity effects of *Yushania alpina* shoots in Sprague Dawley rats.

### 1.3 Hypothesis

*Yushania alpina shoots* extracts contains Pharmacological compounds which have antibacterial activity against *Staphylococcus aureus, Bacillus Cereus, Escherichia Coli* and antifungal properties against *candida albican.*

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Introduction

This chapter contains the taxonomic classification of bamboo plant, medicinal and general uses of bamboo, active ingredients of bamboo extracts and the biochemistry and toxicity of bamboo plant.

## 2.2 Bamboo and the Poaceae family

Plants within the Poaceae family are important because of their industrial uses. The family consists of plants which capture much of the global interest due to their distinctive ecological value, a unique form of life and their wide range of values and uses (Chongtham *et.al.*, 2011). Bamboo plants grow mostly in the subtropical, temperate and tropical regions of the world. Moreover, the plants' diversity enable them to survive and adapt to various climatic conditions. More than 90 genera and 1200 species of bamboo have been described worldwide (Hossain *et.al.*,2016). Out of the 1200 species, 125 are found in India whereas more than 1000 species are found in Asia alone. Similarly, China accounts for more than 300 species with 44 genera (Singha *at.al*, 2013).

The plants grow mainly in forestry regions but may spread to the river banks, roadsides, farmland and rural areas. These plants are characterized by their long hollow stems (figure 1) with evergreen leaves emanating from the branches (Hossain *et.al.*2016). Furthermore, the length of the plants may vary from 30 centimetres long to 40 meters long. The stems of the plants are joined by nodes and oval leaves. In addition, the stems are incredibly flexible and highly tensile such that they can withstand strong winds (Shukla *et.al*., 2012). Today, approximately 2 billion people meet their renewable, low cost, easily accessible, environmentally friendly energy sources due to the widespread bamboo resources

(Chongtham*et.al*, 2011). Additionally, bamboo leaves are used widely in order to provide fodder for animals whereas the shoots have been utilized as food. (Shukla *et.al*, 2012).

**Figure 1: Stems of bamboo plants**

### 2.3 *Yushania alpina* subspecies of Bamboo

*Yushania alpina* species of bamboo resembles the Asian and American bamboo including the *Sarocalamus, Arundinaria*, and *Yushania*. In addition, *Yushania alpina* species is ebracteate due to its open panicle with reduced sheathing branching points. Similarly, the sheaths are reduced to bracts and tufts of hairs with more sessile spikelets that lacks the long pedicel (Stapleton, 2013). The plant's heights can reach up to 20m in their normal natural habitat with tall erect calms that are larger than those of Asian bamboo species. On the same line, the nodes of the culms and the branching differ significantly as compared to the Asian species. *Yushania alpina* branches show more variation in size in relation to the Asian bamboo types. The central branches are stronger and more dominant. Branch orientation is less erect in *Yushania alpina* compared to other *Yushania* species hence appears to be more horizontal. Also, the top parts of the branches contain a sulcate internode due to the development of strong branches. This species has a dense ring of short aerial roots at the lower node's regions of the culm (Stapleton, 2013).

### 2.4 Bamboo shoots and Human health

Bamboo shoots (figure 2) have been shown to exhibit numerous nutritional values. The shoots contain low calories with high fibre and nutrients (Shukla *et.al*, 2012). The main nutritive contents in bamboo shoots constitute in bamboo in bamboo salts and minerals (Li et.al, 2016). Moreover, they also contain a wide range of minerals such as potassium, manganese and phosphorus. Freshly harvested bamboo shoots are a rich source of phosphorus, B6, Vitamin A, and Vitamin E. Similarly, bamboo shoots have been proven to contain 17 amino acids 8 of which are essential amino acids.

**Figure 2: Shoot of *Yushania alpina* species of bamboo**

The fat content in the bamboo shoots ranges from 0.26% and 0.94% whereas the total sugar content averages 2.5%. With regard to minerals, bamboo has a higher mineral content than most of the vegetables apart from spinach and potatoes. Magnesium has a major role in enhancing metabolism, as such, the consumption of bamboo shoots significantly boosts the body's metabolism uses (Ainezzahira1 *et.al*, 2017).

**2.5 Use of Bamboo in Traditional Medicine.**

Exotic bamboo shoots contain glycosides and flavones which have substantial antimicrobial properties. These components can be extracted to manufacture tablets and capsules. The shoots used in traditional Indian medicine have siliceous concentrations of banslochan which have unique healing properties but has been currently replaced by salicylic acid (Chongtham*et.al*.,2011) Furthermore, *Bambusa arundinaria* contains nucleases, glucosides, hetain, choline, urease and cyanogens which are effective against worm infestations, cough and diarrhoeal infections .Bamboo shoots which have been boiled serve as appetizers and may be used in wound cleaning and treatment of maggots' infection sores. Similarly, a mixture of bamboo shoots extracts and palm-jaggery can induce parturition and abortion. *Bambusa vulgaris* shoot sap has been used in the treatment of jaundice as well as preparation of steroidal drugs (Shukla *et.al.,* 2012). Moreover, the shoots of the plant which exhibit antioxidant, anti-aging, anti-free radical activity are used as anticancer agents especially in Asia (Li *et.al.,* 2016).The dietary flavonoids present in bamboo shoots have an anti-proliferative activity which protects the body against cancer and cardiovascular diseases (Park and John, 2009).The Chinese traditional medicine history uses bamboo shavings for the alleviation and curing of stomach ache, vomiting and diaphragm inflammation. Also, the shoots are claimed to protect the heart (Chongtham *et.al*, 2011). In Kenya *Yushania alpina* stem has been used in powdered form among the Marakwet community where its known locally as *Tegaa* and is used in the management of oedema and also as a blood cleanser. (Gabriel. *et.al*., 2017).

Traditionally, bamboo leaves have been used for medicinal purposes in livestock. Among some tribes in India, bamboo leaves have been used during delivery in order to expel the placenta. (Arunbhai .2005). This is also confirmed by traditional Chinese medicine where bamboo shoots were used to ease labour and expulsion of placenta by inducing uterine contractions in livestock. (Jun, 2015). Species of Moso bamboo has been used in traditional medicine to

control fungal infections in plants where studies have shown antifungal activity against some fungal species such as *Fusarium graminearum, Valsa mali and Botrytis cinerea in vitro* (Liao *et.al,*). However, there is scanty data on investigation of antifungal activity against *Candida albican* which is a major causative agent of human and animal diseases.

**2.6 Food uses of Bamboo**

Bamboo shoots are usually harvested when they attain a height of about 15cm.The shoots are washed and eaten as vegetables in soup by mixing with meat or fish (Hossain *et.al.,* 2016). Upon exposure to sunlight, bamboo shoots become bitter due to the build-up of cyanogenic glycosides. Young shoots of clump-forming and running bamboo are both edible (Singhal *et.al*.,). The shoots are a source of low fat, good dietary fibre and calories in humans. Also, the high protein and less fat content coupled with the presence of amino acids potentiate its antioxidants effects in humans (Li *et.al*., 2016). Most of this food is mixed with vegetables and meat for recipes. The nutritional value of bamboo is varied between species hence, the value may vary depending on the stage of processing of the plants. For instance, cooked or boiled bamboo shoots contain a high nutritive value than fresh bamboo shoots (Hossain. *et.al.,* 2016). China and Taiwan are the leading exporters of bamboo shoots which are either fermented, fresh or roasted and are considered culinary treats. Furthermore, the shoots are tender and crisp but can however be canned and frozen (Singhal *et.al*., 2013). Preparation of bamboo shoots involves the removal of the sheaths, cutting the shoots, boiling in water and eventually adding salt. Tender portions of the shoots are steamed to reduce the content of hydrocyanic acid which is aimed at removing bitterness and acidity (Singhal *et.al*., 2013).

**2.7 Phytochemical Composition of Bamboo extracts**

A bamboo extract manufactured through the water-ethanol process followed by membrane filtration chromatographic techniques and vacuum concentration has been processed in most

Asian Countries. The main components in the extract include lactones, flavoids (flavone glycosides, vitexin, orientin, and tricin.) and phenolic compounds. Due to its water solubility properties, the extracts are applied in dietary supplements in capsule form, milk powder and nutraceutical beverages (Shukla *et.al*., 2012).

**2.8 Active constituents of Bamboo**

Previous studies on exotic bamboo species have revealed that the plant contains high levels of acetylcholine which is a neurotransmitter found in the human brain. These compounds contribute significantly to the effects of the plant on the brain. Furthermore, the plant's shoots contain flavoids such as vitexin and orientin which have been utilized as antioxidants. These flavoids are responsible for the reduction of inflammation, inhibition of allergic reactions and promotion of circulation (Shukla *et.al.,* 2012). A study conducted in order to investigate chemical constituents in bamboo shoots isolated various amino acids such as tryptophan, tyrosine, adenosine, guanosine and phenylalanine from shoots of bamboo. In addition, carbohydrates such as fructose, hydrolysate, glucose, and galactose were found to be present (Jia Sun *et.at*.,2015).

The shoots of bamboo contain cyanogenic glycosides which are characteristic of nitrogenous phytoanticipins which are used as a defence mechanism against predators. Hydrogen cyanide is formed by the mechanism of cyanogenic glycosides degradation driven by the enzyme beta –cyanoalaninesynthase (Shukla *et.al*., 2012).

**2.9 Toxic effects of Bamboo in Humans and Animals**

Bamboo shoots contain huge amounts of cyanogenic glycosides which are responsible for their toxic effects in humans. Taxiphyllin (Figure 3) a cyanogenic glycoside, has been found to be the most toxic principle in nearly all the bamboo species. The shoots contain 0.3 %- 0.8% cyanide however due to sequential processing, the cyanide content reduces substantially.

Similarly, incomplete cooking results in glycoside hydrolysis and an increased hydrogen cyanide release. Studies show that there is acute cyanide poisoning in humans due to cyanoglycosides which is manifested by sudden onset of alteration of consciousness and metabolic acidosis. The other symptoms may include lethargy, hyperventilating, vomiting, dark red mucous membranes rapid heart rate and in serious cases it may progress to respiratory failure, shock, coma and sudden death. Hydrogen cyanide formation causes the inhibition of cytochrome oxidase which inhibits oxidative phosphorylation and intracellular oxygen utilization resulting in cardiac arrest. (Nongdam *et.al*., 2014).However, the cyanogenic glycoside's content varies between different species as well as different parts of the plants (Shukla *et.al.*, 2012).

