
**UNIVERSITY OF NAIROBI**

# AN EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY EFFECTS OF NATURAL FOODS AND MEDICINAL PLANTS OF THE ILKISONKO MAASAI COMMUNITY, KENYA

**BY**

**KIMONDO, JULIA WANJIRU, B.PHARM, MSC.**
**U80/98007/2015**

A Thesis submitted in fulfilment of the requirements for award of the degree of Doctor of Philosophy in Pharmacognosy of the University of Nairobi

**2020**

# DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature _____ Date _____

Kimondo, Julia Wanjiru
U80/98007/2015
Department of Pharmacology and Pharmacognosy
School of Pharmacy
University of Nairobi

This thesis is submitted for examination with our approval/knowledge as university supervisors.

Signature Date

**Dr. P. C, Mutai**
Department of Pharmacology and Pharmacognosy
University of Nairobi
P.O. Box 19676-00202, Nairobi, Kenya
Email: peggoty.chepkoech@uonbi.ac.ke _____ _____

**Dr. P. M, Njogu**
Department of Pharmaceutical Chemistry,
University of Nairobi
P.O. Box 19676-00202, Nairobi, Kenya
Email: peter.njogu@uonbi.ac.ke _____ _____

**Prof. C. N, Kimwele**
Department of Veterinary Anatomy and Physiology
University of Nairobi
P.O. Box 30197-00100, Nairobi, Kenya
Email: ckimwele@uonbi.ac.ke _____ _____

## DECLARATION FORM FOR STUDENTS

UNIVERSITY OF NAIROBI

Declaration of Originality Form

Name of Student ___Kimondo Julia Wanjiru_____

Registration Number ___U80/98007/2015_____

College ___of health sciences_____

~~Faculty~~/School/~~Institute~~____of Pharmacy_____

Department _of Pharmacology and Pharmacognosy_____

Course Name __Doctor of Philosophy in Pharmacognosy_____

Title of the work: An Evaluation of Antioxidant and Anti-inflammatory Effects of Natural Foods and Medicinal Plants of the Ilkisonko Maasai Community, Kenya.

DECLARATION

1. I understand what plagiarism is and I am aware of the University's policy in this regard
2. I declare that this _____ Thesis ___ is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.
3. I have not sought or used the services of any professional agencies to produce this work
4. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his/her own work
5. I understand that any false claim in respect of this work shall result in disciplinary action, in accordance with University Plagiarism Policy.

**Signature** _____

**Date** _____

## DEDICATION

This thesis is dedicated to my family who have shown patience and helpfulness above and beyond the call of kinship.

"Knowledge is as wings to man's life, and a ladder for his ascent.
Its acquisition is incumbent upon everyone."
Baháulláh, Epistle to the Son of the Wolf, p. 26

## ACKNOWLEDGEMENTS

I would like to acknowledge my supervisors, Dr. Peggoty Mutai, Dr. Peter Njogu and Prof. Charles Kimwele, for their persistent effort and great guidance without which this work would not have been accomplished. I would especially like to thank them for their encouragement and patience despite my own shortcomings. My proposal would not have come together without the help of my first supervisors, the late Dr. Jacob Miaron, Prof. Grace Thoithi and Prof. Julius Mwangi who started me off so strongly and willingly transferred me to my current supervisors. I would like to acknowledge the contribution of Prof. Charles Kimwele, the late Dr. Miaron and technologists at the department of Veterinary Physiology, Samuel Kamonde, James Mugweru and Peter Irungu, who assisted me in administering questionnaires and in collecting the plants after the survey.

My humble gratitude to Prof. Abiy Yenesew, for allowing me full access to his laboratory and connecting me to Prof. Erdélyi's laboratory in Swedish NMR centre for structure elucidation of my compounds. I would like to thank the postgraduate students at the Department of Chemistry, Carol Chepkirui, Purity Jael, Ivan Kiganda, Richard Oriko, Vincent Kamya, Nicholas and Jeremiah, whom I have picked their brains countless times none of which ruffled their patience or permanently amicable disposition.

I would like to thank the staff at the Department of Pharmacology and Pharmacognosy, University of Nairobi for their administrative assistance. I am extremely grateful to the staff in the School of Pharmacy, Jomo Kenyatta University of Agriculture and Technology (JKUAT) for all their support towards this work.

My appreciation goes to United Nations Development Programme (UNDP) whose grant allowed me to purchase essential materials necessary for my research. Many people besides those mentioned have contributed in meaningful ways to my work and I am forever indebted to them.

# TABLE OF CONTENTS

# GLOSSARY OF ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| ABTS | 2, 2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) |
| COX | Cyclooxygenase |
| CRP | C- reactive protein |
| DPPH | 2, 2- Diphenyl-1-picryl hydrazyl |
| DNA | Deoxyribonucleic acid |
| ET | Electron transfer |
| FRAP | Ferric reducing antioxidant power |
| HAT | Hydrogen atom transfer based assays |
| IL | Interleukin |
| IF | Interferon |
| IR | Infrared |
| MCP-1 | Monocyte chemoattractant protein-1 |
| MS | Mass spectroscopy |
| NCD | non-communicable diseases |
| NF-κB | Nuclear factor *kappa*-light-chain-enhancer of activated B cells |
| NMR | Nuclear magnetic resonance |
| NO | Nitric oxide |
| NSAIDs | Non-steroidal anti-inflammatory drugs |
| ORAC | Oxygen radical absorbance capacity |
| PG | Prostaglandin |
| ROS | Reactive oxygen species |
| RNS | Reactive nitrogen species |
| SALs | Semi-arid lands |
| STI | Sexually transmitted infections |
| TGF-β | Transforming growth factor *beta* |
| TNF-α | Tumour necrosis factor-*alpha* |
| TRAP | The radical trapping antioxidant parameter |
| UNDP | United Nations development programme |

# LIST OF FIGURES

# LIST OF TABLES



# LIST OF EQUATIONS

# ABSTRACT

**Background**

The Ilkisonko Maasai are a pastoralist community in Kajiado County, Kenya that uses indigenous plants for ethnomedicinal interventions.

**Methodology**

An ethnobotanical survey was conducted in Loitokitok sub-county using a semi-structured questionnaire to collect information on plants with antimicrobial, anti-inflammatory, analgesic or adaptogenic activity. Plants were collected and underwent preliminary screening by assessing the phenolic content, flavonoid content and antioxidant activity via the Folin and Ciocalteu's method, aluminium chloride colorimetric method and 2, 2- Diphenyl-1-picryl hydrazyl test respectively. From the assays, plant extracts with the highest antioxidant activity were selected to undergo *in vivo* testing. Anti-inflammatory activity of the methanol extracts of these plants was evaluated via the carrageenan-induced rat paw oedema. After this, one plant was subjected to chromatographic fractionation and isolation of the active ingredients. Spectroscopic techniques were used for structural elucidation of the isolated compounds.

**Results**

The ethnobotanical survey yielded 30 plant species from 21 families and 25 genera reportedly used as food and/or medicine. Some of the commonly treated ailments included body pains, stomach aches, constipation, joint aches, back aches, and sexually transmitted infections. The plants were also used as adaptogens, digestives, and restoratives. In the phytochemical assays, the methanol extracts had a higher phenolic content with the exception of a few plants in which the phenolic content in the water extract is higher. *Acacia nilotica* methanol and water extracts had the highest phenolic content and antioxidant activity, while *Acacia reficiens* had the highest flavonoid content. In the anti-inflammatory assay, the selected plants all exhibited anti-inflammatory activity at early phase of inflammation. *Grewia villosa* extract underwent chromatographic isolation to give two pure compounds.

**Conclusion and recommendations**

This study has shown a positive correlation between the polyphenolic content and the antioxidant effect of plants and hence their health benefits in humans. This might encourage

growing and consumption of these foods. Thus, it is vital to perform a large-scale systematic screening of these herbs to add new knowledge on nutraceuticals. Further, the isolated bioactive compounds may act as starting points in drug development by providing templates for semi-synthetic derivation of more active compounds.

# 1.0. CHAPTER ONE: INTRODUCTION

## 1.1. BACKGROUND

Antioxidant compounds have received increasing attention in the 21st century due to their importance in pharmaceutical, food, and cosmetic industry. In the pharmaceutical industry, antioxidant supplements or antioxidant rich foods may be taken due to their health promoting or disease prevention effects. This has been seen mostly in high income countries where at least half of the population consume natural antioxidant supplements (Bjelakovic *et al.*, 2012). Antioxidants help to maintain the physiological functions of organs by neutralizing free radicals produced in the body. Radicals are molecules with one or more unpaired electrons which makes the molecule reactive. These radicals perform several functions in the body and can be endogenously produced or xenobiotically derived (Kehrer and Klotz, 2015). Inflammation, the biological response to injury, causes *in situ* production of radicals which should eliminate the source of injury. An inflammatory response is usually acute (short term), however in certain cases it can be chronic (long term). Acute inflammation is a short-term beneficial process initiated to mitigate insult (microbial, chemical or physical) and therefore get to a resolution state. It produces free radicals but these are balanced by endogenous antioxidant mechanisms. Chronic inflammation is a long-term state that arises from non-resolution of inflammation. Chronic inflammation provides a continuous source of cell damaging radicals that is associated with chronic conditions such as cardiovascular diseases, metabolic disorders, cancers, Parkinsonism and cataracts. Metabolic disorders are brought about by a number of factors such as a high fat and high sugar diet, which acts as insults, to cause a corresponding increase in inflammation (Li *et al.*, 2018). These conditions are especially seen in the elderly, though younger adults are presenting with the same symptoms especially in the urban setting.

The Ilkisonko Maasai are a Kenyan pastoralist community living in Southern Kenya. They are distinct due to their customs and way of dressing which is still practised up to this day. One of these customs is the use of meat, milk, blood and bone soup for their energy requirements. This diet, especially with high milk consumption, has a high percentage of calories from fat. Due to this high fat diet, the expectation is that the Maasai would have a high incidence of metabolic disorders, on the contrary, they have a low incidence. This observation may be explained by the fact that they routinely include plant parts, potentially

rich in antioxidant content, in their diet which may contribute to this reduced incidence of metabolic disorders (Ole-Miaron, 2003; Christensen *et al.*, 2014).

## 1.2. PLANT DERIVED ANTIOXIDANTS

Compounds with antioxidant activity are present in a variety of forms in our everyday foods. Phenolic compounds are commonly equated with antioxidant activity because they form the majority of antioxidant substances from plants. They are composed of a large number of compounds having a phenolic group in the structure. This enables the compound to neutralise oxidants in the body by taking up the extra electron while remaining stable. Herbal remedies shown to exhibit cholinesterase inhibitory, anti-inflammatory, antimicrobial, or antioxidant activities had a high phenolic content (Natarajan *et al*., 2013).

Flavonoids comprise of a large group within phenolic compounds. Flavonoids have recently aroused considerable interest because of their potential beneficial effects on human health largely due to their potent antioxidant activity. They are relatively lipophilic in nature and have been used as antioxidants in oil emulsions to reduce degradation during storage (Wang *et al.*, 2018). Evidence of *in vivo* activity of dietary flavonoids has also been shown by the link between high intake of foods rich in flavonoids and high plasma concentrations of related flavonoids. High concentrations of active flavonoids in blood have been correlated to a decreased incidence of some cancers (Palma *et al.*, 2017). The antioxidative effects of common spices was related to their polyphenol content. These include spices such as turmeric, clove, red pepper, black pepper, ginger, garlic, onion, and fenugreek containing polyphenols such as curcumin, eugenol, capsaicin, piperine, gingerol, caffeic acid, quercetin derivatives and gallic acid, respectively (Srinivasan, 2005). Phenolic acids and flavonoids are commonly mentioned because they take up a large part of plant phenolics. However, other compounds also show antioxidant activity.

Carotenoids are natural pigments responsible for the bright colours in most edible plants. They include β-carotene which gives the orange/yellow colour to vegetables and fruits such as mango, carrots, pumpkin and oil palm. Another carotenoid is lycopene, a red pigment seen in tomatoes and algae, while dark green leafy vegetables like kales, spinach and broccoli

contain xanthophylls like lutein and zeaxanthin. Carotenoids are efficient scavengers of radicals partly due to their multiple conjugated double bonds (Young and Lowe, 2018).

Another important group of plant compounds are alkaloids. Alkaloids are a nitrogenous group of compounds that can be obtained from plants, animals and marine life. Their most common feature is that they contain nitrogen in the molecule. Their activity as antioxidants is from their ability to form a stable radical due to the presence of the nitrogen and other functional side chain groups (Cushnie *et al*., 2014). Alkaloids are derived from amino acids which also show weak antioxidant activity. Common alkaloids with antioxidant activity include quinine, colchicine, harmane, berberine, ergot and vinca alkaloids (Jung *et al.*, 2009; Tiong *et al.*, 2013).

Phytosterols are plant sterols with close structural similarity to cholesterol. They include β-sitosterol, campesterol, stigmasterol and avenasterol. They naturally occur in nuts, cereals, vegetables and vegetable oils. Phytosterols can be used to enrich foods such as margarine and yoghurt. This is because foods high in phytosterols have cholesterol lowering ability as they compete with cholesterol for absorption in the gut. Their antioxidant activity is mainly seen in bulk oil preparations where they prevent polymerisation reactions in hot oils (Hsu *et al*., 2017).

## 1.3. THE DIET OF THE ILKISONKO MAASAI

The Ilkisonko Maasai are a pastoralist community living mostly in southern Kenya. They depend heavily on the environment as a source of livelihood. Their cattle offer them milk, meat and blood which are their staple foods. There is low consumption of local vegetables in the Maasai diet though there is the occasional intake of herbs in their soups. These herbs are added into the soups they drink or poured on freshly cut meat to preserve it (Ole-Miaron, 2003). That meant that a large proportion of their daily calories came from animal sources with high fat content. Despite this high calorie diet, the Maasai were known to be lean and have lower incidence of non-communicable diseases (NCD) and have a higher life expectancy of 63.5 years, against an average of 59 years in Kenya (Kenya National Bureau of Statistics, 2014).

There were land use changes instigated by the government in the early 1980s which allowed subdivision and sale of Maasai land. This brought an influx of other tribes and their practices into the area which caused a major nutrition transition among the Maasai. By the early 2000s, the Maasai had moved from being pastoralists to agro-pastoralists which resulted in an increased preference for cereals and vegetables commonly eaten by other tribes. The Maasai still obtained at least 30% of their calories from fat (from milk and milk products) which was twice the amount commonly seen in other communities. Due to loss of pastoral land in recent times, the Maasai youth move to urban areas in search of work and take carbohydrate rich foods with hardly any use of herbs and reasonably less physical activity. These lifestyle changes may have brought about an increase in occurrence of NCDs among the urban Maasai because of an increase in calorie rich foods with less physical activity (Prampero and Cerretelli, 1969; Christensen *et al.*, 2012). The rural Maasai have also not been spared. Though there are fewer incidences of NCDs, there is an increase in predisposing conditions such as higher hepatic insulin resistance, higher insulin secretion, lower glucose tolerance and higher cholesterol levels (Christensen *et al.*, 2014).

## 1.4. STUDY JUSTIFICATION

Chronic inflammation has been associated with a number of degenerative pathologies such as cancer, Parkinsonism, diabetes and psoriasis. Most of these disorders are chronic and their prevalence rates are rising quickly in developing countries. Lower age groups, between 30-45 years, are also being affected. There is need to examine how diets can prevent chronic conditions related to inflammation.

The Ilkisonko Maasai are a Kenyan pastoralist community who show a longer life expectancy and lower prevalence of NCDs. Studies have attributed this to a combination of factors including genetic, physical activity and diet (Wagh *et al.*, 2012). This study sought to investigate the role of traditional herbs that they use as medicine and as food additives. In the rural areas, traditional medicine is the primary health care system for some communities and some prefer it to conventional medicine (Kiringe, 2006). These herbs are also used to flavour their meats and soups and also to preserve meat for later use. The traditional herbs are decocted with bone broth to reduce side effects arising from eating too much meat (Ole-Miaron, 2003). These traditional herbs may contribute to improvement of their health. This

study looks at traditional herbs that are specifically used by the Ilkisonko Maasai. Though they share the same beliefs and healing practices with other *Maa* speaking groups, the traditional medicine used is dictated by the plant ecotype in their habitat hence the differences in plant use seen with the Ilkisonko Maasai in Kajiado South.

Though the Maasai have a genetic adaptation that may assist in lowering cholesterol it still does not prevent them from getting chronic diseases related to hypercholesterolemia (Wagh *et al.*, 2012). This was evident when their urban counterparts demonstrated similar incidence of chronic diseases as other tribes (Ngoye *et al*., 2014). The Maasai diet is also not a factor in reducing their rates of inflammatory disorders. This is because they depend mostly on milk and meat products, which gives a high calorie diet. From the 1990s, there was an influx of other tribes into the area prompting the Maasai to progressively add more cereals and vegetables to their diet. Despite this, they still have a high calorie intake, with more than 30% of calories from animal fat, a diet that increases predisposition to chronic diseases (Lawson *et al.*, 2014). However, the incidence of chronic diseases among the Ilkisonko Maasai is still lower than some ethnic communities who take more vegetables or include white meat in their diet (Christensen *et al.*, 2012).

It is important to look into the effects of these traditional herbs used as additives by the Ilkisonko Maasai. These plants grow in the surrounding semi-arid lands (SALs), home to the pastoralist communities. Arid areas have an abundant source of plant medicines that are able to thrive well in such harsh environments (Lamaoui *et al.*, 2018). These conditions encourage the production of secondary metabolites possessing a myriad of biological effects, including antioxidant activity. Plants potentially rich in beneficial metabolites may influence the health that the Ilkisonko Maasai enjoy. Previous work on the Maasai community has found useful medicinal plants (Dambolena *et al.*, 2010). Showing the importance of these plants by documenting their traditional use and demonstrating their pharmacological activity will make it imperative to safeguard plants used as traditional medicine.

## 1.5. ALTERNATIVE HYPOTHESES

1. Traditional plants in the diet of the Ilkisonko Maasai have antioxidant and anti-inflammatory effect.

2. The observed antioxidant and anti-inflammatory effects are attributable to isolable bioactive chemical constituents.

## 1.6. OBJECTIVES

### 1.6.1. General objective

To investigate the anti-oxidative and anti-inflammatory properties of traditional plants used in the diet of the Ilkisonko Maasai of Kajiado County, Kenya.

### 1.6.2. Specific objectives

This study sought to:

a) Conduct an ethnobotanical survey of the traditional herbs used as foods and medicine by the Ilkisonko Maasai community.
b) Determine the total phenolic and flavonoid content of the traditional herbs used by the Ilkisonko Maasai community.
c) Investigate the antioxidant properties of selected traditional herbs and plants used by the Ilkisonko Maasai community.
d) Investigate the anti-inflammatory property in selected herbs and plants.
e) Isolate and characterize phytochemical compounds in plant with antioxidant and anti-inflammatory activity.

## 1.7. SIGNIFICANCE OF THE STUDY

Due to unavailability or inability to afford conventional medicine, traditional medicine is the primary health care system for some rural communities. Other communities have a preference of the traditional medicine over the conventional medicine. Traditional plants also provide a source of food for many in the rural areas. Despite this, traditional plant knowledge is in danger of being lost as it is not only passed down by oral folklore, but is also shrouded in secrecy. There is also an emerging threat to the abundance of traditional plants in some communities due to anthropogenic factors such as increased consumption emanating from

increase in local human population, charcoal burning and land use changes predominantly expansion of agriculture (Kiringe and Okello 2005).

Arid areas have abundant indigenous plants that are able to thrive well in such harsh environments. These plants are adapted to harsh environmental conditions and can support the biodiversity in such arid areas. There is a need to showcase the value of indigenous species in supplementing the nutritional needs of people living in drought prone areas. By showing the benefits of indigenous plants in SALs, their cultivation and propagation can be encouraged. These indigenous plants can be used sustainably as windbreakers, to protect against soil erosion, as food and medicine (Mavhura *et al*., 2015). Due to the harsh conditions, these plants produce higher phytochemical content to cope with the environmental stress (Lamaoui *et al.*, 2018). The wider rural communities harbouring these indigenous plants would benefit from some important secondary metabolites. This would encourage conservation and propagation of these plants rather than the current attempt at modern crop agriculture, which is prone to a high failure rate.

A number of ethnobotanical surveys have focused on documenting plants used in traditional medicine but few have documented the available plants used as both food and medicine. This work investigates a number of indigenous trees and shrubs common in SALs and their benefits to the Maasai and other adjacent communities. Furthermore, no work has been done on the antioxidant and anti-inflammatory effects of traditional food and medicine among the Ilkisonko Maasai. For plants that have already found use in the community, this study seeks to give scientific credence to their traditional use. This will authenticate their value to the community and encourage propagation and conservation of such trees in the area.

# 2.0. CHAPTER TWO: LITERATURE REVIEW

## 2.1. HISTORY OF PLANTS AS SOURCES OF MEDICINE

Plants have played a major role in human life for many centuries. Humans have used different types of plants in everyday life in various ways. One of the most recognisable uses of plants is nutrition. They provide the essential spectrum for a complete diet as good sources of starches, proteins, fibre, vitamins, minerals and many more essential nutrients. Hippocrates philosophy "Let food be thy medicine and medicine be thy food" shows the importance of plants used in nutrition as therapeutic substances. Such plants may be used as spices or flavourings in food but may also be included in herbal formulations in traditional forms of medicine.

Since ancient times, humans have been looking for ways to treat their ailments from the most available resource, nature. This is supported by written evidence from the world over. Indigenous plants and disease prevalence in an area dictated the type of plants used medicinally and how they were used. For example, the plants encountered around the tropics differ from those seen in temperate climates meaning that for a common disease, different plants would be used for a therapeutic intervention. A look at the early systems of medicine shows how different plants may have been used to treat similar diseases or symptoms.

As documented on papyrus leaves by the ancient Egyptian civilisation, one of the earliest medical practices was the traditional African medicine (TAM). Other parts of Africa may have been using TAM though it was not documented but passed on through folklore. Numerous varieties of medicinal plants growing in Africa have been used to treat endemic tropical diseases as well as minor ailments. Such plants include *Anacardium occidentale*, *Adansonia digitata*, *Allium cepa*, *Balanites aegyptiaca*, *Commiphora africana*, *Jatropha curcas*, *Justicia betonica*, *Ruellia patula*, and *Sagittaria guayanensis* among others. All these plants mentioned have not only been used as medicinal agents but also as spices or vegetables (Iwu, 2014).

Ayurveda, one of the earliest Asian traditional medicine that started about 4500 years ago, advocates for the use of plants in balancing the body energy. Each person has their own unique "energy field", comprising of physical, emotional and mental characteristics, and an

imbalance in this energy leads to disease. Balance can be maintained through the right lifestyle and diet choices depending on a person's characteristics. Treatment can be through the use of specific herbs, which can also be incorporated into foods, such as ashwagandha, turmeric, gotu kola, Indian gooseberry, liquorice, guggul, garlic and ginger (Sarkar *et al.*, 2015). Homeopathy arose from the principles of Ayurveda where use of toxic and non-toxic plants offer healing by accentuating the body's response to an injurious agent. It is based on reinforcing of an organism's natural healing power and the ability to restore the energy homoeostasis (Frye, 2003). Herbs used in Ayurveda include *Atropa belladonna*, stinging nettle, *Lycopodium, Strychnos nux-vomica*, and Ipecacuanha. A vegan diet is encouraged in homeopathy as a plant based diet is generally higher in antioxidant content than animal-based food products and is shown to reduce occurrence of some diseases (Venderley and Campbell, 2006).

Traditional Chinese medicine (TCM) began around 2000 years ago and may have been born out of Ayurveda due to the similar plants used in treatment. In TCM, plants are used to negate the effects of the disease on the body such as "warm herbs" to treat a "cold disease" or vice versa. Common plants used in TCM include, but not limited to, liquorice, ginger, ephedra, ginseng, gingko, astralagus, fox glove and nutmeg (Li *et al.*, 2018). Traditional medicine from the Asian continent has added to the wealth of knowledge with books like "Pen T'Sao," about roots and grasses from China and Indian holy books Vedas which involves use of spices abundant in India (Lim, 2012; Sreeramulu *et al.*, 2013). Plant spices are incorporated into foods for prevention and management of certain illnesses. Spices commonly used in Asian medicine include turmeric, black pepper, anise, clove, red pepper, fenugreek, ginger and garlic.

Theophrastus (371-287 BC), regarded as the "father of botany", generated a couple of plant history books with more than 500 medicinal plants. He referred to fragrant hellebore, iris rhizome, pomegranate, false hellebore, mint, cinnamon, aromatic cardamom, monkshood, and so forth. Dioscorides, "the father of pharmacognosy," studied medicinal plants in ancient Rome and wrote the basic *materia medica* of that time. Dioscorides' most appreciated domestic plants include willow, chamomile, garlic, onion, marshmallow, ivy, nettle, sage, common centaury, coriander, parsley, sea onion, and false hellebore (Peduto, 2001). Pliny the Elder (23 AD-79) also looked at European plants to write his book "*Historia naturalis*" with the richest knowledge of medicinal plants at the time. Documented plants used included

*Rosmarinus officinalis*, *Ocimum basilicum*, *Iris germanica*, and *Mentha viridis* in cosmetics, *Alium sativum* as a remedy and *Veratrum album, Cucumis sativus*, *Urtica dioica, Achilea millefolium, Artemisia maritime, Lavandula officinalis* and *Sambuci flos* as spices and also against several injurious insects (Stannard, 1982). From the earlier works of Dioscorides, Theophrastus, and Pliny the elder, more than half the number of plants mentioned have been found to have thousands of pharmacologically active components that can be used as medicine.

## 2.2. INFLAMMATION AS A CAUSE OF DISEASE

Inflammation is a biological response to harmful stimuli (Warrington *et al.*, 2011). The inflammatory process starts a chain of molecular events that occur to help repair tissue after harm such as by infection or injury (Schmid-Schönbein, 2006). Inflammation signals the body to help in pathogen elimination and it is also a physiological signal for angiogenesis, and remodelling of extra-cellular matrix (Cho *et al.*, 2007). The inflammatory process can be short term (acute) or long term (chronic) depending on the duration. Acute inflammation is short term and shows all the five classical symptoms of inflammation such as swelling, redness, pain, warmth and loss of function (Schmid-Schönbein, 2006). An effective acute inflammatory response is necessary and beneficial to the organism as it will lead to elimination of the trigger and repair of the damaged area. Chronic inflammation is a long term process and may occur in the absence of a stimulus with subsequent destruction of the tissue involved. This unresolved and continuous inflammatory process leads to enhanced risk of chronic diseases (Sur *et al.*, 2014). Triggers of inflammation include microorganisms, environmental stimuli such as smoke, dust, and chemicals, tissue necrosis, foreign bodies, and other immune reactions.

Acute inflammation consists of two phases, an early and a late phase, brought about by several mediators. The initial phase of inflammation (1-6 h) involves histamine, serotonin and bradykinin, a vasoactive peptide (di Rosa *et al.*, 1971). Bradykinin is produced from Hageman factor which stimulates the release of more histamine and activates the arachidonic acid pathway to produce prostaglandins (Fernando *et al.*, 2005). Through cyclooxygenase (COX)-2 activation, prostaglandins and related compounds start and sustain the second phase of inflammation (12-24 h) (Guay *et al.*, 2004). Prostaglandins (PGs) are potent modulators of

inflammation and immune responses. They normally regulate immune cell functions though elevated levels of prostaglandin $E_2$ ($PGE_2$) has been found in many pathological states. Prostaglandins increase vascular permeability to allow neutrophils extravasate to the site of injury. Cyclooxygenase inhibitors such as aspirin and indomethacin work well in inhibiting the second phase of inflammation but not the first phase (Guay *et al.*, 2004).

Immune cells such as neutrophils, macrophages/monocytes are the first cells to the site of injury to potentiate acute inflammation by producing mediators and free radicals such as reactive oxygen species (ROS) (Lämmermann *et al.*, 2013) and reactive nitrogen species (RNS). Elevated ROS and RNS levels cause a subsequent increase in different soluble factors such as cytokines, which mediate the movement of immune cells. Cytokines are cell-derived polypeptides that act to interconnect the immune system and host tissue cells (McInnes and Schett, 2007). There are different classes of cytokines grouped according to their functions, though this seems to overlap frequently. There are majorly pro-inflammatory and anti-inflammatory cytokines as regulators of inflammation. Examples of pro-inflammatory cytokines include interleukin (IL)-1α/β, IL-2, IL-6, IL-11, IL-8, tumour necrosis factor (TNF)-α/β, and interferon (IFN)-γ, while anti-inflammatory cytokines include IL-10, transforming growth factor (TGF)-β, and IL-1 receptor antagonist (Turner *et al.*, 2014). Inflammatory cytokines bring more immune cells to the area of injury while anti-inflammatory cytokines increase the expression of other anti-inflammatory cytokines and reduce the production of inflammatory mediators such as $PGE_2$, COX 2 and inducible nitric oxide synthase (NOS) (Doi *et al.*, 2008). Apoptotic cells are ingested by macrophages which triggers the release of inflammation resolving cytokines such as TGF-β and IL-10 (Kennedy and Deleo, 2009). Once the stimulus is removed, resolution of acute inflammation can commence with a return to normal of the cells activated.

There are also important proteins that are involved in the progression or easing of inflammation. They are commonly seen in the development of metabolic syndrome (MS) and include adiponectin, leptin and C-reactive protein (CRP). Metabolic syndrome is a collection of pathological conditions such as, but not limited to, abdominal obesity, insulin resistance, dyslipidemias, hypertension, and prothrombotic and proinflammatory states (Li *et al.*, 2018). Increased glucose and fat intake leads to increased production of radicals that promote chronic inflammation common in MS (Ruggiero *et al.*, 2011). This increase in inflammation is usually preceded by a decrease in adiponectin concentration and an increase in CRP.

Adiponectin is produced by white adipose tissue and it is an anti-inflammatory adipokine associated with lower inflammation states and better outcome in patients with MS. C-reactive protein is a pro-inflammatory protein commonly elevated in inflammatory states. An increase in CRP has a direct correlation with occurrence of diabetes and atherosclerosis (Sur *et al.*, 2014). Metabolic syndrome is a risk factor in the development of diabetes, coronary artery disease and cerebrovascular disease. It is also implicated in many other diseases which show presence of systemic chronic inflammation such as psoriasis, asthma, arthritis, rheumatism, Alzheimer's disease, cataract, cancers and aging (Scalbert *et al.*, 2005).

The ROS produced by the immune cells are highly effective in removing or neutralizing the cause of inflammation. However, the same reactive species may harm the surrounding tissues when the inflammatory process is left active for long (Fig 2.1). Unresolved inflammation is present in patients with immune diseases such as psoriasis and chronic diseases such as diabetes, gout, rheumatoid arthritis, osteoarthritis, cancer, Alzheimer's, and hypertension (Scalbert *et al.*, 2005). This is common where the body endogenous systems of neutralizing the reactive species are overwhelmed or impaired. This is also seen when acute and chronic inflammation coexists over long periods, implying continual re-initiation. The disorders mentioned are mostly seen in the elderly, which could be related to their diet or the fact that the aggressiveness of inflammation tends to increase with age (Harris and Rumbaut, 2001).

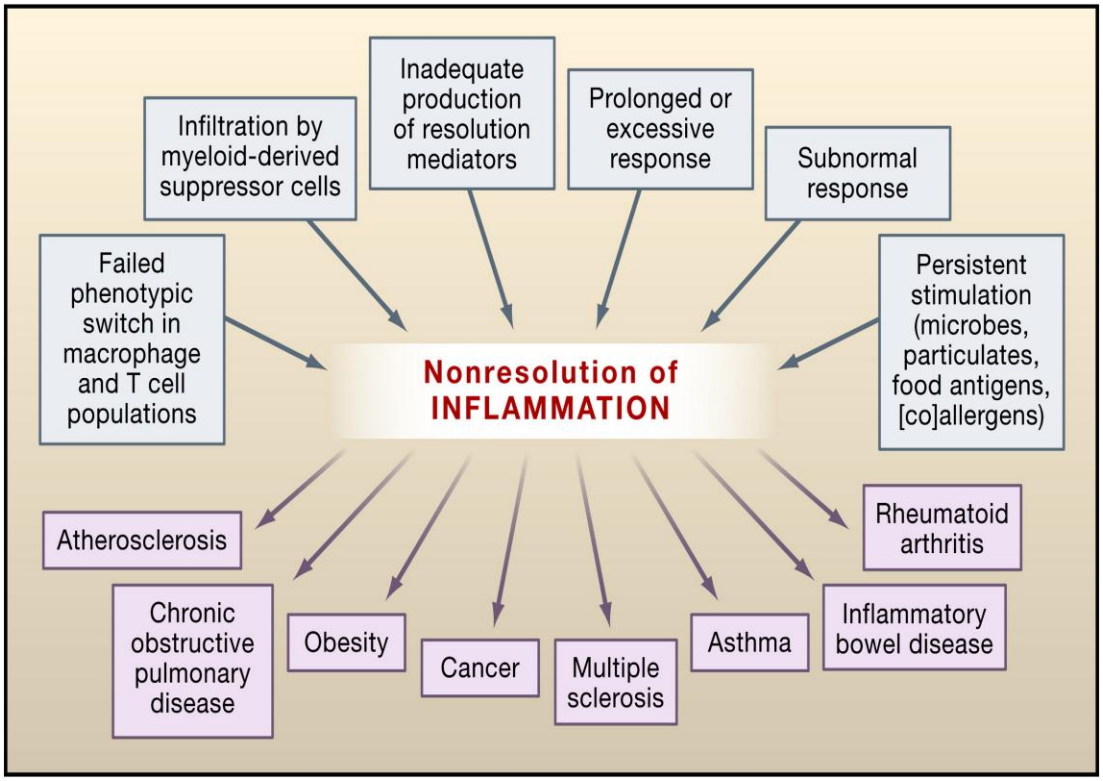



**Figure 2.1: Chronic inflammation and consequences of non-resolution of inflammation** (Nathan and Ding 2010).

Nutrition has a great effect on reducing inflammation as some compounds from plants may act, not only as anti-inflammatory agents but may also promote resolution (Serhan, 2007). Experimental and epidemiological evidence suggests a significant role of diet in the prevention of degenerative diseases (Neuhouser, 2019). Trials of diets enriched in whole grains, and Mediterranean diets enriched in olive oil, wine, raw vegetables and nuts showed reduced levels of inflammation as measured by levels of CRP (Ziech *et al.*, 2012). This was most demonstrable in the French, who partook a staple of Mediterranean diet and had, additionally, a lower incidence of cardiovascular diseases (Scalbert *et al.*, 2005)

## 2.3. ROLE OF OXIDANTS IN THE BODY

Radicals are groups of atoms with one or more unpaired electrons which gives them high chemical reactivity. The ability to oxidise or take electrons from other molecules gives them the name oxidants. In biological systems the most common type of oxidants are those derived from oxygen, such as ROS, though RNS are present in small amounts. Examples of ROS include superoxide anion, hydroxyl radical and peroxide radical while peroxynitrite and

nitrogen dioxide are examples of RNS. These reactive species, usually referred to as ROS/RNS, are often generated as by-products of biological reactions such as mitochondrial oxidative phosphorylation, P450 metabolism, peroxisomes metabolism and activation of neutrophils and macrophages during inflammation. Neutrophils and macrophages can produce high quantities of radicals when the need arises. Reactive species can also be generated from exogenous factors like exposure to drugs, pesticides, smoke, radiation, chlorinated compounds, and polycyclic aromatic hydrocarbons (PAHs), among others (Bartsch and Nair, 2006). The role of reactive species cannot be understated as, at low concentration, they contribute to the immune response, induce a mitogenic response, maintain redox homeostasis and function as signalling molecules (Valko *et al.*, 2007; Wu *et al.*, 2008). Reactive species are involved, at different levels, in the signal transduction cascade; they can function as second messengers and will mediate many biological functions with induction of apoptosis being their major role (Storz, 2007).

Reactive species present a puzzle. Though they have important physiological functions, there is potential for great harm when unregulated, as they are associated with a number of pathologies including cancer (Fig 2.2). This has given rise to the concept of redox homeostasis that refers to the balance between destructive and constructive oxidant levels in the body. There are physiological antioxidative mechanisms that maintain redox homeostasis to protect the cells from the harmful effects of free radicals. In certain conditions, there is an overproduction of ROS/RNS, which may surpass the capacity of antioxidative systems, or there could be a decrease in the effectiveness of cellular antioxidant defence systems. This leads to oxidative stress characterized by increased levels of reactive species (Tudek *et al.*, 2010). In acute conditions, the high levels of reactive species may cause necrosis and on prolonged activity it can induce tissue toxicity by damaging proteins, lipids and DNA (Ziech *et al.*, 2012). Radicals react with DNA bases causing permanent damage and is seen as the first step towards mutagenesis and ageing (Halliwell and Gutteridge, 2004). Free radicals and aldehydes, produced during chronic inflammation, can also induce deadly gene mutation and post-translational modifications of key cancer-related proteins (Block *et al.*, 2015). Oxidative stress plays a role in the pathogenesis of aging, and various inflammatory conditions such as cancer, acute pancreatitis, post ischemic syndrome, atherosclerosis, and diabetic angiopathy (Lobo *et al.*, 2010). Some of these diseases are associated with MS whereby the natural body antioxidant system is crippled and hence more prone to oxidant insults (Jamal *et al.*, 2014). For example, the development of β-cell dysfunction, insulin resistance and later diabetic

complications has been attributed to ROS/RNS oxidative stress. Type 2 diabetic individuals show an imbalance in ROS/RNS generation and neutralisation (Akbar *et al*., 2011).