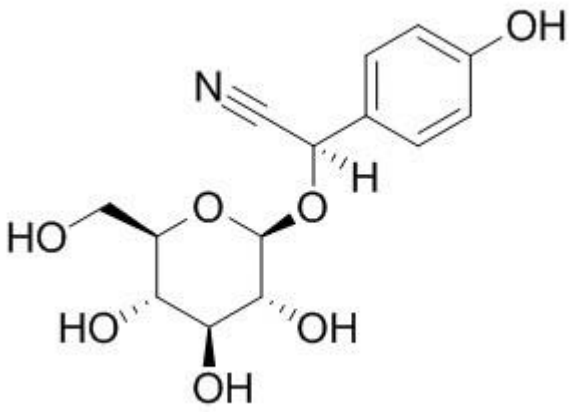


**Figure 3: Structure of Taxiphyllin**

In a previous study done in pregnant rabbits, an aqueous extract of *Bambusa vulgaris* leaves increased the frequency of abortion and caused a decrease in foetal survival rate at doses of 250-500 mg/kg body weight per day (Yakubu *et.al.*,2009). Previous studies have showed that bamboo shoots extracts can cause hypothyroidism-like effects in vivo through inhibition of thyroid peroxidase enzyme (Chandra et.al.,2004). Consumption of bamboo shoots was also associated with cases of hyperuricemia which is a risk factor in cardiovascular diseases during

an epidemiological study done in Taiwan (Chuang et al., 2011). In a study done in Thailand, dietary intake of bamboo shoots was associated with worsening of dyspepsia (Premgamone et al., 2010).

Cyanide poisoning is associated with increased neonatal births, behavioural defects, thyroid dysfunction, and lower birth rates. Studies indicate that the shoot of bamboo may contain as much as 8g/kg of hydrogen cyanide (Nongdam *et.al*., 2014). As such, increased consumption of bamboo shoots may poison consumers, hence proper processing should be observed in order to eliminate excess cyanide.

.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study area

The *Yushania alpina* shoots were obtained from Kipipiri Sub-County in Nyandarua County (Figure 4a and 4b). This study area was arrived at since *Yushania alpina* occur in its natural habitat in this county and has been used by herbalists for medicinal uses. Nyandarua County is located $0^0$ 33' 0'' South, and $36^0$' 0'' East with an average temperature of $14.5^0$ C and an average annual rainfall of 978mm. The County has an altitude of 2303 meters above sea level.

**Figure 3a: Location of Nyandarua County in Kenya**

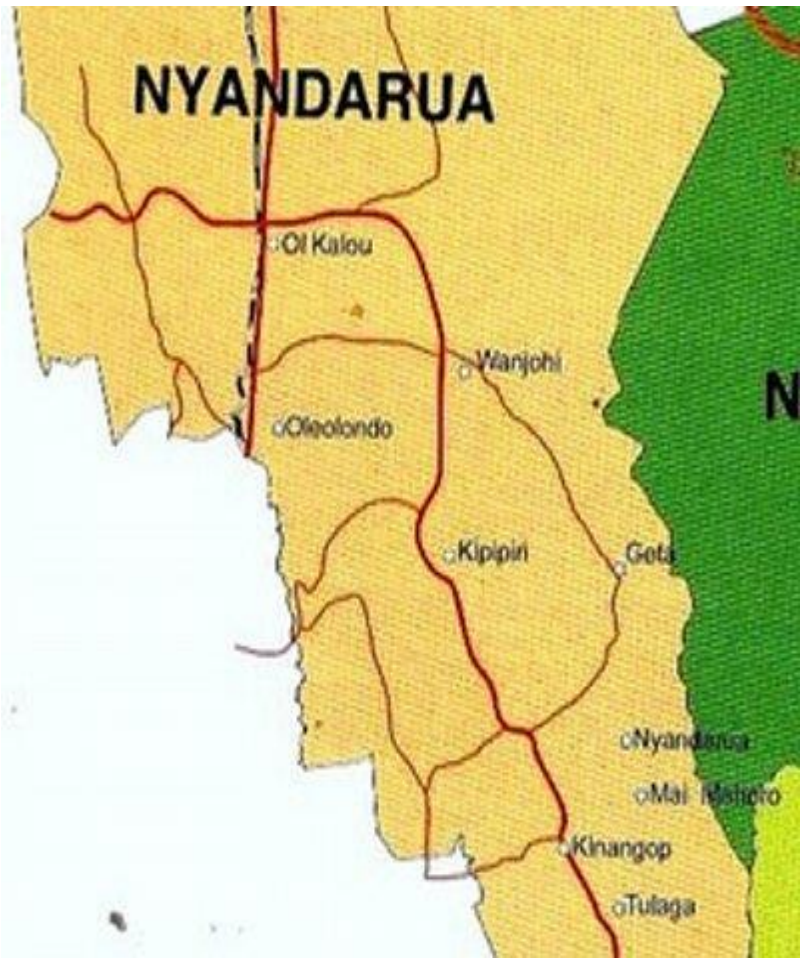


**Figure 4b: Map of Nyandarua County**

### 3.2 Collection of Plant materials

Fresh *Yushania alpina* shoots were collected with the assistance of a renowned herbalist, and sample specimens were authenticated taxonomically at the National Museums of Kenya (NMK/BOT/CTX/1/2), and duplicates were deposited for future reference. The shoots were then transported to the Department of Public Health, Pharmacology and Toxicology of the University of Nairobi where laboratory work was carried out.

The samples were chopped and distributed on a wooden laboratory bench to dry naturally, away from direct sunlight, in a well-ventilated environment for three months. Occasional grabbling was done to deter moisture build-up and facilitate even drying.

### 3.3 Research design

The study used experimental methods.

### 3.4 Acquisition, storage and feeding of laboratory animals

The Sprague Dawley Rats which were used in this study were obtained from the animal house of the Department of Public Health Pharmacology and Toxicology of the University of Nairobi. The rats were kept under standard conditions of ventilation, humidity (55% ±2), temperature (24°C ±1°C) and light (12hour light and 12 hours darkness). The rats were fed on rats' pellets and water ad libitum.

### 3.5 Plant extracts preparation.

The bamboo shoots were air-dried in the shade (Susan *et.al.*,2011) in the Laboratory at Department of Public Health, Pharmacology and Toxicology of the University of Nairobi for three months. The shoots were then grounded into fine powder using an electric plant mill. The powder was weighed using a scientific analytical weighing balance and packed in sterile and clean airtight polythene papers in portions of 300 grams awaiting extraction.

### 3.5.1 Methanolic extract.

A total of 300 grams of the powdered shoots were soaked in one-litre analytical grade methanol in a 2-litre conical flask and then covered with an aluminium foil and shaken for 72 hours (Qing-Wen Zhang et.al.,2018). The menstruum was filtered out by decanting through a Whatman filter paper Number 1. This procedure was repeated several times to exhaust the extraction process. The obtained filtrate was then mixed and concentrated in vacuo at 55°C in a rotary evaporator, transferred into dry clean glass tubes and dried in an oven at 25°C. The extract was then weighed using a simple analytical balance and stored in a refrigerator at 4°C. awaiting further tests.

### 3.5.2 Aqueous extracts.

A total of 850 grams of ground shoots were soaked in three litres of distilled water in a conical flask. The mixture was then heated at 60°C in a water bath for two hours. The extract was then filtered using cotton gauze and then passed through cotton wool. The resultant filtrate was freeze dried using carbon ice with acetone and added to the dry ice in order to enhance quick solidification of extract. The solidified extract was then put in a freeze drier for 48 hours in order to separate the extract and water. The extracts were then placed in brown airtight sample bottles and the extracts were stored at $4^0$ C in a refrigerator. (Wen Zhang *et.al.*,2018)

### 3.6 Culture media preparation

Mueller Hinton Agar (MHA) was used for disc diffusion method while Mueller Hinton Broth (MHB) was used for disc diffusion assay. Tryptic Soy Agar (TSA) was used in the anti-fungal assay using *Candida albican* as the test organism.

### 3.7. Antibacterial Assay

### 3.7.1 Agar well Diffusion

Agar well Diffusion was used as the standard method with *Escherichia coli* (BL21) for gram negative bacteria, *Staphylococcus aureus* serotype 1 (CP1) for gram positive bacteria and

*Bacillus cereous* serotype A for anaerobic bacteria (Manikkuwadura *et.al.,* 2019) . Nine serial concentrations of *Yushania alpina* shoots extracts were prepared in 10% Dimethyl sulfoxide. 800mg/ml, 400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml. Mueller Hinton Agar (MHA) was poured on sterilized plates and allowed to solidify. The plates were then surface inoculated with the test microorganisms using cotton swabs. Four holes about 6 mm in diameter were punched aseptically using a sterile cork borer in each petri dish and numbered 1-8 for each microorganism. 100 ul of the extract from each of the concentration was added to the wells starting from the lowest concentration to the highest concentration. For the control experiment, Cefotaxime antibacterial discs were used since cefotaxime has a broad spectrum activity against gram negative, gram positive and anaerobic bacteria hence would form a good positive control experiment. The plates were then incubated overnight at 37° C to allow for growth. The diameter of inhibition was then measured for both the test plates and the controls using a vernier calliper.

### 3.7.2 Broth dilution test

In this method, *Escherichia coli* (BL21) for gram negative bacteria, *Staphylococcus aureus* serotype 1 (CP1) for gram positive bacteria and *Bacillus cereous* serotype A for anaerobic bacteria were used in the study. (Manikkuwadura et.al., 2019). Mueller Hinton Broth (MHB) was prepared following manufactures specification. Serial dilutions of *Yushania alpina* shoot extracts were then prepared in sample tubes using MHB as the solvent, 800mg/ml, 400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml. Two sets of such dilutions were made for each test microorganism. Each of the sample tube containing the plant extracts was inoculated with 100ul of the test organism and incubated at 37° C overnight. The tubes were then examined after 24 hours for any growth in form of turbidity and results were recorded in the Tables.

### 3.7.3 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The test tubes from broth dilution method which did not record any observable growth were set aside. Mueller Hinton Agar was then prepared and poured into the petri dishes and mixed with the contents of the tube which did not record any observable growth. The tubes were then incubated overnight at 37° C and observed the following day for any signs of microbial growth ( Andrew J.M 2001). The results were then recorded in Tables.

### 3.8 Antifungal Activity against *candida albican*

#### 3.8.1 Agar well diffusion test

Agar well diffusion test (Balouiri *et.al.,* 2016) was used for antifungal susceptibility test with *Candida albican* as the test organism for screening of antifungal activities. The crude methanolic and aqueous extracts of plants were dissolved in Dimethyl sulfoxide (DMSO) and then diluted to final concentrations of 800mg/ml as the stock solution from which serial dilutions of 400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml were prepared for use in the antifungal activity test. The plates containing Tryptic Soy Agar were surface inoculated by spreading the C*andida albican* inoculum over the entire agar surface using sterile swab. A hole with a 6 mm diameter was then punched aseptically with a sterile cork borer and a 100µl volume of the antifungal agent were introduced into the well, where Fluconazole was included as a positive control. The plates were then incubated at 25°C for 24 hours. After 24 hours the Inhibition Zone Diameters (IZD) were measured in millimetres.