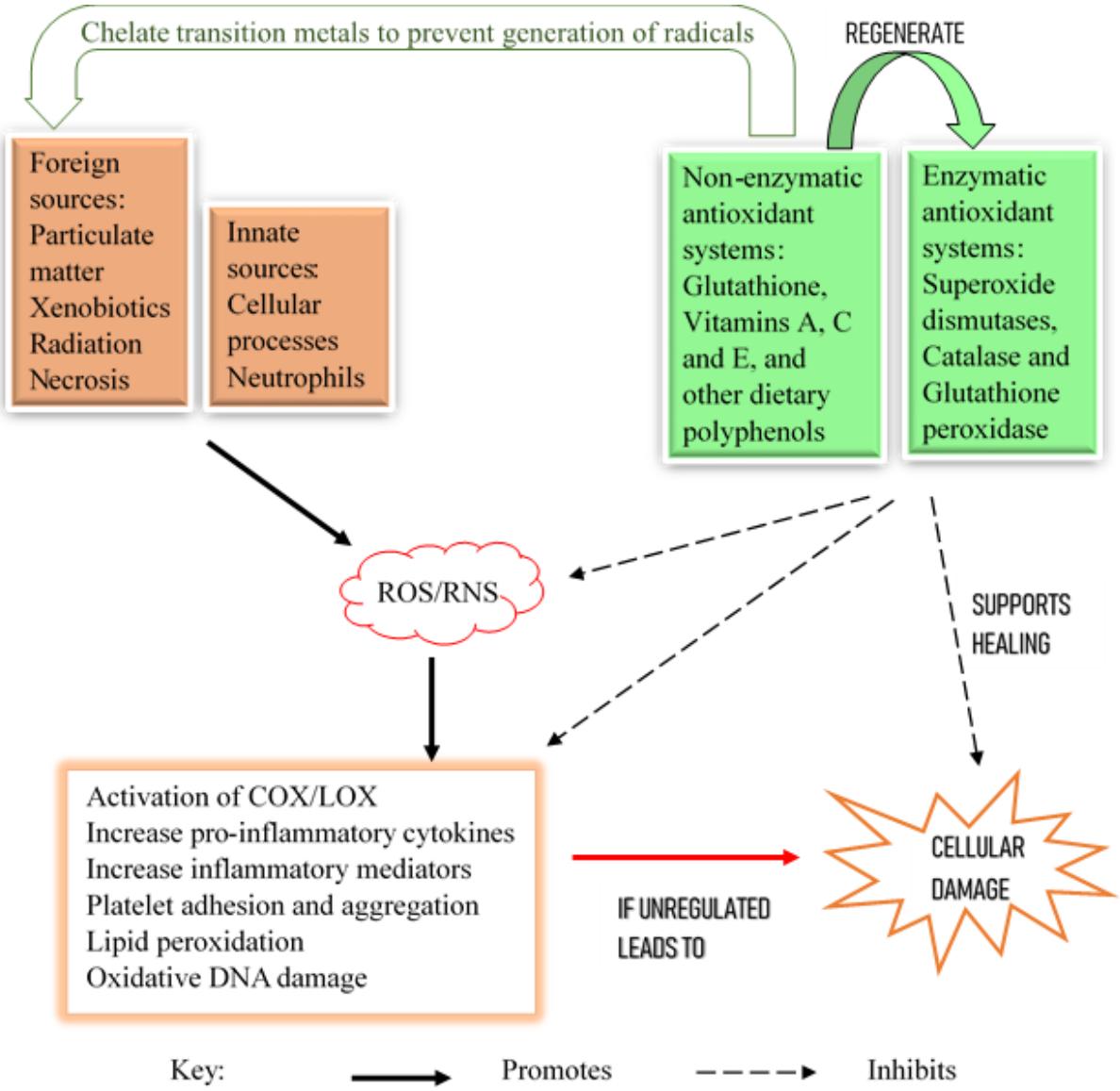



**Figure 2.2: Influence of oxidants and antioxidants on cellular damage**

## 2.4. ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANT SYSTEMS

Radicals produced during normal biological reactions need to be neutralised to avoid damaging cells and other macromolecules. Antioxidants are reducing agents that prevent oxidative reactions by scavenging the ROS/RNS. The human antioxidant system consists of small molecule antioxidants, antioxidant proteins, ROS-metabolizing enzymes, and several regulator proteins that mediate adaptive responses to oxidant stress. There are enzymatic and

non-enzymatic antioxidant systems. The body's first line defence against oxidants are the enzymatic systems namely superoxide dismutases, catalase and glutathione peroxidase. Non-enzymatic antioxidants include glutathione, vitamins A, C and E, and other dietary polyphenols (Younes, 1999). Many antioxidants also elicit adaptive responses such as the induction of enzymes that detoxify ROS/RNS and ROS-promoting chemicals (Ma, 2014). The antioxidant effect of polyphenols can be as direct ROS scavengers like tocopherol and ascorbate, while most antioxidants act indirectly by upregulation of cytoprotective phase two genes, transition metal reducers and chelators, or as chain breaking antioxidants (Yoshida *et al.*, 1999). The cytoprotective genes actually produce endogenous antioxidants like glutathione and thioredoxin. Thus, mammalian anti-oxidation is achieved through both direct (scavenging) and indirect (adaptive) modes of action (Natarajan *et al*., 2013). Antioxidants assist in maintaining redox homeostasis for the health of the organism. They are especially important for cells that are susceptible to ROS/RNS damage such as brain, due to high oxygen utilization, and pancreas. Islet cells have low levels of antioxidant enzymes and are prone to oxidative damage leading to type II diabetes mellitus (Wang and Wang, 2017).

One of the major non-enzymatic antioxidant is glutathione (GSH). It is produced in the cytosol where it undergoes reversible redox conversion to its oxidised form glutathione disulphide (GSSG). GSH is a cofactor of several detoxifying enzymes, it functions as an amino acid carrier, it neutralises hydrogen and lipid peroxides, and is able to regenerate other non-enzymatic antioxidants such as vitamins C and E (Valko *et al.*, 2007). Glutathione is also important in cell growth; its concentration will determine if the cell proceeds towards differentiation, proliferation or apoptosis (Voehringer *et al.*, 2002). Ascorbic acid is another key non-enzymatic antioxidant. It is able to protect and regenerate other antioxidants such as carotenoids and tocopherols. It can also react with several radicals to neutralise them. Ascorbic acid is found in high concentration in certain vegetables and it occurs with other polyphenols where they work synergistically to ensure protection of macromolecules in the body (Fig. 2.2) (Foyer and Noctor, 2011). Phytochemicals present in plant foods and medicines wield health beneficial effects, as they tackle oxidative stress in the body by maintaining a balance between oxidants and antioxidants.

## 2.5. ANTIOXIDANT AND ANTI-INFLAMMATORY COMPOUNDS FROM PLANTS

### 2.5.1. Plant phenolics

Plant phenolics occur as secondary metabolites and are generally involved in protection, as well as contributing to plants' colours (Fig 2.3). Normally, plants produce secondary metabolites to aid in their survival in harsh environments as well as defence against predators. The harsh weather conditions in arid areas are associated with biological traits that allow adaptation in those conditions. The ambient conditions such as poor soil characteristics as well as locust and rodent attacks associated with arid areas, make the plants produce more polyphenolics (above the normal) for protection and survival adaptation (Yordanov *et al*., 2003). Polyphenolics are ubiquitous in all plant organs and are therefore common in plant foods and beverages, and partially responsible for their overall organoleptic properties.

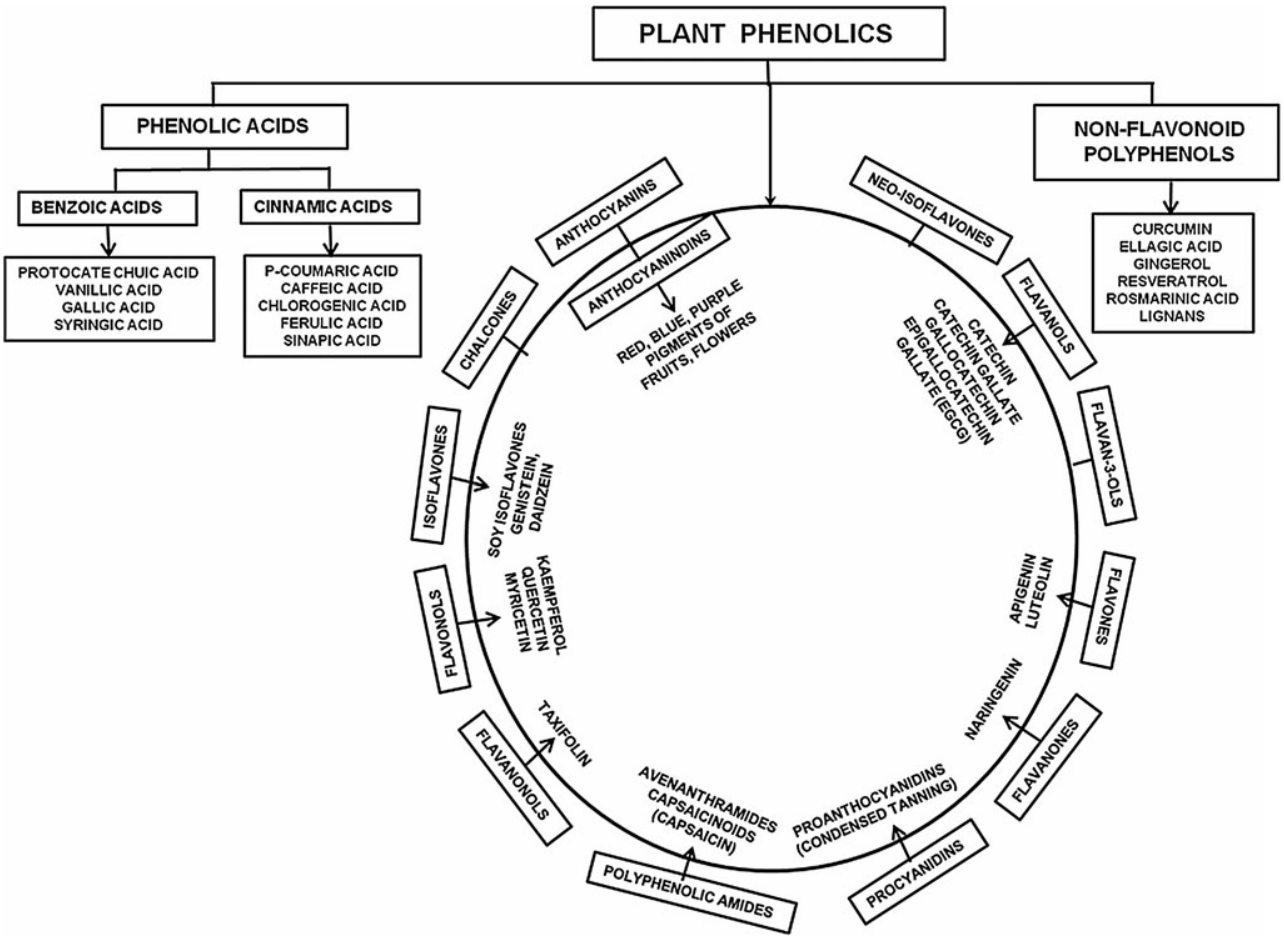



**Figure 2.3: Classification of plant phenolic compounds.**

(Malireddy *et al.*, 2012).

Plant derived antioxidants, such as flavonoids and related phenolic compounds, have multiple biological effects, one of which is their role as non-enzymatic antioxidants (Scalbert *et al.*, 2005). Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups (Dai and Mumper, 2010). Phenolic compounds act as antioxidants by donating a hydrogen atom that will deactivate free radicals. The phenoxyl radical that is formed after losing a hydrogen is more stable than other reactive radicals found in the body such as ROS/RNS. Structure activity relationship of phenolic compounds shows that having more than one active groups (OH or $NH_2$) at *ortho* positions gives the best activity (Fig. 2.4). Catechol has good antioxidant activity due to two hydroxyl groups at *ortho* positions (Bendary *et al.*, 2013). Catechol requires the lowest bond dissociation energy for its hydroxyl groups. Hence, the easier it is for a phenolic compound to lose a hydrogen atom, the more reactive it is as an antioxidant molecule. Aromatic amines have comparable activity to catechol and are likely to give false positive results in phenolic assays (Valgimigli *et al.*, 2008). Catechol, 4-aminophenol and eugenol have high antioxidant activity, unlike phenol, due to the presence of electron donating groups (OH, $NH_2$ or alkyl substituents) at the *ortho* or *para* position (Fig 2.4).

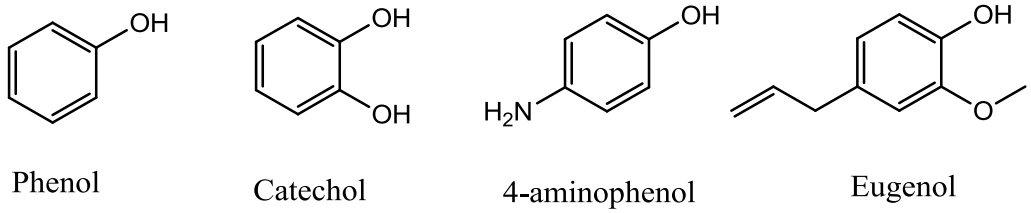



**Figure 2.4: Structures of phenolic and anilinic compounds.**

Plant phenolics include phenolic acids, flavonoids, tannins and the less common stilbenes, coumarins and lignans. Phenolic acids can be divided into three classes: derivatives of hydroxybenzoic acid such as gallic acid, derivatives of hydroxyphenylacetic acid such as homovanillic acid and derivatives of hydroxycinnamic acid such as coumaric, caffeic and ferulic acids (Fig. 2.5) (D'Archivio *et al.*, 2007). Antioxidant activity of phenolic acids is dependent on number and position of hydroxyl groups in the molecule. The more hydroxyl groups present, the higher the antioxidative potential. Cinnamic acid derivatives have better activity than benzoates and phenylacetic acids as the electron withdrawing properties of

carboxylate group affect the hydrogen donating ability of the molecule (Chen *et al.*, 2020). The presence of an ethylene group between the phenol and carboxylate groups in hydroxylated cinnamates, enhances the reducing property of the hydroxyl group. Substitution of 3-hydroxyl group by a methoxy group enhances antioxidant activity making ferulic acid more active than caffeic acid (Fig. 2.5). The methoxy group in ferulic acid increases stabilisation of the resulting radical through electron delocalisation after hydrogen donation (Chen *et al.*, 2020). Caffeic acid is the most abundant phenolic acid in many fruits and vegetables, most often esterified with quinic acid as in chlorogenic acid, which is the major phenolic compound in coffee (Dai and Mumper, 2010). Ferulic acid is commonly present in cereals and is esterified to hemicelluloses in the cell wall, due to this it is found in milled whole grain flour (D'Archivio *et al.*, 2007).

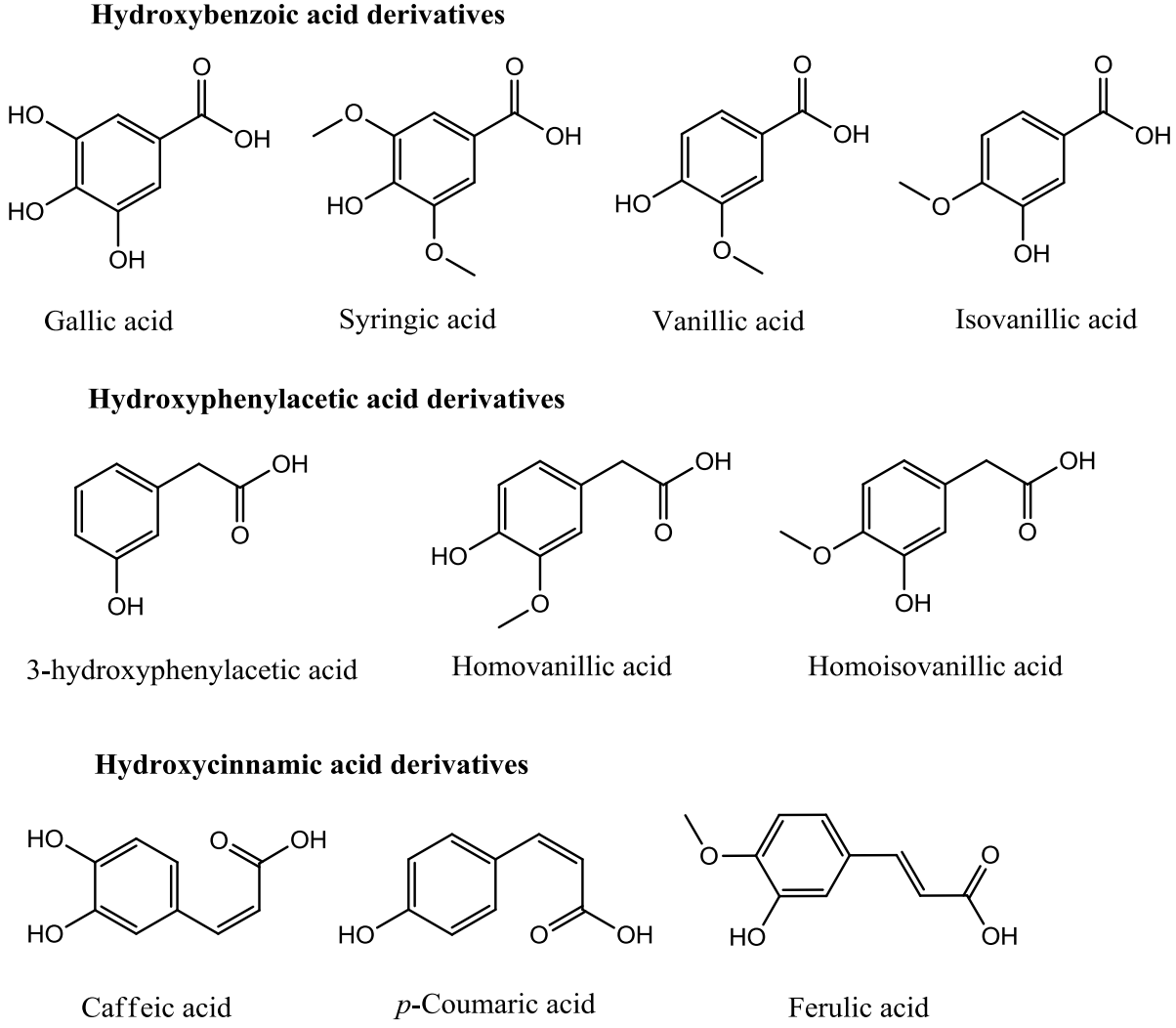



**Figure 2.5: Phenolic acids derived from hydroxybenzoic, hydroxyphenylacetic and hydroxycinnamic acid skeleton.**

Antioxidant activity has been directly related to the total phenolic content. These compounds could be contributors to the high antioxidant activity seen in plants with high phenolic content such as *Geranium wilfordii, Loranthus parasiticus, Polygonum aviculare, Pyrrosia sheaeri, Sinomenium acutum* and *Tripterygium wilfordii* (Natarajan *et al*., 2013). This is also seen in traditional Chinese medicine where the plants commonly used as anticancer agents have high antioxidant activity that is correlated with high phenolic content. Some of these plants are *Rhus chinensis, Acacia catechu, Areca catechu, Scutellaria baicalensis, Rheum officinale* and *Terminalia chebula* (Liao *et al*., 2008; Zhang *et al*., 2017).

*In vivo,* curcumin, a ferulic acid derivative of *Curcuma longa,* has been shown to have anti-inflammatory activity exhibited by inhibiting several inflammatory markers such as thromboxane, PGs and NO, and normalizing antioxidant systems in the liver (Seo *et al*., 2008; Omosa *et al*., 2017). Experimental evidence has shown activity of curcumin in epigenetic modulation to decrease DNA changes that bring about cancers and metabolic disorders (Malireddy *et al.,* 2012). Epigallocatechin gallate reduces expression of COX-2 and soluble inflammatory markers (Fechtner *et al.,* 2017) while umbelliferone, a polyphenol from *Acacia* species, was able to reduce the production of inflammatory markers and increase apoptosis of abnormal cells, which can reduce cancer progression (Muthu *et al*., 2016). *Centella asiatica* used mostly in eastern cuisine is reported to have antioxidant effect *in vitro* due to the high content of phenolic acids (Sreeramulu *et al.,* 2013). Salidroside, a glycosylated phenol from *Rhodiola rosea,* has documented antioxidative properties and acts by preventing the over-activation of oxidative stress-related downstream signalling pathways (Gan *et al.,* 2010; Zhang *et al.,* 2013). Resveratrol, a non-flavonoid polyphenol, found in red wine, were shown to decrease the expression of pro-inflammatory mediators (Chae *et al.,* 2011) and inhibit cytokine production by preventing activation of nuclear factor kappa B (NF-κB) (Yahfoufi *et al.,* 2018).

Flavonoids are some of the common polyphenols in nature. Different compounds with the flavan nucleus exist in different classes such as flavones, flavanones, isoflavones, flavonols, flavanonols, flavan-3-ols, anthocyanidins, biflavones, chalcones, aurones, and coumarins (Fig 2.3) (Pier-Giorgino, 2000). They are made up of three rings and the substitution on different positions on the B ring highly influences antioxidant activity (Fig. 2.6) (Wang *et al*., 2018). Some of the most common flavonoids include quercetin, a flavonol abundant in onion,

broccoli, and apple; naringenin, the main flavanone in grapefruit; catechin, a flavanol found in tea and several fruits; cyanidin-glycoside, an anthocyanin abundant in berry fruits; and daidzein, genistein and glycitein, the main isoflavones in soybean (D'Archivio *et al.*, 2007).

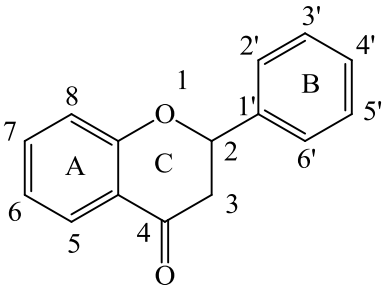



**Figure 2.6: Basic structure for flavonoids.**

Flavonoids combat oxidative stress by acting as antioxidants by way of neutralising radicals or by regenerating enzymatic antioxidants. The arrangement of the B and C rings in flavonoids and their substitutions allows for electron delocalisation after radical scavenging activity. The hydroxyl group at position 3 and an ortho dihydroxyl group in the B-ring favours antioxidant activity. The 2, 3-double bond, in ring C, and the 4-oxo function makes the flavonoid structure more responsive (Palma *et al.*, 2017). This is seen with apigenin and naringenin which only differ in the presence of a 2,3-double bond (present in apigenin only) conferring moderate antioxidant activity to apigenin while naringenin has none (Fig. 2.7). This explains why flavanones and flavanonols have weak antioxidant activity than the more oxidised flavonoids such as flavones and flavonols (Alqasoumi *et al.*, 2016). Quercetin, the most abundant dietary flavonol, is a powerful antioxidant because it has all the right structural features for free radical scavenging activity. Although quercetin and catechin are structurally related (Fig. 2.7) given that they both contain three hydroxyl groups, they differ in the C-ring where quercetin has a 4-keto group and a 2,3 unsaturated bond. This allows for better electron delocalisation with quercetin than catechin, when neutralising radicals (Palma *et al.*, 2017).

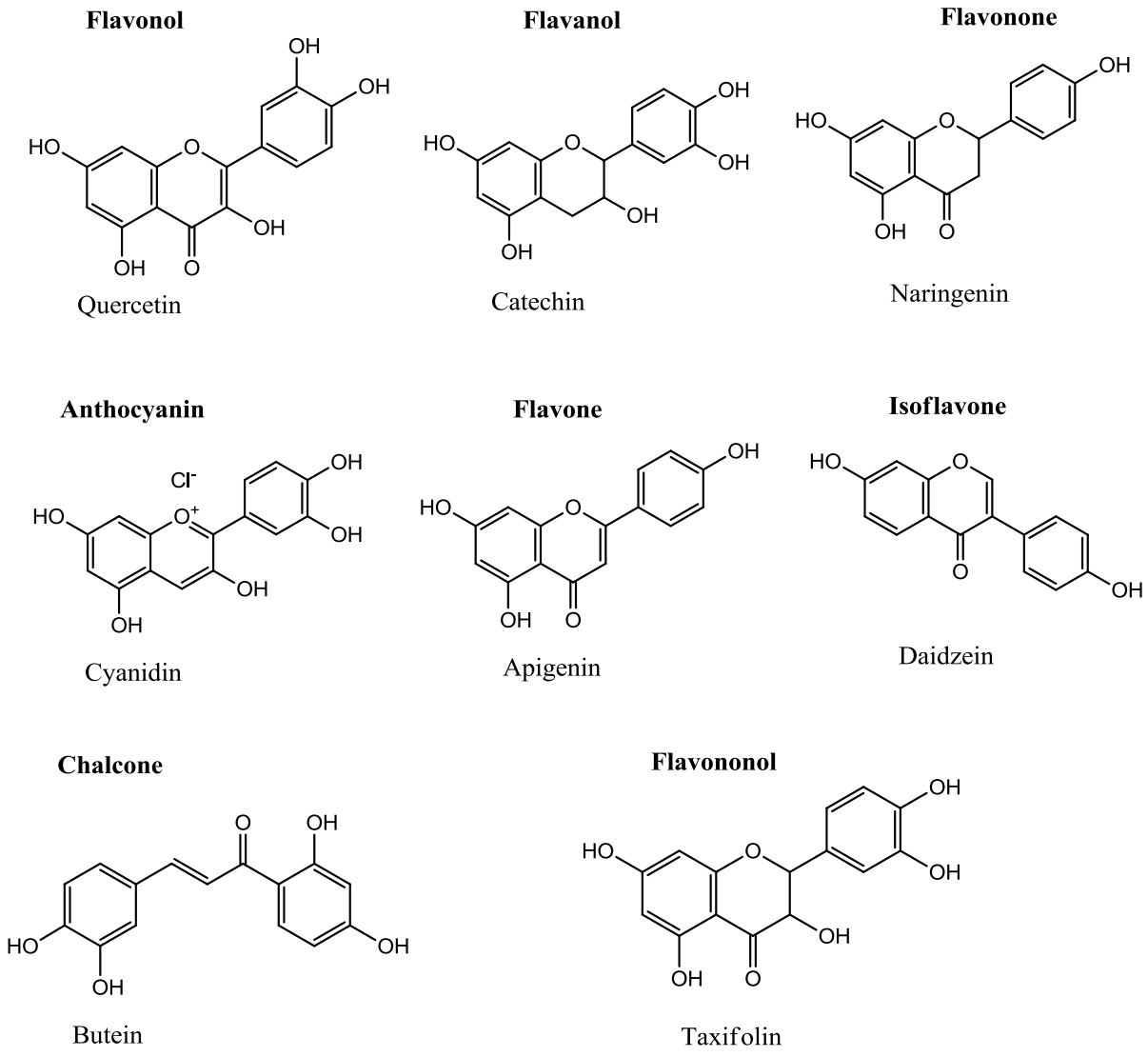



**Figure 2.7: Common flavonoid compounds.**

Flavonoids are able to act in different stages of the inflammation cascade to prevent oxidative stress or delay the occurrence of some diseases. This may be through the reduction in production of radicals (NO) during inflammation by quercetin. Melanoxetin, a flavanol from *Acacia confusa*, inhibited lipopolysaccharide-induced NO production in a concentration-dependent manner, with activity closely similar to that of quercetin (Lin *et al*., 2018). *In vivo* studies have shown the ability of flavonoids such as hesperidin, quercetin to reduce the production of inflammatory mediators that prevent the progression of cancers, metabolic disorders or neurodegenerative illnesses. Flavonoids, like quercetin, may also increase the production of anti-inflammatory proteins and adiponectin, an anti-inflammatory hormone (Fechtner *et al.*, 2017). Extensive research has focused on are soy isoflavones such as genistein and daidzein and their role in cancer prevention due to their effect in lowering

inflammation. Soybean isoflavones reduce the incidence of breast, endometrial and prostate cancers, decrease recurrence and promote survival in cancer patients (Vitolins *et al.*, 2013; Zhong *et al.*, 2018). Flavonoids have been shown to reduce the formation of plaques in atherosclerosis by inhibiting platelet aggregation and improving microcirculation in skin and liver that facilitate excretion of free radicals (Babić *et al.*, 2019). Flavonoid supplementation in a mice model reversed the occurrence of pathological conditions common with inflammation such as insulin resistance (Burke *et al.*, 2018).

### 2.5.2. Amino acids, peptides and alkaloids

Amino acids are organic compounds with an amine and a carboxylic acid group. They are important building blocks for all life forms. Amino acids are present in biological systems where they act as antioxidants. Due to this, they have been incorporated in patented aqueous and oily antioxidant formulations. They are known to act as both antioxidants and pro-oxidants depending on the environmental conditions and their concentrations. Their antioxidant activity comes from the presence of the primary amino and carboxyl groups, while the other side chain functional groups add on to this activity (Aluko, 2015). Amino acids act by chelating metal ions or scavenging free radicals. Examples of strong antioxidant amino acids are histidine, lysine, tyrosine and tryptophan (Fig. 2.8). Histidine has one of the highest antioxidant activity among amino acids due to the imidazole ring (Cushnie *et al*., 2014).

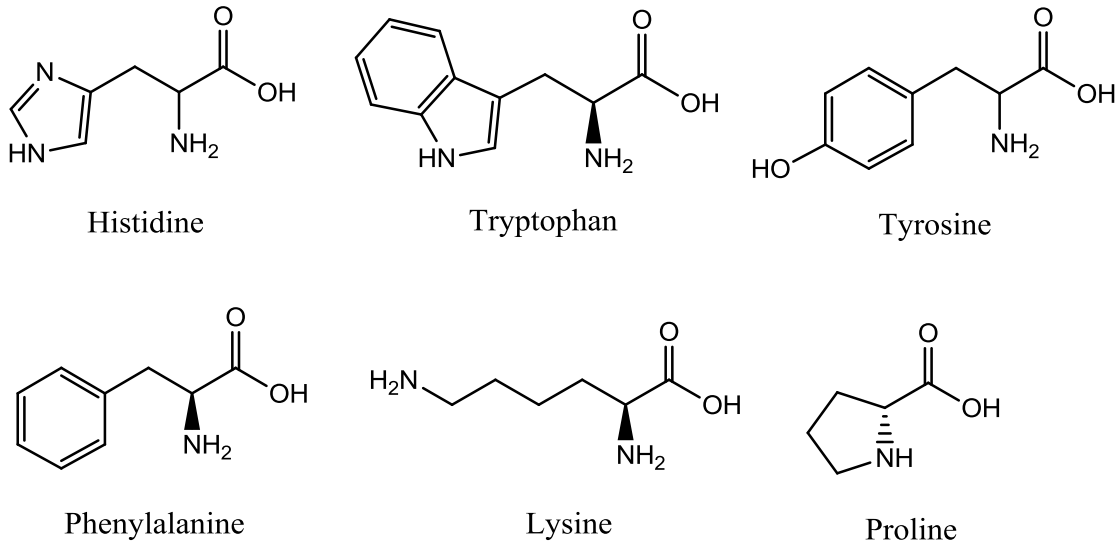



**Figure 2.8: Chemical structures of some amino acids**

Peptides can be obtained from milk, cereals, legumes, meat, eggs and marine organisms. When proteins undergo fermentation, digestion or hydrolysis, they release bioactive peptides that act as antioxidants. Their antioxidant activity depends on the amino acids sequence, the peptide structure and its hydrophobicity (Carbonaro *et al*., 2015). Their antioxidant activity arises through proton donation to the reactive species and the resulting ion is well stabilized by the amino acids in the peptide chain (Tao *et al.*, 2018). Peptides containing histidine have the highest antioxidant activity though the sequence of the amino acids in the peptide structure also affects activity (Jiang *et al.,* 2018).

Alkaloids are compounds possessing one or more nitrogen atom(s) in the molecule preferably from an amino acid. They possess significant pharmacological activity and are of diverse chemistry. A number of alkaloids have been identified from nature and have provided lead compounds for drug discovery and development of conventional medicines. Alkaloids can be classified according to their carbon skeletons such as indole, quinoline, isoquinoline and pyridine like alkaloids. They can also be classified according to their biochemical precursors which are ornithine, tyrosine, lysine, phenylalanine and tryptophan some of which show strong antioxidant activity (Cushnie *et al*., 2014). Different alkaloidal skeletons have certain structural features that support antioxidant activity. These include the presence of a secondary amine group (Colchicine), phenolic moiety (morphine), conjugated ring system (harmine) which allow for favourable electron delocalisation when neutralizing radicals (Tiong *et al.*, 2013). The effect of alkaloids as antioxidants has been best seen in their anti-inflammatory activity. Isoquinoline, quinoline and indole alkaloids show significant anti-inflammatory activity when assessed through the carrageenan-induced paw oedema (Souto *et al.*, 2011). Quinolizidine alkaloids matrine and oxymatrine, the natural *N*-oxide form of matrine, were able to reduce the plasma levels of IFN-γ and IL-2 in rats when given orally and prevent further injury of the colon due to inflammation by increasing IL-10 (Wu *et al.*, 2016). Matrine and oxymatrine also show antiprotozoal activity against several protozoan parasites (Zhang *et al.*, 2016). Vindolicine, an indole alkaloid, showed strong antioxidant and hypoglycaemic activity in mouse pancreatic cells (Tiong *et al.*, 2013). Matrine, berberine and colchicine have anticancer and antioxidant effects when used in TCM clinical practice (Zhang *et al*., 2017).

## 2.6. KENYAN PLANTS WITH KNOWN ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY

Most often, knowledge on African traditional medicine is passed down through folklore, thereby facing the danger of being lost in favour of conventional medicine. Despite this, African traditional medicine has gained renewed interest in the healthcare services throughout the continent and more indigenous plants are being researched on. Previous ethnobotanical studies of medicinal plants confirm the rational use of diverse formulations against many diseases by different ethnic communities. Herbs with high antioxidant activity were routinely used to manage diseases of inflammation, with the inflammation either due to microbes or chronic conditions non-infectious conditions such as rheumatism (Skrinjar and Nemet, 2009; Ravipati *et al.*, 2012). Examples of herbs used traditionally as antimicrobial agents with antioxidative properties are *Leonotis mollisima* which was used to manage conjunctivitis and intestinal disorders (Maroyi, 2013). On the other hand, the stem and leaf decoctions of *Leonotis leonurus* have been used to manage upper respiratory conditions, hasten wound healing and as an adaptogen. The presence of the various diterpenes and flavonoids may contribute to its good antioxidative capacity and anti-inflammatory activity (Jimoh *et al*., 2010). Another plant from the same family (Lamiaceae), *Plectranthus barbatus*, was subtly used in venereal diseases and human immunodeficiency virus, acquired immunodeficiency syndromme (HIV/AIDS). This property comes from its antioxidant and antiviral activity *in vitro* (Ole-Miaron, 2003; Kapewangolo and Meyer, 2018). Plants from the genus *Solanum* have a wide range of use as foods that exhibit antioxidant activity though the toxicity of their fruits limits their use. They have been found useful in managing fever, upper respiratory tract infections and abdominal pain (Elekofehinti *et al.*, 2013). *Solanum dasyphyllum* leaves and root extract, used to induce labour and treat backache, were found to have high antioxidant activity (Odongo *et al.*, 2017, 2018). *Vepris eugenifolia* is adept in treating malaria, contains alkaloids with moderate antioxidative ability which could reduce the systemic complications that arise due to oxidative stress brought on by the parasite (Kiplimo *et al*., 2012; Percário *et al.*, 2012). *Camelia sinensis* is renowned for producing tea with high antioxidant activity and has been shown to have antimicrobial activity (Almajano *et al.*, 2008).

Africa has more than 30 species of the genus Rhus, whose common name is Sumac. *Rhus vulgaris* has been used in treating coughs, colds, diarrhoea, abdominal pain, and gonorrhoea. The branchlets of this tree are used as toothbrushes while the root decoction is used as a medicine for hookworms (Orwa *et al.*, 2009; Odongo *et al.*, 2018). Odongo *et al*. found *R. vulgari*s to have high antioxidant and anti-inflammatory activities that may be related to its traditional medicinal use (Odongo *et al.*, 2017). Other plants, known to treat parasitic infections or immune diseases of unknown origin (now assumed to have been due to cancerous lesions) have also been found to have high antioxidant activity and include *Xeoderris sthulmannii*, *Tamarindus indica*, *Parinari curatellifollia*, *Ozoroa insignis* and *Ficus platyphylla* (Ole-Miaron, 2003; Lamien-Meda *et al.*, 2008).

Herbs with high antioxidant activity are also known to be active in cancer prevention, from *in vivo* studies and also act as adaptogens (Duthie *et al*., 2000). *Phyllanthus schimperi* leaf and root bark is used for general body illness and the leaf paste is applied on skin cancers. *In vitro* studies have reported good antioxidant and anti-inflammatory activity of the methanolic extract of *P. schimperi* (Odongo *et al.*, 2018). *Prunus africana* is used traditionally to manage malaria, prostate cancer, benign prostatic hyperplasia and to improve appetite. To increase their potency, they are usually decocted with certain plants used in treating cancers such as *Harungana madagascariensis*, *Spathodea capanulata* and *Justicia betonica*, among others (Ochwang'i *et al.*, 2014). *Prunus africana* bark extract has strong antioxidant activity with accompanied high total flavonoid and phenolic content (Madivoli *et al.*, 2018). *Harungana madagascarensis* is used to treat colorectal, skin and breast cancer, diarrhoea, sore throat, venereal diseases and can be used to interrupt menses (Masinde, 2010; Ochwang'i *et al.*, 2014). The methanolic extract of *H. madagascarensis* had high total phenolic and flavonoid content with high antioxidant activity. Its stem bark extract stimulated the release of NO, which is efficient in eliminating microbes and cancerous cells (Iwalewa *et al.*, 2009). Plants from the genus *Acacia* grow in arid areas and are commonly used by communities living in such areas mostly as tonics, to soothe body aches and as an appetizer among many other uses. They contain high concentration of polyphenolic compounds in their bark, which is boiled, and the decoction used to flavour their soups (Ole-Miaron, 2003). *Acacia macrostachya* a hardy shrub has been known as a cure for snake bites and recently, as an antitumour agent (Sawadogo *et al.*, 2012).

## 2.7. THE ILKISONKO MAASAI

The Maasai are a Nilotic ethnic group who originated from the north (the scarp of Kerio, the present day Sudan) and migrated south after a dry spell. They have lived near the game parks in Southern Kenya and Northern Tanzania for more than 2000 years (McCabe *et al*., 1992). The Kenyan Maasai live mostly in the southern part and number about 1,189,522 according to the 2019 national census. They speak *Maa* language, a Nilo-Saharan tongue, with two dialects, spoken by the Maasai, Samburu and Ilcamus people (Finke, 2000). They are a nomadic community living in arid and SALs favourable for nomadic pastoralism. They depend heavily on the environment to obtain food, shelter and medicine. There are eight Maasai clusters within Kajiado County namely: Ilkisonko, Ilmatapato, Inkidongi, Ilkaputiei, Ilpurko, Ildamat, Illoodokilani and Ilkeekonyokie. The Ilkisonko Maasai live near or around Loitokitok. As pastoralists, they consume milk, blood and meat as their staple meals while vegetables were rarely incorporated into their diet (Johns *et al.*, 1994). They are considered a marginalized community due to their geographical location and because they have set traditions and cultures preserved throughout the ages, which has attracted considerable interest.

The Maasai are a patrilineal society where clan membership is inherited from the father. In the clan, indigenous education is practiced, where the elderly teach the young on spiritual, social and economic aspects of living so as to prepare them for a future adult life. The Maasai have also been known to practise African traditional medicine (Kiringe and Okello, 2005). Medicinal plants knowledge is part of indigenous knowledge and is passed on by folklore. It is available to those who stay in close contact with the community. The commonly used trees and shrubs are from the genus *Acacia*, *Balanites*, *Commiphora*, *Omorcarpum, Terminalia*, *Strychnos* and *Ximenia* (Muthee *et al*., 2011). Their medicinal plant knowledge includes the use of barks, roots, and fruits to complement their diet, preserve food, prevent illness or treat certain conditions in both humans and livestock. The Maasai live in drought prone areas which encourage increased production of secondary metabolites, such as polyphenols (Lamaoui *et al.*, 2018). Inclusion of these plants with a higher than normal quantity of antioxidant molecules into their diet might contribute to the lower numbers of inflammatory conditions witnessed with the Maasai living in rural areas (Christensen *et al.*, 2014).

In the modern era, the Maasai are educating more of their children, leaving few to harness this important information. Youths in the villages have moved into urban areas or bigger towns in search of opportunities and this erodes the cultural importance of traditional knowledge instilled into them since childhood. Traditional medicine use is also under threat from modernisation of Maasai lands, such as subdivision and sale for agricultural use, which had been left majorly for pastoral use and could support a diverse number of plants (Johns *et al.*, 1994). The influence of modernisation was seen as the most serious threat to local medicinal plant knowledge.

## 2.8. PRINCIPLES OF EXTRACTION AND CHARACTERISATION OF PHYTOCHEMICALS

### 2.8.1. Importance of extraction in phytochemical screening studies

Extraction assists in releasing the active constituents from the biomass through the use of appropriate solvents. The type of extraction method chosen and the solvents used are usually determined by how the indigenous community used the plant and the type of constituents present. Normally, solid liquid extraction is most popular with maceration, percolation and decoction being the type of extraction methods commonly used. Maceration is preferred in the laboratory setting to avoid the destruction of any thermolabile compounds.

In the community setting, water and ethanol are the most common solvents though this is not mirrored in research. Water is a good solvent as it can dissolve many constituents from the biomass, it is safe and readily available but it is nearly impossible to remove with the rotary evaporator common in most laboratories. When a substantial amount of water is left in the extract, it can encourage the growth of microorganisms, rendering the extract inferior. Ethanol, with its high polarity, can be used in the laboratory setting though methanol is preferred. Despite their similar polarities methanol has been found to have better extraction properties especially for phenolic compounds due to its higher solvation and consequent extraction of polyphenols (Boeing *et al.*, 2014). Efficient extraction to obtain the compounds in the plant allow researchers to gauge appropriately which compounds are present and if they have the accompanying activity.

Freeze drying, also called lyophilisation, is a process of removing a solvent from a medium by converting it to a solid through crystallization and directly to vapour. It is usually done with water as the solvent, at low vapour pressures and low temperatures to facilitate crystallization. Due to the low temperatures used, heat sensitive compounds and colour of the products are preserved. It produces a powder that can be easily reconstituted and one with good shelf stability especially if moisture content is well controlled during the drying process. It has high maintenance costs and requires skilled personnel to handle the equipment.

### 2.8.2. Total phenolic content

The methods of quantifying total phenolic content in plant samples are based on the reaction of phenolic compounds with a colorimetric reagent, which allows measurement in the visible portion of the spectrum. The Folin–Ciocalteu (F–C) assay has been standardized for use in measurement of antioxidant capacity of food products and dietary supplements. The F–C assay is a redox reaction that relies on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes to form blue complexes that are determined spectrophotometrically at approximately 760 nm. This technique is accurate for certain groups of phenolic compounds which change colour according to their unit mass and reaction kinetics (Blainski *et al*., 2013). The long wavelength also reduces interference due to the colour of the sample, hence it is commonly used for plant extracts. It gives excellent linear correlation when compared against different antioxidant capacity assays (Gallego *et al.*, 2013). Other compounds in the extracts such as sugars, sulphites, ascorbic acid can cause an additive effect leading to higher than anticipated levels. In wines, sulphur and sulphur dioxide is usually incorporated and these will give an additive effect. In such a scenario, a more specific test for that compound is performed and this is negated from the F-C assay to get the true value. Despite this, the F-C assay provides an easily reproducible method in the analysis of total phenols in a sample (Ainsworth and Gillespie, 2007). Standards used include gallic acid, tannic acid, and ferulic acid depending on the sample type. Gallic acid is the most commonly used standard.

### 2.8.3. Total flavonoid content

Aluminium chloride colorimetric method is normally used to quantify the total flavonoid content in plant parts or extracts. The basic principle of aluminium chloride colorimetric method is that aluminium chloride forms acid stable complexes with the keto group (C-4) and either the C-3 or the C-5 hydroxyl group of flavones and flavonols (Fig. 2.6). This is due to the presence of an aromatic ring with a catechol group where certain positions (C-3, C-4 or C-5) are unsubstituted. Once aluminium chloride is added, a yellow complex is formed which turns red on adding sodium hydroxide. This solution is mixed and measured against a blank at 510 nm. Standard catechin solutions of various concentrations are used to build up the calibration curve. Catechin is sometimes preferred over quercetin as it has better solubility and polarity than quercetin (Ismail *et al.*, 2017). This polarity will enable it to be effective as an antioxidant in more lipophilic than hydrophilic systems due to the polar paradox theory. According to the polar paradox theory, polar antioxidants are more effective in lipophilic media, such as oils, while nonpolar antioxidants are more effective in relatively hydrophilic media, such as oil-in-water emulsions (Palma *et al.*, 2017).

### 2.8.4. Principle of Antioxidant Assays

Antioxidant capacity assays may be generally classified as hydrogen atom transfer (HAT) based assays and electron transfer (ET). The majority of HAT assays are kinetics-based in which the antioxidant and substrate compete for peroxyl radicals thermally produced through the decomposition of azo compounds. Hydrogen atom transfer-based assays include oxygen radical absorbance capacity (ORAC) and the radical trapping antioxidant parameter (TRAP) assays. Electron transfer−based assays are able to measure the capacity of an antioxidant in the reduction of an oxidant, which changes colour when reduced. Electron transfer assays include the 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS), cupric ion reducing antioxidant capacity (CUPRAC), 2,2-diphenyl-1-picryl-hydrazyl (DPPH), Folin-Ciocalteu, β-carotene–linoleic acid assay and ferric reducing antioxidant power (FRAP) methods (Huang *et al*., 2005). Within the EAT assays, antioxidant activity of a sample can be quantified by its radical scavenging activity (ORAC, ABTS and DPPH) or the reduction process of the antioxidant with the reagent (CUPRAC and FRAP). In a reaction, the stable chromogen radical, DPPH, receives an electron or a hydrogen from a capable molecule causing it to

change colour from purple to yellow. This change in absorbance is taken as the antioxidant capacity of the molecule and is measured by spectrophotometry. A review on the antioxidant activity (AOA) methods showed that DPPH is widely used due to its simplicity, stability, accuracy, and reproducibility (Siddhuraju and Becker, 2007). DPPH has been suggested to be one of the most commonly accepted assays for the estimation of AOA in plant foods. Electron transfer assays, such as the DPPH assay, have also shown good correlation of phenolic content and antioxidant activity (Huang *et al*., 2005). Its only disadvantage is that it is not applicable to lipophilic antioxidant components.

### 2.8.5. Inflammation assessment on paw oedema

In inflammation research, several experimental models have been used to look at the effect of inflammation on the mammalian body. The anti-inflammatory activity of compounds can be assessed *in vitro* in cells or *in vivo* in rodents and mammals. In animal models, this is done by inducing the five cardinal signs of inflammation: swelling, heat, hypersensitivity, redness, and loss of function. Use of carrageenan, egg albumin, histamine, cotton pellet or complete Freund's adjuvant injected in the paw can elicit inflammation. The first three elicit short-term response while the last two generate a more prolonged response (Zhao *et al.*, 2018). Carrageenan is usually preferred as it gives an acute response and it is highly reproducible (Necas and Bartosikova, 2013). The drugs under investigation can be administered, orally or applied to the inflamed area, and their ability to reduce or prevent the cardinal signs of inflammation assessed. Drugs used as reference are non-steroidal anti-inflammatory drugs (NSAIDs) as they inhibit an important step in inflammation (Guay *et al.*, 2004). Oedema is frequently used to assess for inflammation on the animals as it can be easily measured through different methods. Oedema is seen by paw swelling which may be measured by use of volume displacement, width measurement, measuring the diameter or circumference of the paw before and after inflammation (Sharma *et al*., 2004). These all deal with an influx of cells and substances that may be mitigated by use of anti-inflammatory agents. Pain or hypersensitivity, which will accompany the oedema, cannot be assessed by these methods.

### 2.8.6. Principles of carrageenan-induced rat paw oedema

Carrageenan is a sulphated polygalactan with 15 to 40% of ester-sulphate content and an average relative molecular mass well above 100 kDa. It is extracted from red algae of the family Rhodophyceae. It is formed by alternate units of d-galactose and 3,6-anhydro-galactose joined by α-1,3 and β-1,4-glycosidic linkage. Carrageenan is classified into various types such as λ, κ, ι, ε, μ, all containing 22 to 35% sulphate groups (Necas and Bartosikova, 2013). Lambda-carrageenan is non gelling and commonly used in inducing an inflammatory response in rodents.

An injection of carrageenan into the paw of rats induces inflammation and later oedema that is biphasic. In the early phase after carrageenan injection, happening within 2 h, there is oedema brought about by mediators such as histamine and bradykinin but unrelated to the dose of carrageenan. The second phase shows an increase in oedema and swelling peaking at 2-6 h post-carrageenan injection (Sakat *et al.*, 2014). Carrageenan is known to activate production of the pro-inflammatory cytokine, IL-8, and causes generation of ROS/RNS (Bhattacharyya *et al.*, 2011). Mediators of inflammation such as histamine, serotonin, and bradykinin increase the vascular permeability at the site of injury leading to infiltration of neutrophils. These cells then produce other mediators such as ROS/RNS, which continue to act as mediators of inflammation and to remove the offending agent. ROS/RNS is produced in both the early and late phases of inflammation (Salvemini *et al.*, 1996). The production and release of NO by NOSs are thought to contribute to tissue injury and inflammation-induced hyperalgesia and oedema (Omote *et al.*, 2001).

# 3.0. CHAPTER THREE: METHODOLOGY

## 3.1. ETHNOBOTANICAL SURVEY

### 3.1.1. Study site

The ethnobotanical survey was conducted in three selected locations (Rombo, Kimana and Entonet) that comprise Loitokitok sub-county, Kajiado (Figure 3.1). Kajiado County is situated in the southern part of the former Rift Valley Province. It has an area of about 21,297 $km^2$ with inhabitants close to a million. Plains and occasional volcanic hills characterize the general topography of the county. The county was predominantly inhabited by the Maasai up to the 1960s but is now culturally rich as other Kenyan tribes and foreigners have resettled there after subdivision of communal lands (Campbell *et al.*, 2000). Kajiado county is peri-urban with about 40% of the population living below the poverty line with a poverty gap of about 13% (Kenya National Bureau of Statistics, 2018) in spite of the county being endowed with natural resources such as Amboseli Reserve, and arable land suitable for agriculture (Walker *et al.*, 1981). Kajiado County borders the highest mountain in Africa, Mt. Kilimanjaro, which has generated the fertile volcanic soils in the area. The main economic activities are tourism in the neighbouring national parks, commercial farming, and peasant livestock farming.

Kajiado South sub-county, also known as Loitokitok is one of the five constituencies in Kajiado County, the others being Kajiado North, Kajiado East, Kajiado West and Kajiado Central constituencies. Loitokitok has an approximate area of 6,411 $km^2$ and has five administrative locations, namely Entonet/Lenkisi, Mbirikani/Eselen, Keikuku, Rombo and Kimana (Seno and Tome, 2013). Rainfall pattern in Loitokitok is bimodal with the long rains falling between March and May and the short rains between October and December. Although Loitokitok abuts Mt. Kilimanjaro and the lush forest surrounding it, heavy rains are only experienced in the mountainous zones and much less around Loitokitok. The soils are fine, inherently fertile volcanic clays, very prone to erosion. These soils support bushland vegetation and open grassland suitable for agro-pastoralism and wildlife. Loitokitok was chosen because a majority of the Ilkisonko Maasai reside in this area and still practice many aspects of their culture including traditional medicine. Rombo and Kimana were chosen as

they are under the Endangered Ecosystems list of Kenya, while Entonet was included as it is in the nearby area (Misana *et al*., 2003).

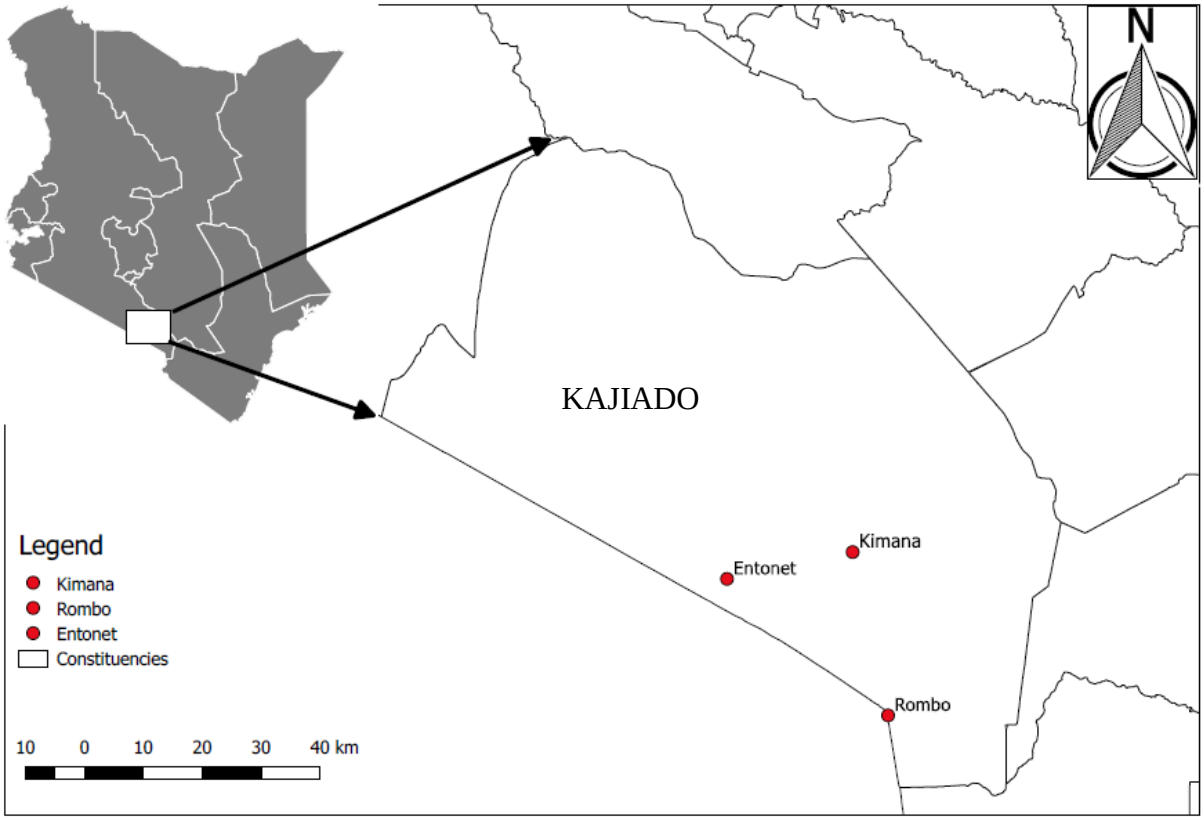



**Figure 3.1: The three sampling points in Loitokitok, Kajiado County.**

The Flora of Tropical East Africa demarcates Kenya into seven plant distribution zones (K1–K7). K1 represents the northern part, K2 represents the northwest part and K3 represents the western part of Kenya (or Rift Valley Province). The central part of Kenya is designated K4, the southwest part of Kenya (the former Nyanza Province) is designated K5, while K6 represents the "Maasai Province" which is located in the southern part of Kenya, and K7 represents the coastal region. Loitokitok sub-county is in the K6 zone and has mainly open grassland (Zhou *et al.*, 2017). The most frequently occurring trees in the Maasai Province are the Acacias, which are widely interspersed within the grassland. Though it is a semi-arid region, Loitokitok has great plant biodiversity, which is slowly disappearing due to intermittent drought, overgrazing and population increase. Commercial farming has made the sub-county considerably wealthier than before but this investment is localized and many areas of Loitokitok remain poor.

### 3.1.2. Study population

The key informants (KIs) were individuals locally recognised as knowledgeable on plants used as food and medicine and were identified with the assistance of the village elders. The KIs were selected through guided sampling so as to obtain useful and reliable data. The KIs had to be above 18 years and must have lived in Loitokitok for not less than 3 years. This initial selection was also based on the willingness of the KIs to give voluntary information through interviews and interact with researchers during consultative meetings. After selection, a second meeting was set up where the researchers introduced the project to the KIs and gave a detailed overview of what would be required from them during the interview. A minimum of 40 KIs were used for this study.

### 3.1.3. Study design

This was a qualitative cross sectional study. It involved data collection on medicinal plants at a specific point in time (Levin, 2006).

### 3.1.4. Data collection

The data collection tool was a semi-structured questionnaire consisting of open and close-ended questions which were administered through face-to-face interviews. The questionnaire was designed in such a manner as to collect data on plants used as food and medicine among the Ilkisonko Maasai community. Pretesting of the semi-structured questionnaire was conducted with seven KIs not involved in the study. The questionnaire (Appendix 4) had 14 questions which were administered in the local dialect of the Maasai language, *Maa*, or the national language, *Swahili*, depending on the respondent's preference. The questions asked to the respondents, and discussions during the survey focused on the following aspects:

i. Types of medicinal plant species found within the county that were recognized by the community to be of medicinal value but are also taken as food.
ii. Part(s) used such as roots, bark, leaves, fruits and flowers.
iii. Human ailments and conditions treated or managed using these plants.
iv. The preparation and administration of the named plants.

The plants mentioned were identified by a taxonomist using the Flora of Tropical East Africa (Zhou *et al.*, 2017). A voucher specimen was deposited at the University of Nairobi Herbarium (NAI).

### 3.1.5. Study approval

Ethical approval for this study was obtained from Kenyatta National Hospital-University of Nairobi Research Ethics Review Committee (KNH/ERC/A/173) (Appendix 1). Permission to conduct the study in Loitokitok was obtained from the respective sub-county administrators in Rombo, Kimana and Entonet. Written consent was sought from the study participants after information was provided to them on the purpose, benefits and risks associated with the study (Appendix 2 and 3).

### 3.1.6. Data Analysis

Ethnobotanical data was summarized by descriptive statistics using Microsoft® Excel spreadsheets.

## 3.2. PHYTOCHEMICAL SCREENING

### 3.2.1. Materials, reagents and equipment

Methanol, Folin Ciocalteu's phenol reagent, tannic acid ACS, sodium nitrite ($NaNO_2$) analytical reagent, catechin analytical standard, sodium carbonate ($Na_2CO_3$) powder, sodium hydroxide (NaOH) pellets, and aluminium chloride hexahydrate ($AlCl_3.6H_2O$) powder were obtained from Sigma Aldrich Co. (St Louis, MO, USA).

Shimadzu 1800 UV-Vis spectrophotometer (Shimadzu Inc., Kyoto) was used for all spectrophotometric determinations while Büchi Rotavapor R-200 rotary evaporator (Büchi Labortechnik, Flawil) was used to evaporate extracting solvents from plant extracts to complete dryness. Lyophilisation was carried out using Modulyo Freeze Dryer (Edwards, England) for all water extracts. Grinding of plant parts was done by multifunctional grinder GRT-06B (Yongkang Tiange Electric Co., Zhejiang).

### 3.2.2. Plant collection and preparation of extracts

The medicinal plants identified in the ethnobotanical survey were collected in Loitokitok in December 2014, catalogued and a voucher specimen deposited in the University of Nairobi Herbarium. The parts of the plant traditionally used were dried under shade at ambient temperature for a week and pulverized to fine powder using a hammer mill. The powdered plants were packaged in brown paper bags, placed in covered plastic buckets and stored on bench tops at room temperature to protect from light and moisture until their extraction.

Methanol extraction was carried out using maceration. The crude plant powder was weighed (100 gm) into a glass beaker. Previously distilled general grade 80% v/v methanol was added to the beaker at a solvent: feed ratio of 6:1. The set up was left to stand at room temperature for 72 h, with occasional stirring. The extracts were filtered, concentrated using a rotary evaporator, and dried further in an oven at 40°C for 24 h.

Water extraction was carried out using decoction. The crude plant powder was weighed (100 gm) into a glass beaker. Distilled water was added to the beaker at a solvent: feed ratio of 10:1 and heated at 60°C for 20 min. The extracts were then filtered and concentrated using a freeze drier. The dry extracts were stored in glass vials and placed under refrigeration at 4°C awaiting phytochemical screening (Sultana *et al*., 2009).

### 3.2.3. Determination of total phenolic content

The total phenolic content of the extracts was determined by colorimetric spectrophotometry using the Folin and Ciocalteu's method (Singleton and Rossi Jr, 1965). Each extract was dissolved in distilled water to give a concentration of 0.5 mg/mL. A 0.5 mL aliquot of the extract was added to 2.5 mL of 1N Folin–Ciocalteu reagent. The mixture was then shaken and allowed to stand for 6 min, before addition of 2.0 mL of 7.5 % w/v $Na_2CO_3$. The solution was then adjusted with distilled water to a final volume of 6 mL and mixed thoroughly. Serial dilutions of the tannic acid standard was prepared at a concentration range of 50-250 µg/mL and a 0.5 mL aliquot of the standard treated as the sample. After incubation in the dark for 2 h, absorbance at 765 nm was determined spectrophotometrically versus a blank containing 1.5 mL distilled water, 2.5 mL of 1N Folin–Ciocalteu reagent and 2.0 mL of 7.5 % w/v

$Na_2CO_3$. The total phenolic contents of the extracts were expressed as milligrams of tannic acid equivalents per gram of extract from a calibration curve (Appendix 5) prepared using tannic acid standard (Ainsworth and Gillespie, 2007; Mishra *et al.*, 2013). All samples were analyzed in triplicate.

### 3.2.4. Determination of total flavonoid content

Aluminium chloride colorimetric method was used to measure the total flavonoid content in the extracts (Lin and Tang, 2006). The extracts were dissolved in methanol to give a concentration of 0.5 mg/mL. A 0.5 mL aliquot of the test extract was mixed with 0.75 mL of 5% w/v $NaNO_2$. After 5 min, 0.15 mL of 10% w/v $AlCl_3$ was added, and 6 min later, 0.5 mL of 1M NaOH was incorporated into the mixture. The solution was then adjusted with distilled water to a final volume of 3 mL, mixed thoroughly and incubated for 60 min at room temperature. Catechin was used to make the standard calibration curve. Standard solutions (6.25-100 µg/mL) of catechin were prepared by serial dilutions in methanol and a 0.5 mL aliquot of the standard treated as the sample. The absorbance of the reaction mixture was then measured spectrophotometrically against a blank at 510 nm. The blank constituted 0.75 mL of 5% w/v $NaNO_2$, 0.15 mL of 10% w/v $AlCl_3$, 0.5 mL of 1M NaOH and 1.6 mL distilled water. The concentration of total flavonoid content in the test sample was calculated from the calibration plot (Appendix 5) and expressed as milligram of catechin equivalent per gram of extract (mg CE/g E) (Atanassova *et al.*, 2011). All the determinations were carried out in triplicate.

## 3.3. ANTIOXIDANT ACTIVITY

### 3.3.1. Materials, reagents and equipment

Methanol, ascorbic acid standard and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Aldrich Co. (St Louis, MO, USA).

Grinding of plant parts was done by multifunctional grinder GRT-06B (Yongkang Tiange Electric Co., Zhejiang). Shimadzu 1800 UV-Vis spectrophotometer (Shimadzu Inc., Kyoto) was used for all spectrophotometric determinations while Büchi Rotavapor R-200 rotary

evaporator (Büchi Labortechnik, Flawil) was used to evaporate extracting solvents from plant extracts to complete dryness. Lyophilisation was carried out using Modulyo Freeze Dryer (Edwards, England) for all water extracts.

### 3.3.2. Procedure of antioxidant assay

The assay for antioxidant activity was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as the oxidant. The choice was based on its reproducibility as previously described in the literature (Mishra *et al.*, 2013). The test solutions of the plant extracts were prepared in 95% v/v methanol for the methanol extracts and in distilled water for the water extracts, to obtain concentrations in the range 12.5-400 µg/mL. Ascorbic acid was used as a standard and serial dilutions at 3.125-100 µg/mL concentration range were prepared using methanol. The oxidant was prepared by dissolving 3.94 mg of DPPH in 100 mL of 95% v/v methanol just before use and stored in the dark to minimize degradation. A 200 µL aliquot of the test/standard solution was placed in a vial and 2800 µL of DPPH added. The mixture was kept in the dark for 30 min and optical density measured spectrophotometrically against a blank at 517 nm. The blank constituted only 2800 µL of DPPH with 200 µL methanol or distilled water added as a replacement for the sample/standard. The assay was done in triplicate.

The antioxidant activity of the test extracts was expressed as half-maximal inhibitory concentration ($IC_{50}$), which is the concentration (expressed in µg/mL) of sample required to cause a 50% reduction in DPPH radicals. Extract concentration providing 50% inhibition ($IC_{50}$) was calculated from the graph that plotted inhibition percentage against concentration (Appendix 6). Inhibition of free radical of DPPH in percentage terms (percentage inhibition-PI %) was calculated as shown in Equation 1.

$$PI\ \% = (A_{blank} - A_{sample}/A_{blank}) \times 100 \qquad \text{Equation 1}$$

Where $A_{blank}$ is the absorbance of the control reaction (containing all reagents except the sample) while $A_{sample}$ is the absorbance of the test extract (or the standard).

### 3.3.3. Statistical Analysis

All the experiments for determination of total phenolic content, flavonoid content, and antioxidant activity were conducted in triplicate. The values are expressed as the mean ± standard deviation (SD). The statistical package for social sciences (SPSS) version 16 was used for statistical analysis of the results. Differences in quantity or activity between methanol and water extracts were determined using the paired *t*-test. A *p* value less than 0.05 ($p<0.05$) was considered statistically significant. Correlation between total phenols and total flavonoid content and antioxidant activity was determined using Pearson's chi-squared test.

## 3.4. CARRAGEENAN-INDUCED RAT PAW OEDEMA ASSAY

### 3.4.1. Materials, reagents and equipment

The following materials were obtained from Sigma Aldrich Co. (St Louis, MO, USA): λ-carrageenan (22049), diclofenac sodium salt, methanol and dimethyl sulfoxide (DMSO). Normal saline solution was bought from Dawa Limited (Nairobi, Kenya). Plethysmometer (LE 7500, Panlab Harvard) was used to measure the rat paw oedema. Grinding of plant parts was done by multifunctional grinder GRT-06B (Yongkang Tiange Electric Co., Zhejiang). Büchi Rotavapor R-200 rotary evaporator (Büchi Labortechnik, Flawil) was used to evaporate extracting methanol from the plant extracts.

### 3.4.2. Ethical considerations

Ethical approval for this study was obtained from Kenyatta National Hospital-University of Nairobi Research Ethics Review Committee (KNH/ERC/A/173) (Appendix 1 and 2). The animal care and use protocol was approved by the Small Animal Facility for Research and Innovation (SAFARI) Animal Ethics Committee, Jomo Kenyatta University of Agriculture and Technology.

### 3.4.3. Experimental animals

Adult Wistar rats weighing 190-250 grams were obtained from SAFARI. They were kept at a maximum of five per cage ($435 \times 290 \times 150$ mm) in a well-ventilated animal house with room temperature maintained at 20-25°C with a 12 h light/dark cycle. The animals had *ad libitum* access to rat chow (Unga Feeds™) and clean drinking water. Animal beddings comprising of saw dust, was changed once daily. The rats were allowed to habituate for one week before experimentation. Prior to the carrageenan-induced rat paw oedema test, they were fasted overnight but provided with water *ad libitum*. All the tests were carried out during the daytime in a quiet laboratory setting with ambient illumination and temperature similar to those of the animal house. All effort was made to minimize animal suffering and to reduce the number of animals used.

### 3.4.4. Carrageenan induced rat paw oedema

Carrageenan induced rat paw oedema was carried out as previously described (Ganga *et al*., 2012). Nine groups of five adult Wistar rats each, received either methanol plant extracts (400 mg/kg body weight), diclofenac (20 mg/kg body weight), or vehicle control (distilled water) in 3% w/w DMSO orally 1 h before the carrageenan injection (Paviaya *et al.*, 2013). Acute inflammation was induced by the sub-plantar administration of 0.1 ml of 1% w/v carrageenan in normal saline in the right hind paw of the rats (Adedapo *et al*., 2008). The paw volume was measured (up to the tibio-tarsal junction) at time 0 (immediately before the injection), 1, 2, 3, 4 and 24 h after carrageenan injection by the volume displacement method using a digital plethysmometer. An increase in the volume of the paw was taken as an indication of oedema. The percentage inhibition of the inflammation was calculated using Equation 2.

$$\textbf{Percentage inhibition} = (D_0 - D_t)/D_0 * 100 \qquad \text{Equation 2}$$

Where $D_0$ is the average inflammation (hind paw oedema) of the control group of rats at a given time and $D_t$ is the average inflammation of the drug treated (i.e. extracts or reference Diclofenac) rats at the same time (Pérez González *et al.*, 2013; Meshram *et al.*, 2016).

### 3.4.5. Statistical analysis

Data were expressed as mean paw volume. The descriptive statistics were the standard deviation of the mean and the corresponding percentage inhibition of inflammation calculated. The difference in response was analysed using ANOVA followed by the *post hoc* Tukey's test. A $p$-value of $< 0.05$ was considered significant.

## 3.5. ISOLATION OF PURE COMPOUNDS

### 3.5.1. Reagents, materials and equipment

#### 3.5.1.1. Reagents

Analytical grade or general grade dichloromethane, *n*-hexane, ethyl acetate, acetone, toluene, dichloromethane and methanol were obtained from Kobian Chemicals, Nairobi Kenya. For general grade, the solvents were double distilled before use. Iodine crystals were obtained from Alpha Chemika, Mumbai and methanol-$d_4$ and chloroform-$d_4$ were sourced from Merck KGaA, Darmstadt.

#### 3.5.1.2. Materials

Analytical thin layer chromatography (TLC) was performed on aluminium pre-coated plates of silica gel 60 $F_{254}$ (Merck & Co. Inc., New Jersey) with a 0.2 mm layer thickness. Preparative thin layer chromatography (PTLC) was performed using normal phase silica gel 60 $F_{254}$ (Merck & Co. Inc., New Jersey) coated on glass plates (20 × 20). Silica gel for column chromatography (60-120 mesh, Alpha Chemika, Mumbai) was used for open column chromatography. Size-exclusion chromatography was carried out using Sephadex® LH-20 (Merck & Co. Inc., New Jersey). Potassium bromide used in Fourier-transform infrared spectrometer was obtained from Sigma Aldrich Co. (St Louis, MO, USA).

#### 3.5.1.3. Equipment

Pulverisation of plant part was performed by multifunctional grinder GRT-06B (Yongkang Tiange Electric Co., Zhejiang). Solvent evaporation was performed on a Büchi Rotavapor R-200 rotary evaporator (Büchi Labortechnik, Flawil). Locally made glass columns (90 cm by 50 mm diameter or 60cm by 25mm diameter) were used for open column chromatography. Detection was performed using a handheld UV Lamp ENF-260C 365nm/254nm (Spectronics Corp. New York). NMR spectra were run on a Bruker Avance III HD 800 spectrometer (Bruker BioSpin AG, Fallanden, Switzerland) using the residual solvent peak as the reference and equipped with a TXO cryogenic probe. Melting points were measured on a Büchi Melting Point M-565 apparatus (Büchi Labortechnik, Flawil). Fourier-transform infrared (FTIR) spectra were scanned on a Shimadzu 8400 FTIR-Spectrophotometer (Shimadzu Inc., Kyoto). Ultraviolet-visible (UV-Vis) spectra were recorded on a Shimadzu 1800 UV-Vis spectrophotometer (Shimadzu Inc., Kyoto). The electron ionization mass spectrometry (EI-MS) spectra were recorded on a Micromass GC-TOF mass spectrometer (Micromass, Wythenshawe, Waters Inc., Manchester), using direct inlet, and 70 eV ionization voltage.

### 3.5.2. Plant material

Plant selection for phytochemical isolation was based on anti-inflammatory activity using the carrageenan-induced rat paw oedema assay. The stem and root bark of the selected plant was collected from Loitokitok in January 2015 and a voucher specimen was deposited at the University of Nairobi Herbarium (NAI). The collected material was dried at room temperature for a week, then pulverised to a powder and stored in a brown paper bag at room temperature.

### 3.5.3. Extraction of plant constituents

Ground stem and root bark (1000 g) was packed in a glass conical flask and extracted by maceration with methanol and dichloromethane in a 1:1 ratio (2.5 L). The extract was filtered using Whatman No. 1 filter paper and concentrated to dryness under reduced pressure at low temperature. This procedure was repeated three times for exhaustive extraction.

### 3.5.4. Fractionation of the crude plant extract

A large portion of the semi dried sample (40 g) was adsorbed onto silica by mixing the sample with a few mL of dichloromethane and silica gel 60. This mixture was dried under reduced pressure and then ground on a mortar and pestle to a fine powder. Meanwhile, silica gel for column chromatography (400 g) was suspended in hexane to make a slurry. The slurry was packed into a glass column (90 cm by 50 mm diameter) and left to equilibrate for a few hours. The adsorbed sample was loaded unto the packed column and fractionation commenced using a gradient of hexane and ethyl acetate. Fractions were collected at a fixed volume (400 mL), reduced under pressure to about 30 mL volume and transferred to marked glass vials. Initially, 100% hexane was used, and the hexane concentration was reduced to 50% hexane in 5% increments, and finally 100% ethyl acetate was used. Later, ethyl acetate/methanol was used as eluent, where 10% increments of methanol were used. The process yielded a total of 350 fractions. Aliquots of all the fractions were spotted on TLC plates and developed in appropriate mobile phases. Visualization of the TLC spots was achieved under UV light and by exposure to iodine and the bands marked. As per the TLC plate results, fractions were combined to give seven major fractions (JK1, JK2, JK3, JK4, JK5, JK6 and JK7).

JK2 fraction eluted with 5% ethyl acetate in hexane was cleaned with sephadex LH-20. The sephadex column was prepared by shaking sephadex (150 g) with methanol and dichloromethane (1:1). After shaking, the sephadex column was left to slowly settle for an hour. The sample diluted with methanol was loaded gently unto the packed column. The column was eluted with methanol and dichloromethane (1:1) and allowed to run until clear. It was purified further using silica gel preparative TLC (Hexane:ethyl acetate 9:1) to yield a pure compound.