#### 3.8.2 Broth dilution test

*Candida albican* was used as the test organism for testing of antifungal activity of *Yushania alpina* aqueous and Methanolic extracts. Mueller Hinton Broth was prepared using manufacturer's specification. Serial dilutions of *Yushania alpina* shoot extracts were then

prepared in sample tubes by using Mueller Hinton Broth as the solvent, 800mg/ml, 400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml. Two set of such dilutions were made for each test microorganism. Each of the sample tube containing the plant extract was then inoculated with 100ul of the test microorganism and was incubated at 25° C overnight. The tubes were then examined for any growth of the organism. The data collected was qualitative as it involved observation to check whether there was turbidity which signified growth of microorganism or lack of it which implied the extracts had inhibited the growth of microorganism. The results were recorded down.

### 3.9 Testing Sub-Acute Oral Toxicity

In testing for sub-acute oral toxicity, Organisation for Economic Co-operation and Development (OECD) guidelines 407 was used with Sprague Dawley Rats as the laboratory animals.

#### 3.9.1 Weighing and allocation to treatment groups

The animals were weighed using a simple analytical balance (Mettler PM 4600, Switzerland). They were then allocated randomly to treatment groups and the rats were labeled at the bottom of the tail. Four groups comprising five animals for each sex were formed and housed in separate cages throughout the duration of the study. The four groups were as follows;

i. Treatment group one received 1000mg/kg
ii. Treatment group two received 200mg/kg
iii. Treatment group three received 40mg/kg
iv. Control group received distilled water

The rats were weighed and their weights were recorded at the beginning of the experiment.

#### 3.9.2 Treatment with the plant extract and observation

The animals were observed for five days before the commencement of the study in order to allow them acclimatize to the lab conditions.The weights of the animals were used to calculate

the specific dose for each rat. The aqueous extracts were then used to prepare solutions for the three dose levels. The rats were fasted overnight prior to the start of the treatment and then the extracts were administered orally through oral gavage once daily for twenty-eight days. All the animals were observed daily for any signs of toxicity, including feed and water intake, behavioural and neurological changes, abnormal secretions, and motor function changes, among other clinical symptoms of toxicity. The rats were also observed for mortality and were weighed on day, 7, 14, 21 and 28.

### 3.9.3 Study termination

After 28-day study, the rats were observed for one extra day for any delayed symptoms. They were then fasted overnight and a gas chamber containing lethal dose of Diethyl ether was used for euthanasia. Two sets of blood samples were obtained through capillary bleeding through the eye. One set was collected in sample bottles containing Ethylene diamine tetra-acetic acid (EDTA) for the purpose of haematological testing while the other set was collected in plain sample bottles and centrifuged in order to obtain serum for biochemistry work. The concentration of haematological and biochemistry parameters was measured using automated haematological and biochemical analysers at Mama Lucy County Hospital.

The animals were then fastened on a dissection board and were sacrificed for macroscopic examination and harvesting organs for histopathology. This was carried out by opening the abdominal and thoracic cavities. Internal membranes, intestines were carefully observed for any abnormality, general observation was also carried out on liver, kidneys, spleen and gonads and any colour, morphological changes or presence of any lesions were noted and recorded. The kidneys, spleen and liver were harvested and stored in 10 % formalin and they were taken to Histology laboratory for Histopathological examination.

### 3.9.4 Histopathology

Histopathology work was carried out at the Department of Veterinary Pathology, Microbiology and Parasitology. Necropsies were conducted in the rats by using Mayo's scissors and a thumb forceps in order to check for cytotoxicity. A 10% of neutral buffered formalin was used to fix the necropsy samples. The samples were then processed, blocked with paraffin wax, cut into 5ul microtome sections and then stained with Eosin and Haematoxylin and Masons Tris-chrome stains. The tissue sections were examined under a light microscope using x40 and x100 oil immersion objective lens, any morphological changes, and toxicological signs such as inflammation, ulceration, cell atrophy, cell infiltration, or deranged architecture, discolouration, dead cells, among others were recorded. Micrographs were taken using a microscope linked camera.

### 3.9.5 Disposal of carcases

Once the dissection process was completed for all the groups, the carcases were transferred to the Department of Veterinary Pathology, Microbiology and Parasitology and disposed through burying into the disposal pit.

### 3.10 Data processing, Statistical Analysis and Reporting.

Qualitative data were recorded and presented in prose and in the form of photographs and micrographs. Quantitative data was entered into a Microsoft spreadsheet (Microsoft 365) and transferred to Minitab version 21.1 software (State College Pennsylvania; Minitab, Inc. www.minitab.com) for statistical analysis. The data was analysed descriptively, and the results were expressed as $\bar{x} \pm SEM$ of replicate measurements or observations. After that, inferential statistics were performed using a One-Way Analysis of Variance (ANOVA) to determine significant differences among means. Unpaired student t-test was also perfomed to compare the differences between two independent variables. Values with $P<0.05$ were considered significant.

### 3.11 Ethical consideration

Relevant approvals for the study were obtained from Faculty Biosafety, Animal Use and Ethics Committee and National Commission for Science Technology and Innovation (NACOSTI). Confidentiality was also observed throughout the study.

# CHAPTER FOUR

# RESULTS

## 4.1 Testing of anti-bacterial activity

The results of antibacterial activity of *Yushania alpina* shoots extracts were recorded and are shown in Tables 1 to Table 3.

**Table 1: Inhibition zone diameter of bacteria by *Yushania alpina* shoots extracts**

| **Micro-organism** | **Extracts** | **Extracts concentration in mg/ml** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 800 | 400 | 200 | 100 | 50 | 25 | 12.5 | 6.25 | 3.125 |
| | | **Inhibition zone diameters in millimeters** | | | | | | | | |
| *S.Aureus* | methanol | 8.5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | aqueous | 11.5 | 11.5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| *E. coli* | methanol | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | aqueous | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| *B. cereous* | methanol | 13.5 | 9,5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | aqueous | 12.5 | 10.5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |

Anti-bacterial activity was recorded against *B cereous* and *S. aureus as shown by the inhibition diameter shown above.* The activity was however less compared to that of the positive control.

**Table 2: Anti-bacterial activity of *Yushania alpina* activity against selected bacteria.**

| Micro-organism | Extracst | Anti-microbial activity |
|---|---|---|
| *S.aureus* | Methanol | + |
| | Aqueous | + |
| *E. coli* | Methanol | - |
| | Aqueous | - |
| *B.cereous* | Methanol | + |
| | Aqueous | + |

In both methanolic and aqueous extracts considerable activity was recorded in concentration of 400 mg/ml and 800 mg/ml in *staphylococcus aureus* and *bacillus cereous*. However, both extracts did not show any antibacterial activity against *Escherichia. coli.*

**Table 3: Interpretation of antibacterial activity**

| Symbol | Diameter | Classification |
|---|---|---|
| - | < 6 mm | No activity |
| + | 10- 15 mm | Activity |
| ++ | 15 - 20 mm | Good activity |
| +++ | >20mm | Very good activity |

### 4.2 Testing of anti-fungal activity

The findings of anti-fungal activity of *Yushania alpina* shoots extracts against *Candida albican* are shown in Table 4.

**Table 4: Zone of inhibition of fungal growth by *Yushania alpina* shoots extracts.**

| Micro-organism | Extracts | Extracts concentration in mg/ml | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 800 | 400 | 200 | 100 | 50 | 25 | 12.5 | 6.25 | 3.125 |
| | | Inhibition zone diameters in millimeters | | | | | | | | |
| *C. albicans* | methanol | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | aqueous | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |

There was no anti-fungal activity against *Candida albican* which was detected across the concentration ranges for both aqueous and methanolic extracts as indicated on the table above where there was no zone of inhibition.

### 4.3 Sub- acute toxicity testing

#### 4.3.1 Wellness parameters

In this study, all the experimental rats (both sex) administered with the test extract appeared normal throughout the 28-day study period. Also, the treated rats did not exhibit any clinical, behavioural, motor, or neurological signs of extract-induced toxicity. No morbidity or mortality was observed in extract-treated rats, even at 1000 mg/Kg BW, during the 28-day experimentation period. Thus, the $LD_{50}$ of the studied plant extract was deemed >1000 mg/Kg BW.

#### 4.3.2 Feed and water intake

The results revealed no significant differences in the average daily feed intake in experimental rats (both sex) treated with the test extract at all dose levels ($P>0.05$; Table 5). Similarly, the average daily feed intake recorded in the male control group rats was comparable to those recorded in rats (male) administered 40 mg/Kg BW and 200 mg/Kg BW, respectively, of the test extract ($P>0.05$; Table 5). Besides, the difference in average feed intake in female experimental rats treated with 40 mg/Kg BW of the study extract was significantly lower than

that recorded for the female control group rats (P<0.05; Table 5). Male rats' average daily feed intakes were significantly higher than those recorded for female rats (P<0.05; Table 5).

**Table 5: Average daily feed intake**

| Treatment | Average daily feed intake (g) | |
|---|---|---|
| | **Male** | **Female** |
| Control | $114.62 \pm 3.54^{a}_{a}$ | $90.07 \pm 2.00^{a}_{b}$ |
| 40 mg/Kg BW of *Y. alpina* | $106.07 \pm 2.42^{ab}_{a}$ | $80.20 \pm 1.36^{b}_{b}$ |
| 200 mg/Kg BW of *Y. alpina* | $105.89 \pm 2.89^{ab}_{a}$ | $86.67 \pm 1.05^{ab}_{b}$ |
| 1000 mg/Kg BW of *Y. alpina* | $101.76 \pm 2.58^{b}_{a}$ | $86.00 \pm 2.73^{ab}_{b}$ |

Values are presented as $\bar{x} \pm SEM$ of replicate measurements (28 days). Means with similar superscript alphabets within the same column are not significantly different (P>0.05; One-Way ANOVA); Means with different subscripts alphabets within the same row are significantly different (P<0.05; Unpaired student t-test); Control group rats received distilled water (10 ml/Kg BW; *orally*).

The results showed no significant differences among the average daily water intakes recorded in male experimental rats (P>0.05; Table 6). Generally, the average daily water intake by the male experimental rats was significantly higher than those of female rats (P<0.05; Table 6). Besides, no significant differences in the average daily water intakes were observed between the female control rats and those (female) treated with 200 mg/Kg BW and 1000 mg/Kg BW of the extract, and among those treated with the test extract at all doses (P>0.05; Table 6)

**Table 6: Average daily water intake**

| Treatment | Average daily water intake (ml) | |
|---|---|---|
| | **Male** | **Female** |
| Control | $143.21 \pm 6.83^{a}_{a}$ | $90.07 \pm 2.00^{a}_{b}$ |
| 40 mg/Kg BW of *Y. alpina* | $137.86 \pm 7.01^{a}_{a}$ | $80.20 \pm 1.36^{b}_{b}$ |
| 200 mg/Kg BW of *Y. alpina* | $146.07 \pm 7.52^{a}_{a}$ | $86.67 \pm 1.05^{ab}_{b}$ |
| 1000 mg/Kg BW of *Y. alpina* | $128.75 \pm 8.73^{a}_{a}$ | $86.00 \pm 2.73^{ab}_{b}$ |

Values are presented as $\bar{x} \pm SEM$ of replicate measurements (28 days). Means with similar superscript alphabets within the same column are not significantly different (P>0.05; One-Way ANOVA); Means with different subscript alphabets within the same row are significantly different (P<0.05; Unpaired student t-test); Control group rats received 10 ml/Kg BW of distilled water orally.

### 4.4 Effects of *Yushania alpina* extracts on the weight gain by the Sprague Dawley rats during the 28-day study in comparison with the control group of rats.

Weekly analysis of body weights of experimental rats (both sex) treated orally with the aqueous shoot extract of Y. alpina and respective control group rats revealed no significant differences each week (P>0.05; Table 7). Moreover, the average body weights of the experimental rats (both sex) increased normally during the experimental period, with significantly higher body weights recorded in the fourth week compared with those recorded in week 0 (baseline) (P<0.05; Table 7, Figure 5 and Figure 6)

Notably, the differences in body weights of the control group rats (male and female) and those treated with 40 mg/Kg BW (male and female), 1000 mg/Kg BW (male), and 200 mg/Kg BW (female) of the test extract in the second week (week 2) and fourth week (week 4) were

insignificant (P>0.05; Table 7). Additionally, the differences in body weights recorded for all the experimental rats (male and female) between the first (week 1) and the second week (week 2) were insignificant.

The weight of the rats taken at 7-day interval was tabulated and the results were used to calculate means for each group. The means were then compared with those from the control group of rats and the results are shown in Table 7

**Table 7: Weekly body weights**

| Treatment group | Bodyweight (g) | | | | |
|---|---|---|---|---|---|
| | **Week 0** | **Week 1** | **Week 2** | **Week 3** | **Week 4** |
| **Male Rats** | | | | | |
| Control | $183.81\pm8.47^{a}_{c}$ | $215.60\pm10.90^{a}_{bc}$ | $293.30\pm13.80^{a}_{ab}$ | $261.10\pm12.50^{a}_{ab}$ | $272.20\pm11.30^{a}_{a}$ |
| 40 mg/Kg BW of *Y. alpina* | $191.16\pm9.31^{a}_{b}$ | $230.30\pm16.90^{a}_{ab}$ | $241.20\pm17.80^{a}_{ab}$ | $259.70\pm15.50^{a}_{a}$ | $274.90\pm14.00^{a}_{a}$ |
| 200 mg/Kg BW of *Y. alpina* | $193.07\pm5.71^{a}_{d}$ | $226.42\pm5.49^{a}_{c}$ | $241.55\pm4.69^{a}_{bc}$ | $258.21\pm4.87^{a}_{ab}$ | $273.39\pm3.64^{a}_{a}$ |
| 1000 mg/Kg BW of *Y. alpina* | $188.32\pm4.54^{a}_{c}$ | $224.21\pm7.06^{a}_{bc}$ | $244.68\pm9.98^{a}_{ab}$ | $261.00\pm10.70^{a}_{ab}$ | $277.73\pm9.90^{a}_{a}$ |
| **Female Rats** | | | | | |
| Control | $177.31\pm6.20^{a}_{c}$ | $204.90\pm8.10^{a}_{b}$ | $229.30\pm6.65^{a}_{ab}$ | $244.11\pm5.08^{a}_{a}$ | $255.41\pm4.85^{a}_{a}$ |
| 40 mg/Kg BW of *Y. alpina* | $182.16\pm9.66^{a}_{b}$ | $206.10\pm10.40^{a}_{ab}$ | $228.90\pm13.10^{a}_{ab}$ | $243.70\pm13.50^{a}_{a}$ | $253.40\pm12.30^{a}_{a}$ |
| 200 mg/Kg BW of *Y. alpina* | $175.48\pm8.03^{a}_{b}$ | $209.86\pm9.82^{a}_{ab}$ | $225.60\pm11.20^{a}_{a}$ | $238.20\pm11.20^{a}_{a}$ | $250.00\pm11.40^{a}_{a}$ |
| 1000 mg/Kg BW of *Y. alpina* | $171.78\pm3.92^{a}_{d}$ | $203.15\pm5.75^{a}_{c}$ | $225.50\pm5.79^{a}_{bc}$ | $243.36\pm6.71^{a}_{ab}$ | $254.05\pm6.01^{a}_{a}$ |

Values are expressed as $\bar{x} \pm SEM$ of replicate measurements. Means with similar superscript alphabets within the same column and those with the same subscript alphabets within the same row are not significantly different (P>0.05, One-Way ANOVA); Control group rats received 10 ml/Kg BW of distilled water orally.

The mean weight gain of each treatment group of rats was calculated and compared with those of control group of rats. The data was then presented by using multiple lines graph as shown in Figures 5 and 6.

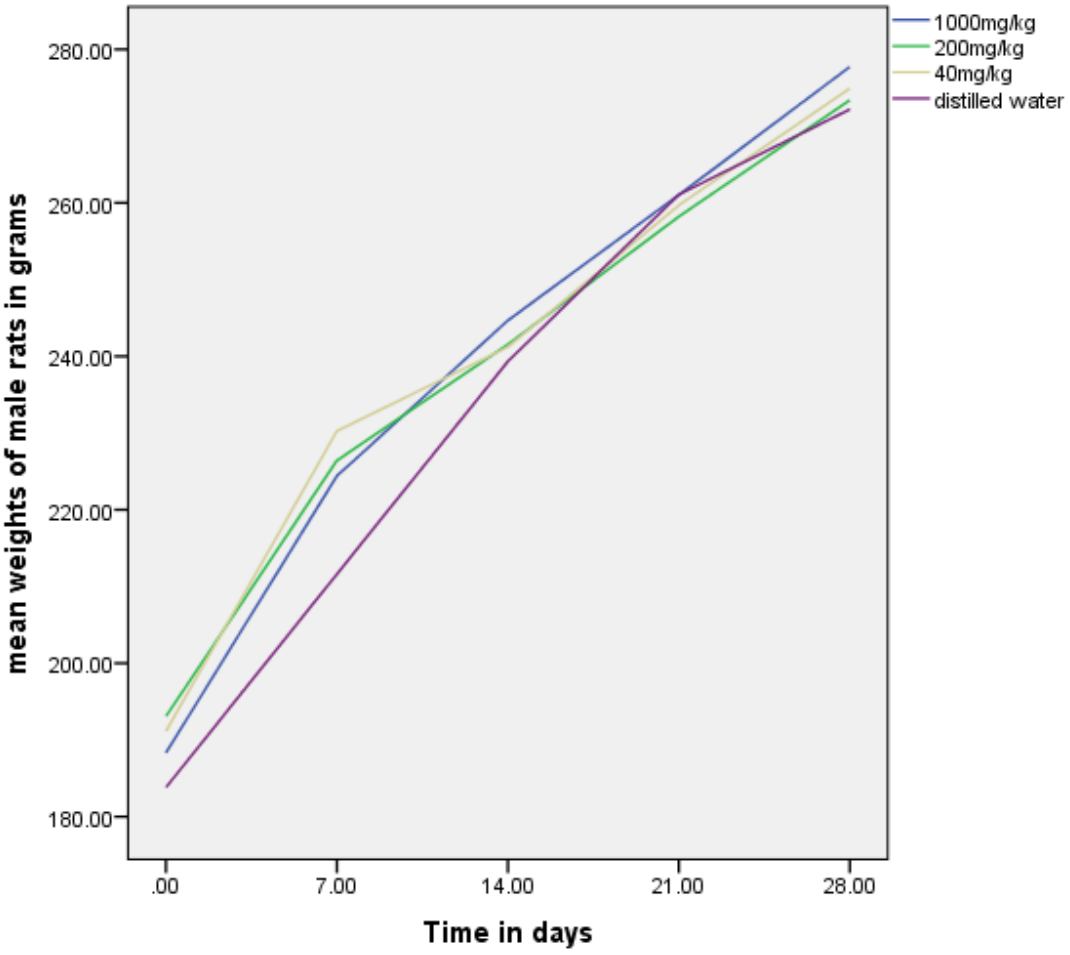


**Figure 5: Mean weight gain curves of male Sprague Dawley rats at 7-day intervals during the 28 days period**

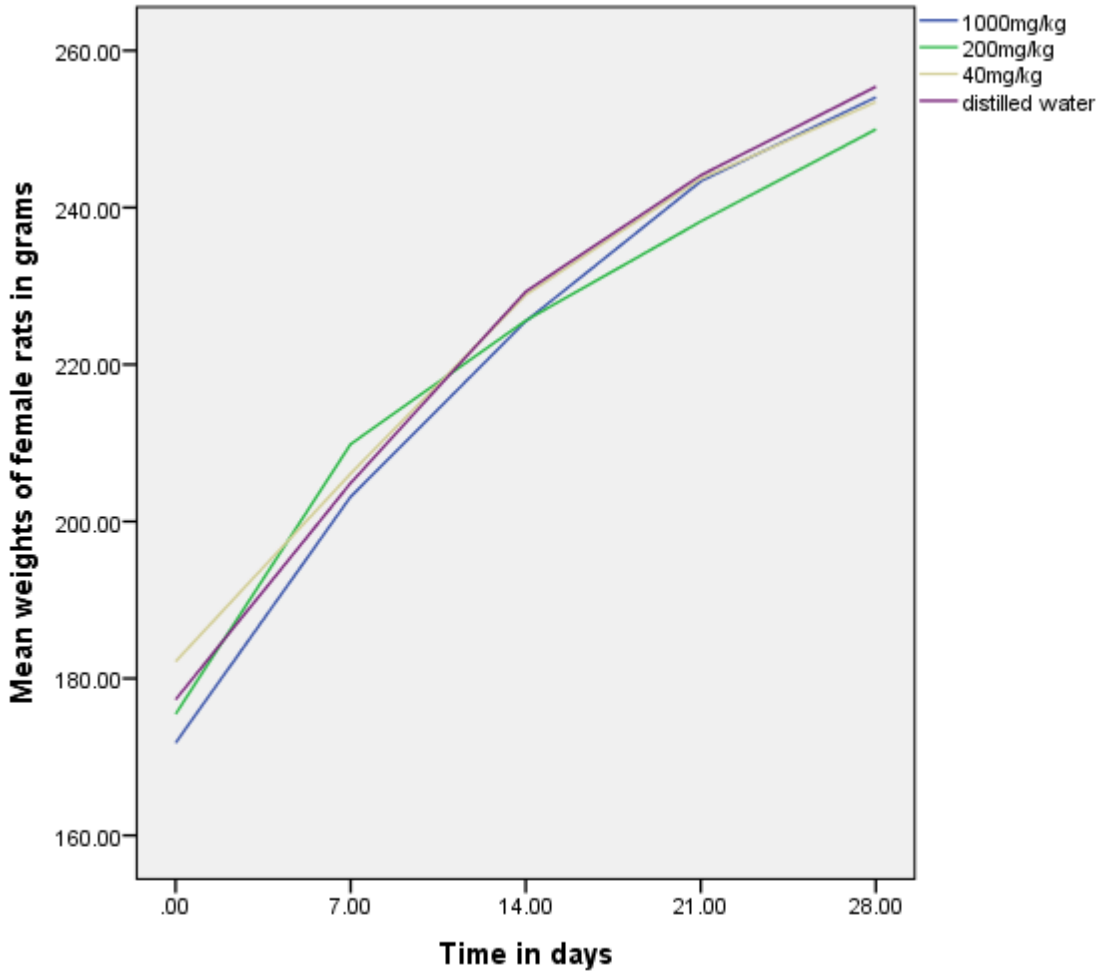


**Figure 6: Mean weight gain curves of female Sprague Dawley rats during the 28 days period.**

The data collected on weight changes of both male and female rats when analysed in order to compare the means of the treatment groups (1000 mg/kg, 200 mg/kg and 40 mg/ kg) did not produce any significant differences. All the treatment groups produced a normal growth curve which compared well with that from the control group of rats.