The last major fraction, JK7, eluted with 30-70% methanol was repeatedly passed through sephadex. This was done to separate the main compound from a brown pigment that eluted in great quantities with it. Further purification was done by subjecting fraction JK7 to a smaller column (60cm by 25mm diameter). This column was prepared by wet slurry method using silica gel (65 g) and the sample was loaded after adsorbing it onto silica gel. The column was

eluted with ethyl acetate-methanol in an increasing gradient elution. Another pure compound was obtained at 10% methanol in ethyl acetate.

Structural elucidation of pure compounds obtained was by nuclear magnetic resonance (NMR) spectroscopy, mass spectroscopy (MS) and infrared (IR) spectroscopy. Assignments were derived from (1D and 2D) the proton and carbon-13 NMR ($^{1}$H-NMR and $^{13}$C-NMR), correlation spectroscopy (COSY), distortionless enhancement by polarization transfer (DEPT), heteronuclear single quantum coherence spectroscopy (HSQC), and heteronuclear multiple bond coherence (HMBC) spectra. Chemical shifts were recorded in δ (ppm).

A descriptive flow chart of isolation is presented in Figure 3.2 below.

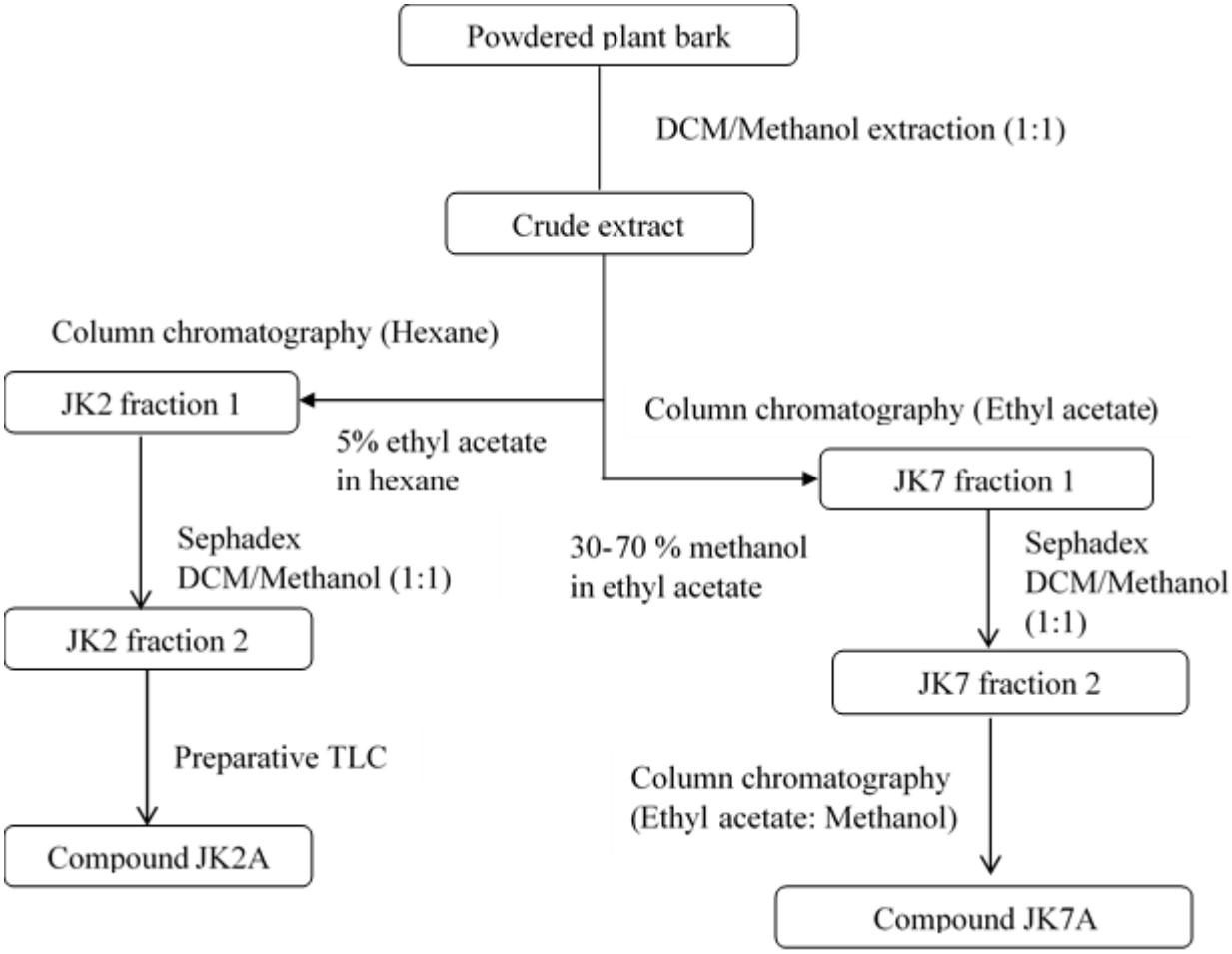



**Figure 3.2: Flow chart on isolation of the powdered plant bark.**

### 3.5.5. Procedure for antioxidant activity of isolated compound

The assay for antioxidant activity was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as the oxidant. The choice was based on its reproducibility as previously described in the literature (Mishra *et al.*, 2013). The test solutions of the pure compound prepared in 95% v/v methanol, to obtain concentrations in the range 250- 2000 µg/mL. Ascorbic acid was used as a standard and serial dilutions at 6.25-75 µg/mL concentration range were prepared using methanol. The oxidant was prepared by dissolving 3.94 mg of DPPH in 100 mL of 95% v/v methanol just before use and stored in the dark to minimize degradation. A 200 µL aliquot of the test/standard solution was placed in a vial and 2800 µL of DPPH added. The mixture was kept in the dark for 30 min and optical density measured spectrophotometrically against a blank at 517 nm. The blank constituted only 2800 µL of DPPH with 200 µL methanol added as a replacement for the sample/standard. The assay was done in triplicate.

The antioxidant activity of the pure compound was expressed as half-maximal inhibitory concentration ($IC_{50}$), which is the concentration (expressed in µg/mL) of sample required to cause a 50% reduction in DPPH radicals. Extract concentration providing 50% inhibition ($IC_{50}$) was calculated from the graph that plotted inhibition percentage against concentration (Appendix 5). Inhibition of free radical of DPPH in percentage terms (percentage inhibition-PI %) was calculated as shown in Equation 1.

$$PI\ \% = (A_{blank} - A_{sample}/A_{blank}) \times 100 \qquad \text{Equation 3}$$

Where $A_{blank}$ is the absorbance of the control reaction (containing all reagents except the sample) while $A_{sample}$ is the absorbance of the pure compound (or the standard).

# 4.0. CHAPTER FOUR: RESULTS AND DISCUSSION

## 4.1. ETHNOBOTANICAL SURVEY

The survey identified 48 respondents recommended by the local authorities or chiefs in the area. All the 48 respondents interviewed were males above 35 years of age. A high number of respondents (60%) were above 45 years of age. A majority of the interviewees (90%) were still practising pastoralism, and 77% of the respondents had no formal education (Fig 4.1). The number of respondents in our study were more than the number of herbalists (30) interviewed by Muthee *et al*. in a previous study done in Loitokitok as they were targeting herbalists while we were targeting persons knowledgeable in plants used as food and medicine. In the same study by Muthee *et al*., the interviews were conducted away from the homestead and likewise male respondents were the majority at 87.5% similar to this study. (Muthee *et al.*, 2011). The exclusively male respondents in our study may have been due to the time of the interviews, which were all conducted in the morning, at the chief's camp in Loitokitok town, a time when most women would be at home tending to the family's needs or doing chores. The absence of women could also be due to the patriarchal nature of the Maasai where the male elders speak on behalf of the community (Gneezy et al., 2009). The large percentage of pastoralists is similar to a study done by Ole Seno in Loitokitok where 65% of the respondents were uneducated pastoralists (Seno and Tome, 2013). Since pastoralism provides for the Maasai families, there seems to be little need for formal education which feeds a cycle of illiteracy in the community.

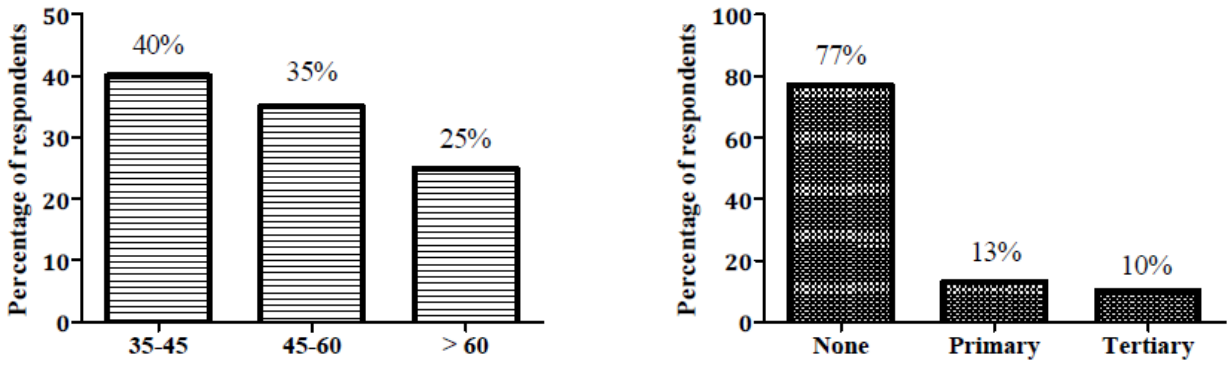



**Figure 4.1: Age and education level of the respondents**

All the respondents admitted to taking traditional medicine with or without conventional drugs. In addition, the study showed that 73% of respondents preferred traditional medicine (TM) to conventional medicine which is in agreement with previous observation by Kiringe (Kiringe, 2006) as shown in Fig 4.2.

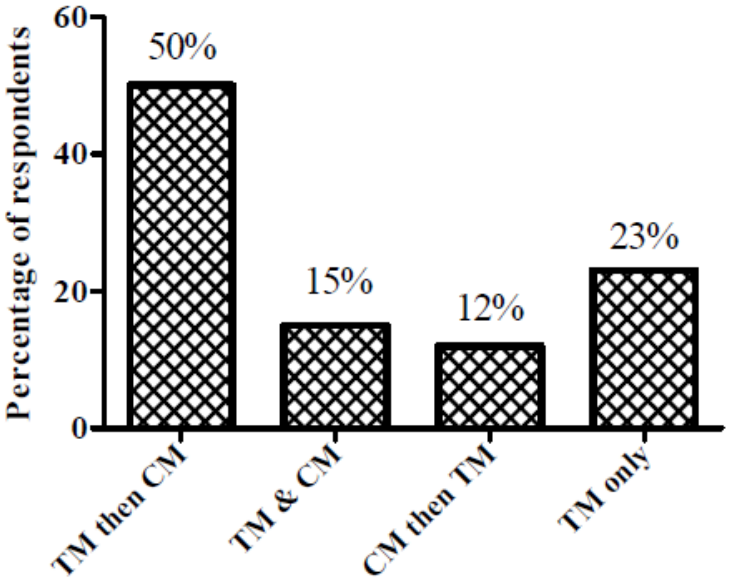



**Figure 4.2: The preference with which the respondents take the medicinal plants.**
TM- Traditional medicine and CM- Conventional medicine.

At least half of the respondents ingested traditional foods or medicines in the early stages of the disease, but when ineffective the locals resorted to conventional medicine (Fig 4.2). Traditional medicine was seen as preventive and starting with it is thought to inhibit any disease causing agent and/or remove any offending cause. The Maasai believe that what causes disease usually comes from the outside and can be treated by use of purgatives (Johns *et al.*, 1994). Traditional medicines may also be relatively cheaper and easily available since they are sold in the local market. It is only when there is no improvement with the TM that conventional medicines are sought. In TM, symptoms are treated whereas in conventional medicine a diagnostic procedure identifies a disease or condition and treats the causative agent. Since symptoms appear earlier and are usually common among different diseases, this could bring the notion that traditional knowledge may have a more expansive repertoire to help identify health problems earlier than conventional medicine. There is also rapidly increasing awareness of the potential and curative abilities of alternative medicines, especially from the use of medicinal plants, as well as the inadequate access to conventional medicine and physicians, and the high cost for conventional drugs (Busia, 2005).

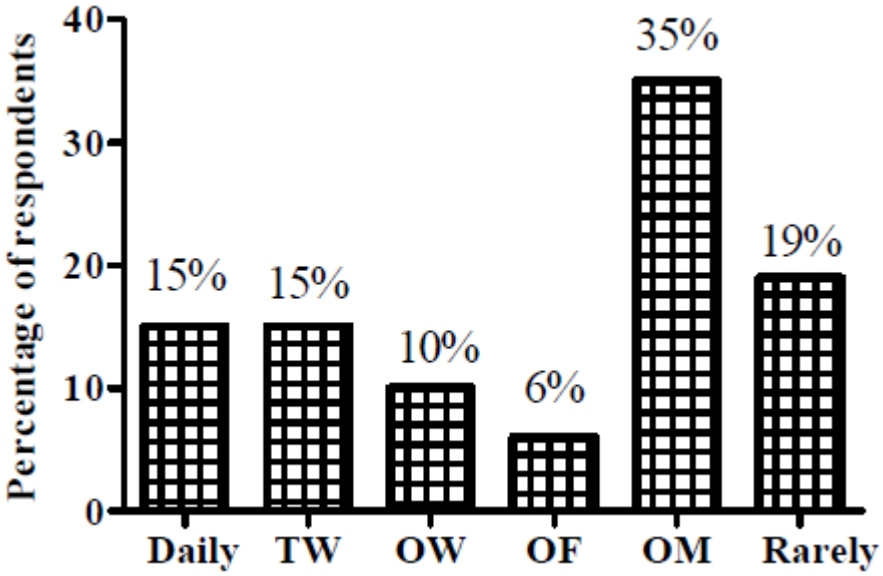



**Figure 4.3: Frequency of plant use**

TW- thrice weekly, OW- once weekly, OF- once a fortnight and OM- once a month.

At least 81% of the respondents use indigenous foods and medicinal plants once a month or more (Figure 4.3). Use of indigenous vegetables by the Maasai increased after an influx of other tribes in the area who regularly consume indigenous vegetables (Hansen *et al.*, 2011). Herbs used as food additives by the Maasai are usually consumed with meat, where the decoction of the herb is used to flavour the meat broth. The Maasai usually boil the traditional plants and mix the decoction with either bone soup or milk before drinking (Ole-Miaron, 2003). This would point towards the presence of heat stable hydrophilic and lipophilic compounds that act as adaptogens and medicines. Adaptogens promote tolerance to stress and facilitate homeostasis (Liao *et al.*, 2018). The decoction is taken as an adaptogen when the herder has a long-distance trek in search of pasture.

The most common route of administration was oral (78%) while 19% of the plants were used as topical preparations and only *Ocimum gratissimum* (3%) was used as an inhalant. Oral route was the most practical and a review on plant use by the Maasai showed oral route to be the most commonly used followed by topical preparations and inhalations (Nankaya *et al.*, 2020). This is related to the physical and chemical characteristics of the bioactive compounds in these plants, where phytochemicals such as phenols and alkaloids are taken orally while terpenoids are best administered topically (Cao *et al.*, 2008).

A total of 30 species from 21 families and 25 genera were reportedly used as food and/or medicine. Other studies performed in the same region have encountered many more plant species such as 155 plant species from Sekenani valley and 80 plant species from Loitokitok district (Muthee *et al.*, 2011). Out of the 155 plant species from Sekenani valley, only 39 plant species were reportedly used as medicinal plants (Bussmann *et al.*, 2006). Muthee *et al* included all plants mentioned by less than three respondents while this study included plant species mentioned by three or more informants. This is so as to obtain more reliable data due to the dynamic nature of ethnobotanical information (Johns *et al.*, 1994).

All the respondents mentioned the plant names in the local *Maa* dialect. The most commonly encountered family was Fabaceae with a total of eight plants, followed by Anacardiaceae and Solanaceae comprising two plants each (Table 4.1). Fabaceae was also cited as the most commonly encountered family by Nankaya *et al* and correlates with the flora frequently encountered in Loitokitok (K6 zone) (Zhou *et al.*, 2017; Nankaya *et al.*, 2020).

**Table 4.1: Plants* used as food and/or medicine by the Ilkisonko Maasai community with their corresponding traditional use and pharmacological activity**

| Family and Scientific name | Local Maasai name (Maa) | Uses | Reported traditional uses | Reported pharmacological/ chemical activity | Part used | Number of mentions | Habit |
|---|---|---|---|---|---|---|---|
| Amaranthaceae *Achyranthes aspera* L. | Olerubat | Teeth and new wounds, back and knee aches, conjunctivitis | Used to treat malaria and toothache (Bussmann *et al*., 2006; Kareru(b) *et al*., 2007) | Anti-inflammatory effect (Vetrichelvan and Jegadeesan, 2003) | Whole plant | 3 | Herb |
| Anacardiaceae | | | | | | | |
| *Rhus natalensis* Krauss | Olmusigiyoi | Strengthener, respiratory disorders, stomachic, malaria | Used as food (Orwa *et al*., 2009). Roots used for digestive disorders and gonorrhea (Arbonnier, 2004) | Water and methanol extracts showed antiplasmodial activity (Gathirwa *et al.*, 2008) | Root, fruit, stem | 3 | Tree |
| *Sclerocarya birrea* (A. Rich.) Hochst. | Oloisuki | Cold and flu in children, edema of limbs, respiratory disorders, joint pains, tonic in cows, food | Used in inflammatory disorders, malaria, nausea, tonic for man and cattle food, timber, dye (Arbonnier, 2004) | The methanol extract exhibited antifungal activity (Hamza *et al.*, 2006) | Root/ Stem bark | 8 | Tree |
| Apocynaceae *Carissa spinarum* L. | Olamuriaki | Food, colds | Used as a gastrointestinal remedy (Maundu *et al.*, 2001). | Isolated compounds were found to have antioxidant effects, while the extract had antibacterial effect. (Sanwal and Chaudhary, 2011; | Fruit | 5 | Shrub |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | Wangteeraprasert *et al.*, 2012) | | | |
| Asphodelaceae *Aloe secundiflora* Engl. | Osukuroi | Tonic, respiratory problems, East Coast fever, wounds, headache | Used for chest pain, headaches, malaria, rheumatism and topically on wounds (Masinde, 2010; Muthee *et al.*, 2011) | The leaf exudate has antibacterial property which may be due to the presence of aloenin. (Rebecca *et al.*, 2003; Wagate *et al.*, 2010) | Leaf sap | 13 | Herb |
| Canellaceae *Warburgia ugandensis* (Sprague) subsp. *ugandensis* | Osokonoi | Diarrhea, respiratory problems, stomach ache, malaria | Used by the Maasai to treat respiratory disorders (Muthee *et al.*, 2011), as a tonic and aphrodisiac (Kiringe, 2006) | The crude extract showed antibacterial, antifungal effect and antioxidant molecules were obtained (Olila *et al.*, 2001; Manguro *et al.*, 2003) | Root/ Stem bark | 14 | Tree |
| Capparaceae *Maerua triphylla* A. Rich. | Olamalogi | Food, cleaning wounds, aphrodisiac, headache, tonic | Leaf paste is used to clean boils (Hassan-Abdallah *et al.*, 2013) | No reported pharmacological activity | Stem bark, leaf | 3 | Tree |
| Combretaceae *Combretum molle* R. Br. ex G. Don. | Olmaroroi | Sexually transmitted infections (STIs), backache | Used to treat respiratory disorders and backache (Muthee *et al.*, 2011) | Anti-inflammatory triterpenoids are present (Ponou *et al.*, 2008) | Root | 3 | Tree |
| Fabaceae | | | | | | | |
| *Acacia drepanolobium* Harms ex Sjöstedt | Eluai | Retained placenta in cows, postpartum pain in humans, fertility, tonic | Used to expel retained placenta (Maundu *et al.*, 2001; Kiringe, 2006) | Presence of tannins and proanthocyanidins (Kusano *et al.*, 2011) | Stem bark | 15 | Shrub |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Acacia nilotica* (L.) Willd. | Enkiloriti | Strengthener/ tonic, appetizer, body aches, stomachic, stamina, stimulant/excitant | Antioxidant (Sultana *et al*., 2007) | Umbelliferone has antioxidant activity (Singh *et al.*, 2010), niloticane has anti inflammatory and antibacterial effect (Eldeen *et al*., 2010) | Stem/ Root bark | 20 | Tree |
| *Acacia mellifera* (M. Vahl) Benth. | Oiti | Postpartum tonic, appetizer, sore throat, East Coast fever | Stomachache (Johns *et al*., 1999), appetizer (Kiringe, 2006) | Lupanes isolated have cytotoxic activity while its triterpenoids have antibacterial activity (Mutai *et al*., 2009) | Stem bark | 4 | Tree |
| *Acacia reficiens* subsp. *misera* (Vatke) Brenan | Olchurrai | Strengthener, appetizer, tonic/adaptogen, laxative | Spice and condiment, fodder, aphrodisiac (Masinde, 2010) | No reported activity | Root/ Stem bark | 16 | Tree |
| *Acacia nubica* Benth. | Oldepe | STIs, postpartum tonic, facilitate lactation, rejuvenation | Tonic and joint pains (Johns et al., 1994), postpartum (Kiringe, 2006) | Root bark contains triterpenes with antifungal activity (Elfadil *et al*., 2015) | Root bark | 5 | Tree |
| *Albizia anthelmintica* Brongn. | Olmukutan | Purgative, dewormer, antimalarial, tonic, food for goats | To induce vomiting in malaria (Kiringe, 2006) and treat fevers (Johns *et al*., 1999) | Contains triterpenes with potent analgesic and antioxidant activity (Mohamed *et al*., 2013) | Root/ Stem bark | 24 | Tree |
| *Acacia robusta* Burch. | Olmumunyi | Retained placenta in cows and humans | To remove placenta after birth (Kiringe, 2006) | The methanol extract exhibited antifungal activity (Hamza *et al.*, 2006) | Root bark | 22 | Tree |
| *Ormocarpum kirkii* S. Moore | Enkokirisian joi | Stops postpartum bleeding, prevents | Cuts and wounds (Muthee *et al*., | Presence of biflavonoids with | Root, fruit | 3 | Shrub |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | abortion | 2011) | antimicrobial activity (Dhooghe *et al.*, 2010) | | | |
| Labiatae *Ocimum gratissimum* L. | Olemoran | Treatment of colds, headache, fragrant | Used for bronchitis and malaria (Kareru(b) *et al*., 2007) | Extracts were found to have anti-inflammatory effects (Chiu *et al.*, 2012) | Leaf | 5 | Herb |
| Malvaceae *Grewia villosa* Willd. | Olmankulai | Food, galactagogue, strength/ tonic, stomachache in children | Used as an anticancer (Kareru(b) *et al*., 2007), for oral hygiene (Bussmann *et al.*, 2006) | The fruits and leaves are highly nutritive and the root contains harman alkaloids (Goyal, 2012; Saleem *et al*., 2012) | Root, stem, fruit | 25 | Shrub |
| Olacaceae *Ximenia americana* L. | Enkamai | Stomachache in kids, food, tonic, constipation | Stomachaches (Muthee *et al.*, 2011) used in Mali as a tonic (Le *et al.*, 2012) and to manage HIV related symptoms in Kenya (Nagata *et al.*, 2011) | Leaves contain sambunigrin which is toxic to parasites (Orwa *et al*., 2009). Presence of gallic acid, gallotannins and flavanols in the leaves (Le *et al.*, 2012) | Root, fruit | 25 | Tree |
| Oleaceae *Olea capensis* L. | Oloiren | Preserve milk, tonic, cold and fever, East Coast fever | For respiratory ailments (Muthee *et al.*, 2011) | The plant extract contains triterpenoids with antibacterial activity (Bamuamba *et al*., 2008) | Stem | 5 | Tree |
| Polygonaceae *Oxygonum sinuatum* (Hochst. & Steud ex Meisn.) | Enkaisijoi | Tonsillitis, food, conjunctivitis | Used to treat gonorrhea (Kareru(a) *et al*., 2007) | Antibacterial and anti-inflammatory activity (Matu and Van Staden, 2003) | Whole plant | 4 | Herb |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Dammer | | | | | | | |
| Rhamnaceae *Rhamnus prinoides* L. | Olkonyil | STIs, back and joint aches, arthritis, aids in digestion, tonic | The plant was observed by Muthee *et al*., (2011) to treat STIs and some parasitic infections | Extract contain laxative anthraquinones and has antiplasmodial activity (Muregi *et al.*, 2007; Berhanu, 2014) | Root | 16 | Shrub |
| Salvadoraceae *Salvadora persica* L. | Oremit | Eye infections, worms, malaria, stomachache, constipation, tonic, cold, teeth hygiene, respiratory infections | Used for abdominal disturbances (Masinde, 2010) | Antimicrobial properties and contains flavonoids with known antioxidant effect (Halawany, 2012) | Root, stem | 41 | Shrub |
| Sapindaceae *Pappea capensis* Eckl. & Zeyh. | Oltimigomi | Strengthens, food, fertility, stomach ache, stamina | Used for stomachaches, as an aphrodisiac and an adaptogen (Kokwaro, 2009; Muthee *et al*., 2011; Johns *et al*., 1999) | Leaf and stem bark extracts have antioxidant activity (Karau *et al*., 2012) | Stem bark | 17 | Tree |
| Simaroubaceae *Harrisonia abyssinica* Oliv. | Enkisarang' atuny | Arthritis, STIs | Arthritis (Johns *et al*., 1999; Kareru(a) *et al*., 2007) | Bark and root produced compounds with potent anti-microbial activity (Balde *et al*., 1995; Lee *et al*., 2014) | Root, fruit, leaf | 7 | Shrub |
| Solanaceae | | | | | | | |
| *Solanum incanum* L. | Entulelei | Oral hygiene, strength, stomachache, sore | Used for throat infections (Muthee *et al*., 2011), also for | Anti-tumor glycoalkaloids (Lin *et al*., 2000) | Fruit, stem | 4 | Herb or soft wooded |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | throat | symptoms of diabetes (Moshi and Mbwambo, 2002) | | | | shrub |
| *Withania somnifera* (L.) Dunal. | Olesayiet | Blood tonic and rejuvenator, back and joint aches, galactagogue, appetite and tonic for calves | Treats symptoms related to diabetes and also used as a tonic (Masinde, 2010; Keter and Mutiso, 2012) | Antioxidant and anti-inflammatory effect of the withanolides (Yang *et al.*, 2013) | Root bark | 10 | Shrub |
| Urticaceae *Urtica massaica* Mildbr. | Enjamejoi | Food, stomachache | Stomachache (Masinde, 2010) | Contains acetylcholine and histamine which affect smooth muscle (Maitai *et al*., 1980) | Whole plant | 4 | Herb |
| Verbenaceae *Lippia kituiensis* Vatke | Osinoni | Respiratory problems, measles, protects cattle from ectoparasites | Management of chronic pain (Wambugu *et al.*, 2011) | Contains essential oil which has acaricidal activity (Kosgei, 2014) | Leaf | 5 | Shrub |
| Vitaceae *Cyphostemma nodiglandulosum* (Th. Fr. Jr) | Enkilenya | STIs, tonic, galactagogue, stomachache | No reports | No reported pharmacological activity | Whole plant | 3 | Herb |

*Plants outlined in Table 4.1 above were only those mentioned three or more times by the respondents.

*Salvadora persica* was mentioned by 85% of the respondents due to its importance in dental hygiene and also as a medicine (Halawany, 2012). Other plants that were commonly mentioned by respondents were *Grewia villosa* (52%), *Ximenia americana* (52%), *Albizia anthelmintica* (50%), *Acacia robusta* (46%) and *Acacia nilotica* (42%) as evident in Table 4.1. The trees and shrubs mentioned above are commonly found in the arid areas north and south of Kenya and are used as fodder for livestock. The commonly mentioned plants were all used as restoratives except for *Acacia robusta*.

In this study, the common ailments treated were stomach aches, constipation, back aches, joint aches, body pains and STIs (Table 4.1). Stomach aches may be due to consumption of raw milk, meat and blood that is practiced in rituals by the Maasai (Bussmann *et al.*, 2006). This can lead to brucellosis and worm infestation symptomized by frequent stomach aches. This was seen in a different study where Maasai pre-schoolers had high levels of inflammatory proteins that pointed towards intestinal damage (Lawson *et al.*, 2014). Joint aches, swollen joints and back pain are associated with brucellosis though the same symptoms are common in the elderly or could also be due to the nomadic lifestyle practiced by Maasai communities. Constipation, a common ailment seen in the study, may arise due to decreased food and fibre intake seen among pastoralists due to food insecurity as they live in drought prone areas (Houghton *et al.*, 2019). A high number of sexual partners coupled with polygamy may be the reason why more than a quarter of the plants mentioned in the study are used to treat STIs or for libido enhancement (Talle, 2007). In addition, the Maasai use plants as digestives and restoratives, which is not common in many cultures except for pastoralists who usually take the plants after long treks in search of pasture and water for their animals. Most of the traditional uses were also supported by similar studies as illustrated in Table 4.1.

Sixteen families (53%) from each of the studies by Bussman *et al*. (2006) and Muthee *et al* (2011) are similar to those cited in this study as their study area was the same County. At least 33% of the plants identified were used as both food and medicine in agreement with plants such as *Ximenia* sp., *Carissa* sp., *Cyphostemma* sp., and *Grewia* sp. previously cited as sources of food (Bussmann *et al.*, 2006), while the remaining 67% were used as medicine. Amongst the thirty plants included in Table 4.1, nine plants (30%) found use in management of livestock conditions such as retained placenta, East Coast fever and as restoratives. This is confirmed by the indigenous knowledge that the Maasai possess due to their close association with cattle (Ole-Miaron, 2003).

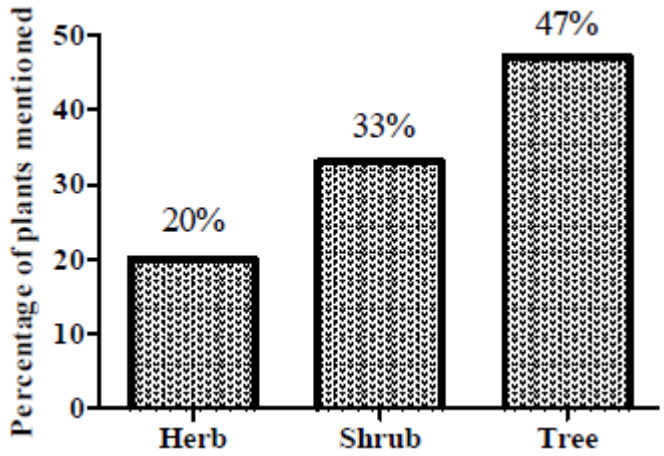



**Figure 4.4: The predominant growth forms in the area.**

As shown in Figure 4.4, the growth forms encountered were tree (47%), shrub (33%) and herb (20%). The area studied is under the K6 vegetation zone which comprises mostly of grassland interspersed with Acacia (Zhou *et al.*, 2017). The family most commonly encountered was Fabaceae, having mainly trees and shrubs.

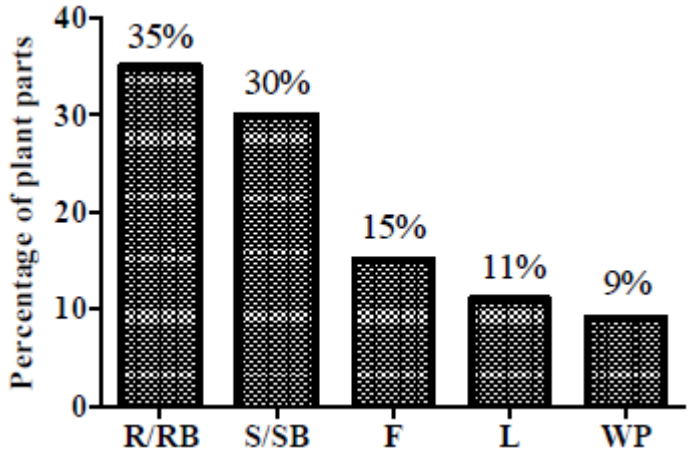



**Figure 4.5: The plant parts used as food and medicine.**

R/RB- root/root bark, S/SB- stem/stem bark, F- fruit, L- leaves, WP- whole plant.

Figure 4.5 shows that the root/root bark was the most commonly used part of the plant (35%), followed by the stem/stem bark (30%), fruit (15%), leaves (11%) and whole plant (9%). A review on the medicinal plants used by the Maasai cited roots to be the most commonly used plant part (Nankaya *et al.*, 2020) Roots are known to have highly potent compounds which remain stable for a longer time, than compounds in other parts of the plant, after harvesting (Haichar *et al.*, 2014). This may be useful if the plant material is to be stored for later use.

Harvesting of roots has serious consequences to sustainable land use, as good harvesting practices are normally not adhered to leading to loss of plant life (Ssegawa and Kasenene, 2007). Leaves are the least used plant parts possibly because the Maasai are known to consume very little vegetable in their diet while fruits are mainly eaten by children when in season (Hansen *et al.*, 2011).

Documentation of the medicinal plants and their uses is important in preserving the indigenous knowledge. Further research in these plants could provide novel compounds that could be used as leads in drug discovery.

## 4.2. PHYTOCHEMICAL SCREENING

### 4.2.1. Total phenolic content

The total phenolic content in the extracts as assayed by the Folin-Ciocalteu's reagent is presented in Table 4.2.

**Table 4.2: Total phenolic content expressed as Tannic acid equivalents (TAE) in the studied methanol and water plant extracts**

| | | TAE in mg/g of extract | | |
|---|---|---|---|---|
| CODE | SAMPLE | Methanol extract | Water extract | *p*-value |
| AN | *Acacia nilotica* (L.) Willd. | 237.3 | 149.7 | ** |
| AB | *Acacia robusta* Burch. | 183.4 | 113.7 | ** |
| XA | *Ximenia americana* L. | 156.1 | 147.4 | ns |
| AR | *Acacia reficiens* subsp. *misera* (Vatke) | 153.9 | 130.4 | * |
| RN | *Rhus natalensis* Krauss | 148.7 | 121.3 | ** |
| GV | *Grewia villosa* Willd. | 136.8 | 117.7 | ns |
| AD | *Acacia drepanolobium* Harms | 126.5 | 107.6 | * |
| RP | *Rhamnus prinoides* L. | 97.9 | 95.3 | ns |
| OK | *Ormocarpum kirkii* S. Moore | 66.0 | 78.2 | ** |
| MT | *Maerua triphylla* A. Rich. | 75.9 | 54.1 | ** |
| OC | *Olea capensis* L. | 62.9 | 84.1 | ** |
| LU | *Lippia kituiensis* Vatke | 77.9 | 62.5 | ** |
| PC | *Pappea capensis* Eckl. & Zeyh. | 56.7 | 124.1 | ** |
| RS | *Rhamnus staddo* A. Rich. | 63.6 | 52.7 | ** |

| | | | | |
|---|---|---|---|---|
| AM | *Acacia mellifera* (M. Vahl) Benth | 63.1 | 50.8 | ** |
| HH | *Harrisonia abyssinica* Oliv. | 64.3 | 50.8 | ** |
| OS | *Oxygonum sinuatum* Dammer | 65.0 | 50.6 | ** |
| CS | *Carissa spinarum* L. | 67.8 | 45.1 | ** |
| WS | *Withania somnifera* (L.) Dunal. | 60.2 | 41.4 | ** |
| SI | *Solanum incanum* L. | 66.5 | 41.2 | ** |
| CN | *Cyphostemma nodiglandulosum* (Th.Fr. Jr.) | 59.4 | 38.0 | ** |
| AU | *Acacia nubica* Benth. | 58.4 | 37.4 | ** |
| ZX | *Zanthoxylum chalybeum* Engl. | 60.7 | 36.4 | ** |
| AA | *Achyranthes aspera* L. | 54.9 | 35.7 | ** |
| AS | *Aloe secundiflora* Engl. | 35.5 | 64.2 | ** |
| UM | *Urtica massaica* Mildbr. | 56.4 | 33.7 | ** |
| AH | *Albizia anthelmintica* Brogn. | 60.0 | 33.5 | ** |
| WU | *Warburgia ugandensis* (Sprague) *subsp. ugandensis* | 54.1 | 31.1 | ** |

The last column indicates any statistically significant differences in phenolic content between the methanol and water extracts (* $p < 0.05$; ** $p < 0.001$; ns -not significant).

The methanol extracts had higher levels of phenolic content compared to the water extracts with the exception of *P. capensis*, *O. kirkii*, *O. capensis*, and *A. secundiflora* where the converse was true. This is consistent with other studies where methanol has been found to be more efficient in extraction of polyphenols (Dai and Mumper, 2010). All the plant extracts analysed showed a significant ($p<0.05$) difference in total phenolic content extracted by the different solvents except for *X. americana*, *G. villosa* and *R. prinoides* (Table 4.2). In the methanol extracts, *A. nilotica* had the highest phenolic content (237.3±1.83) while *A. secundiflora* had the lowest (35.5±0.19) mg TAE/g of extract. In the water extracts, *A. nilotica* also had the highest (149.7±0.60) while *W. ugandensis* had the lowest phenolic content (31.1±0.18) mg TAE/g of extract. This is in agreement with Aadil (Aadil *et al.*, 2014) where the methanolic fraction of *A. nilotica* had the highest polyphenol content when they extracted the wood lignin of *A. nilotica* using different solvents. This is supported by its use as an alternative to *Acacia mearnsii* (black wattle) in production of tannins for the leather industry because of its high tannin content (Mugedo and Waterman, 1992).