### 4.5 Gross macroscopic examination and Histopathology

Results from macroscopic and histopathological examination were recorded in form of photographs and are shown in Plates 1 to 4.


**Plate 1: Liver, spleen and kidneys from male rats in group 1 in the control group of rats.**


**Plate 22: Liver, spleen and kidneys from the male rats**

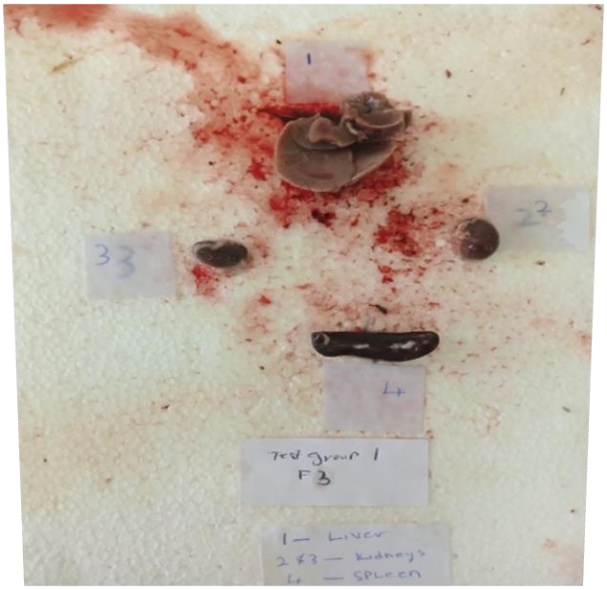


**Plate 33: Liver, spleen and kidneys from female rat in group one of male rat in rats .**


**Plate 4: Liver, spleen and kidneys from the control group of rats.**

The macroscopic and histopathological examinations of the harvested organs and tissues showed normal results with no signs of tissue injury at cellular level except for group two which showed chronic kidney injury characterized by chronic renal interstitial fibrosis, multifocal areas of connective tissue proliferation in the renal interstitial spaces.

### 4.6 Effects of treatment of Sprague Dawley rats with *Yushania alpina* extracts on the weight of selected organs harvested after the study.

This study observed insignificant differences in weights of the liver, spleen, and right kidney, respectively, of all the experimental rats (both sex) (P>0.05; Table 8). Likewise, the weights of the right kidneys of male rats were comparable to those of the female rats (P>0.05; Table 8).

**Table 8: Selected organ weights**

| Treatment group | Organ Weights | | | |
|---|---|---|---|---|
| | **Liver** | **Spleen** | **Kidney (L)** | **Kidney (R)** |
| **Male Rats** | | | | |
| Control | 8.53±0.49[a] | 1.08±0.09[a] | 0.91±0.09[a] | 0.90±0.08[a] |
| 40 mg/Kg BW of *Y. alpina* | 8.54±0.59[a] | 0.97±0.08[a] | 0.89±0.06[ab] | 0.89±0.07[a] |
| 200 mg/Kg BW of *Y. alpina* | 8.06±0.68[a] | 1.16±0.11[a] | 0.89±0.05[ab] | 0.84±0.04[a] |
| 1000 mg/Kg BW of *Y. alpina* | 7.99±0.55[a] | 1.20±0.10[a] | 0.85±0.05[ab] | 0.88±0.04[a] |
| **Female Rats** | | | | |
| Control | 8.13±0.28[a] | 1.02±0.08[a] | 0.88±0.02[ab] | 0.88±0.02[a] |
| 40 mg/Kg BW of *Y. alpina* | 7.25±0.51[a] | 0.85±0.11[a] | 0.74±0.05[ab] | 0.74±0.08[a] |
| 200 mg/Kg BW of *Y. alpina* | 6.25±0.57[a] | 0.95±0.06[a] | 0.67±0.03[b] | 0.72±0.03[a] |
| 1000 mg/Kg BW of *Y. alpina* | 7.12±0.59[a] | 0.90±0.04[a] | 0.73±0.02[ab] | 0.68±0.02[a] |

Values are expressed as $\bar{x} \pm SEM$ of replicate experiments. Means with similar superscript alphabets within the same column are not significantly different (P>0.05, One-Way ANOVA); Control group rats received distilled water (10 ml/Kg BW; *orally*).

### 4.7 Haematological parameters

The WBC, RBC, Hb, MCHC, and HCT concentrations were comparable in all experimental rats (male and female) ($P>0.05$; Table 9). Similarly, significant differences in platelets concentrations were observed among the control group rats (male and female), extract-treated female rats (all dose levels), and extract-treated male rats (40 mg/ Kg BW and 200 mg/Kg BW) ($P>0.05$; Table 9). Likewise, the differences in platelet levels recorded in rats (both sex) treated with 200 mg/Kg BW and 1000 mg/Kg BW of the extract and their respective control group rats (both sex) were insignificant ($P>0.05$; Table 9).

**Table 9: Haematological traits**

| Treatment group | Hematologic traits | | | | | |
|---|---|---|---|---|---|---|
| | WBC ($10^9$/L) | RBC ($10^{12}$/L) | Hb(g/dL) | MCHC(g/dL) | HCT (%) | PLT ($10^9$/L) |
| **Male Rats** | | | | | | |
| Control | 11.34±2.38[a] | 11.44±1.92[a] | 10.98±1.74[a] | 16.10±0.71[a] | 70.30±12.70[a] | 970.00±351.00[ab] |
| 40 mg/Kg BW of *Y. alpina* | 13.75±1.01[a] | 12.60±0.10[a] | 12.10±0.26[a] | 14.92±0.14[a] | 81.78±1.85[a] | 843.00±126.00[b] |
| 200 mg/Kg BW of *Y. alpina* | 12.89±1.40[a] | 11.69±0.75[a] | 11.54±0.52[a] | 15.78±0.45[a] | 74.38±4.49[a] | 1068.00±102.00[ab] |
| 1000 mg/Kg BW of *Y. alpina* | 11.24±1.51[a] | 12.51±0.75[a] | 11.30±0.43[a] | 14.04±0.67[a] | 81.68±6.42[a] | 1623.00±127.00[a] |
| **Female Rats** | | | | | | |
| Control | 11.20±2.24[a] | 10.80±0.84[a] | 12.82±0.99[a] | 19.66±2.92[a] | 67.58±4.33[a] | 905.00±82.00[ab] |
| 40 mg/Kg BW of *Y. alpina* | 12.63±1.91[a] | 13.89±2.78[a] | 11.66±0.72[a] | 13.60±1.72[a] | 97.20±23.20[a] | 1279.00±99.10[ab] |
| 200 mg/Kg BW of *Y. alpina* | 13.17±3.32[a] | 12.46±1.31[a] | 12.60±0.83[a] | 16.62±3.23[a] | 81.32±7.39[a] | 1123.00±81.70[ab] |
| 1000 mg/Kg BW of *Y. alpina* | 16.42±4.31[a] | 10.61±1.31[a] | 13.74±1.24[a] | 20.28±3.77[a] | 72.90±7.25[a] | 790.00±103.00[b] |

Values are presented as $\bar{x} \pm SEM$ of replicate experiments. Means with similar superscript alphabets within the same column are not significantly different (P>0.05, One-Way ANOVA); Control group rats received 10 ml/Kg BW of distilled water orally; WBC: White blood cells; RBC: Red blood cells; Hb: Haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; HCT: Haematocrit; PLT: Platelets.

### 4.8 Biochemical parameters

I determined the biochemical parameters of experimental rats treated with the test extract to assess its safety. The serum urea levels in experimental rats (both sex) which received the test extract (at all dose levels) were comparable to those of the respective control group rats ($P>0.05$; Table 10). Notably, extract-treated male rats had significantly higher concentrations of serum urea levels than those of female rats (control and extract-treated groups) ($P<0.05$; Table 10).

The results further showed significantly higher serum creatinine concentrations in female rats orally administered with 40 mg/Kg BW of the test extract than those recorded in male rats treated with the same extract (200 mg/Kg BW) ($P<0.05$; Table 10). The serum creatinine levels in the extract-treated rats and their respective controls were comparable ($P>0.05$; Table 10).

Besides, the alkaline phosphatase levels in all the extract-treated experimental rats (both sex), except the male rats, which received 1000 mg/Kg BW of the extract, were comparable ($P>0.05$; Table 10). Notably, the male rats which received 1000 mg/Kg BW of the extract orally had significantly higher alkaline phosphatase levels than those recorded in all the female rats ($P<0.05$; table 10).

The results showed no significant differences in the alanine transferase, aspartate transaminase, and serum albumin levels among all the experimental rats ($P>0.05$; Table 10). Likewise, the total protein levels recorded in male rats were comparable and those of female rats in this study, as shown in Table 10 ($P>0.05$).