Panossian and Wikman (Panossian and Wikman, 2010) have shown that phenolics contribute to the adaptogenic effect of some plants. Findings from this study indicate that eight (*A. nilotica, A. robusta, X. americana, R. natalensis, G. villosa, A. drepanolobium, R. prinoides* and *P. capensis*) of the top nine plants (except for *A. reficiens*) that were used as tonics are high in total phenols. Other communities have also used the same plants as tonics such as the Mali using the roots of *X. americana* (Le *et al.*, 2012). This could explain why the Ilkisonko Maasai have routinely used these eight plants as adaptogens as they may derive their beneficial health effects from the observed high polyphenol content.

### 4.2.2. Total flavonoid content

Flavonoids are a component of polyphenols that contain 15 carbon flavanol nucleus. The flavonoid content measured using the aluminium colourimetric method is presented in Table 4.3.

**Table 4.3: Total flavonoid content expressed as Catechin equivalents (CE) in the studied methanol and water plant extracts**

| | | Catechin equivalent in mg/g of extract | | |
|---|---|---|---|---|
| CODE | SAMPLE | Methanol extract | Water extract | *p*-value |
| AN | *Acacia nilotica* (L.) Willd. | 118.32 | 83.75 | ** |
| AB | *Acacia robusta* Burch. | 68.99 | 27.62 | ** |
| XA | *Ximenia americana* L. | 119.02 | 88.84 | ** |
| AR | *Acacia reficiens* subsp. *misera* (Vatke) | 130.62 | 99.80 | ** |
| RN | *Rhus natalensis* Krauss | 70.58 | 35.38 | ** |
| GV | *Grewia villosa* Willd. | 127.17 | 91.68 | ** |
| AD | *Acacia drepanolobium* Harms | 35.12 | 16.53 | ** |
| RP | *Rhamnus prinoides* L. | 57.06 | 29.34 | ** |
| OK | *Ormocarpum kirkii* S. Moore | 12.22 | 17.80 | ** |
| MT | *Maerua triphylla* A. Rich. | 50.11 | 0.58 | ** |
| OC | *Olea capensis* L. | 50.35 | 51.72 | ns |
| LU | *Lippia kituiensis* Vatke | 70.22 | 45.62 | ** |
| PC | *Pappea capensis* Eckl. & Zeyh. | 35.38 | 71.48 | ** |
| RS | *Rhamnus staddo* A. Rich. | 18.42 | 7.62 | ** |
| AM | *Acacia mellifera* (M. Vahl) Benth | 33.65 | 8.43 | ** |

| | | | | |
|---|---|---|---|---|
| HH | *Harrisonia abyssinica* Oliv. | 18.64 | 7.27 | ** |
| OS | *Oxygonum sinuatum* Dammer | 30.51 | 15.09 | ** |
| CS | *Carissa spinarum* L. | 32.89 | 19.19 | ** |
| WS | *Withania somnifera* (L.) Dunal. | 20.40 | 6.87 | ** |
| SI | *Solanum incanum* L. | 15.98 | 17.59 | ** |
| CN | *Cyphostemma nodiglandulosum* (Th.Fr. Jr.) | 19.68 | 4.73 | ** |
| AU | *Acacia nubica* Benth. | 9.44 | 0.27 | ** |
| ZX | *Zanthoxylum chalybeum* Engl. | 8.90 | 3.12 | ** |
| AA | *Achyranthes aspera* L. | 25.52 | 7.00 | ** |
| AS | *Aloe secundiflora* Engl. | 8.32 | 9.11 | * |
| UM | *Urtica massaica* Mildbr. | 8.29 | 1.21 | ** |
| AH | *Albizia anthelmintica* Brogn. | 4.29 | 0.25 | ** |
| WU | *Warburgia ugandensis* (Sprague) *subsp. ugandensis* | 8.94 | 3.82 | ** |

The last column indicates any statistically significant differences in flavonoid content between the methanol and water extracts (* $p < 0.05$; ** $p < 0.001$; ns -not significant).

The flavonoid content was higher in the methanol extracts compared to the water extracts with the exception of *P. capensis*, *O. kirkii*, *O. capensis*, *S. incanum* and *A. secundiflora* where the converse was observed (Table 4.3). This is similar to the pattern seen in phenolic content and in other studies where methanol extraction yields a higher quantity of polyphenols (Dai and Mumper, 2010). The poor solubility of flavonoids in water may explain the difference in the concentration between the water and methanol extracts (Miguel *et al.*, 2014). All the plant extracts analysed showed a significant ($p<0.05$) difference in total flavonoid content extracted by the different solvents except for *O. capensis*. In both the methanol and water extracts, *A. reficiens* had the highest (130.6±1.78 and 99.8±1.73 mg CE/g of extract, respectively) while *A. anthelmintica* had the lowest flavonoid content (4.3±0.21 and 0.3±0.01 mg CE/g of extract, respectively). Flavonoids, commonly seen in the plants studied, are known to increase enzymes which are critical to phase II detoxification system that reduce the reactive oxygen species and thereby protect against carcinogenesis (Ziech *et al.*, 2012). The top eight plants high in total flavonoid content (*A. nilotica, A. robusta, X. americana, A. reficiens, R. natalensis, G. villosa, P. capensis and L. kituiensis*) are closely similar to the plants with high total phenolic content.

This study indicates that methanol extraction of polyphenols is more efficient than aqueous extraction, a finding that has been supported by other studies (Dai and Mumper, 2010). This is counter to the traditional mode of preparation. The Ilkisonko Maasai prepare most of their decoctions with water before adding them to soups or milk. These decoctions have been documented as safe when prepared as such for all ages (Maundu *et al.*, 2001). The use of water in preparing traditional herbs may give a safer decoction since a lower quantity of tannins is extracted, which can have anti-nutritive qualities if used in high concentration.
All the respondents mentioned the use of heat when preparing the traditional herbs. Use of heat helps to break down cell walls and facilitate the diffusion of phytochemicals from the plant solids to the solution. Steaming, boiling and broiling are also the preferred methods of cooking as most polyphenols present in foods are water-soluble (hydrophilic) (Özcan and Arslan, 2011). Even though the Maasai prepare soups using heat, some phenolic compounds still retain activity even under high moisture and heat (Beta and Hwang, 2018) while flavonoids are stable under heat and even after metabolism (Hu *et al.*, 2018).

From the ethnobotanical survey (Table 4.1), the plants in this study have also been used in illnesses whose aetiology is microbial such as STIs, stomach ache and respiratory disorders. Plants with antimicrobial activity are characterized by high phenolic and flavonoid content and consequently the high antioxidant activity which could contribute to their antimicrobial activity (Scio *et al.*, 2012). There were variations in the polyphenol content in plants from the same genus (*Acacia* spp.) which could be due to intrinsic genetic species differences, or differences in environmental factors, time of collection and/or storage conditions (Suzuki *et al.*, 2014). It has been previously reported that the amount of phenolics may vary considerably in some plants due to geographical variations and environmental factors such as humidity and, temperature as well as aging which invariably influence the antioxidant activity (Ernst *et al.*, 1991).

## 4.3. ANTIOXIDANT ACTIVITY OF THE PLANT EXTRACTS

The DPPH-based antioxidant activity of the studied plant extracts is shown in Table 4.4. The DPPH assay for antioxidant activity is based on the ability of the antioxidant to scavenge the DPPH cation radical with ascorbic acid used as a standard. The $IC_{50}$ value indicates the

degree of antioxidant activity and it is defined as the concentration of substrate that causes 50% reduction in the DPPH colour. The lower the $IC_{50}$ value, the higher the antioxidant activity (Kedare and Singh, 2011).

**Table 4.4: Antioxidant activity of methanol and water extracts of the studied plants**

| | Half maximal inhibitory concentration ($IC_{50}$) in µg/ml | | |
|---|---|---|---|
| CODE | SAMPLE | Methanol extracts | Water extracts |
| AA | Ascorbic acid standard | 50.32 | |
| AN | *Acacia nilotica* (L.) Willd. | 54.6 | 103.0 |
| AB | *Acacia robusta* Burch. | 76.8 | 251.6 |
| XA | *Ximenia americana* L. | 82.5 | 179.1 |
| AR | *Acacia reficiens* subsp. *misera* (Vatke) | 114.7 | 186.1 |
| RN | *Rhus natalensis* Krauss | 125.5 | 185.3 |
| GV | *Grewia villosa* Willd. | 107.5 | 241.9 |
| AD | *Acacia drepanolobium* Harms | 117.5 | 188.5 |
| RP | *Rhamnus prinoides* L. | 253.0 | 377.3 |
| OK | *Ormocarpum kirkii* S. Moore | 1891.5 | 981.8 |
| MT | *Maerua triphylla* A. Rich. | 207.6 | 367.5 |
| OC | *Olea capensis* L. | 548.2 | 447.6 |
| LU | *Lippia kituiensis* Vatke | 391.1 | 475.5 |
| PC | *Pappea capensis* Eckl. & Zeyh. | 378.6 | 208.8 |
| RS | *Rhamnus staddo* A. Rich. | 698.9 | 1300.6 |
| AM | *Acacia mellifera* (M. Vahl) Benth | 577.4 | 1548.0 |
| HH | *Harrisonia abyssinica* Oliv. | 376.3 | 664.8 |
| OS | *Oxygonum sinuatum* Dammer | 1177.1 | 666.4 |
| CS | *Carissa spinarum* L. | 761.5 | 1181.6 |
| WS | *Withania somnifera* (L.) Dunal. | 659.4 | 1406.5 |
| SI | *Solanum incanum* L. | 540.9 | 1178.4 |
| CN | *Cyphostemma nodiglandulosum* (Th.Fr. Jr.) | 6218.8 | 3452.7 |
| AU | *Acacia nubica* Benth. | 6229.0 | 11297.5 |
| ZX | *Zanthoxylum chalybeum* Engl. | 1694.8 | 3389.8 |
| AA | *Achyranthes aspera* L. | 839.8 | 1168.5 |
| AS | *Aloe secundiflora* Engl. | 7279.7 | 3953.9 |

| | | | |
|---|---|---|---|
| UM | *Urtica massaica* Mildbr. | 2376.0 | 6329.8 |
| AH | *Albizia anthelmintica* Brogn. | 14829.3 | 45524.0 |
| WU | *Warburgia ugandensis* (Sprague) *subsp. ugandensis* | 2039.1 | 1368.5 |

For each plant, the antioxidant activity of the methanol extract was higher than that of water extract except a few such as *O. kirkii*, *O. capensis*, *O. sinuatum*, *C. nodiglandulosum*, *A. secundiflora* and *W. ugandensis*. The difference in $IC_{50}$ values in the methanol and water extracts (higher value compared to the lower value) is anywhere between 1.2 to 3.3 times, showing a substantial difference in activity due to the solvent used in extraction. Previous studies indicate that antioxidant activity is higher in solvents with lower polarity since they dissolve higher molecular weight phenols giving the plant extract stronger antioxidant effects (Tian *et al.*, 2009). *Acacia nilotica* had the highest antioxidant activity in both the methanol and water extracts with $IC_{50}$ values of 54.6 µg/mL and 103.0 µg/mL, respectively. *Albizia anthelmintica* had the lowest antioxidant activity in both the methanol and water extracts (14,829.3 µg/mL and 45,524.0 µg/mL) respectively. The phenols, in plants with high polyphenolic content, act as strong antioxidants. However, as the polyphenolic content decreases other compounds with weak antioxidant potential may show activity which could have been masked by high amount of polyphenols.

With a few exceptions, the phenolic and flavonoid content closely mirrored the antioxidant activity. At least eight of nine plants with the highest total phenolic, total flavonoid and antioxidant activity (*A. nilotica*, *A. robusta*, *X. americana*, *R. natalensis*, *G. villosa*, *A. drepanolobium*, *R. prinoides* and *P. capensis*) had been mentioned more than 15 times by the respondents, with the exception of *R. natalensis* (Tables 4.1, 4.2, 4.3 and 4.4). The most commonly used plants by the Ilkisonko Maasai have high polyphenolic content which could contribute to their medicinal properties.

Table 4.5 shows the Pearson's linear correlation between the three assays for the top nine plants. The correlation coefficient between the total phenolic content and flavonoid content is higher (r=0.621) than that between antioxidant activity and flavonoid content (r=-0.597). This is because they are both polyphenols and share structural similarities which dictate activity.

**Table 4.5: Pearson's correlation between total phenolic content, total flavonoid content and antioxidant activity for the top nine plants with the highest total phenolic content**

| | Correlation coefficient (r) n= **18** | | |
|---|---|---|---|
| Variable | Phenolic content | Flavonoid content | Antioxidant activity |
| Phenolic content | 1 | 0.621 | -0.841 |
| Flavonoid content | 0.621 | 1 | -0.597 |
| Antioxidant activity | -0.841 | -0.597 | 1 |

There is a stronger negative correlation between the $IC_{50}$ with total phenolic content (r=-0.841) and flavonoid content (r=-0.597), in the top nine plants (*A. nilotica*, *A. robusta*, *X. americana*, *R. natalensis*, *G. villosa*, *A. drepanolobium*, *R. prinoides* and *P. capensis*) with the highest total phenolic contents (Table 4.5) (Appendix 6). The observed negative correlation between the total phenolic/flavonoid content with the antioxidant $IC_{50}$ value is consistent with a research finding by Farasat *et al*. which shows that the higher the phenolic/flavonoid content, the lower the $IC_{50}$ value, and hence the higher the antioxidant activity per gram of extract (Farasat *et al.*, 2014). Other studies have confirmed the direct relationship between phenolic content and antioxidant activity (Lamien-Meda *et al.*, 2008) and this lends credence that the observed antioxidant activity in the plants with the highest total phenolic content may be largely due to the phenolic content in the extracts. Phenolics have strong antioxidant activity through chelation and free radical scavenging activity on mostly hydroxyl and peroxyl radicals. Also, the Folin- Ciocalteu's method which is used to measure total phenolic content in a sample is considered an alternative antioxidant capacity assay because its basic mechanism is oxidation/reduction reaction (Ainsworth and Gillespie, 2007). The mechanism of action of phenols and the similarity in its assay to antioxidant activity may give this direct relationship observed.

The significant negative correlation between antioxidant activity and flavonoids (r=-0.597), in the top nine plants, implies that the flavonoids in the extracts may contribute, to a certain extent, to the antioxidant activity (Procházková *et al*., 2011).

## 4.4. CARRAGEENAN-INDUCED RAT PAW OEDEMA ASSAY

Anti-inflammatory activity measured using the carrageenan-induced paw oedema has given good correlation of anti-inflammatory drug activity used for inflammatory diseases in humans (Necas and Bartosikova, 2013). As previously indicated in Section 3.4.5, nine selected plant extracts were assessed for their anti-inflammatory potential by the carrageenan-induced rat paw oedema assay and the results summarised in Table 4.7. Methanol extract was used because it showed consistently higher total phenolic content, total flavonoid content and antioxidant activity for most plants studied. A dosage of 400 mg/kg body weight was used for the extracts while diclofenac was administered at 20 mg/kg body weight.

**Table 4.6: Anti-inflammatory effects of select plant extracts on carrageenan-induced rat paw oedema.**

| | **Mean paw volume in mL ± SD (% inhibition, n=5) at the respective times** | | | | | |
|---|---|---|---|---|---|---|
| **CODE** | **0 h** | **1 h** | **2 h** | **3 h** | **4 h** | **24 h** |
| **C** | 0.86±0.11 | 1.24±0.21 | 1.38±0.22 | 1.45±0.22 | 1.56±0.22 | 1.39±0.14 |
| **DC** | 0.92±0.07 | 1.10 ±0.08* (52.9) | 1.17±0.07* (52.7) | 1.20±0.08* (54.1) | 1.16±0.08* (66.2) | 1.38±0.06 (14.3) |
| **RN** | 0.78±0.06 | 1.00±0.06* (41.9) | 1.16±0.06 (28.6) | 1.28±0.06 (15.5) | 1.29±0.09 (27.3) | 1.08±0.07* (43.8) |
| **AD** | 1.08±0.08 | 1.31±0.08* (40.3) | 1.46±0.06 (27.1) | 1.53±0.04 (23.7) | 1.57±0.03* (30.4) | 1.34±0.06* (50.9) |
| **AN** | 0.95±0.12 | 1.12±0.12* (55.5) | 1.23±0.11* (45.8) | 1.35±0.14* (32.8) | 1.46±0.16* (27.8) | 1.40±0.11 (14.3) |
| **AR** | 0.91±0.12 | 1.11±0.12* (47.6) | 1.28±0.15* (30.5) | 1.36±0.15 (24.7) | 1.47±0.17 (20.7) | 1.35±0.15 (17.4) |
| **AB** | 0.87±0.08 | 1.11±0.08* (37.2) | 1.23±0.05 (31.7) | 1.32±0.09 (24.7) | 1.32±0.09* (36.7) | 1.20±0.04* (37.4) |
| **GV** | 1.09±0.13 | 1.25±0.13* (58.6) | 1.41±0.17* (39.3) | 1.52±0.19 (28.4) | 1.62±0.19 (25.6) | 1.33±0.16* (55.1) |
| **XA** | 0.90±0.04 | 1.08±0.06* (54.5) | 1.19±0.06* (45.0) | 1.23±0.06* (44.6) | 1.25±0.06* (50.6) | 1.34±0.04 (17.4) |

KEY: C- negative control; DC- positive control (diclofenac); RN- *Rhus natalensis*; AD- *Acacia drepanolobium*; AN- *Acacia nilotica*; AR- *Acacia reficiens*; AB- *Acacia robusta*; GV- *Grewia villosa*; XA- *Ximenia americana*. * Statistically significant $p < 0.05$.

An injection of carrageenan has been shown to induce biphasic paw oedema in rats (Patrono and Baigent, 2014). Mediators such as serotonin and histamine are responsible for the swelling, redness and pain experienced during the early phase of inflammation. These mediators then induce the release of cyclooxygenase (COX) 2 and its products, which start the second phase of inflammation (Fernando *et al*, 2005). Diclofenac, the positive control, showed significant anti-inflammatory activity, which consistently rose from the first to the fourth hour, with a maximum percentage inhibition (PI) of 66.2% at 4 h. This is in agreement with a previous study showing a similar PI after oral administration of diclofenac (20 mg/kg) (Sakat *et al*, 2014). The mean paw volume of the positive control was also significantly different from all other treatment groups at four hours. The fourth hour is key because it is associated with the highest production in prostaglandin (PG) synthesis and also the highest accumulation of diclofenac in inflamed tissues (Schweitzer *et al*, 2009). Diclofenac is an arylacetic acid non-steroidal anti-inflammatory drug commonly used for symptomatic treatment of moderate pain and inflammation. It is an unspecific COX inhibitor that works well in lowering inflammation from the 1$^{st}$ to the 6$^{th}$ hour, similar to the results in Table 4.7. COX enzyme is responsible for generating PGs which mediate pain, fever and inflammation. At a later phase, diclofenac ceases to be useful as it has a short half-life of 6 hours (Schweitzer *et al*, 2009) as illustrated by its weak activity at 24 h with a PI of 14.3%.

*Acacia nilotica* and *X. americana* significantly inhibited paw oedema from 1 h to 4 h. The PI values for *A. nilotica*, *A. reficiens* and *X. americana* consistently declined from 1 h to 4 h, but only the PIs of *A. nilotica* and *X. americana* were significantly different from the negative control at all the four time points. (*A. nilotica*- 55.5%, 45.8%, 32.8% and 27.8%; *X. americana*- 54.5%, 45.0%, 44.6% and 50.6%). This is in concurrence with previously observed *in vitro* anti-inflammatory activity of methanol extracts of *A. nilotica* and *X. americana* which exhibited significant inhibitory activity on inflammation (Khan Tabassum *et al.*, 2015; Shettar *et al.*, 2015). Effective inhibition of COX 1 and COX 2 (96%) was present when using *Acacia nilotica* ethanol bark extracts (Eldeen *et al*., 2005), which could explain its activity in lowering inflammation during the 2$^{nd}$ hour. Anti-inflammatory activity

may also be due to phytochemicals from these two plants. Noteworth, niloticane, a diterpene from the bark of *A. nilotica*, has been reported to exhibit good COX-2 inhibitory activity (Eldeen *et al*, 2010) while polysaccharide rich fractions from *X. americana* have anti-inflammatory and anti-nociceptive activity (da Silva-Leite *et al*, 2017). Further, a topical ointment of *X. americana* branch extract exhibited significant anti-inflammatory activity in a study conducted in Brazil (Neto Júnior *et al*, 2019).

All extracts (400 mg/kg) showed significant inhibition of carrageenan rat paw oedema at 1 h compared to the negative control. Most extracts (*R. natalensis*, *A. drepanolobium*, *A. robusta* and *G. villosa*) showed a constant decline in PI from 1 h to 3 h or 4 h except for *A. nilotica*, *A. reficiens* and *X. americana* which had their lowest PI at 24 h (14.3%, 17.4% and 17.4% respectively). The maximum PI for the extracts was observed as *G. villosa* (58.6% at 1 h), *A. nilotica* (55.5% at 1 h), *X. americana* (54.5% at 1 h), *A. drepanolobium* (50.9% at 24 h), *A. reficiens* (47.6% at 1 h), *R. natalensis* (43.8% at 24 h) and *A. robusta* (37.4% at 24 h). The following extracts showed their highest PIs of inflammation at 1 h and 24 h, respectively: *R. natalensis* (41.9% and 43.8%), *A. drepanolobium* (40.3% and 50.9%), *A. robusta* (37.2% and 37.4%) and *G. villosa* (58.6% and 55.1%) (Table 4.7). This could point to residual antioxidant activity of the extracts as ROS/RNS concentrations reach high concentrations at the site of inflammation several hours after inflammation was initiated (Bhattacharyya *et al.*, 2011). In a previous study, extracts from *Rhus vermiciflua* and polyphenols from *Acacia* were able to rejuvenate internal antioxidant systems such as catalase and superoxide dismutase in rats (Ikarashi *et al.*, 2018; Lee *et al.*, 2019). D-pinitol a cyclitol present in Fabaceae, protected the pancreas from oxidative damage by increasing the level of enzymatic antioxidants (Sivakumar and Subramanian, 2009). The ROS/RNS on site, act to eliminate the offending agent but causes more damage if resolution does not occur soon (Kennedy and Deleo, 2009). Anti-inflammatory activity 24 h later could also show the presence of substances from these plants that promote resolution of inflammation. It could also mean the presence of more active metabolites generated *in vivo* after administration of the extract. For instance, wine flavonoids have been found to retain activity even after metabolism as compared to diclofenac metabolites which are weakly active (Davies and Anderson, 1997; Fernandes *et al.*, 2017).

Extracts with the highest total phenolic content did not necessarily show the highest anti-inflammatory activity, which is common with other studies (Gutiérrez *et al.*, 2014).

Flavonoids may play a part since all the four extracts (*A. nilotica, A. reficiens, G. villosa and X. americana*) showing significant anti-inflammatory activity at the first hour, had the highest flavonoid content (Table 4.3). Gutiérrez *et al*. showed that the alkaloidal fraction had the best anti-inflammatory activity despite having low polyphenol content (Gutiérrez *et al.*, 2014). This could be the case with *G. villosa*, which showed good anti-inflammatory activity at 1 h and 24 h. Similar to our observations, a study by Paviaya *et al*. showed inhibition of the carrageenan-induced biphasic inflammatory response in rats using a methanolic extract of *G. asiatica* (Paviaya *et al.*, 2013). Though *G. villosa* was among the top nine plants with high total phenolic and flavonoid content, *G. villosa* methanol extract also contains lignans and alkaloids which may facilitate activity in both the early and late phases of inflammation (Ullah *et al*., 2012).

In treatment of common ailments and metabolic disorders, anti-inflammatory activity may be more relevant than antioxidant activity. Inflammation, cited as one of the six hallmarks of the priority cancer condition, may be better countered by the use of natural compounds which are relatively safer and have a multi-pronged effect in cancer prevention. As a consequence of this, they are being adopted in the emerging integrative medicine approach in cancer chemotherapy (Block *et al.*, 2015).

## 4.5. ISOLATION OF PURE COMPOUNDS

### 4.5.1. *Grewia villosa*

*Grewia villosa* (Tiliaceae) was chosen for phytochemical isolation as it showed significant anti-inflammatory inhibitory activity both at 1 h and 24 h after administration of carrageenan.

The genus *Grewia* comprises of about 150 species of small trees and shrubs distributed in tropical and subtropical regions of Africa and Asia where they have been used for decades as both food and medicine. The fruits are eaten or made into a juice for pregnant or lactating mothers (Mohammed *et al.*, 2018). The young leaves and seeds have an appreciable amount of crude protein and iron to make good fodder for livestock (Saleem *et al*., 2012).

**Figure 4.6: Figures depicting *Grewia villosa* branch and flower**
(Wikipedia, 2020)

The bark and leaf extract of *G. mollis* have been used to treat wounds, fever, and cough in Ethiopia (Mohammed *et al.,* 2018). *Grewia bicolor* is used in delayed afterbirth and as a tranquilizer (Jaspers *et al.,* 1986). *Grewia villosa* is a deciduous shrub, which reaches a height of about 3 m. It grows in arid areas of Africa and India, usually on riverbanks, stony ground or under the shade of larger trees. In Kenya, *G. villosa* is found in the arid south and northern parts of the country (Ullah *et al*., 2012). Traditionally, *G. villosa* has been used to manage cough and its fruits are considered healthy for women and children (Bashir *et al*., 1987; Saleem *et al*., 2012). Classes of compounds isolated from *Grewia* species include flavonoid glycosides, anthocyanins, sterols, triterpenes, lignans, lactones and harman alkaloids (Ullah *et al*., 2012).

### 4.5.2. Extraction yield

Repeated extraction of *G. villosa* stem and root bark (1000 g) yielded 43.11 g of solid material (4.3 % of the total plant powder).

### 4.5.3. Structure elucidation of isolated compounds

#### 4.5.3.1. 1-(-4-(amino)phenyl)ethanone

The structure was elucidated using data from 1D ($^{1}H$, $^{13}C$ and DEPT) and 2D-NMR (COSY, NOESY, HSQC and HMBC) experiments (Table 4.8, Appendix 7-11).

**Table 4.7: $^{1}H$, $^{13}C$ NMR Data of Compound JK2A in $CDCl_3$ at 500MHz and 125 MHz (δ in ppm, J in Hz)**

| Position | $^{13}C$ NMR | $^{1}H$ NMR | | HMBC | $^{13}C$ NMR[b] |
|---|---|---|---|---|---|
| | δ | δ, (multiplicity), *J* | | | δ |
| 1 | 135.2 | | | | 134.9 |
| 1a | 198.0 | | | | 197.9 |
| 1b | 26.7 | 2.58, (s) | | 1a, 1*, 2/6* | 25.8 |
| 2 | 128.7 | 7.89, (d), 8.4 | H-3 | 1a, 3/5, 4 | 130.1 |
| 3 | 127.2 | 7.30, (d), 8.2 | H-2 | 1, 2/6, 4*, 1a* | 126.3 |
| 4 | 153.8 | | | | 154.4 |
| 5 | 127.2 | 7.30, (d), 8.2 | H-6 | 1, 2/6, 4*, 1a* | 126.3 |
| 6 | 128.7 | 7.89, (d), 8.4 | H-5 | 1a, 3/5, 4 | 127.1 |

$^{13}C$ NMR[b] column outlines chemical shift values of 1-(-4-(amino) phenyl)ethanone from literature (Penner and Wasylishen, 1989).

Compound JK 2A (Figure 4.7) was obtained as light yellow crystals, chemical formula $C_8H_9NO$, with a molecular mass of 135.07 and a melting point 106°C.

The presence of two doublets each integrating for two aromatic protons was evident from the $^{1}H$ NMR spectrum of the compound; $\delta_H$ 7.89 (d, J = 8.4 Hz, H-2, H-6) and $\delta_H$ 7.30 (d, J = 8.2 Hz, H-3, H-5). This indicated the presence of a 1,4 substituted benzene ring and was supported by the presence of $A_2X_2$ spin system. Using HSQC spectrum the aromatic protons were attached to $\delta_C$ 128.7 (C-2, C-6) and $\delta_C$ 127.2 (C-3, C-5). The $^{13}C$ NMR showed the presence of a carbonyl carbon $\delta_C$ 198.0 (C-1a); typical of a ketone or aldehyde, a methyl group $\delta_C$ 26.7 (C-1b) and two tertiary carbons; $\delta_C$ 135.2 (C-1) and $\delta_C$ 153.8 (C-4). The methyl protons $\delta_H$ 2.58 (s) showed HMBC correlation with the carbonyl carbon hence the carbonyl group was confirmed to be that of a ketone and the substituent on C-1 of the aromatic ring. Due to the downfield chemical shift of C-4; the substituent is an electronegative element (O or N). The substituent was confirmed to be an amino group due to the IR peak at 3317$cm^{-1}$

and a carbonyl peak at 1680 $cm^{-1}$ (Appendix 13) The compound was confirmed to be 1-(-4-(amino) phenyl)ethanone (Figure 4.7), a chemical intermediate common in plants and has been used as a botanical marker (Kaškoniene and Venskutonis, 2010).

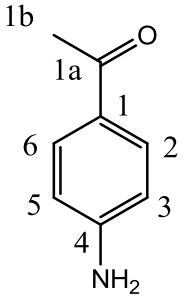



**Figure 4.7:** Compound JK2A

#### 4.5.3.2. 1-Methyl-4,9-dihydro-3H-β-carbolin-7-ol

The structure was elucidated using data from 1D ($^{1}H$, $^{13}C$ and DEPT) and 2D-NMR (COSY, NOESY, HSQC and HMBC) experiments (Table 4.9, Appendix 14-18).

**Table 4.8: $^{1}H$, $^{13}C$ NMR Data of Compound JK7A in $CD_3OD$ at 500 MHz and 125 MHz (δ in ppm, *J* in Hz)**

| Position | $^{13}C$ NMR | $^{1}H$ NMR | HMBC |
|---|---|---|---|
| | δ | δ, (multiplicity), *J* | |
| 1 | 165.9 | | |
| 3 | 43.1 | 4.03, (t), 8.6 | 1, 4 |
| 4 | 20.3 | 3.37, (t), 8.4 | 4a, 4b, 3 |
| 4a | 127.8 | | |
| 4b | 120 | | |
| 5 | 124 | 7.69, (d), 9.5 | 8, 4b, 4a, 8a, 7 |
| 6 | 115.8 | 6.93, (d), 9.5 | 4b, 5*, 8a* |
| 7 | 161.4 | | |
| 8 | 97 | 6.94, (s) | 6, 4b, 8a* |
| 8a | 145.6 | | |
| 9a | 126.4 | | |
| 1-Me | 18.4 | 2.80, (s) | 1, 9a |

$^{13}C$ NMR$^{c}$ and $^{1}H$ NMR$^{c}$ column outlines chemical shift values of 1-Methyl-4,9-dihydro-3H-β-carbolin-7-ol.

Compound JK7A (Figure 4.8) was obtained as a yellow solid with the chemical formula $C_{12}H_{12}N_2O$ consistent with the molecular ion peak m/z 200.09 on MS (Appendix 21) Compound JK7A had its melting point at 100-105°C and $UV_{max}$ at 373 nm (Appendix 20). The $^{1}H$ NMR spectra showed the presence of aromatic protons which are between 7.69 and 6.93 ppm. These two ortho coupled aromatic protons are at $\delta_H$ 7.69 (d, J = 9Hz, H-5) and $\delta_H$ 6.93 (d, J = 9Hz, H-6) while the third aromatic proton is at $\delta_H$ 6.94 (s H-8). The $^{1}H$ NMR spectra showed two sets of down shifted methylene protons at $\delta_H$ 4.03 (t, J = 8.6 Hz, H-3) and $\delta_H$ 3.37 (t, J = 8.4, H-4) and methyl protons at $\delta_H$ 2.80 (s) due to closeness to an electronegative amine group. $^{13}C$ NMR and HSQC spectra were used to assign the corresponding carbons as follows: $\delta_C$ 124 (C-5), $\delta_C$ 115.8 (C-6), $\delta_C$ 97(C-8), $\delta_C$ 43.1 (C-3), $\delta_C$ 20.3 (C-4) and $\delta_C$ 18.4 (1-Me) respectively. The $^{13}C$ NMR spectrum further revealed the presence of five quaternary carbons $\delta_C$ 165.9 (C-1), $\delta_C$ 145.6 (C-8a), $\delta_C$ 126.4 (C-9a), $\delta_C$ 127.8 (C-4a) and $\delta_C$ 120 (C-4b). There was an additional oxygenated quaternary carbon at $\delta_C$ 161.5 (C-7) attached to an hydroxyl group which gave an IR signal at 3490 cm-1 (Appendix 19). The NMR assignments were further confirmed using HMBC spectrum where the methyl protons $\delta_H$ 2.80 (s) showed correlation with C-1 ($^{2}J$) and C-9a ($^{3}J$). The aromatic proton $\delta_H$ 6.94 (s, H-8) showed correlation with C-7 ($^{2}J$) and C-4a ($^{3}J$). The other aromatic proton $\delta_H$ 7.69 (d, J = 9Hz, H-5) showed correlation with C-4a ($^{3}J$) and the methylene proton $\delta_H$ 4.03 (t, J = 8.6 Hz, H-3) showed correlation with C-1. The spectral data confirmed the compound to be an indole alkaloid with an IUPAC name 1-Methyl-4,9-dihydro-3H-β-carbolin-7-ol and common name harmalol (Figure 4.8). This compound is a harman alkaloid previously isolated from *Peganum harmala* (Moloudizargari *et al.*, 2013) and was also isolated from the same plant root, *G. villosa* (Bashir *et al.*, 1987).

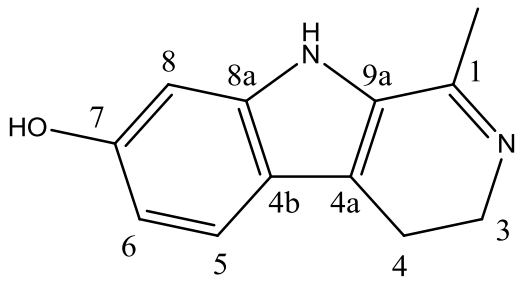



**Figure 4.8:** Compound JK7A

Harman alkaloids are indole alkaloids with diverse structures which exhibit a wide range of biological activities. Harman alkaloids derive their name from the plant species where they were first isolated, *Peganum harmala* (Moloudizargari *et al.*, 2013). They have also been isolated in high levels from other plants such as *Passiflora edulis* and *Banisteriopsis caapi* (Abourashed *et al*., 2003; Callaway, 2005). Harman alkaloids are endogenously produced in animal tissues and have been detected in alcoholic drinks, meat, coffee and tobacco smoke (Piechowska *et al*,. 2019). Harman alkaloids have demonstrated certain biological activities such as antianxiety, hypotensive, anti-inflammatory, antidiabetic, anticancer antinociceptive and hallucinogenic effects (Khan *et al*., 2017). Harmalol is a 3,4-dihydro-β-carboline with a $C_7$ alkyl substitution common in naturally occurring β-carbolines (Piechowska *et al*,. 2019).

### 4.5.4. Antioxidant activity of harmalol

**Table 4.9: Antioxidant activity expressed in terms of IC50 of harmalol**

| **S. No.** | **Sample** | **Half maximal inhibitory concentration ($IC_{50}$) in µg/ml** |
|---|---|---|
| 1. | Ascorbic acid standard | 50.32 |
| 2. | Harmalol (JK7A) | 115.22 |
| 3. | *Grewia villosa* (Methanol extract) | 107.50 |

Harmalol showed closely similar antioxidant activity to the methanol extract (115.22 for harmalol and 107.50 for *G. villosa*). Harmalol is a Harman alkaloid detected in the roots of *G. villosa* and isolated in this study. Harman alkaloids contain an intact β-carboline skeleton and are sometimes referred to as simple β-carbolines. These β-carbolines show different modifications that give rise to several compounds with slightly different activities. Examples of simple β-carbolines include norharman, Harman, harmol, harmine, harmaline and harmalol (Piechowska *et al*,. 2019). β-carbolines are derived from tryptophan which is known to have antioxidative activity by scavenging ROS and forming a stable indole radical (Cushnie *et al*., 2014). The antioxidative activities of β-carbolines may form a similar stable radical which gives them significant antioxidative activity demonstrated in several studies (Cho *et al*., 1995). The structure of the β-carbolines gives rise to different levels of antioxidant activities. An example is dehydrogenation of the pyridyl ring (e.g. harmalol to harmol, harmaline to

harmine) resulted in a significant decrease in antioxidant activity while replacement of the hydroxyl group by a methoxyl group also decreases the antioxidant effect (e.g. harmalol to harmaline, harmol to harmine). Due to this harmalol was found to have the highest antioxidant activity among the β-carbolines tested (Moura *et al.*, 2007). Harmalol is able to pass the blood brain barrier and may have a protective effect on neuronal cells to delay the onset of neurodegenerative diseases (Lee *et al.*, 2000).