**Table 10: Biochemical parameters**

| Treatment group | Serum biochemical parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| | **U (mmol/L)** | **C(µmol/L)** | **ALP (U/L)** | **ALT(U/L)** | **AST(U/L)** | **P (g/L)** | **A (g/dL)** |
| **Male Rats** | | | | | | | |
| Control | 10.40±0.44$^{a}$ | 61.53±1.62$^{ab}$ | 152.40±12.50$^{ab}$ | 80.20±7.81$^{a}$ | 270.40±15.10$^{a}$ | 72.35±1.34$^{ab}$ | 3.67±0.08$^{a}$ |
| 40 mg/Kg BW of *Y. alpina* | 10.58±0.84$^{ab}$ | 63.45±1.85$^{ab}$ | 208.20±30.00$^{ab}$ | 64.20±2.58$^{a}$ | 242.00±11.20$^{a}$ | 67.16±1.72$^{b}$ | 3.45±0.21$^{a}$ |
| 200 mg/Kg BW of *Y. alpina* | 10.51±0.72$^{ab}$ | 58.35±1.02$^{b}$ | 171.00±16.50$^{ab}$ | 63.80±5.28$^{a}$ | 311.00±34.90$^{a}$ | 68.96±1.63$^{ab}$ | 3.47±0.09$^{a}$ |
| 1000 mg/Kg BW of *Y. alpina* | 12.21±0.49$^{ab}$ | 62.94±1.75$^{ab}$ | 245.60±43.90$^{a}$ | 73.20±13.80$^{a}$ | 288.40±37.90$^{a}$ | 68.63±3.20$^{ab}$ | 3.34±0.15$^{a}$ |
| **Female Rats** | | | | | | | |
| Control | 7.72±0.60$^{b}$ | 61.53±3.04$^{ab}$ | 81.20±19.10$^{b}$ | 72.80±9.21$^{a}$ | 213.60±9.93$^{a}$ | 76.79±1.71$^{ab}$ | 3.85±0.13$^{a}$ |
| 40 mg/Kg BW of *Y. alpina* | 8.96±0.56$^{b}$ | 70.52±3.64$^{a}$ | 112.80±43.90$^{b}$ | 55.20±6.50$^{a}$ | 234.60±36.90$^{a}$ | 77.73±4.36$^{a}$ | 3.76±0.18$^{a}$ |
| 200 mg/Kg BW of *Y. alpina* | 8.35±0.71$^{b}$ | 61.68±1.41$^{ab}$ | 151.80±19.90$^{ab}$ | 63.00±5.30$^{a}$ | 263.40±17.40$^{a}$ | 76.21±0.17$^{ab}$ | 3.64±0.12$^{a}$ |
| 1000 mg/Kg BW of *Y. alpina* | 8.35±0.71$^{b}$ | 61.68±1.41$^{ab}$ | 151.80±19.90$^{ab}$ | 63.00±5.30$^{a}$ | 263.40±17.40$^{a}$ | 76.21±0.17$^{ab}$ | 3.64±0.12$^{a}$ |

Values are presented as $\bar{x} \pm SEM$ of replicate experiments. Means with similar superscript alphabets within the same column are not significantly different (P>0.05, One-Way ANOVA); Control group rats received 10 ml/Kg body weight of distilled water orally; U: Urea; C: Creatinine; ALP: Alkaline Phosphate; ALT: Alanine aminotransferase; AST: Aspartate transaminase; P: Total protein; A: Albumin.

# CHAPTER FIVE

# DISCUSSION, CONCLUSIONS AND RECOMENDATIONS

## 5.1 Discussion

The results obtained from the antibacterial study of *Yushania alpina* shoots extracts showed significant activity against gram positive bacteria which were represented by *Staphylococcus aureus* and also spore forming *Bacillus cereous*. In *Staphylococcus aureus* the lowest concentration for aqueous and methanolic extracts in which antibacterial activity was recorded was 200mg/kg and 400 mg/kg respectively while for *Bacillus cereous* it was 400 mg/kg for both Methanolic and aqueous extract concentrations. This is a good indication that *Yushania alpina* shoots have a great potential for antimicrobial activity. However, there was no any quantifiable activity recorded against gram negative *Escherichia coli.*

These results agree with a study done on the antibacterial activity of bamboo (Tsuzuki *et.al.*,2011) which was carried out on Australian bamboo culms extract which found antibacterial activity against gram positive bacteria and no antibacterial activity against *E. Coli* by the aqueous extract of the bamboo plant. In this study, the antibacterial activity could be attributed to aromatic and phenolic functional groups which are known to be present in bamboo species. These results are however inconsistent with the findings of another study on antimicrobial properties of developed bamboo (Graça *et. al.*, 2018) which confirmed significant antibacterial activity against *E. coli*. The results also support claims where extracts made from bamboo shoots have been used in the management of wounds, boils and respiratory infections considering that one of the most common causative agents in such infections is *Staphylococcus aureus*. Results from this study agree closely to those from the screening of antimicrobial properties of several bamboo species where the diameter of the zones of inhibition of S. *aureus* by the crude extracts ranged between 9.3 mm to 10.7 mm.

However, the activity from the test results was small when compared to that of the control experiment where a broad-spectrum antibiotic Cefotaxime was used. From the two experiment, broth dilution assay and agar diffusion assay minimum inhibitory concentration and minimum bactericidal concentration for *S. aureus* was calculated as 200mg/kg and 800mg/kg respectively. For *Bacillus cereus* both MIC and MBC were determined as 400mg/kg and 800mg/kg respectively. From these results it was observed that though both Methanolic and aqueous extract of *Yushania alpina* shoots extracts had marked antibacterial activity against both gram positive and spore forming bacilli and the activity was more pronounced in the former than the latter.

Previous studies have attributed this antibacterial activity of bamboo to the lignin component in the bamboo plant which is an aromatic gummy material comprising p-hydroxyphenyl functional groups as well as p-coumaric groups which is esterified in the polymer system. (Tsuzuki *et.al.*,2011) Fourier transform infrared spectroscopy (FTIR) studies have revealed presence of carbonyl and aromatic functional groups in bamboo extracts. Phenolic groups cause denaturing and aggregation of proteins in bacteria leading to their anti-bactericidal activity. In another study 2,6-dimethoxy-p benzoquinone has been isolated in bamboo plant which was responsible for its activity against *Staphylococcus aureus*. (Akinobu *et al.,* 2011)

Results from both Broth Dilution Test and Agar Diffusion Assay performed on *Candida albicans* did not indicate any observable anti-fungal activity against the microorganism. The fungal growth in the tubes containing the *Yushania alpina* shoots extracts was comparable to the one found in the negative control where no extract was added. There was no inhibition diameter in the agar diffusion test across all the range of extracts concentrations. These results are different from the study carried out in order to investigate antimicrobial properties of several species of bamboo which found considerable antifungal activity against *Candida albican* with a minimum inhibitory concentration of 800 mg/ml though this activity was

attributed to the microorganisms hosted in the bamboo. Similar results were obtained in a study on anti-fungal-producing bacteria from bamboo powder (Reynald *et.al.,* 2016).

This study did not observe any extract-induced behavioral, neurological, or other clinical signs of toxicity in Sprague Dawley rats (male and female) throughout the 28-day experimentation period. Earlier studies show that the absence of clinical signs of toxicity in experimental animals may indicate the safety of the administered agent. Also, the experimental rats' daily water and feed intake remained normal during the study period, depicting the noninterference by the administered extract. Thus, our observations suggest the safety of the aqueous shoot extract of *Yushania. alpina,* depicting its therapeutic potential. Bodyweight is an important anthropometric measure for assessing the toxicity of administered drug agents. Reductions in body weight gain, or lack thereof, are associated with potential exposure to toxic agents, which may cause adverse effects. In this study, the body weights of experimental rats treated with the aqueous shoot extract of *Yushania alpina* were comparable to those of the control group rats and increased normally during the experimentation period. These findings demonstrate the nontoxicity of the study extract in Sprague Dawley rats, which may signify safety in humans, as previously reported.

The liver and kidney are prone to drug or toxicant-induced cell damage due to their fundamental roles in metabolism and excretion. The spleen is the principal organ of the lymphatic system, which plays an essential role in producing and regulating crucial immunity elements. Intact drugs, exogenous chemical toxicants, or their metabolites might cause toxicity to these organs (liver, kidney, and spleen) depending on their dose and period of exposure, resulting in characteristic histopathological changes. Damage to these organs may result in deleterious consequences, including death. Gross macroscopic and histopathological examination of these organs did not show changes in form of shape, size, colour or any lesions.

Statistical analysis of organs weights and comparison of their means with those of the control group of rats by using Statistical Package for Social Sciences did not show significant changes. Also, analysis of weight gain from data collected from the study indicated that there was a normal growth curve for the three treatment groups which was not significantly different from the control group of rats. These results are in agreement with the results obtained from the rats in the control group. A study carried out in order to evaluate the toxicity of bamboo leaves (Baiyi Lu *et.al.*,2018) using Sprague Dawley rats concluded that bamboo was non-toxic at NOEL value of >10g/kg .

The results from Biochemical analysis of blood samples which were collected on the following parameters; Creatinine, Blood urea, Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), and Gamma-glutamyl transpeptidase (GGT) , Total protein and Albumin levels showed all values were within the normal ranges. Furthermore, the results from the three test groups of rats (1000mg/kg, 200mg/ kg and 40mg/kg) were not significantly different from the results obtained from the samples collected from the control group of rats. This implies that, treatment with *Yushania alpina* shoots extracts did not lead to alteration of the Biochemical parameters. These results are in agreement with studies done previously on other bamboo species where Biochemistry results from the studies showed that the values were within the normal ranges and were similar to those found in the control experiments.

There were no significant Haematological changes in the following parameters; Haemoglobin concentration, Red blood cell count (RBC), White blood cell count (WBC), Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC). All these parameters had values within the normal ranges and when their means were compared with those from the control experiment using independent t-test at 95% confidence level, there was no significant difference in their

which was an indicator of lack of toxic effects by the extracts in the rats after 28-day treatment at the given doses of the extracts.

**5.2 Conclusions**

I. The results which were obtained from the study demonstrate that *Yushania alpina* shoots extracts had significant activity against *Staphylococcus aureus* and against *Bacillus cereous* and more potency was observed in the former than the later. The antibacterial activity observed was dose dependent which changed gradually from bacteriostatic to bactericidal with increase in concentrations of the extracts it had however no activity against *E. coli* as shown in the findings.

II. On the second objective of determining activity of *Yushania Alpina* against candida albicans it can be concluded that both methanolic and aqueous shoots extracts have no activity against the tested microorganism at the concentration range of the experiment.

III. It can be concluded that *Yushania alpina* shoots extracts had low toxicity in Sprague Dawley rats within the concentration range tested since it did not produce significantly different results in the parameters tested for sub-acute toxicity from the results obtained from the control group of rats.

**5.3 Recommendations**

It is recommended that, *Yushania alpina* shoots extracts be studied further in depth in order to come up with appropriate dosage levels in order to have bamboo shoots extracts used in the preparations for the management of infections caused by susceptible microorganisms in vivo.

Further studies should also focus on other fungal species in order to determine whether *Yushania alpina* shoots extracts have any antifungal activity.

Chronic toxicity studies should also be conducted in animals on the extracts in order to determine whether shoots extracts have any chronic toxicity, characterise the expected adverse effects and categorise their safety profile.

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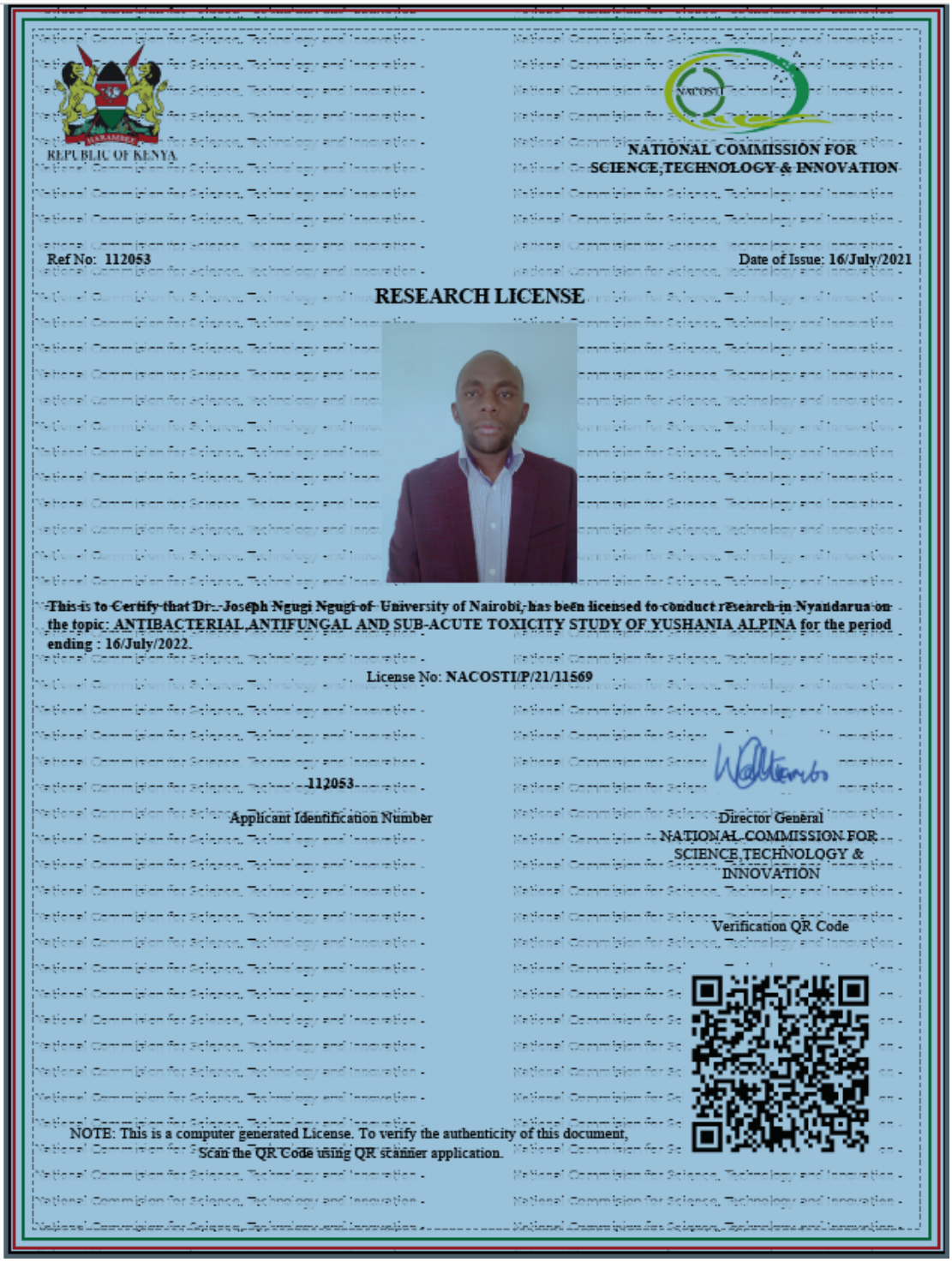
# APPENDICES

## APPENDIX 1: RESEARCH LICENCE



REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 112053

Date of Issue: 16/July/2021

RESEARCH LICENSE

This is to Certify that Dr. Joseph Ngugi Ngugi of University of Nairobi, has been licensed to conduct research in Nyandarua on the topic: ANTIBACTERIAL ANTIFUNGAL AND SUB-ACUTE TOXICITY STUDY OF YUSHANIA ALPINA for the period ending : 16/July/2022.

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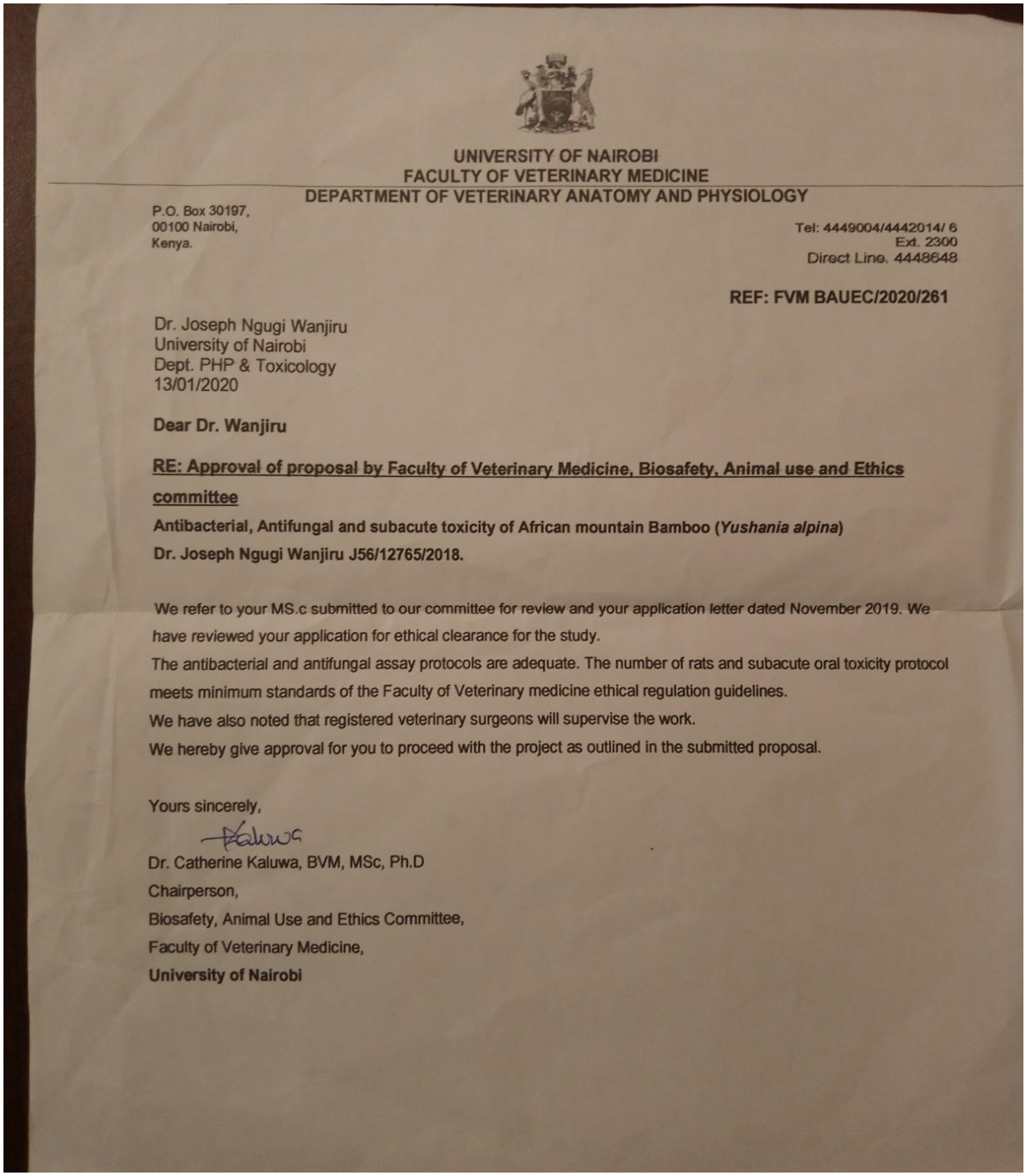
# APPENDIX 2: ETHICS APPROVAL LETTER



UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
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Kenya.

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Ext. 2300
Direct Line. 4448648

**REF: FVM BAUEC/2020/261**

Dr. Joseph Ngugi Wanjiru
University of Nairobi
Dept. PHP & Toxicology
13/01/2020

**Dear Dr. Wanjiru**

**RE: Approval of proposal by Faculty of Veterinary Medicine, Biosafety, Animal use and Ethics committee**

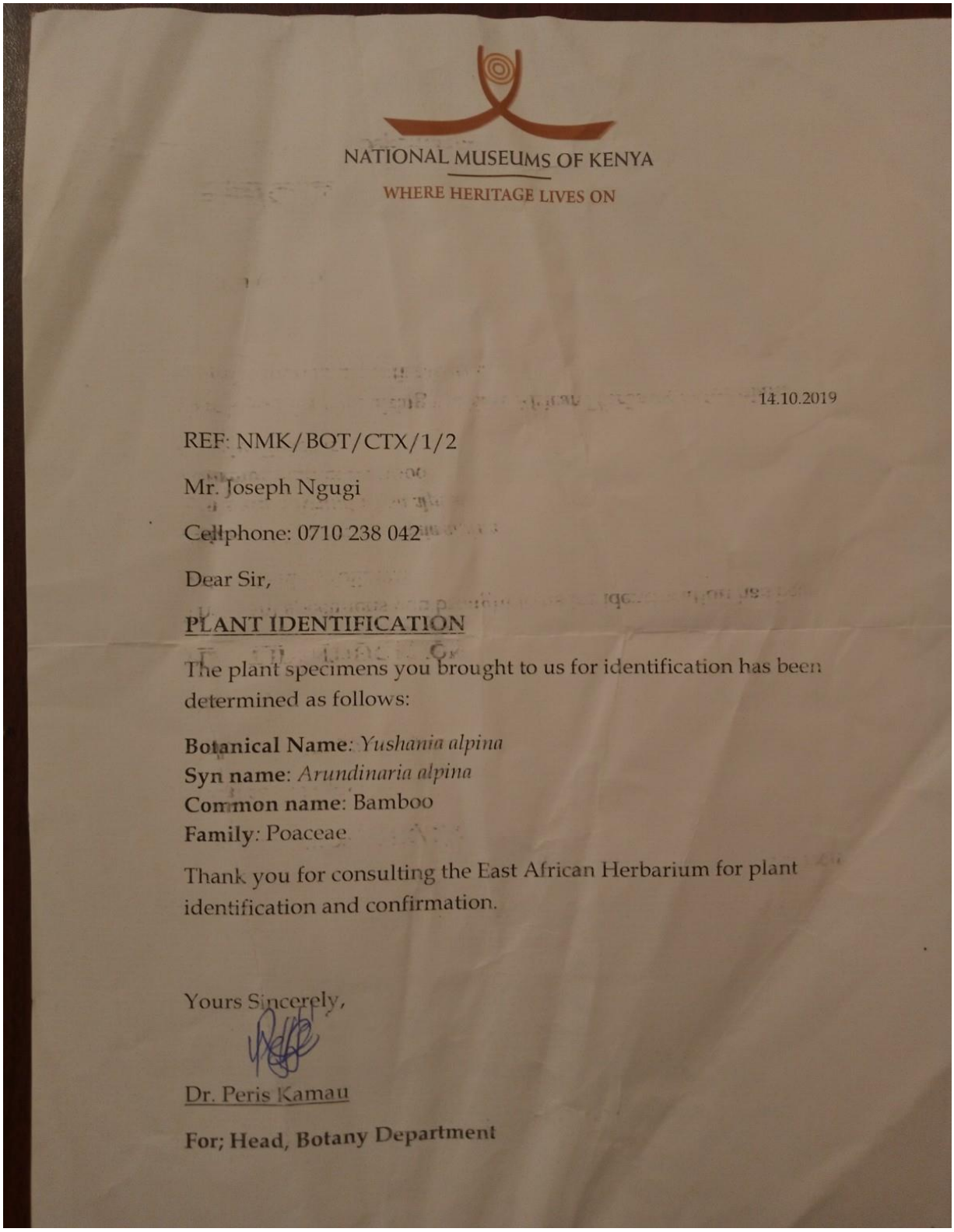
**Antibacterial, Antifungal and subacute toxicity of African mountain Bamboo (*Yushania alpina*)**
**Dr. Joseph Ngugi Wanjiru J56/12765/2018.**