A descriptive flow chart of the research carried out in this thesis is pictorially presented in Figure 4.9.

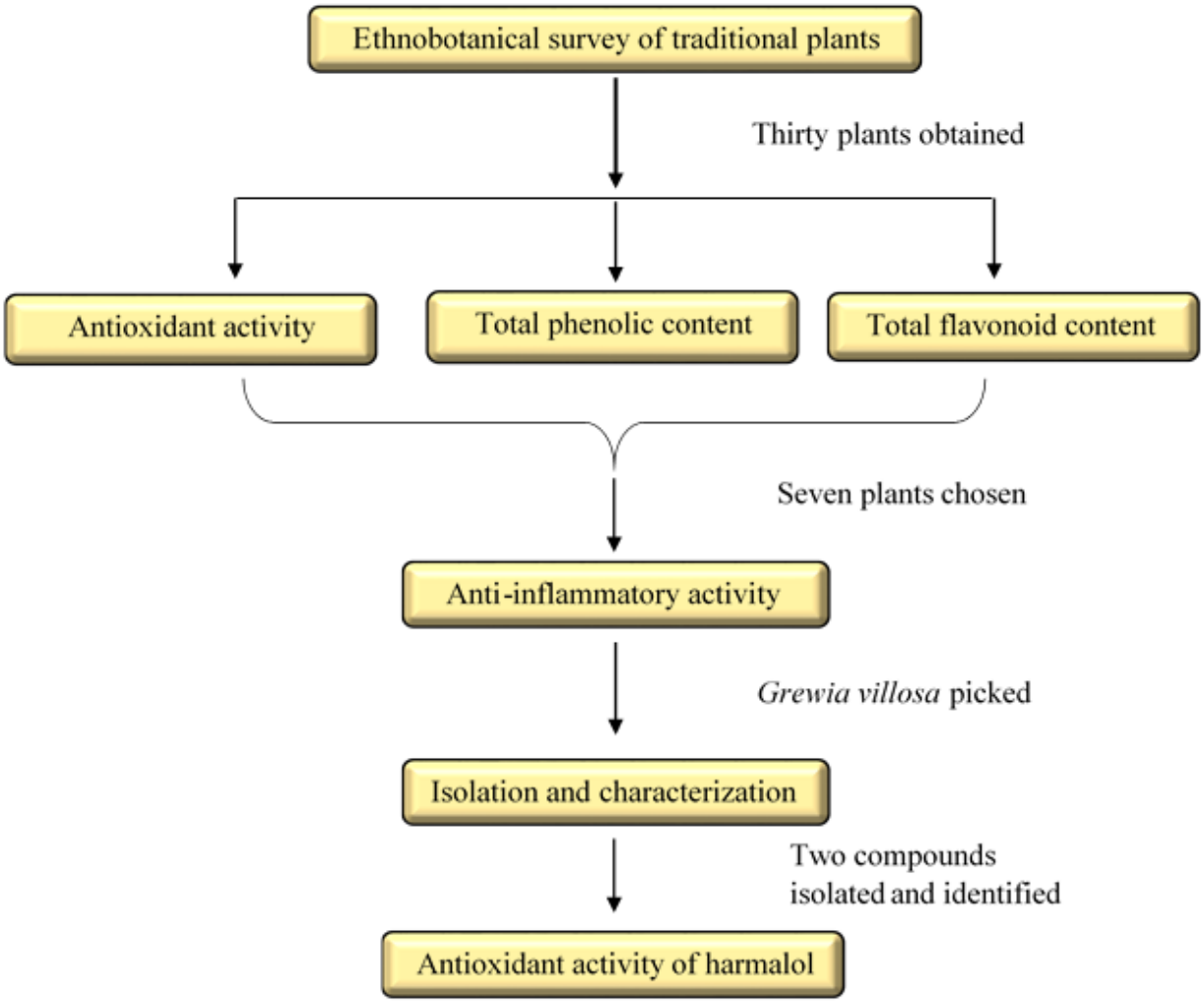



**Figure 4.9: Flow chart of work done.**

## 4.6. STUDY LIMITATIONS

There are several limitations in this study to consider. The field research was conducted during one season, which could give different concentration of phytochemicals when compared to other seasons. Although the laboratory assays are effective at determining potential biological functions, they are not able to determine *in vivo* activity. Therefore, conclusions cannot be drawn on the effectiveness of the antioxidant and anti-inflammatory actions in the body, especially because interactions between phytochemicals and cells *in vivo* are complex and not completely understood. Also, the bioavailability of the phytochemicals needs to be assessed as well before any *in vivo* conclusions can be drawn. In structure elucidation of harmalol although it has previously been isolated, the relevant reference spectra could not be obtained in this study. This is because previous reports used HPLC-MS and not NMR or were reported in old inaccessible literature published in 1978.

## 4.7. STUDY RELEVANCE

The importance of this study was the collation and pharmacological validation of plant species used culturally by the Ilkisonko Maasai in an area where traditional knowledge and plants are facing several threats. Most often, knowledge of traditional medicine is passed down from generation to generation through folklore thereby facing the danger of being lost. In addition, there are emerging threats to local medicinal plants due to anthropogenic factors such as charcoal burning, increased consumption emanating from increase in human population and land use changes particularly expansion of agriculture (Kiringe and Okello, 2005). Further, phytochemical assays, antioxidant activity and anti-inflammatory activity have not been performed on some plant extracts such as *Maerua triphylla*, *Acacia reficiens* and *Cyphostemma nodiglandulosum*. The results from this study have contributed new information to the body of knowledge on the total phenolic and flavonoid content and antioxidant activity of the studied plant species. This study also affirms the importance of using ethnobotanical approach in search of new phytochemicals with potential for application in human therapy.

It is hoped that this study will be relevant in encouraging the use of traditional plants as sources of healthy food. Traditional plants with potential to produce both food and medicine

can be a potential resource for poverty alleviation. Further, the safety of these plants can be deduced from their long-term use as food or food additives by the Ilkisonko Maasai. The use of these plants among the Ilkisonko Maasai is similar to their use in other African communities such as in Uganda, Tanzania, Ethiopia, Sudan, Zimbabwe and South Africa where they have been used ethnomedicinally over several generations in all age groups and by all sexes, indicating their relative safety (Johns *et al.*, 1999; Musa *et al.*, 2011; Tabuti *et al.*, 2012; Maroyi, 2013, 2017; Berhanu, 2014). The community and ultimately the wider society could benefit from the findings of this study by targeted use of these medicinal plants.

The health benefits of traditional plants used as food and medicine is indicated by the high polyphenolic content and significant anti-inflammatory activity. It is hoped that these findings may prompt research onto the assessment and determination of potential rich sources of phytochemical compounds in other plant produce given the importance of dietary habits and food components to health.

# 5.0. CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS

**Conclusion**

This study explored the properties and health benefits of traditional plants used as food and medicine by the Ilkisonko Maasai community in Kenya. It showed that there was a wide variety of plant species available in the area that are utilised in their diet for treatment and health promoting effects. At least 10 plant species from this study also had a dietary context, in aiding digestion or flavouring tea and as a food source. Previous studies performed in Loitokitok sub-county or nearby areas surveyed plants used in treatment of specific diseases (Bussmann *et al.*, 2006; Muthee *et al.*, 2011) but not those included in the diet as food or food additives. This gives the false impression that the Maasai survive on meat and grain while in reality the inclusion of decoctions from locally available medicinal plants may supplement their dietary needs. The use of traditional medicine by the Ilkisonko Maasai community was found to be prevalent and preferred to conventional medicine.

The study found that the most commonly mentioned/used medicinal plants were characterized by high phenolic content, high flavonoid content with attendant elevated antioxidant activity and significant anti-inflammatory effects. This implies a pharmacological basis underlying the selection of plants used in traditional medicine. Further, the findings by this study that the ethnobotanical plants used displayed high polyphenol content, antioxidant and anti-inflammatory properties, showed that these plant species contribute to the health of the Ilkisonko Maasai. The anti-inflammatory activity focused on plants with high antioxidant activity arising from the attendant high total phenolic content (r=-0.841), though plant extracts with the highest total phenolic content did not have the highest anti-inflammatory activity. This indicates that phytochemicals targeted in the study's phytochemical assays may not be the only contributors to the anti-inflammatory activity of the studied plants. Harmalol, a β-carboline alkaloid isolated from *G. villosa*, was found to have significant antioxidant activity from this study. Harmalol and other β-carbolines alkaloids displayed a protective effect against oxidative damage to neuronal cells, microsomal lipids, hyaluronic acid, and collagen to protect against some age-related conditions such as Alzheimer's and cancer (Lee *et al.*, 2000; Moura *et al.*, 2007).

**Recommendations for future research**

From this thesis work, the following are recommended for future research:

- Collection of plant samples in all five administrative locations of Loitokitok sub-county to obtain a better understanding of the type of plants in the area used as foods or medicine.
- Investigations of antioxidant and anti-inflammatory activities using different assays to obtain a better understanding of the pharmacological activities of the plants in this study.
- Assessment of *in vitro* and *in vivo* anti-inflammatory activity of all the extracts (water and methanol) and correlate their anti-inflammatory activity with their antioxidant activity.
- Determining whether the inclusion of certain extracts from this study in the diets of laboratory animals leads to higher concentration of antioxidant compounds and reduced levels of inflammatory markers in blood.
- Isolation of bioactive constituents to add on to the knowledge on phytochemistry of all the plants in the study.
- Investigate any synergistic antioxidant activity between the studied plants and any isolated bioactive compounds since traditional medicine usually involves a combination of herbs.
- Investigation of antioxidant and anti-inflammatory activities of the non-polar fraction.

**Research outputs**

Three journal publications arising from this thesis research have already been published.

1. Kimondo, J., Miaron, J., Mutai, P., & Njogu, P. (2015). Ethnobotanical survey of food and medicinal plants of the Ilkisonko Maasai community in Kenya. *Journal of Ethnopharmacology*, *175*, 463–469. https://doi.org/10.1016/j.jep.2015.10.013
2. Kimondo, J., Mutai, P., Njogu, P., & Kimwele, C. (2019). Evaluation of the Antioxidant Activity of Nine Plants Used Medicinally by the Ilkisonko Maasai Community of Kenya. *Free Radicals and Antioxidants*, *9*(1), 29–34. https://doi.org/10.5530/fra.2019.1.6
3. Julia Kimondo, Peggoty Mutai, Peter Njogu, and Charles Kimwele (2020). Anti-inflammatory activity of selected plants used by the Ilkisonko Maasai, Kenya. *African Journal of Pharmacology and Therapeutics, 9*(2): 39-43.

A further one manuscript on the phytochemistry work is under preparation.

# 6.0. REFERENCES


Aadil, K. R., Barapatre, A., Sahu, S., Jha, H. and Tiwary, B. N. (2014) 'Free radical scavenging activity and reducing power of *Acacia nilotica* wood lignin', *International Journal of Biological Macromolecules*, 67, pp. 220–227.

Abourashed, E. A., Vanderplank, J. and Khan, I. A. (2003) 'High-speed extraction and HPLC fingerprinting of medicinal plants - II. Application to harman alkaloids of genus *Passiflora'*, *Pharmaceutical Biology*, 41(2), pp. 100–106.

Adedapo, A. A., Sofidiya, M. O., Masika, P. J. and Afolayan, A. J. (2008) 'Anti-inflammatory and analgesic activities of the aqueous extract of *Acacia karroo* stem bark in experimental animals', *Basic and Clinical Pharmacology and Toxicology*, 103(5), pp. 397–400.

Ainsworth, E. A. and Gillespie, K. M. (2007) 'Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent.', *Nature protocols*, 2, pp. 875–877.

Akbar, S., Bellary, S. and Griffiths, H. R. (2011) 'Dietary antioxidant interventions in type 2 diabetes patients: A meta-analysis', *British Journal of Diabetes and Vascular Disease*, 11(2), pp. 62–68.

Almajano, M. P., Carbó, R., Jiménez, J. A. L. and Gordon, M. H. (2008) 'Antioxidant and antimicrobial activities of tea infusions', *Food Chemistry*, 108(1), pp. 55–63.

Alqasoumi, S. I., Basudan, O. A., Alam, P. and Abdel-Kader, M. S. (2016) 'Antioxidant study of flavonoid derivatives from the aerial parts of *Rhus natalensis* growing in Saudi Arabia', *Pakistan Journal of Pharmaceutical Sciences*, 29(1), pp. 97–103.

Aluko, R. E. (2015) 'Amino acids, peptides, and proteins as antioxidants for food preservation', in *Handbook of Antioxidants for Food Preservation*, pp. 105–140.

Arbonnier, M. (2004) *Trees, Shrubs and Lianas of West African Dry Zones*. Editions Quae.

Atanassova, M., Georgieva, S. and Ivancheva, K. (2011) 'Total Phenolic and Total Flavonoid Contents , Antioxidant Capacity and Biological Contaminants in Medicinal Herbs', *Journal of the University of Chemical Technology and Metallurgy*, 46, pp. 81–88.

Babić, I., Bojić, M., Maleš, Ž., Zadro, R., Gojčeta, K., Duka, I., Rimac, H. and Jukić, I. (2019) 'Influence of flavonoids' lipophilicity on platelet aggregation', *Acta pharmaceutica (Zagreb, Croatia)*, 69(4), pp. 607–619.

Balde, A. M., Pieters, L., De Bruyne, T., Geerts, S., Vanden Berghe, D. and Vlietinck, A. (1995) 'Biological investigations on *Harrisonia abyssinica*.', *Phytomedicine : International Journal of Phytotherapy and Phytopharmacology*, 1(4), pp. 299–302.

Bamuamba, K., Gammon, D. W., Meyers, P., Dijoux-Franca, M. G. and Scott, G. (2008) 'Anti-mycobacterial activity of five plant species used as traditional medicines in the Western Cape Province (South Africa).', *J Ethnopharmacol*, 117(2), pp. 385–390.

Bartsch, H. and Nair, J. (2006) 'Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer: Role of lipid peroxidation, DNA damage, and repair', *Langenbeck's Archives of Surgery*, 391(5), pp. 499–510.

Bashir, A. K., Ross, M. F. and Turner, T. D. (1987) 'The alkaloids of *Grewia villosa* root', *Fitoterapia*, 58(2), pp. 141–142.

Bendary, E., Francis, R. R., Ali, H. M. G., Sarwat, M. I. and El Hady, S. (2013) 'Antioxidant and structure–activity relationships (SARs) of some phenolic and anilines compounds', *Annals of Agricultural Sciences*, 58(2), pp. 173–181.

Berhanu, A. (2014) 'Microbial profile of tella and the role of gesho (*Rhamnus prinoides*) as bittering and antimicrobial agent in traditional tella (beer) production', *International Food Research Journal*, 21(1), pp. 357–365.

Beta, T. and Hwang, T. (2018) 'Influence of heat and moisture treatment on carotenoids, phenolic content, and antioxidant capacity of orange maize flour', *Food Chemistry*, 246, pp. 58–64.

Bhattacharyya, S., Borthakur, A., Anbazhagan, A. N., Katyal, S., Dudeja, P. K. and Tobacman, J. K. (2011) 'Specific effects of BCL10 serine mutations on phosphorylations in canonical and noncanonical pathways of NF-κB activation following carrageenan', *American Journal of Physiology - Gastrointestinal and Liver Physiology*, 301(3), pp. 475–486.

Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G. and Gluud, C. (2012)

‘Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases’, *Sao Paulo Medical Journal*, 14(3), p. CD007176.

Blainski, A., Lopes, G. C. and De Mello, J. C. P. (2013) ‘Application and analysis of the Folin Ciocalteu method for the determination of the total phenolic content from *Limonium brasiliense* L.’, *Molecules*, 18(6), pp. 6852–6865.

Block, K. I., Gyllenhaal, C., Lowe, L., Amedei, A., Ruhul Amin, A. R. M., Amin, A., Aquilano, K., Arbiser, J., *et al.* (2015) ‘Designing a broad-spectrum integrative approach for cancer prevention and treatment’, *Seminars in Cancer Biology*. Elsevier Ltd, 35, pp. S276–S304.

Boeing, J. S., Barizão, É. O., e Silva, B. C., Montanher, P. F., de Cinque Almeida, V. and Visentainer, J. V. (2014) ‘Evaluation of solvent effect on the extraction of phenolic compounds and antioxidant capacities from the berries: Application of principal component analysis’, *Chemistry Central Journal*, 8(1), p. 48.

Burke, A. C., Sutherland, B. G., Telford, D. E., Morrow, M. R., Sawyez, C. G., Edwards, J. Y., Drangova, M. and Huff, M. W. (2018) ‘Intervention with citrus flavonoids reverses obesity and improves metabolic syndrome and atherosclerosis in obese Ldlr / mice’, *Journal of Lipid Research*, 59(9), pp. 1714–1728.

Busia, K. (2005) ‘Medical provision in Africa - Past and present’, *Phytotherapy Research*, pp. 919–923.

Bussmann, R. W., Gilbreath, G. G., Solio, J., Lutura, M., Lutuluo, R., Kunguru, K., Wood, N. and Mathenge, S. G. (2006) ‘Plant use of the Maasai of Sekenani Valley, Maasai Mara, Kenya’, *Journal of Ethnobiology and Ethnomedicine*, 2, p. 22.

Callaway, J. C. (2005) ‘Various alkaloid profiles in decoctions of *Banisteriopsis caapi*’, *Journal of Psychoactive Drugs*, 37(2), pp. 151–5.

Campbell, D. J., Gichohi, H., Mwangi, A. and Chege, L. (2000) ‘Land use conflict in Kajiado District, Kenya’, *Land Use Policy*, 17(4), pp. 337–348.

Cao, X., Yu, L. and Sun, D. (2008) ‘Drug Absorption Principles’, in *Biopharmaceutics Applications in Drug Development*. Springer, pp. 75–100.

Carbonaro, M., Maselli, P. and Nucara, A. (2015) 'Structural aspects of legume proteins and nutraceutical properties', *Food Research International*, 76(1), pp. 19–30.

Chae, H. S., Park, H. J., Hwang, H. R., Kwon, A., Lim, W. H., Yi, W. J., Han, D. H., Kim, Y. H., *et al.* (2011) 'The effect of antioxidants on the production of pro-inflammatory cytokines and orthodontic tooth movement', *Molecules and Cells*, 32(2), pp. 189–196.

Chen, J., Yang, J., Ma, L., Li, J., Shahzad, N. and Kim, C. K. (2020) 'Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids', *Scientific Reports*, 10(1), pp. 1–9.

Chiu, C. C., Huang, C. Y., Chen, T. Y., Kao, S. H., Liu, J. Y., Wang, Y. W., Tzang, B. S. and Hsu, T. C. (2012) 'Beneficial effects of *Ocimum gratissimum* aqueous extract on rats with CCl 4 -Induced acute liver injury', *Evidence-based Complementary and Alternative Medicine*, 2012, pp. 1–9.

Cho, C., Koh, Y. J., Han, J., Sung, H., Lee, H. J., Morisada, T., Schwendener, R. A., Brekken, R. A., *et al.* (2007) 'Angiogenic Role of LYVE-1 – Positive Macrophages in Adipose Tissue', *Circulation Research*, 100, pp. e47–e57.

Cho, I. S., Shin, Y. K. and Lee, C. S. (1995) 'Effects of harmaline and harmalol on the oxidative injuries of hyaluronic acid, lipid and collagen by Fe2+ and H2O2', *Korean Journal of Pharmacology*, 31, pp. 345–353.

Christensen, D. L., Faurholt-Jepsen, D., Boit, M. K., Mwaniki, D. L., Kilonzo, B., Tetens, I., Kiplamai, F. K., Cheruiyot, S. C., *et al.* (2012) 'Cardiorespiratory fitness and physical activity in Luo, Kamba, and Maasai of rural Kenya', *American Journal of Human Biology*, 24(6), pp. 723–729.

Christensen, D. L., Faurholt-Jepsen, D., Faerch, K., Mwaniki, D. L., Boit, M. K., Kilonzo, B., Tetens, I., Friis, H., *et al.* (2014) 'Insulin resistance and beta-cell function in different ethnic groups in Kenya: The role of abdominal fat distribution', *Acta Diabetologica*, 51(1), pp. 53–60.

Cushnie, T. P. T., Cushnie, B. and Lamb, A. J. (2014) 'Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities', *International Journal of Antimicrobial Agents*, pp. 377–386.

D'Archivio, M., Filesi, C., Di Benedetto, R., Gargiulo, R., Giovannini, C. and Masella, R. (2007) 'Polyphenols, dietary sources and bioavailability', *Annali dell'Istituto Superiore di Sanita*, 43(4), pp. 348–361.

Dai, J. and Mumper, R. J. (2010) 'Plant phenolics: extraction, analysis and their antioxidant and anticancer properties.', *Molecules (Basel, Switzerland)*, 15(10), pp. 7313–52.

Dambolena, J. S., Zunino, M. P., López, A. G., Rubinstein, H. R., Zygadlo, J. A., Mwangi, J. W., Thoithi, G. N., Kibwage, I. O., *et al.* (2010) 'Essential oils composition of *Ocimum basilicum* L. and *Ocimum gratissimum* L. from Kenya and their inhibitory effects on growth and fumonisin production by *Fusarium verticillioides*', *Innovative Food Science and Emerging Technologies*, 11, pp. 410–414.

Davies, N. M. and Anderson, K. E. (1997) 'Clinical Pharmacokinetics of Diclofenac', *Clinical Pharmacokinetics*, 33(1997), pp. 184–213.

Dhooghe, L., Maregesi, S., Mincheva, I., Ferreira, D., Marais, J. P. J., Lemière, F., Matheeussen, A., Cos, P., *et al.* (2010) 'Antiplasmodial activity of (I-3,II-3)-biflavonoids and other constituents from *Ormocarpum kirkii*', *Phytochemistry*, 71, pp. 785–791.

Doi, H., Nishida, K., Yorimitsu, M., Komiyama, T., Kadota, Y., Tetsunaga, T., Yoshida, A., Kubota, S., *et al.* (2008) 'Interleukin-4 downregulates the cyclic tensile stress-induced matrix metalloproteinases-13 and cathepsin b expression by rat normal chondrocytes', *Acta Medica Okayama*, 62(2), pp. 119–126.

Duthie, G. G., Duthie, S. J. and Kyle, J. A. M. (2000) 'Plant polyphenols in cancer and heart disease : implications as nutritional antioxidants', *Nutrition Research reviews*, 44(13), pp. 79–106.

Eldeen, I. M. S., Elgorashi, E. E. and Van Staden, J. (2005) 'Antibacterial, anti-inflammatory, anti-cholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine', *Journal of Ethnopharmacology*, 102(3), pp. 457–64.

Eldeen, I. M. S., Van Heerden, F. R. and Van Staden, J. (2010) 'In vitro biological activities of niloticane, a new bioactive cassane diterpene from the bark of *Acacia nilotica* subsp. *kraussiana'*, *Journal of Ethnopharmacology*, 128(3), pp. 555–60.

Elekofehinti, O. O., Kamdem, J. P., Bolingon, A. A., Athayde, M. L., Lopes, S. R., Waczuk, E. P., Kade, I. J., Adanlawo, I. G., *et al.* (2013) 'African eggplant (*Solanum anguivi* Lam.) fruit with bioactive polyphenolic compounds exerts in vitro antioxidant properties and inhibits Ca2+-induced mitochondrial swelling', *Asian Pacific Journal of Tropical Biomedicine*, 3, pp. 757–766.

Elfadil, H., Fahal, A., Kloezen, W., Ahmed, E. M. and van de Sande, W. (2015) 'The In Vitro Antifungal Activity of Sudanese Medicinal Plants against *Madurella mycetomatis*, the Eumycetoma Major Causative Agent', *PLOS Neglected Tropical Diseases*, 9(3), p. e0003488.

Ernst, W. H. O., Kuiters, A. T., Nelissen, H. J. M. and Tolsma, D. J. (1991) 'Seasonal variation in phenolics in several savanna tree species in Botswana', *Acta Botanica Neerlandica*, 40(1), pp. 63–74.

Farasat, M., Khavari-Nejad, R. A., Nabavi, S. M. B. and Namjooyan, F. (2014) 'Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian gulf', *Iranian Journal of Pharmaceutical Research*, 13(1), pp. 163–170.

Fechtner, S., Singh, A., Chourasia, M. and Ahmed, S. (2017) 'Molecular insights into the differences in anti-inflammatory activities of green tea catechins on IL-1β signaling in rheumatoid arthritis synovial fibroblasts', *Toxicology and Applied Pharmacology*, 15(329), pp. 112–120.

Fernandes, I., Pérez-Gregorio, R., Soares, S., Mateus, N., De Freitas, V., Santos-Buelga, C. and Feliciano, A. S. (2017) 'Wine flavonoids in health and disease prevention', *Molecules*, 22(2), p. 292.

Fernando, A. N., Fernando, L. P., Fukuda, Y. and Kaplan, A. P. (2005) 'Assembly, activation, and signaling by kinin-forming proteins on human vascular smooth muscle cells', *American Journal of Physiology-Heart and Circulatory Physiology*, 289(1), pp. H251-7.

Finke, J. (2000) 'Maasai- Introduction', in *Traditional Music and Cultures of Kenya*. Dallas: Camerapix Publishers International. Available at: kenya@bluegecko.org.

Foyer, C. H. and Noctor, G. (2011) 'Ascorbate and glutathione: The heart of the redox hub', *Plant Physiology*, pp. 2–18.

Frye, J. C. (2003) ‘Herbal and homeopathic medicine: Understanding the difference’, *Seminars in Integrative Medicine*, pp. 158–166.

Gallego, M. G., Gordon, M. H., Segovia, F. J., Skowyra, M. and Almajano, M. P. (2013) ‘Antioxidant properties of three aromatic herbs (rosemary, thyme and lavender) in oil-in-water emulsions’, *Journal of the American Oil Chemists' Society*, 2013(90), pp. 1559–1568. doi: 10.1007/s11746-013-2303-3.

Gan, R. Y., Kuang, L., Xu, X. R., Zhang, Y., Xia, E. Q., Song, F. L. and Li, H. Bin (2010) ‘Screening of natural antioxidants from traditional Chinese medicinal plants associated with treatment of rheumatic disease’, *Molecules*, 15(9), pp. 5988–5997.

Ganga, R. B., Madhu, K. P. and Vijaya, R. A. D. (2012) ‘Investigation of antioxidant and anti-inflammatory activity of leaves of *Dalbergia paniculata* (Roxb)’, *Asian Pacific Journal of Tropical Medicine*, 5(6), pp. 455–458.

Gathirwa, J. W., Rukunga, G. M., Njagi, E. N. M., Omar, S. A., Mwitari, P. G., Guantai, A. N., Tolo, F. M., Kimani, C. W., *et al.* (2008) ‘The in vitro anti-plasmodial and in vivo anti-malarial efficacy of combinations of some medicinal plants used traditionally for treatment of malaria by the Meru community in Kenya’, *Journal of Ethnopharmacology*, 115(2), pp. 223–231.

Goyal, P. K. (2012) ‘Phytochemical and pharmacological properties of the genus Grewia: A review’, *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(Suppl. 4), pp. 72–78.

Guay, J., Bateman, K., Gordon, R., Mancini, J. and Riendeau, D. (2004) ‘Carrageenan-induced paw edema in rat elicits a predominant prostaglandin E2 (PGE2) response in the central nervous system associated with the induction of microsomal PGE2 synthase-1’, *Journal of Biological Chemistry*, 279(23), pp. 24866–24872.

Gutiérrez A, D. M., Bah, M., Garduño R, M. L., Mendoza D, S. O. and Serrano C, V. (2014) ‘Anti-inflammatory and antioxidant activities of methanol extracts and alkaloid fractions of four Mexican medicinal plants of Solanaceae’, *African Journal of Traditional, Complementary and Alternative Medicines*, 11(3), pp. 259–267.

Haichar, F. el Z., Santaella, C., Heulin, T. and Achouak, W. (2014) ‘Root exudates mediated

interactions belowground', *Soil Biology and Biochemistry*, 77, pp. 69–80.

Halawany, H. S. (2012) 'A review on miswak (*Salvadora persica*) and its effect on various aspects of oral health', *Saudi Dental Journal*, 24(2), pp. 63–69.

Halliwell, B. and Gutteridge, J. M. C. (2004) 'Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts', *Archives of Biochemistry and Biophysics*, 246(2), pp. 501–514.

Hamza, O. J., van den Bout-van den Beukel, C. J., Matee, M. I., Moshi, M. J., Mikx, F. H., Selemani, H. O., Mbwambo, Z. H., Van der Ven, A. J. A. M., *et al.* (2006) 'Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infections', *Journal of Ethnopharmacology*, 108(1), pp. 124–132.

Hansen, A. W., Christensen, D. L., Larsson, M. W., Eis, J., Christensen, T., Friis, H., Mwaniki, D. L., Kilonzo, B., *et al.* (2011) 'Dietary patterns, food and macronutrient intakes among adults in three ethnic groups in rural Kenya', *Public Health Nutrition*, 14(9), pp. 1671–1679.

Harris, N. R. and Rumbaut, R. E. (2001) 'Age-related responses of the microcirculation to ischemia-reperfusion and inflammation', *Pathophysiology*, 8(1), pp. 1–10.

Hassan-Abdallah, A., Merito, A., Hassan, S., Aboubaker, D., Djama, M., Asfaw, Z. and Kelbessa, E. (2013) 'Medicinal plants and their uses by the people in the Region of Randa, Djibouti', *Journal of Ethnopharmacology*, 148(2), pp. 701–713.

Houghton, L. A., Brown, R. C., Beaumont, S., Jennings, S., Bailey, K. B., Haszard, J. J., Erhardt, J., Daniels, L., *et al.* (2019) 'Micronutrient status differs among Maasai and Kamba preschoolers in a supplementary feeding programme in Kenya', *Maternal and Child Nutrition*, 15(3), p. e12805.

Hsu, C. C., Kuo, H. C. and Huang, K. E. (2017) 'The effects of phytosterols extracted from *Diascorea alata* on the antioxidant activity, plasma lipids, and hematological profiles in Taiwanese menopausal women', *Nutrients*, 9(12), p. 1320.

Hu, Y., Li, Y., Zhang, W., Kou, G. and Zhou, Z. (2018) 'Physical stability and antioxidant activity of citrus flavonoids in arabic gum-stabilized microcapsules: Modulation of whey

protein concentrate', *Food Hydrocolloids*, 77(2018), pp. 588–597.

Huang, D., Boxin, O. U. and Prior, R. L. (2005) 'The chemistry behind antioxidant capacity assays', *Journal of Agricultural and Food Chemistry*, 53(6), pp. 1841–1856.

Ikarashi, N., Toda, T., Hatakeyama, Y., Kusunoki, Y., Kon, R., Mizukami, N., Kaneko, M., Ogawa, S., *et al.* (2018) 'Anti-hypertensive effects of Acacia polyphenol in spontaneously hypertensive rats', *International Journal of Molecular Sciences*, 19(3), p. 700.

Ismail, H. F., Hashim, Z., Soon, W. T., Rahman, N. S. A., Zainudin, A. N. and Majid, F. A. A. (2017) 'Comparative study of herbal plants on the phenolic and flavonoid content, antioxidant activities and toxicity on cells and zebrafish embryo', *Journal of Traditional and Complementary Medicine*, 7(4), pp. 452–465.

Iwalewa, E. O., Naidoo, V., Bagla, V. and Eloff, J. N. (2009) 'Nitric oxide producing effect of six extracts from *Harungana madagascariensis* Lam . ex Poiret ( Guttiferae ) stem bark', 3(1), pp. 14–21.

Iwu, M. M. (2014) *Handbook of African medicinal plants*, *CRC Press*.

Jamal, O., Aneni, E. C., Shaharyar, S., Ali, S. S., Parris, D., McEvoy, J. W., Veledar, E., Blaha, M. J., *et al.* (2014) 'Cigarette smoking worsens systemic inflammation in persons with metabolic syndrome', *Diabetology & Metabolic Syndrome*, 6(1), p. 79.

Jaspers, M. W. J. M., Bashir, A. K., Zwaving, J. H. and Malingre, T. M. (1986) 'Investigation of *Grewia bicolor* Juss', *Journal of Ethnopharmacology*, 17(3), pp. 205–211.

Jiang, Y., Zhang, M., Lin, S. and Cheng, S. (2018) 'Contribution of specific amino acid and secondary structure to the antioxidant property of corn gluten proteins', *Food Research International*, 105, pp. 836–844.

Jimoh, F. O., Adedapo, A. A. and Afolayan, A. J. (2010) 'Comparison of the nutritional value and biological activities of the acetone, methanol and water extracts of the leaves of *Solanum nigrum* and *Leonotis leonorus*', *Food and Chemical Toxicology*, 48(3), pp. 964–71.

Johns, Mahunnah, R. L. A., Sanaya, P., Chapman, L. and Ticktin, T. (1999) 'Saponins and phenolic content in plant dietary additives of a traditional subsistence community, the Batemi of Ngorongoro District, Tanzania', *Journal of Ethnopharmacology*, 66(1), pp. 1–10.

Johns, T., Mhoro, E. B., Sanaya, P. and Kimanani, E. K. (1994) 'Herbal remedies of the Batemi of Ngorongoro District, Tanzania: a quantitative appraisal', *Economic Botany*, 48(1), pp. 90–95.

Jung, H. A., Min, B. S., Yokozawa, T., Lee, J. H., Kim, Y. S. and Choi, J. S. (2009) 'Anti-Alzheimer and antioxidant activities of *Coptidis rhizoma* alkaloids', *Biological and Pharmaceutical Bulletin*, 32(8), pp. 1433–8.

Kapewangolo, P. and Meyer, D. (2018) 'Plectranthus barbatus; Antioxidant, and Other Inhibitory Responses Against HIV/AIDS', in *HIV/AIDS: Oxidative Stress and Dietary Antioxidants*, pp. 149–159.

Karau, G. M., Njagi, E. N. M., Machocho, A. K. and Wangai, L. N. (2012) 'Phytonutrient, Mineral Composition and In vitro Antioxidant Activity of Leaf and Stem Bark Powders of *Pappea capensis* (L.)', *Pakistan Journal of Nutrition*, 11(2), pp. 123–132.

Kareru, P G, Gachanja, A. N., Keriko, J. M. and Kenji, G. M. (2007) 'Antimicrobial activity of some medicinal plants used by herbalists in Eastern province, Kenya.', *African Journal of Traditional, Complementary, and Alternative Medicines*, 5(1), pp. 51–55.

Kareru, P.G., Kenji, G. M., Gachanja, A. N., Keriko, J. M. and Mungai, G. (2007) 'Traditional medicines among the Embu and Mbeere people of Kenya', *African Journal of Traditional, Complementary and Alternative Medicines*, 4(1), pp. 75–86.

Kaškoniene, V. and Venskutonis, P. R. (2010) 'Floral Markers in Honey of Various Botanical and Geographic Origins: A Review', *Comprehensive Reviews in Food Science and Food Safety*, 9(6), pp. 620–634.

Kedare, S. B. and Singh, R. P. (2011) 'Genesis and development of DPPH method of antioxidant assay.', *Journal of food science and technology*, 48(4), pp. 412–22.

Kehrer, J. P. and Klotz, L. O. (2015) 'Free radicals and related reactive species as mediators of tissue injury and disease: Implications for Health', *Critical Reviews in Toxicology*, 45(9), pp. 765–98.

Kennedy, A. D. and Deleo, F. R. (2009) 'Neutrophil apoptosis and the resolution of infection', *Immunologic Research*, 43(1–3), pp. 25–61.

Kenya National Bureau of Statistics (2014) *Kenya Demographic and Health Survey 2014*.

Kenya National Bureau of Statistics (2018) *Economic Survey 2018*. Available at: http://www.knbs.or.ke.

Keter, L. K. and Mutiso, P. C. (2012) 'Ethnobotanical studies of medicinal plants used by Traditional Health Practitioners in the management of diabetes in Lower Eastern Province, Kenya', *Journal of Ethnopharmacology*, 139(1), pp. 74–80.

Khan, H., Patel, S. and Kamal, M. A. (2017) 'Pharmacological and Toxicological Profile of Harmane-β-Carboline Alkaloid: Friend or Foe', *Current Drug Metabolism*, 18(9), pp. 853–857.

Khan Tabassum, A., Anjaria Jeet, K., Vanish, D. and Gohel Anjali, K. (2015) 'Bioactivity guided fractionation and anti-inflammatory activity of *Acacia nilotica* pods', *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(7), pp. 380–383.

Kiplimo, J. J., Shahidul Islam, M. and Koorbanally, N. A. (2012) 'Ring A-seco limonoids and flavonoids from the Kenyan *Vepris uguenensis* Engl. and their antioxidant activity', *Phytochemistry*, 83, pp. 136–143.

Kiringe, J. W. (2006) 'A survey of traditional health remedies used by the Maasai of Southern Kaijiado District, Kenya', *Ethnobotany Research & Applications*, 4, pp. 61–73.

Kiringe, J. W. and Okello, M. M. (2005) 'Use and availability of tree and shrub resources on Maasai communal rangelands near Amboseli, Kenya', *African Journal of Range and Forage Science*, 22(1), pp. 37–45.

Kosgei, J. C. (2014) 'Phytochemical screening, cytotoxicity studies and Larvicidal activity of hexane extract of *Lippia kituiensis* against *Rhipicephalus appendiculatus*', *Journal of Biomedical and Pharmaceutical Research*, 3(4), pp. 105–110.