We refer to your MS.c submitted to our committee for review and your application letter dated November 2019. We have reviewed your application for ethical clearance for the study.
The antibacterial and antifungal assay protocols are adequate. The number of rats and subacute oral toxicity protocol meets minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines.
We have also noted that registered veterinary surgeons will supervise the work.
We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Dr. Catherine Kaluwa, BVM, MSc, Ph.D
Chairperson,
Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine,
**University of Nairobi**

## APPENDIX 3:*YUSHANIA ALPINA* PLANT IDENTIFICATION



NATIONAL MUSEUMS OF KENYA

WHERE HERITAGE LIVES ON

14.10.2019

REF: NMK/BOT/CTX/1/2

Mr. Joseph Ngugi

Cellphone: 0710 238 042

Dear Sir,

PLANT IDENTIFICATION

The plant specimens you brought to us for identification has been determined as follows:

**Botanical Name**: *Yushania alpina*
**Syn name**: *Arundinaria alpina*
**Common name**: Bamboo
**Family**: Poaceae

Thank you for consulting the East African Herbarium for plant identification and confirmation.

Yours Sincerely,

Dr. Peris Kamau

**For; Head, Botany Department**

## APPENDIX 4: PLATES SHOWING ANTI-BACTERIAL ACTIVITY

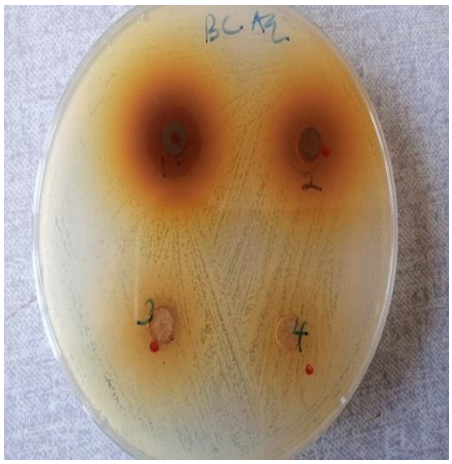



**Plate 5**: Zone of inhibition on *Bacillus cereous* by *alpina Yushania alpina* shoots extracts

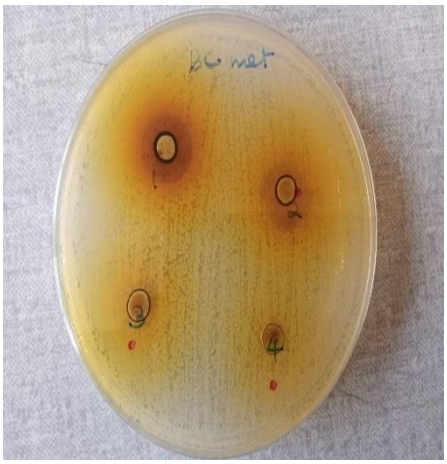



**Plate** 6**:** Zone of inhibition on *Bacillus cereous* by *Yushania Yushania alpina* shoots


Plate 7: Zone of inhibition on *Bacillus cereous* by cefotaxime shoots

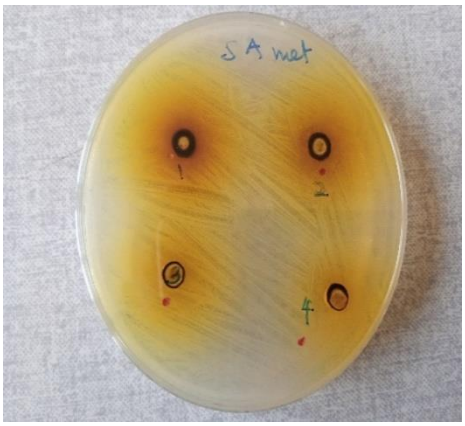



Plate 8: Zone of inhibition on *S. aureus* by *Yushania alpina* extract

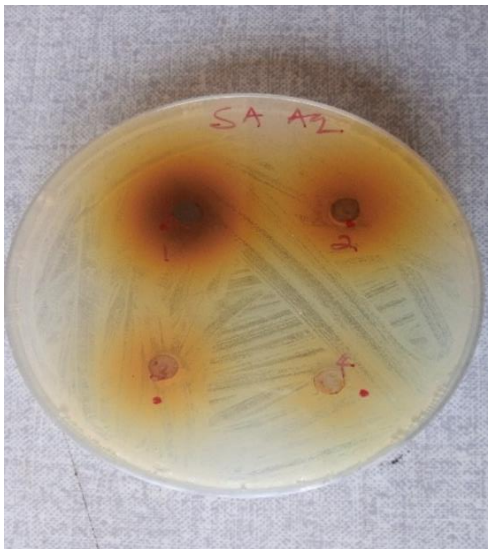



Plate 9: Zone of inhibition on *S. aureus* by *Yushania alpina* shoots extract

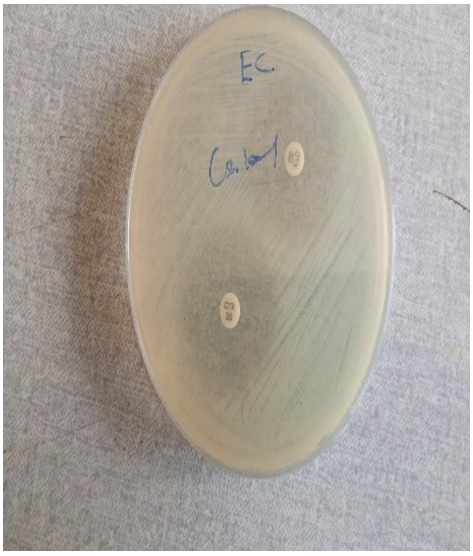



Plate 10: Pone of inhibition on *E.coli* by Cefotaxime

.


**Plate 11: Zone of inhibition on *E.coli* by *Yushania alpina* shoots extract**

## APPENDIX 5: PLATES SHOWING ANTIFUNGAL ACITIVITY OF YUSHANIA ALPINA SHOOTS EXTRACTS AGAINST CANDIDA ALBICAN.

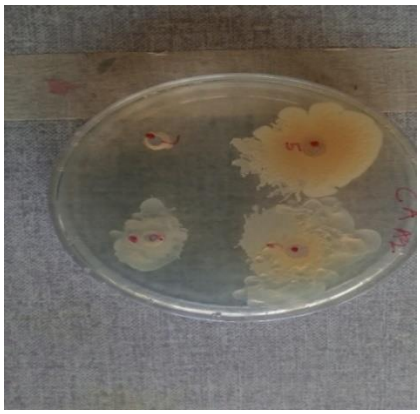


Plate 12: *Candida albicans* incubated in *Yushania alpina* shoots extracts.

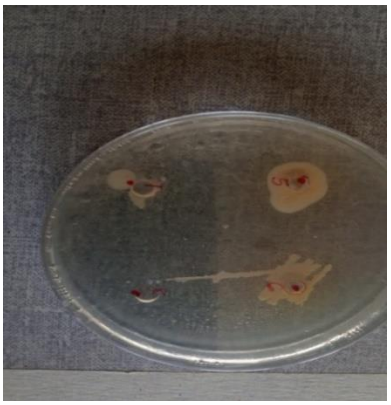


Plate 13: *Candida albicans* incubated in *Yushania alpina* .
shoots extracts.

## APPENDIX 6: PUBLISHED ARTICLE

Hindawi
Journal of Toxicology
Volume 2022, Article ID 6283066, 11 pages
https://doi.org/10.1155/2022/6283066

*Research Article*

# Subacute Toxicity Effects of the Aqueous Shoot Extract of *Yushania alpina* (K. Schum.) W.C.Lin in Sprague Dawley Rats: An Appraisal of Its Safety in Ethnomedicinal Usage

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Received 15 April 2022; Accepted 30 July 2022; Published 25 August 2022

Academic Editor: Zongming Ren



Plant-based medicines have effectively managed several ailments in humans and animals since prehistoric times. However, the pharmacologic efficacy and safety of many plants currently used in traditional medicine have not been explored empirically, which raises serious public health concerns, derailing further research and their integration into the conventional healthcare system. Despite the longstanding ethnomedicinal usage of *Yushania alpina* shoot extract to treat inflammation, microbial infections, and diarrhoea, among other diseases, there is insufficient scientific data to appraise its toxicity profile and safety. Accordingly, we investigated the subacute toxicity of the aqueous shoot extract of *Y. alpina* in Sprague Dawley rats (both sexes) for 28 days based on the Organisation for Economic Cooperation and Development guideline 407. In this study, all the experimental rats treated orally with 40 mg/Kg BW, 200 mg/Kg BW, and 1000 mg/Kg BW of the aqueous shoot extract of *Y. alpina* remained normal, like the control group rats, and did not show any clinical signs of subacute toxicity, and no morbidity or mortality was recorded. Besides, the weekly body weight gains and the haematological and biochemical parameters of experimental rats orally administered with the studied plant extract at the tested doses and in the control group were comparable ($P > 0.05$). No pathologic alterations in internal organs were observed following necroscopy. Further, the differences in weights of the liver, kidney, and spleen of experimental rats which were subacutely treated with the studied plant extract and the control rats were insignificant ($P > 0.05$). Moreover, no histopathological changes were observed in tissue sections of the liver, kidney, and spleen obtained from all the experimental rats. Our findings demonstrate that the aqueous shoot extract of *Y. alpina* may be safe as it does not elicit subacute toxicity in Sprague Dawley rats. Further toxicological and pharmacological studies using other model animals and in clinical setups are encouraged to fully appraise the efficacy and safety of the studied plant extract.

## APPENDIX 7: PLAGIARISM REPORT

STUDY OF ANTIMICROBIAL ACTIVITY AND SUB-ACUTE TOXICITY IN RATS OF AFRICAN MOUNTAIN BAMBOO (YUSHANIA ALPINA)

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