Kusano, R., Ogawa, S., Matsuo, Y., Tanaka, T., Yazaki, Y. and Kouno, I. (2011) 'α-amylase and lipase inhibitory activity and structural characterization of *Acacia* bark proanthocyanidins', *Journal of Natural Products*, 74(2), pp. 119–128.

Lamaoui, M., Jemo, M., Datla, R. and Bekkaoui, F. (2018) 'Heat and drought stresses in crops and approaches for their mitigation', *Frontiers in Chemistry*, 19(6), p. 26.

Lamien-Meda, A., Lamien, C. E., Compaoré, M. M. Y., Meda, R. N. T., Kiendrebeogo, M., Zeba, B., Millogo, J. F. and Nacoulma, O. G. (2008) 'Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso', *Molecules*, 13(3), pp. 581–594.

Lämmermann, T., Afonso, P. V., Angermann, B. R., Wang, J. M., Kastenmüller, W., Parent, C. A. and Germain, R. N. (2013) 'Neutrophil swarms require LTB4 and integrins at sites of cell death in vivo', *Nature*, 498(2013), pp. 371–375.

Lawson, D. W., Mulder, M. B., Ghiselli, M. E., Ngadaya, E., Ngowi, B., Mfinanga, S. G. M., Hartwig, K. and James, S. (2014) 'Ethnicity and child health in northern Tanzania: Maasai pastoralists are disadvantaged compared to neighbouring ethnic groups', *PLoS ONE*, 9(10), p. e110447.

Le, N. H. T., Malterud, K. E., Diallo, D., Paulsen, B. S., Nergård, C. S. and Wangensteen, H. (2012) 'Bioactive polyphenols in *Ximenia americana* and the traditional use among Malian healers', *Journal of Ethnopharmacology*, 139(3), pp. 858–862.

Lee, C. S., Han, E. S., Jang, Y. Y., Han, J. H., Ha, H. W. and Kim, D. E. (2000) 'Protective effect of harmalol and harmaline on MPTP neurotoxicity in the mouse and dopamine-induced damage of brain mitochondria and PC12 cells', *Journal of Neurochemistry*, 75(2), pp. 521–31.

Lee, H. Y., Lee, G. H., Yoon, Y. and Chae, H. J. (2019) '*R. verniciflua* and *E. ulmoides* extract (ILF-RE) protects against chronic CCL 4 -induced liver damage by enhancing antioxidation', *Nutrients*, 11(2), p. 382.

Levin, K. A. (2006) 'Study design III: Cross-sectional studies', *Evidence-Based Dentistry*, 7(1), pp. 24–5.

Li, Y., Zhao, L., Yu, D., Wang, Z. and Ding, G. (2018) 'Metabolic syndrome prevalence and its risk factors among adults in China: A nationally representative cross-sectional study', *PLoS ONE*, 13(6), p. e0199293.

Liao, H., Banbury, L. K. and Leach, D. N. (2008) 'Antioxidant activity of 45 Chinese herbs and the relationship with their TCM characteristics', *Evidence-based Complementary and Alternative Medicine*, 5(4), pp. 429–434.

Liao, L. Y., He, Y. F., Li, L., Meng, H., Dong, Y. M., Yi, F. and Xiao, P. G. (2018) 'A preliminary review of studies on adaptogens: Comparison of their bioactivity in TCM with that of ginseng-like herbs used worldwide', *Chinese Medicine*, 13, p. 57.

Lim, T. K. (2012) *Edible medicinal and non-medicinal plants, Volume 2, Fruits*.

Lin, H. Y., Chang, T. C. and Chang, S. T. (2018) 'A review of antioxidant and pharmacological properties of phenolic compounds in *Acacia confusa*', *Journal of Traditional and Complementary Medicine*, 8(4), pp. 443–450.

Lin, J. Y. and Tang, C. Y. (2006) 'Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation', *Food Chemistry*, 101(1), pp. 140–147.

Lin, Y. L., Wang, W. Y., Kuo, Y. H. and Chen, C. F. (2000) 'Nonsteroidal constituents from *Solanum incanum* L', *Journal of the Chinese Chemical Society*, 47(1), pp. 247–251.

Lobo, V., Patil, A., Phatak, A. and Chandra, N. (2010) 'Free radicals, antioxidants and functional foods: Impact on human health', *Pharmacognosy Reviews*, 4(8), pp. 118–126. doi: 10.4103/0973-7847.70902.

Ma, Q. (2014) 'Advances in Mechanisms of Anti-oxidation', *Discovery Medicine*, 17(93), pp. 121–130.

Madivoli, E. S., Maina, E. G., Kairigo, P. K., Murigi, M. K., Ogilo, J. K., Nyangau, J. O., Kimani, P. K. and Kipyegon, C. (2018) 'In vitro antioxidant and antimicrobial activity of *Prunus africana* (Hook. f.) Kalkman (bark extracts) and *Harrisonia abyssinica* Oliv. extracts (bark extracts): A comparative study', *Journal of Medicinal Plants for Economic Development*, 2(1), p. a39.

Maitai, C. K., Talalaj, S., Njoroge, D. and Wamugunda, R. (1980) 'Effect of extract of hairs from the herb *Urtica massaica*, on smooth muscle', *Toxicon*, 18(2), pp. 225–229.

Malireddy, S., Kotha, S. R., Secor, J. D., Gurney, T. O., Abbott, J. L., Maulik, G., Maddipati, K. R. and Parinandi, N. L. (2012) 'Phytochemical antioxidants modulate mammalian cellular epigenome: Implications in health and disease', *Antioxidants and Redox Signaling*, 17(2), pp. 327–339.

Manguro, L. O., Ugi, I., Lemmen, P. and Hermann, R. (2003) 'Flavonol glycosides of *Warburgia ugandensis* leaves', *Phytochemistry*, 64(4), pp. 891–896.

Maroyi, A. (2013) 'Traditional use of medicinal plants in south-central Zimbabwe : review and perspectives', *Journal of Ethnobiology and Ethnomedicine*, 9(1), p. 31.

Maroyi, A. (2017) 'Review of ethnomedicinal uses, phytochemistry and pharmacological properties of *Euclea natalensis* A.DC', *Molecules*, 22(12), p. 2128.

Masinde, S. (2010) 'Medicinal Plants of East Africa, 3rd edn.', *African Journal of Ecology*. 3rd edn. Nairobi: University of Nairobi Press, 48(4), pp. 1143–1144.

Matu, E. N. and Van Staden, J. (2003) 'Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya', *Journal of Ethnopharmacology*, 87(1), pp. 35–41.

Maundu, P., Berger, D., ole Saitabau, C., Nasieku, J., Kipelian, M., Mathenge, S. G. and Morimoto, Y. (2001) 'Ethnobotany of the Loita Maasai: Towards Community Management of the Forest of the Lost Child Experiences from the Loita Ethnobotany Project.', in *People and Plants Working Paper 8*. Paris (Experiences from the Loita Ethnobotany project), pp. 1–47.

Mavhura, E., Manatsa, D. and Mushore, T. (2015) 'Adaptation to drought in arid and semi-arid environments: Case of the Zambezi Valley, Zimbabwe', *Jamba: Journal of Disaster Risk Studies*, 7(1), p. 144.

McCabe, J., Perkin, S. and Schofield, C. (1992) 'Can Conservation and Development be Coupled among Pastoral People? An Examination of the Maasai of the Ngorongoro Conservation Area, Tanzania', *Human Organization*, 51(4), pp. 353–367.

McInnes, I. B. and Schett, G. (2007) 'Cytokines in the pathogenesis of rheumatoid arthritis', *Nature Reviews Immunology*, 7, pp. 429–442.

Meshram, G. G., Kumar, A., Rizvi, W., Tripathi, C. D. and Khan, R. A. (2016) 'Evaluation of the anti-inflammatory activity of the aqueous and ethanolic extracts of the leaves of *Albizzia lebbeck* in rats', *Journal of Traditional and Complementary Medicine*. Elsevier Ltd, 6(2), pp. 172–175.

Miguel, M. G., Nunes, S., Dandlen, S. A., Cavaco, A. M. and Antunes, M. D. (2014) 'Phenols, flavonoids and antioxidant activity of aqueous and methanolic extracts of propolis (*Apis mellifera L.*) from Algarve, South Portugal', *Food Science and Technology (Campinas)*, 34(1), pp. 16–23.

Misana, S. B., Majule, A. and Lyaruu, H. (2003) 'Linkages between changes in land use, biodiversity and land degradation on the slopes of Mount Kilimanjaro, Tanzania', *LUCID working papers*. Nairobi, Kenya (LUCID), 38, pp. 1–30.

Mishra, K. K., Pal, R. S., Arunkumar, R., Chandrashekara, C., Jain, S. K. and Bhatt, J. C. (2013) 'Antioxidant properties of different edible mushroom species and increased bioconversion efficiency of *Pleurotus eryngii* using locally available casing materials', *Food Chemistry*, 138(2–3), pp. 1557–1563.

Mohamed, T. K., Nassar, M. I., Gaara, A. H., El-Kashak, W. A., Brouard, I., & El-Toumy, S. A., Mohamed, T. K., Nassar, M. I., Gaara, A. H., El-Kashak, W. A., Brouard, I. and El-Toumy, S. A. (2013) 'Secondary metabolites and bioactivities of *Albizia anthelmintica*.', *Pharmacognosy research*, 5(2), pp. 80–5.

Mohammed, N., Sati, E., Abdallah, F. and Ahmed, M. (2018) 'Botanical overview and chemical composition of some *Grewia* spp', *Open Science Journal*, 3(1).

Moloudizargari, M., Mikaili, P., Aghajanshakeri, S., Asghari, M. and Shayegh, J. (2013) 'Pharmacological and Therapeutic effects of *Peganum harmala* and its main alkaloids', *Pharmacognosy Reviews*.

Moshi, M. J. and Mbwambo, Z. H. (2002) 'Experience of Tanzanian Traditional Healers in the Management of Non-insulin Dependent Diabetes Mellitus', *Pharmaceutical Biology*, 40(7), pp. 552–560.

Moura, D. J., Richter, M. F., Boeira, J. M., Pêgas Henriques, J. A. and Saffi, J. (2007) 'Antioxidant properties of β-carboline alkaloids are related to their antimutagenic and antigenotoxic activities', *Mutagenesis*, 22(4), pp. 293–302.

Mugedo, J. Z. A. and Waterman, P. G. (1992) 'Sources of tannin: Alternatives to wattle (*Acacia mearnsii*) among indigenous Kenyan species.', *Economic Botany*, 46, pp. 55–63.

Muregi, F. W., Ishih, A., Miyase, T., Suzuki, T., Kino, H., Amano, T., Mkoji, G. M. and Terada, M. (2007) 'Antimalarial activity of methanolic extracts from plants used in Kenyan ethnomedicine and their interactions with chloroquine (CQ) against a CQ-tolerant rodent parasite, in mice', *Journal of Ethnopharmacology*, 111(1), pp. 190–195.

Musa, M. S., Abdelrasool, F. E., Elsheikh, E. A., Ahmed, L. A. M. N., Mahmoud, A. L. E. and Yagi, S. M. (2011) 'Ethnobotanical study of medicinal plants in the Blue Nile State, South-eastern Sudan', *Journal of Medicinal Plants Research*, 5(17), pp. 4287–4297.

Mutai, C., Bii, C., Rukunga, G., Ondicho, J., Mwitari, P., Abatis, D., Vagias, C., Roussis, V., *et al.* (2009) 'Antimicrobial activity of pentacyclic triterpenes isolated from *Acacia mellifera*', *African Journal of Traditional, Complementary and Alternative Medicines*, 6, pp. 42–48.

Muthee, J. K., Gakuya, D. W., Mbaria, J. M., Kareru, P. G., Mulei, C. M. and Njonge, F. K. (2011) 'Ethnobotanical study of anthelmintic and other medicinal plants traditionally used in Loitoktok district of Kenya', *Journal of Ethnopharmacology*, 135(1), pp. 15–21.

Muthu, R., Selvaraj, N. and Vaiyapuri, M. (2016) 'Anti-inflammatory and proapoptotic effects of umbelliferone in colon carcinogenesis', *Human and Experimental Toxicology*, 35(10), pp. 1041–54.

Nagata, J. M., Jew, A. R., Kimeu, J. M., Salmen, C. R., Bukusi, E. A. and Cohen, C. R. (2011) 'Medical pluralism on Mfangano Island: Use of medicinal plants among persons living with HIV/AIDS in Suba District, Kenya', *Journal of Ethnopharmacology*, 135, pp. 501–509.

Nankaya, J., Gichuki, N., Lukhoba, C. and Balslev, H. (2020) 'Medicinal plants of the Maasai of Kenya: A review', *Plants*, 9(1), p. 44.

Natarajan, S., Shunmugiah, K. P. and Kasi, P. D. (2013) 'Plants traditionally used in age-related brain disorders (dementia): An ethnopharmacological survey', *Pharmaceutical Biology*, pp. 492–523. doi: 10.3109/13880209.2012.738423.

Nathan, C. and Ding, A. (2010) 'Nonresolving inflammation.', *Cell*. Elsevier Inc., 140(6), pp. 871–82. doi: 10.1016/j.cell.2010.02.029.

Necas, J. and Bartosikova, L. (2013) ‘Carrageenan: A review’, *Veterinarni Medicina*, 58(4), pp. 187–205. doi: 10.17221/6758-VETMED.

Neto Júnior, J. de C. S., Estevão, L. R. de M., Ferraz, A. A., Simões, R. S., Vieira, M. G. F. and Evêncio-Neto, J. (2019) ‘Ointment of *Ximenia americana* promotes acceleration of wound healing in rats’, *Acta Cirurgica Brasileira*, 34(3), p. e201900307.

Neuhouser, M. L. (2019) ‘The importance of healthy dietary patterns in chronic disease prevention’, *Nutrition Research*, 70, pp. 3–6.

Ngoye, A., Petrucka, P. and Buza, J. (2014) ‘Differences in Hypertension Risk Factors between Rural Maasai in Ngorongoro and Urban Maasai in Arusha Municipal: A Descriptive Study’, *Journal of Applied Life Sciences International*, 1(1), pp. 17–31.

Ochwang’i, D. O., Kimwele, C. N., Oduma, J. A., Gathumbi, P. K., Mbaria, J. M. and Kiama, S. G. (2014) ‘Medicinal plants used in treatment and management of cancer in Kakamega County, Kenya’, *Journal of Ethnopharmacology*. Elsevier, 151(3), pp. 1040–1055.

Odongo, E., Mungai, N., Mutai, P., Karumi, E., Mwangi, J., Okalebo, F. and Simiyu, J. (2017) ‘Antioxidant and anti-inflammatory activities of selected medicinal plants from western Kenya.’, *African Journal of Pharmacology and Therapeutics*, 6(4), pp. 178–182.

Odongo, E., Mungai, N., Mutai, P., Karumi, E., Mwangi, J. and Omale, J. (2018) ‘Ethnobotanical survey of medicinal plants used in Kakamega County, western Kenya’, *Applied Medical Research*, 4(1), p. 22. doi: 10.5455/amr.20180315095706.

Ole-Miaron, J. O. (2003) ‘The Maasai ethnodiagnostic skill of livestock diseases: A lead to traditional bioprospecting’, *Journal of Ethnopharmacology*, 84, pp. 79–83.

Olila, D., Olwa-Odyek and Opuda-Asibo, J. (2001) ‘Antibacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis*, Ugandan medicinal plants.’, *Afr Health Sci*, 1(2), pp. 66–72.

Omosa, L. K., Midiwo, J. O. and Kuete, V. (2017) ‘Curcuma longa: Therapeutic Potential Against Metabolic, Inflammatory, Infectious and Systemic Diseases’, in *Medicinal Spices and Vegetables from Africa*, pp. 425–435.

Omote, K., Hazama, K., Kawamata, T., Kawamata, M., Nakayaka, Y., Toriyabe, M. and

Namiki, A. (2001) ‘Peripheral nitric oxide in carrageenan-induced inflammation’, *Brain Research*, 912(2), pp. 171–5.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., Anthony, S. and Simons, A. (2009) ‘A tree reference and selection guide’, in *Agroforestree Database*, pp. 1–5.

Özcan, M. M. and Arslan, D. (2011) ‘Antioxidant effect of essential oils of rosemary, clove and cinnamon on hazelnut and poppy oils’, *Food Chemistry*, 129(1), pp. 171–174.

Palma, M., Robert, P., Holgado, F., Velasco, J. and Márquez-Ruiz, G. (2017) ‘Antioxidant Activity and Kinetics Studies of Quercetin, Epicatechin and Naringenin in Bulk Methyl Linoleate’, *Journal of the American Oil Chemists’ Society*, 94, pp. 1189–1196.

Panossian, A. and Wikman, G. (2010) ‘Effects of Adaptogens on the Central Nervous System and the Molecular Mechanisms Associated with Their Stress—Protective Activity’, *Pharmaceuticals*, 3(1), pp. 188–224.

Patrono, C. and Baigent, C. (2014) ‘Nonsteroidal anti-inflammatory drugs and the heart’, *Circulation*, 129, pp. 907–916. doi: 10.1161/CIRCULATIONAHA.113.004480.

Paviaya, U., Wanjari, M., Thenmozhi, S., Kumar, P. and Balakrishnan, B. (2013) ‘Analgesic and anti-inflammatory activity of root bark of *Grewia asiatica* Linn. in rodents’, *Ancient Science of Life*, 32(3), pp. 150–155.

Peduto, V. A. (2001) ‘The mandrake root and the *Viennese Dioscorides*’, *Minerva anestesiologica*, 67(10), pp. 751–766.

Penner, G. H. and Wasylishen, R. E. (1989) ‘A carbon-13 CP/MAS nuclear magnetic resonance study of several 1,4-disubstituted benzenes in the solid state’, *Canadian Journal of Chemistry*, 67(3), pp. 525–534.

Percário, S., Moreira, D. R., Gomes, B. A. Q., Ferreira, M. E. S., Gonçalves, A. C. M., Laurindo, P. S. O. C., Vilhena, T. C., Dolabela, M. F., *et al.* (2012) ‘Oxidative stress in Malaria’, *International Journal of Molecular Sciences*, 13(12), pp. 16346–16372.

Pérez González, C., Serrano Vega, R., González-Chávez, M., Zavala Sánchez, M. A. and Pérez Gutiérrez, S. (2013) ‘Anti-inflammatory activity and composition of *Senecio salignus* Kunth’, *BioMed Research International*, 2013, p. 814693.

Piechowska, P., Zawirska-Wojtasiak, R. and Mildner-Szkudlarz, S. (2019) ‘Bioactive β-carbolines in food: A review’, *Nutrients*, 11(4), p. 814.

Pier-Giorgino, P. (2000) ‘Flavonoids as antioxidants’, *Journal of Natural Products*, 63, pp. 1035–1042.

Ponou, B. K., Barboni, L., Teponno, R. B., Mbiantcha, M., Nguelefack, T. B., Park, H. J., Lee, K. T. and Tapondjou, L. A. (2008) ‘Polyhydroxyoleanane-type triterpenoids from *Combretum molle* and their anti-inflammatory activity’, *Phytochemistry Letters*, 1(4), pp. 183–187.

Prampero, P. E. and Cerretelli, P. (1969) ‘Maximal Muscular Power (Aerobic and Anaerobic) in African Natives’, *Ergonomics*, 12(1), pp. 51–59.

Procházková, D., Boušová, I. and Wilhelmová, N. (2011) ‘Antioxidant and prooxidant properties of flavonoids’, *Fitoterapia*, pp. 513–523. doi: 10.1016/j.fitote.2011.01.018.

Ravipati, A. S., Zhang, L., Koyyalamudi, S. R., Jeong, S. C., Reddy, N., Bartlett, J., Smith, P. T., Shanmugam, K., *et al.* (2012) ‘Antioxidant and anti-inflammatory activities of selected Chinese medicinal plants and their relation with antioxidant content’, *BMC Complementary and Alternative Medicine*, 12, p. 173.

Rebecca, W., Kayser, O., Hagels, H., Zessin, K. H., Madundo, M. and Gamba, N. (2003) ‘The phytochemical profile and identification of main phenolic compounds from the leaf exudate of *Aloe secundiflora* by high-performance liquid chromatography-mass spectroscopy’, *Phytochemical Analysis*, 14(2), pp. 83–86.

Di Rosa, M., Giroud, J. P. and Willoughby, D. A. (1971) ‘Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine’, *The Journal of Pathology*, 104(1), pp. 15–29.

Ruggiero, C., Ehrenshaft, M., Cleland, E. and Stadler, K. (2011) ‘High-fat diet induces an initial adaptation of mitochondrial bioenergetics in the kidney despite evident oxidative stress and mitochondrial ROS production’, *American Journal of Physiology - Endocrinology and Metabolism*, 300(6), pp. E1047–E1058.

Sakat, S. S., Mani, K., Demidchenko, Y. O., Gorbunov, E. A., Tarasov, S. A., Mathur, A. and

Epstein, O. I. (2014) ‘Release-active dilutions of diclofenac enhance anti-inflammatory effect of diclofenac in carrageenan-induced rat paw edema model’, *Inflammation*, 37(1), pp. 1–9.

Saleem, N. A., Nour, M. El and Adam., K. M. (2012) ‘The Nutritive Value of Leaves and Fruits of Three Grewia Species under Semi-Arid Environment’, *Journal of Agricultural Science and Technology*, 2, pp. 956–964.

Salvemini, D., Wang, Z. Q., Wyatt, P. S., Bourdon, D. M., Marino, M. H., Manning, P. T. and Currie, M. G. (1996) ‘Nitric oxide: A key mediator in the early and late phase of carrageenan-induced rat paw inflammation’, *British Journal of Pharmacology*, 118(4), pp. 829–38.

Sanwal, R. and Chaudhary, A. K. (2011) ‘Wound healing and antimicrobial potential of *Carissa spinarum* Linn. in albino mice’, *Journal of Ethnopharmacology*, 135, pp. 792–796.

Sarkar, P., Lohith, K. D. H., Dhumal, C., Panigrahi, S. S. and Choudhary, R. (2015) ‘Traditional and ayurvedic foods of Indian origin’, *Journal of Ethnic Foods*, 2(3), pp. 97–109.

Sawadogo, W. R., Le Douaron, G., Maciuk, A., Bories, C., Loiseau, P. M., Figadère, B., Guissou, I. P. and Nacoulma, O. G. (2012) ‘In vitro antileishmanial and antitrypanosomal activities of five medicinal plants from Burkina Faso’, *Parasitology Research*, 110(5), pp. 1779–1783.

Scalbert, A., Manach, C., Morand, C., Rémésy, C. and Jiménez, L. (2005) ‘Dietary polyphenols and the prevention of diseases’, *Critical Reviews in Food Science and Nutrition*, 45(4), pp. 287–306.

Schmid-Schönbein, G. W. (2006) ‘Analysis of Inflammation’, *Annual Review of Biomedical Engineering*, 8(1), pp. 93–151.

Schweitzer, A., Hasler-Nguyen, N. and Zijlstra, J. (2009) ‘Preferential uptake of the non steroid anti-inflammatory drug diclofenac into inflamed tissues after a single oral dose in rats’, *BMC Pharmacology*, 9(5), pp. 1–9.

Scio, E., Mendes, R. F., Motta, E. V. S., Bellozi, P. M. Q., Aragao, D. M. O., Mello, J., Fabri, R. L., Moreira, J. R., *et al.* (2012) ‘Antimicrobial and antioxidant activities of some plant

extracts.', in *Phytochem. Nutraceuticals: Global Approaches Their Role Nutr. Health*, pp. 21–42.

Seno, S. K. O. and Tome, S. (2013) 'Socioeconomic and ecological viability of pastoralism in Loitokitok district, Southern Kenya', *Nomadic Peoples*, 17(1), pp. 66–86.

Seo, K. Il, Choi, M. S., Jung, U. J., Kim, H. J., Yeo, J., Jeon, S. M. and Lee, M. K. (2008) 'Effect of curcumin supplementation on blood glucose, plasma insulin, and glucose homeostasis related enzyme activities in diabetic db/db mice', *Molecular Nutrition and Food Research*, 52(9), pp. 995–1004.

Serhan, C. N. (2007) 'Resolution Phase of Inflammation: Novel Endogenous Anti-Inflammatory and Proresolving Lipid Mediators and Pathways', *Annual Review of Immunology*, 21(2), pp. 325–332.

Sharma, J. N., Samud, A. M. and Asmawi, M. Z. (2004) 'Comparison between plethysmometer and micrometer methods to measure acute paw oedema for screening anti-inflammatory activity in mice', *Inflammopharmacology*, 12(1), pp. 89–94.

Shettar, A. K., Kotresha, K., Kaliwal, B. B. and Vedamurthy, A. B. (2015) 'Evaluation of in vitro antioxidant and anti-inflammatory activities of *Ximenia americana* extracts', *Asian Pacific Journal of Tropical Disease*, 5(11), pp. 918–923.

Siddhuraju, P. and Becker, K. (2007) 'The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts', *Food Chemistry*, 101(1), pp. 10–19.

Da Silva-Leite, K. E. S., Assreuy, A. M. S., Mendonça, L. F., Damasceno, L. E. A., De Queiroz, M. G. R., Mourão, P. A. S., Pires, A. F. and Pereira, M. G. (2017) 'Polysaccharide rich fractions from barks of *Ximenia americana* inhibit peripheral inflammatory nociception in mice: antinociceptive effect of *Ximenia americana* polysaccharide rich fractions', *Brazilian Journal of Pharmacognosy*, 27(3), pp. 339–345.

Singh, R., Singh, B., Singh, S., Kumar, N., Kumar, S. and Arora, S. (2010) 'Umbelliferone - An antioxidant isolated from *Acacia nilotica* (L.) Willd. Ex. Del.', *Food Chemistry*, 120(3), pp. 825–830.

Singleton, V. L. and Rossi Jr, J. A. (1965) ‘Colorimetry of total phenolics with phosphomolybdic-phosphothungstic acid reagents’, *American Journal of Enology & Viticulture*, 16(3), pp. 144–158.

Sivakumar, S. and Subramanian, S. P. (2009) ‘D-pinitol attenuates the impaired activities of hepatic key enzymes in carbohydrate metabolism of streptozotocin-induced diabetic rats’, *General Physiology and Biophysics*, 28(3), pp. 233–241.

Skrinjar, M. and Nemet, N. (2009) ‘Antimicrobial effects of spices and herbs essential oils’, *Acta Periodica Technologica*, (40), pp. 195–209.

Souto, A. L., Tavares, J. F., Da Silva, M. S., De Diniz, M. F. F. M., De Athayde-Filho, P. F. and Barbosa Filho, J. M. (2011) ‘Anti-inflammatory activity of alkaloids: An update from 2000 to 2010’, *Molecules*, 16(1), pp. 8515–8534.

Sreeramulu, D., Reddy, C. V. K., Chauhan, A., Balakrishna, N. and Raghunath, M. (2013) ‘Natural antioxidant activity of commonly consumed plant foods in India: Effect of domestic processing’, *Oxidative Medicine and Cellular Longevity*, 2013, p. 369479.

Srinivasan, K. (2005) ‘Spices as influencers of body metabolism: An overview of three decades of research’, *Food Research International*, 38(1), pp. 77–86.

Ssegawa, P. and Kasenene, J. M. (2007) ‘Medicinal plant diversity and uses in the Sango bay area, Southern Uganda’, *Journal of Ethnopharmacology*, 113(3), pp. 521–540.

Stannard, J. (1982) ‘Medicinal plants and folk remedies in Pliny, Historia naturalis.’, *History and philosophy of the life sciences*, 4, pp. 3–23.

Storz, P. (2007) ‘Reactive oxygen species in tumor progression’, *Frontiers in Bioscience*, 10, pp. 1881–1896.

Sultana, B., Anwar, F. and Ashraf, M. (2009) ‘Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts.’, *Molecules*, 14(6), pp. 2167–2180.

Sultana, B., Anwar, F. and Przybylski, R. (2007) ‘Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. trees’, *Food Chemistry*, 104(3), pp. 1106–1114.

Sur, G., Floca, E., Kudor-Szabadi, L., Sur, M. L., Sur, D. and Samasca, G. (2014) 'The relevance of inflammatory markers in metabolic syndrome.', *Maedica*, 9(1), pp. 15–18.

Suzuki, N., Rivero, R. M., Shulaev, V., Blumwald, E. and Mittler, R. (2014) 'Abiotic and biotic stress combinations', *New Phytologist*, 203(1), pp. 32–43.

Tabuti, J. R. S., Kukunda, C. B., Kaweesi, D. and Kasilo, O. M. J. (2012) 'Herbal medicine use in the districts of Nakapiripirit, Pallisa, Kanungu, and Mukono in Uganda', *Journal of Ethnobiology and Ethnomedicine*, 8(1), p. 35.

Talle, A. (2007) '"Serious Games": Licences and Prohibitions in Maasai Sexual Life', *Africa: The Journal of the International African Institute*, 77(3), pp. 351–370.

Tao, J., Zhao, Y. Q., Chi, C. F. and Wang, B. (2018) 'Bioactive Peptides from Cartilage Protein Hydrolysate of Spotless Smoothhound and Their Antioxidant Activity In Vitro', *Marine Drugs*, 16(4), p. 100.

Tian, F., Li, B., Ji, B., Yang, J., Zhang, G., Chen, Y. and Luo, Y. (2009) 'Antioxidant and antimicrobial activities of consecutive extracts from *Galla chinensis*:The polarity affects the bioactivities', *Food Chemistry*, 113(1), pp. 173–179.

Tiong, S. H., Looi, C. Y., Hazni, H., Arya, A., Paydar, M., Wong, W. F., Cheah, S. C., Mustafa, M. R., *et al.* (2013) 'Antidiabetic and antioxidant properties of alkaloids from *Catharanthus roseus* (L.) G. Don', *Molecules*, 18, pp. 9770–9784.

Tudek, B., Winczura, A., Janik, J., Siomek, A., Foksinski, M. and Oliński, R. (2010) 'Involvement of oxidatively damaged DNA and repair in cancer development and aging', *American Journal of Translational Research*, 2(3), pp. 254–284.

Turner, M. D., Nedjai, B., Hurst, T. and Pennington, D. J. (2014) 'Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease', *Biochimica et Biophysica Acta - Molecular Cell Research*, 1843(11), pp. 2563–2582.

Ullah, W., Uddin, G. and Siddiqui, B. S. (2012) 'Ethnic uses, pharmacological and phytochemical profile of genus *Grewia'*, *Journal of Asian Natural Products Research*, 14(2), pp. 186–195.

Uri Gneezy, Kenneth L., J. A. (2009) 'Gender Differences in Competition: Evidence From a

Matrilineal and a Patriarchal Society', *Econometrica*, 77(5), pp. 1637–1664.

Valgimigli, L., Amorati, R., Fumo, M. G., DiLabio, G. A., Pedulli, G. F., Ingold, K. U. and Pratt, D. A. (2008) 'The unusual reaction of semiquinone radicals with molecular oxygen', *Journal of Organic Chemistry*, 73(5), pp. 1830–1841.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M. and Telser, J. (2007) 'Free radicals and antioxidants in normal physiological functions and human disease', *International Journal of Biochemistry and Cell Biology*, 39(1), pp. 44–84.

Venderley, A. M. and Campbell, W. W. (2006) 'Vegetarian diets: Nutritional considerations for athletes', *Sports Medicine*, 36(4), pp. 293–305.

Vetrichelvan, T. and Jegadeesan, M. (2003) 'Effect of alcohol extract of *Achyranthes aspera* Linn. on acute and subacute inflammation', *Phytotherapy Research*, 17(1), pp. 77–79.

Vitolins, M. Z., Griffin, L., Tomlinson, W. V., Vuky, J., Adams, P. T., Moose, D., Frizzell, B., Lesser, G. J., *et al.* (2013) 'Randomized trial to assess the impact of venlafaxine and soy protein on hot flashes and quality of life in men with prostate cancer', *Journal of Clinical Oncology*, 31(32), pp. 4092–4098.

Voehringer, D. W., Hirschberg, D. L., Xiao, J., Lu, Q., Roederer, M., Lock, C. B., Herzenberg, L. A., Steinman, L., *et al.* (2002) 'Gene microarray identification of redox and mitochondrial elements that control resistance or sensitivity to apoptosis', *Proceedings of the National Academy of Sciences*, 97(6), pp. 2680–2685.

Wagate, C. G., Mbaria, J. M., Gakuya, D. W., Nanyingi, M. O., Kareru, P. G., Njuguna, A., Gitahi, N., Macharia, J. K., *et al.* (2010) 'Screening of some Kenyan medicinal plants for antibacterial activity', *Phytotherapy Research*, 24(1), pp. 150–153.

Wagh, K., Bhatia, A., Alexe, G., Reddy, A., Ravikumar, V., Seiler, M., Boemo, M., Yao, M., *et al.* (2012) 'Lactase Persistence and Lipid Pathway Selection in the Maasai', *PLoS ONE*, 7(9), p. e44751.

Walker, B. H., Ludwig, D., Holling, C. S. and Peterman, R. M. (1981) 'Stability of Semi-Arid Savanna Grazing Systems', *The Journal of Ecology*, 69(2), p. 473.

Wambugu, S. N., Mathiu, P. M., Gakuya, D. W., Kanui, T. I., Kabasa, J. D. and Kiama, S. G.

(2011) ‘Medicinal plants used in the management of chronic joint pains in Machakos and Makueni counties, Kenya’, *Journal of Ethnopharmacology*, 137(2), pp. 945–955.

Wang, J. and Wang, H. (2017) ‘Oxidative Stress in Pancreatic Beta Cell Regeneration’, *Oxidative Medicine and Cellular Longevity*, 2017, p. 1930261.

Wang, Li, Q. and Bi, K. shun (2018) ‘Bioactive flavonoids in medicinal plants: Structure, activity and biological fate’, *Asian Journal of Pharmaceutical Sciences*, 13(1), pp. 12–23.

Wangteeraprasert, R., Lipipun, V., Gunaratnam, M., Neidle, S., Gibbons, S. and Likhitwitayawuid, K. (2012) ‘Bioactive compounds from *Carissa spinarum*’, *Phytotherapy Research*, 26(10), pp. 1496–1499.

Warrington, R., Watson, W., Kim, H. L. and Antonetti, F. R. (2011) ‘An introduction to immunology and immunopathology’, *Allergy, Asthma & Clinical Immunology*, 7(S1), p. S1.

Wikipedia (2020) *Grewia villosa*, *Wikipedia*. Available at: https://en.wikipedia.org/wiki/Grewia_villosa (Accessed: 14 August 2020).

Won Jeong Lee, Jae Sun Moon, Sung In Kim, Young Tae Kim, Oyekanmi Nash, Yong-Sun Bahn, S. U. K. (2014) ‘Inhibition of the Calcineurin Pathway by Two Tannins, Chebulagic Acid and Chebulanin, Isolated from *Harrisonia abyssinica* Oliv.’, *Journal of Microbiology and Biotechnology*, 24(10), pp. 1377–1381.

Wu, C., Xu, Z., Gai, R. and Huang, K. (2016) ‘Matrine ameliorates spontaneously developed colitis in interleukin-10-deficient mice’, *International Immunopharmacology*, 36, pp. 256–262.

Wu, J. H., Tung, Y. T., Chien, S. C., Wang, S. Y., Kuo, Y. H., Shyur, L. F. and Chang, S. T. (2008) ‘Effect of phytocompounds from the heartwood of *Acacia confusa* on inflammatory mediator production’, *Journal of Agricultural and Food Chemistry*, 56(5), pp. 1567–1573.

Yahfoufi, N., Alsadi, N., Jambi, M. and Matar, C. (2018) ‘The immunomodulatory and anti-inflammatory role of polyphenols’, *Nutrients*, 10(11).

Yang, Z., Garcia, A., Xu, S., Powell, D. R., Vertino, P. M., Singh, S. and Marcus, A. I. (2013) ‘*Withania somnifera* Root Extract Inhibits Mammary Cancer Metastasis and Epithelial to Mesenchymal Transition’, *PLoS ONE*, 8(9).

Yordanov, I., Velikova, V. and Tsonev, T. (2000) ‘Plant responses to drought, acclimation, and stress tolerance’, *Photosynthetica*, 38(2), pp. 171–186.

Yoshida, N., Yoshikawa, T., Yamaguchi, T., Naito, Y., Tanigawa, T., Murase, H. and Kondo, M. (1999) ‘A novel water-soluble vitamin E derivative protects against experimental colitis in rats.’, *Antioxidants & redox signaling*, 1(4), pp. 555–562.

Younes, M. (1999) ‘Free Radicals and Reactive Oxygen Species’, in *Toxicology*, pp. 111–125.

Young, A. J. and Lowe, G. L. (2018) ‘Carotenoids—antioxidant properties’, *Antioxidants*, 7(2), p. 28.

Zhang, J. K., Yang, L., Meng, G. L., Yuan, Z., Fan, J., Li, D., Chen, J. Z., Shi, T. Y., *et al.* (2013) ‘Protection by Salidroside against Bone Loss via Inhibition of Oxidative Stress and Bone-Resorbing Mediators’, *PLoS ONE*, 8(2).

Zhang, X., Jin, L., Cui, Z., Zhang, C., Wu, X., Park, H., Quan, H. and Jin, C. (2016) ‘Antiparasitic effects of oxymatrine and matrine against *Toxoplasma gondii* in vitro and in vivo’, *Experimental Parasitology*, 165, pp. 95–102.

Zhang, Y., Liang, Y. and He, C. (2017) ‘Anticancer activities and mechanisms of heat-clearing and detoxicating traditional Chinese herbal medicine’, *Chinese Medicine*, 12(12), p. 20.

Zhao, J., Maitituersun, A., Li, C., Li, Q., Xu, F. and Liu, T. (2018) ‘Evaluation on analgesic and anti-inflammatory activities of total flavonoids from *Juniperus sabina*’, *Evidence-based Complementary and Alternative Medicine*, 2018, p. 7965306.

Zhong, X. shan, Ge, J., Chen, S. wei, Xiong, Y. quan, Ma, S. juan and Chen, Q. (2018) ‘Association between Dietary Isoflavones in Soy and Legumes and Endometrial Cancer: A Systematic Review and Meta-Analysis’, *Journal of the Academy of Nutrition and Dietetics*, 118(4), pp. 637–651.

Zhou, Y., Liu, B., Mbuni, Y., Yan, X., Mwachala, G., Hu, G. and Wang, Q. (2017) ‘Vascular flora of Kenya, based on the Flora of Tropical East Africa’, *PhytoKeys*, 2017(90), pp. 113–126.

Ziech, D., Anestopoulos, I., Hanafi, R., Voulgaridou, G. P., Franco, R., Georgakilas, A. G., Pappa, A. and Panayiotidis, M. I. (2012) 'Pleiotrophic effects of natural products in ROS-induced carcinogenesis: The role of plant-derived natural products in oral cancer chemoprevention', *Cancer Letters*, 327(1–2), pp. 16–25.

# 7.0. APPENDICES

## Appendix 1; Ethical approvals for the study

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

KENYATTA NATIONAL HOSPITAL APPROVED 16 APR 2015 KNH/UoN-ERC P.O. BOX 20723

Ref: KNH-ERC/A/173

16th April, 2015

J.W. Kimondo
Dept. of Pharmacology and Pharmacognosy
School of Pharmacy
University of Nairobi

Dear Kimondo

**Research Proposal: An Evaluation of Antioxidant and Anti-Inflammatory Effects of Natural Foods and Medicinal Plants of the Ilkisonko Maasai Community (P48/01/2015)**

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 16th April 2015 to 15th April 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chair, KNH/UoN-ERC
The Dean, School of Pharmacy
The Chair, Dept. of Pharmacology and Ihamacognosy
Supervisors: Dr. P.C. Mutai, Dr. J.O.O. Miaron, Dr. P.M. Njogu

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref. No.KNH/ERC/R/116

25th June, 2019

J.W. Kimondo
Reg. No.U80/98007/2015
Dept. of Pharmacology and Pharmacognosy
School of Pharmacy
College of Heath Sciences
University of Nairobi

Dear Julia

**Re: Approval of Annual Renewal – An evaluation of Antioxidant and Anti-inflammatory effects of natural foods and Medicinal plants of the Ilkisonko Maasai Community from April 2016 to April 2020' (P48/01/2015)**

Refer to your communication received on 18th June, 2019.

This is to acknowledge receipt of your study progress report and hereby grant you annual extension approval for ethics research protocol P48/01/2015.

The approval dates are 16th April 2019 – 15th April 2020.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN- ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.

g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

**PROF. M.L. CHINDIA**
**SECRETARY, KNH-UON ERC**

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chairperson, KNH-UoN ERC
The Dean, School of Pharmacy, UoN

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref. No.KNH/ERC/R/119

23rd July 2020

Kimondo Julia Wanjiru
PhD Candidate
Reg. No.U80/98007/2015
Dept. of Pharmacology and Pharmacognosy
School of Pharmacy
College of Health Sciences
University of Nairobi

Dear Julia

**Re: Approval of Annual Renewal – An evaluation of Antioxidant and Anti-Inflammatory Effect of natural foods and medicinal plants of the Ilkisonko Maasai Community, Kenya from April 2020 to April 2021 (P48/01/2015)**

Refer to your communication received on 10th July 2020.

This is to acknowledge receipt of the study progress report and hereby grant annual extension of approval for ethical research protocol P48/01/2015.

The approval dates are 16th April 2020 – 15th April 2021.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH- UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH- UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN-Ethics & Research Committee for each batch of shipment.

g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

**PROF. M.L. CHINDIA**
**SECRETARY, KNH-UON ERC**

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chairperson, KNH-UoN ERC
The Dean, School of Pharmacy, UoN

**Appendix 2; Independent Consent Information Document used in the study**

**Title of the research study**: An Evaluation of Antioxidant and Anti-inflammatory Effects of Natural Foods and Medicinal Plants of The Ilkisonko Maasai community.
**Jina la utafiti**: Tathmini ya mimea inayotumika kama vyakula na dawa katika jamii ya Ilkisonko Wamasai Vinavyozuia Vioksidishaji na Kuvimba.

**Principal investigator**: Julia Wanjiru Kimondo, University of Nairobi.
**Mpelelezi mkuu**: Julia Wanjiru Kimondo, Chuo Kikuu cha Nairobi.

**Lead supervisor**: Dr. Jacob Olongida Ole-Miaron, University of Nairobi.
**Kiongozi msimamizi**: Dr. Jacob Olongida Ole-Miaron, Chuo Kikuu cha Nairobi.

**Study Location**: Loitokitok sub-county.
**Eneo la Masomo**: Loitokitok Ndogo-Kata.

**Purpose of the Study**: The study aims to document the traditional plants used as food and medicine, in humans as well as in animals, among the Ilkisonko Maasai in Loitokitok sub-county.
**Madhumuni ya Utafiti**: Utafiti unalenga kurekodi mimea ya kienyeji ambayo hutumika kama chakula na dawa, kwa binadamu na wanyama, miongoni mwa Ilkisonko Wamasai katika Loitokitok Ndogo-Kata.

**Potential Benefits of the study**: The findings of the study will show health benefits of indigenous plants used by the Ilkisonko Maasai, hence encourage their consumption and cultivation. This can become a source of income to the community. The farmers and pastoralists will also be educated on sustainable land practices.
**Faida ya utafiti**: Matokeo ya utafiti yatasaidia kuonyesha faida kwa afya ya mimea ya kienyeji, hivyo kuhamasisha matumizi yao na kilimo. Hii yanaweza kuwa chanzo cha mapato kwa jamii. Wakulima na wafugaji pia wataelimishwa juu ya njia endelevu ya ardhi.

**Potential Risk of the Study**: This study has no potential risk.
**Uwezekano Hatari wa Utafiti**: Utafiti huu hauna uwezekano wa hatari.

**Appendix 3; Participant Consent Form used in the study**

**Title of the research study:** An Evaluation of Antioxidant and Anti-inflammatory Effects of Natural Foods and Medicinal Plants of the Ilkisonko Maasai Community
**Jina la utafiti**: Tathmini ya mimea inayotumika kama vyakula na dawa katika jamii ya Ilkisonko Wamasai Vinavyozuia Vioksidishaji na Kuvimba

**Principal investigator**: Julia Wanjiru Kimondo, University of Nairobi.
**Mpelelezi mkuu**: Julia Wanjiru Kimondo, Chuo Kikuu cha Nairobi.

**Lead supervisor**: Dr. Jacob Olongida Ole-Miaron, University of Nairobi.
**Kiongozi msimamizi**: Dr. Jacob Olongida Ole-Miaron, Chuo Kikuu cha Nairobi.

**Study Location**: Loitokitok sub-county, Kajiado County.
**Eneo la Masomo**: Loitokitok Ndogo-Kata.

**Purpose of the Study**: The purpose of this study is to document the traditional plants used as food and medicine in humans as well as in animals among the Ilkisonko Maasai in Loitokitok sub-county.
**Sababu ya Utafiti**: Utafiti unalenga kurekodi mimea ya kienyeji ambayo hutumika kama chakula na dawa, kwa binadamu na wanyama, miongoni mwa Ilkisonko Wamasai katika Loitokitok Ndogo-Kata.

**Potential Benefits of the study**: This study has several benefits to the community and humanity as a whole. First, it is going to help us document the traditional plants used as food and medicines that promote well being. Second, it will assist us to preserve the environment, increase its resilience while nurturing the beneficial plants. Third, it can encourage cultivation of beneficial plants which can act as a source of income for the community.
**Faida wa Utafiti**: Utafiti huu una faida kadhaa kwa jamii na ubinadamu kwa jumla. Kwanza, huenda ukatusaidia kurekodi mimea ya kale ambayo hutumika kama chakula na dawa yanayo kuza ustawi. Pili, itatusaidia kuhifadhi mazingira, kuongeza ujasiri wake kwa kulea mimea ya faida. Tatu, inaweza kuhimiza kilimo cha mimea ya faida ambayo tutaweza kuyafanya kama chanzo cha mapato kwa jamii

**Potential Risk of the Study**: There is no risk associated with your participation in this study.
**Uwezekano Hatari wa Utafiti**: Hakuna hatari inayohusiana na ushiriki wako katika utafiti huu.

**Contact**: If you have any questions about this study you may ask me now or contact:
Julia Wanjiru Kimondo, P.O. BOX 1136-90100, Machakos and Telephone 0704598539 OR
Jacob Olongida Ole Miaron, P.O Box 29053 - 00625 Nairobi and Telephone 0733123561
**Mawasiliano:** Kama una maswali yeyote kuhusu utafiti huu unaweza kuniuliza sasa au wasiliana na: Julia Wanjiru Kimondo, Sanduku la Posta 1136-90100, Machakos na Simu ya Mkononi 0704598539 AU
Jacob Olongida Ole Miaron, Sanduku la Posta 29053-00625, na Simu ya Mkononi 0733123561

**Approval of the study**: This study will be approved by:
Kenyatta National Hospital and University of Nairobi - Ethics and Research Committee
P. O. BOX 19676 - 00202, Nairobi, Kenya
(254-020) 2726300 Ext 44355
**Idhini ya utafiti**: Utafiti huu utapitishwa na:
Hospitali ya Taifa ya Kenyatta na Chuo Kikuu cha Nairobi - Maadili na Kamati ya Utafiti
Sanduku la Posta 19676-00202, Nairobi, Kenya
(254-020) 2726300 Ext 44355

Should you agree to participate in this study, please sign your name below, indicating that you understand the nature of the study, your responsibilities, inconveniences associated with voluntary participation and that all your questions and concerns have been answered satisfactorily.
Kama umekubali kushiriki katika utafiti huu, tafadhali weka saini na jina lako, kuonyesha ya kwamba umeelewa utafiti huu, majukumu yako, usumbufu uliohusishwa na ushiriki wa hiari na kwamba maswali yako yote yamejibiwa.

**Consent**: I have been fully informed of the purpose of study as well as the benefits and risks associated with it. I had the opportunity to ask questions which were satisfactorily answered. I therefore consent to voluntarily participate in the study.

**Ridhaa**: Nimefahamishwa kikamilifu kuhusu utafiti huu pamoja na faida na hatari zinazohusiana nao. Nilikuwa na fursa ya kuuliza maswali ambayo yalijibiwa vyema. Hivyo basi, mimi nimekubali kushiriki kwa hiari katika utafiti huu.

Name of participant/ Jina la mshiriki……

Signature/ thumb print of participant/ Saini ya mshiriki…….……..

Name of researcher/research assistant /Jina la mtafiti / msaidizi wa utafiti……

Signature /Saini……Date / Tarehe …….

**Appendix 4; Questionnaire used in the study to collect data**

AN EVALUATION OF ANTIOXIDANT AND ANTIINFLAMMATORY EFFECTS OF NATURAL FOODS AND MEDICINAL PLANTS OF THE ILKISONKO MAASAI COMMUNITY

TATHMINI YA MIMEA INAYOTUMIKA KAMA VYAKULA NA DAWA KATIKA JAMII YA ILKISONKO WAMASAI VINAVYOZUIA VIOKSIDISHAJI NA KUVIMBA

Section A/ Sehemu A

1. Name of Interviewer/ Jina la Muulizaji: _____ _____
2. Location/ Eneo_____ Form Number/Namba ya fomu ☐

Section B/ Sehemu B

CLIENT IDENTIFICATION DETAILS / MAELEZO YA KUMTAMBUA ANAYE JIBU

(To be filled by interviewer for eligible clients) / (Litajazwa na muulizaji)

**1.** Initials of Client / Herufi ya Anaye jibu swali _____ _____

Unique Study Number / Namba ya kipekee ☐

**2. Age/ Umri**_____Years/ Miaka

**3. Sex / Jinsia** ☐Male / Mwanaume ☐ Female / Mwanamke

**4.** Highest level of formal Education / Kiwango cha Elimu rasmi

☐ No Formal Education / Hauna Elimu rasmi

☐ Primary Complete / Umekamili shule ya msingi

☐ Secondary Complete / Umekamili shule ya sekondari

☐ Tertiary and above / Umekamili Elimu ya juu

**5.** Occupation/ Kazi

☐ Unemployed/Bila ajira ☐ Self Employed/Umejiajiri

☐ Salaried/Unapata mshahara ☐ Student/Mwanafunzi

☐ Other (Specify)/ Nyingine (taja) _____

Section C/ Sehemu C

TRADITIONAL PLANTS USED (Filled by Interviewer)

MIMEA YA KIENYEJI INAYOTUMIKA (Itajazwa na Muulizaji)

**6.** Do you use wild or semi domesticated plants that have been used in your custom and traditions or are part of a traditional diet? /

Je, umetumia mimea pori ambayo hutumika katika desturi na mila yako au ni sehemu ya mlo wa kienyeji?

☐ Yes / Ndiyo ☐ No / Hapana

**7.** If yes, how often / Kama ndiyo, ni kwa mara ngapi

☐ Daily / Kila siku

☐ Thrice weekly / Mara tatu kila wiki

☐ Once weekly / Mara moja kwa wiki

☐ Once every two weeks / Mara moja kila wiki mbili

☐ Once a month / Mara moja kwa mwezi

☐ Rarely / Mara chache

**8.** I use traditional foods for the following reasons: /

Mimi hutumia vyakula vya kienyeji kwa sababu zifuatazo:

☐ Nutrition / Kujilisha

☐ Keeps diseases away / Kuzuia magonjwa

☐ To treat certain ailments / Kutibu maradhi fulani

☐ For general wellbeing / Kwa ustawi wa mwili kijumla

☐ Other / Nyingine

Answer / Jibu: _____

**9.** Regarding traditional foods, fill in the table below stating the plant used (local name), reason for use, part used, method of preparation and availability.

Kuhusu vyakula vya kienyeji, jaza katika jedwali ifuatayo kwa kusema mmea unaotumika (jina la kiasilia), kwa sababu gani unatumika, sehemu ambayo hutumika, maandalizi, mmea wa kienyeji au kigeni na upatikanaji.

| Plant/ Mmea | Reason for use /matumizi | Part used/ sehemu ambayo hutumika | Method of preparation/ maandalizi | Indigenous or exotic/ kienyeji au kigeni | Availability/ upatikanaji |
|---|---|---|---|---|---|
| | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | |

**10.** Regarding traditional plant foods used for treatment, fill in the table below stating the plant used (local name), ailment treated, part used, method of preparation, length of use and availability.

Kuhusu vyakula vya kienyeji vinavyo tumika kwa matibabu, jaza katika jedwali ifuatayo kwa kusema mmea unaotumika (jina la kiasilia), maradhi inayotibu, sehemu inayotumika, maandalizi na upatikanaji.

| Plant/ Mmea | Ailment treated/ Maradhi inayotibu | Part used/ Sehemu inayotumika | Method of preparation/ Maandalizi | Length of use/ Muda wa matumizi | Indigenous or exotic/ kienyeji au kigeni |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |

**11.** Regarding traditional plant foods used as ethnoveterinary medicine, fill in the table below stating the plant used (local name), reason for use, part used and method of preparation.

Kuhusu vyakula vya kienyeji vinavyo tumika kwa kutibu wanyama, jaza katika jedwali ifuatayo kwa kusema mmea unaotumika (jina la kiasilia), maradhi inayotibu, sehemu inayotumika, maandalizi na upatikanaji.

| Plant/ Mmea | Ailment treated /Maradhi inayotibu | Part used/ Sehemu inayotumika | Method of preparation/ Maandalizi | Length of use/ Muda wa matumizi | Indigenous or exotic/ Kienyeji au kigeni |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |

**12.** For treatment, do you use; Wakati wa matibabu, unatumia;

- ☐ Traditional medicine alone/ Dawa ya kienyeji peke yake
- ☐ In combination with western medicine/ Dawa ya kienyeji pamoja na ya kisasa

**13.** How do you use them / Unazitumia namna gani?

I. ☐ I use traditional medicine first and if it fails I go to the western medicine

Kwanza mimi hutumia dawa za kienyeji, ikishindwa, natumia dawa ya kisasa

II. ☐ I use traditional and western medicine together

Mimi hutumia dawa za kienyeji na dawa za kisasa pamoja

III. ☐ I use western medicine first and then if it fails I go to traditional foods

Kwanza mimi hutumia dawa za magharibi, ikishindwa, natumia dawa za kienyeji

IV. ☐ For the traditional medicine, I use one kind of herb

Kwa dawa za kienyeji, mimi hutumia aina moja ya mimea

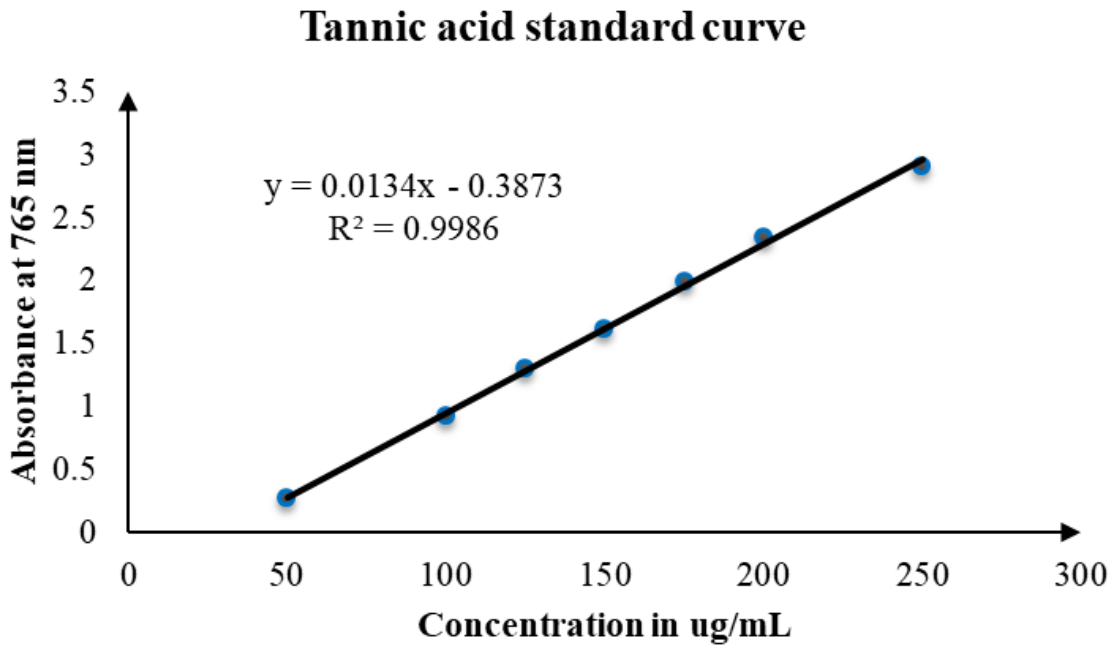
V. ☐ For the traditional foods, I use multiple herbs.

Kwa dawa za kienyeji, mimi hutumia aina nyingi ya mimea

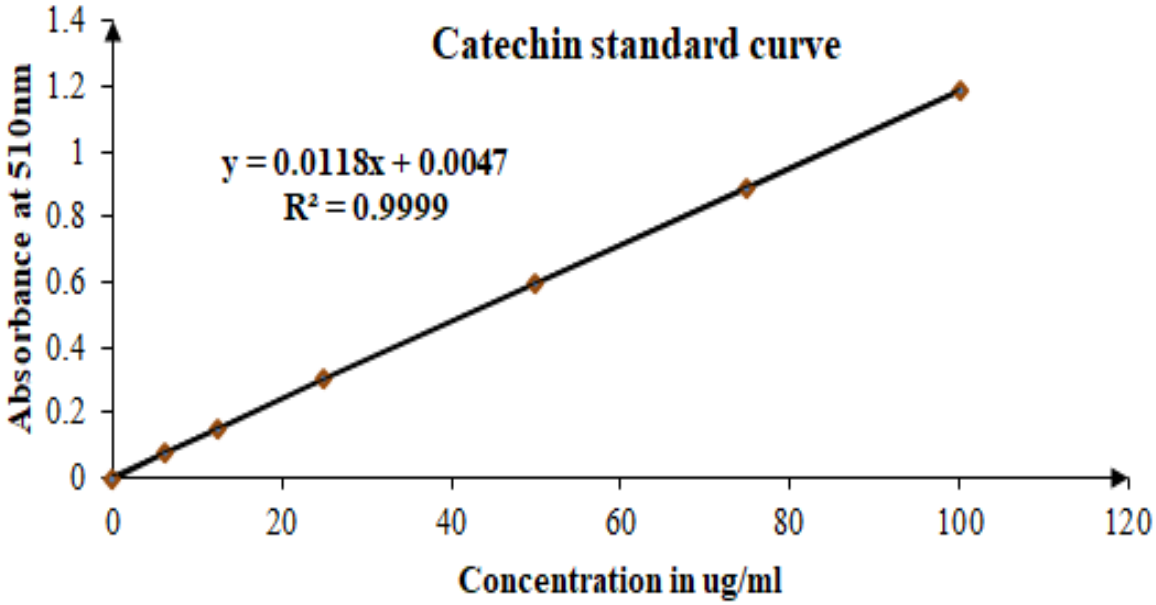
**14.** Clarify the conditions under which treatment of I, II or III occurs (is it dependent on seriousness, acute disease, chronic diseases?)

Fafanua hali ambayo matibabu ya magonjwa kwa sehemu I, II au III huwa inategemea (Je, hutegemea uzito wa ugonjwa, ugonjwa papo hapo, magonjwa sugu?).

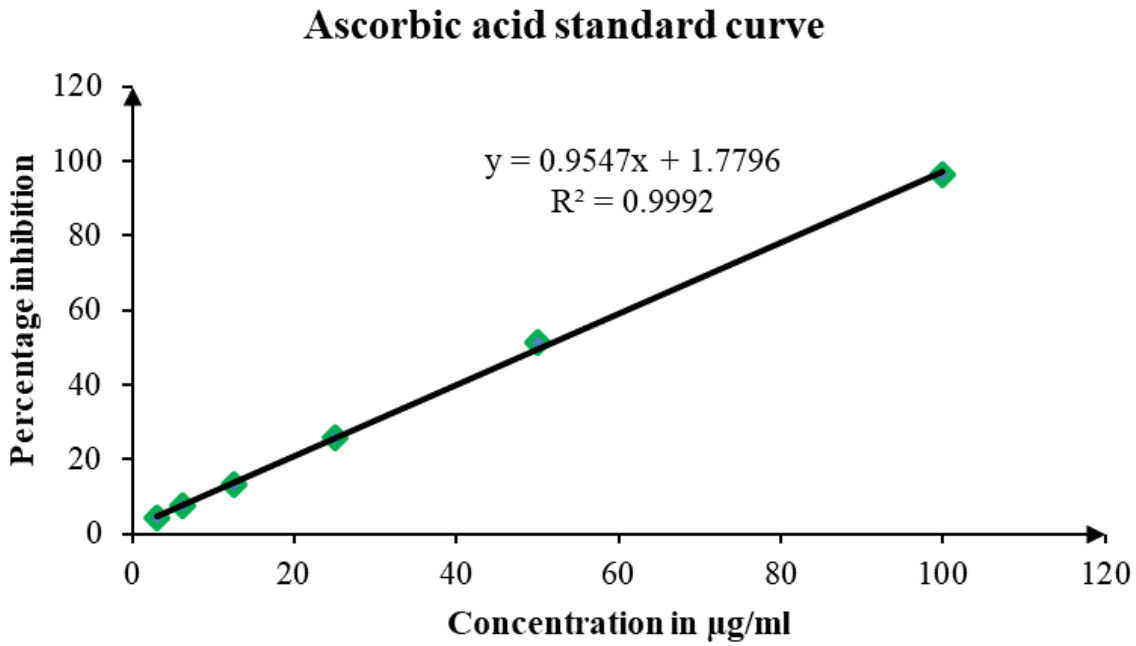
**Appendix 5; Standard curves used in the phytochemical screening**




Tannic acid standard curve used to calculate total phenolic content

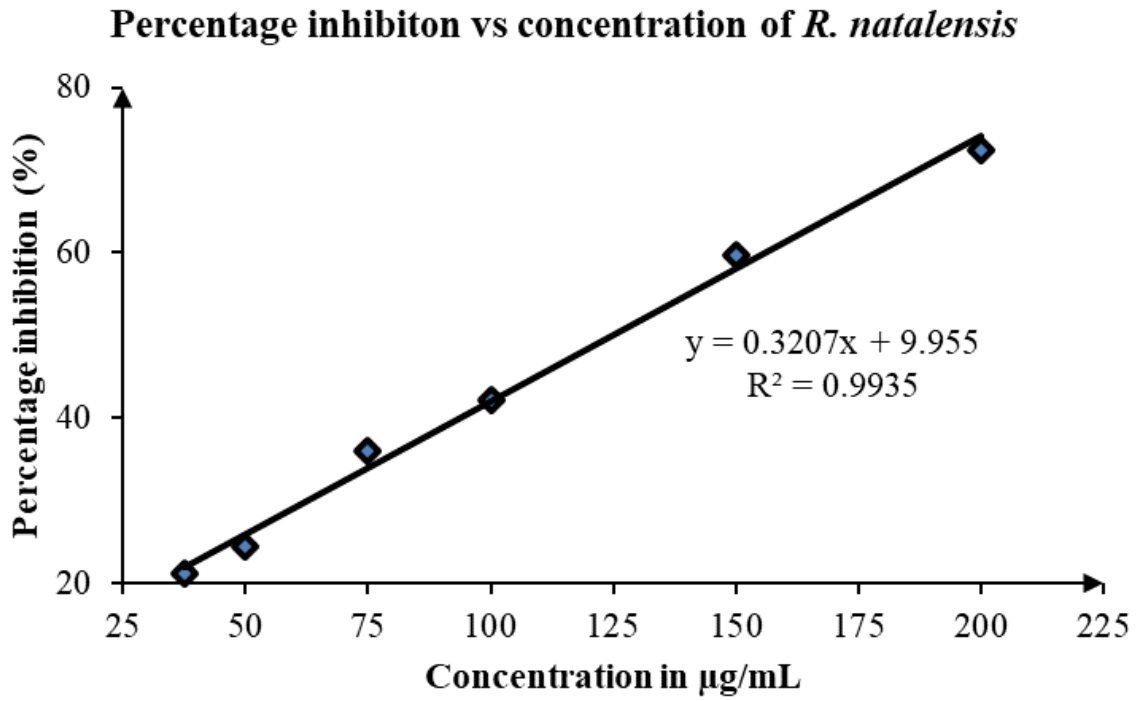



Catechin standard curve used to calculate total flavonoid content

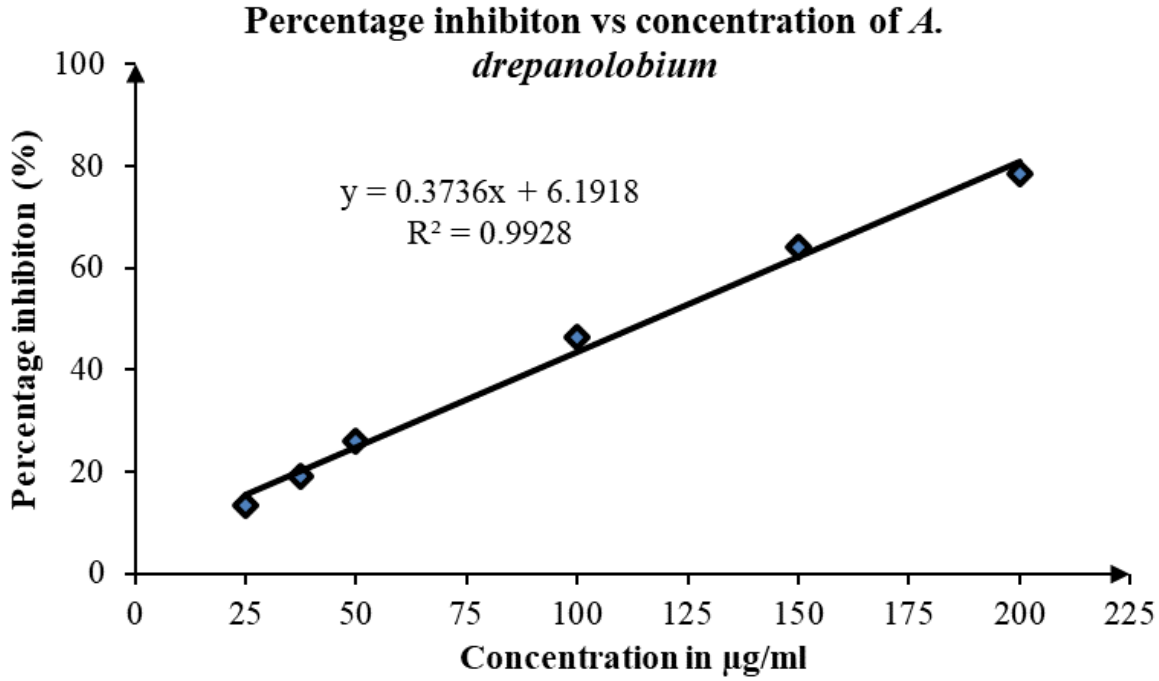



Ascorbic acid standard curve used to compare the antioxidant activity of several extracts

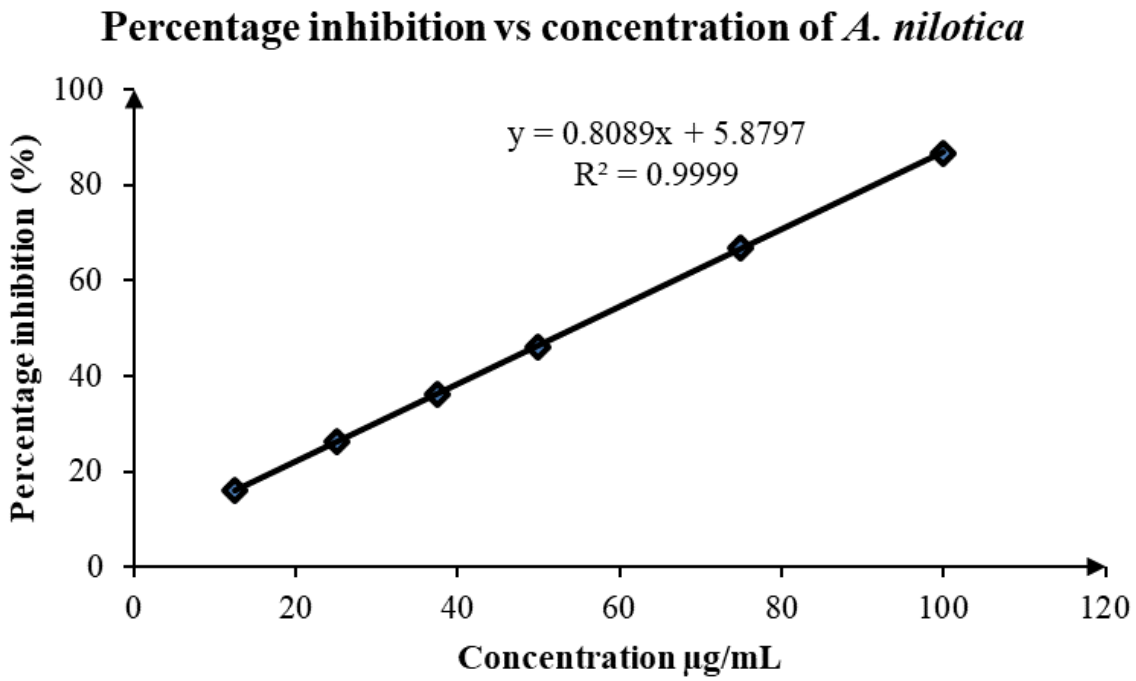
**Appendix 6; Percentage inhibition versus concentration curves for selected plant extracts from the study and scatter plots**



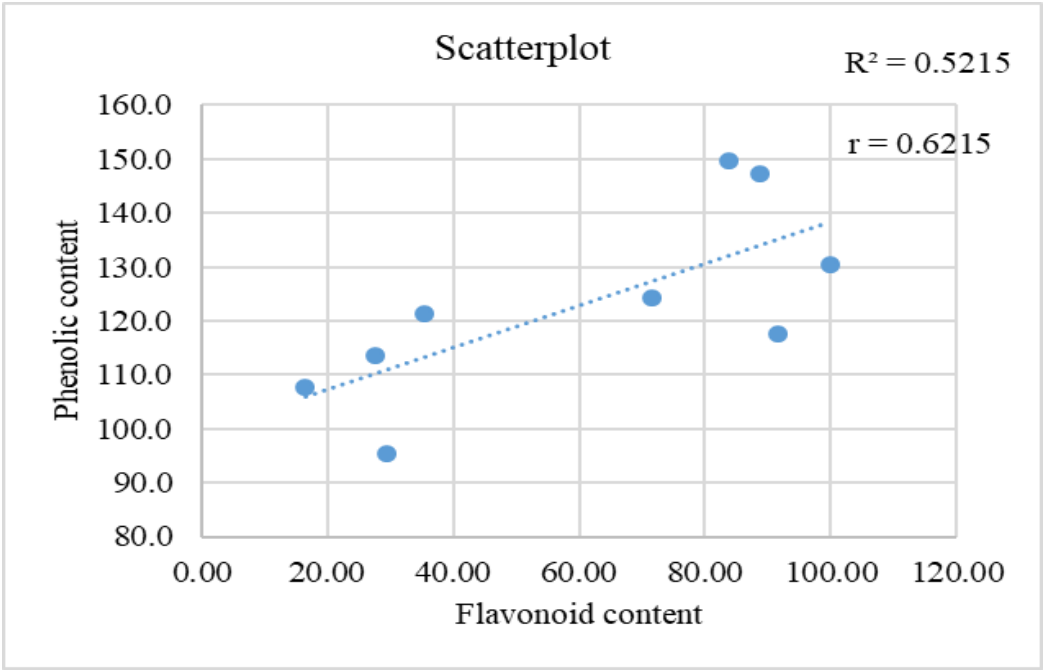

Percentage inhibition versus concentration of *Rhus natalensis* to calculate its antioxidant activity.

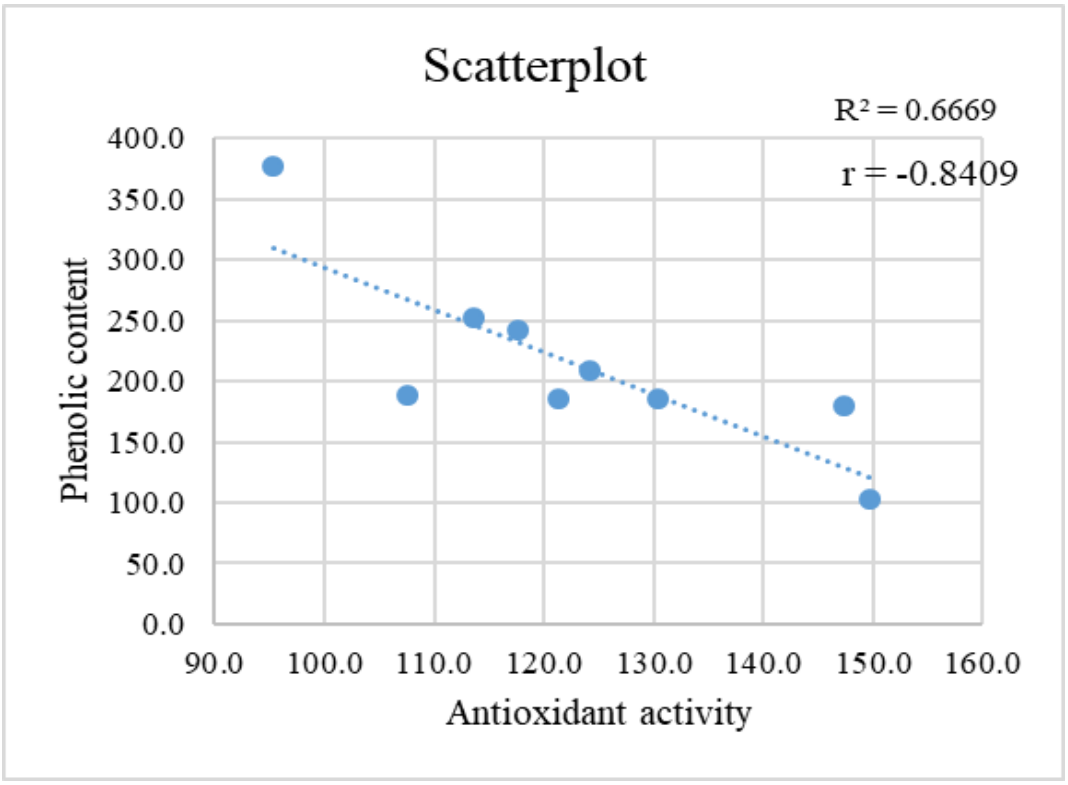


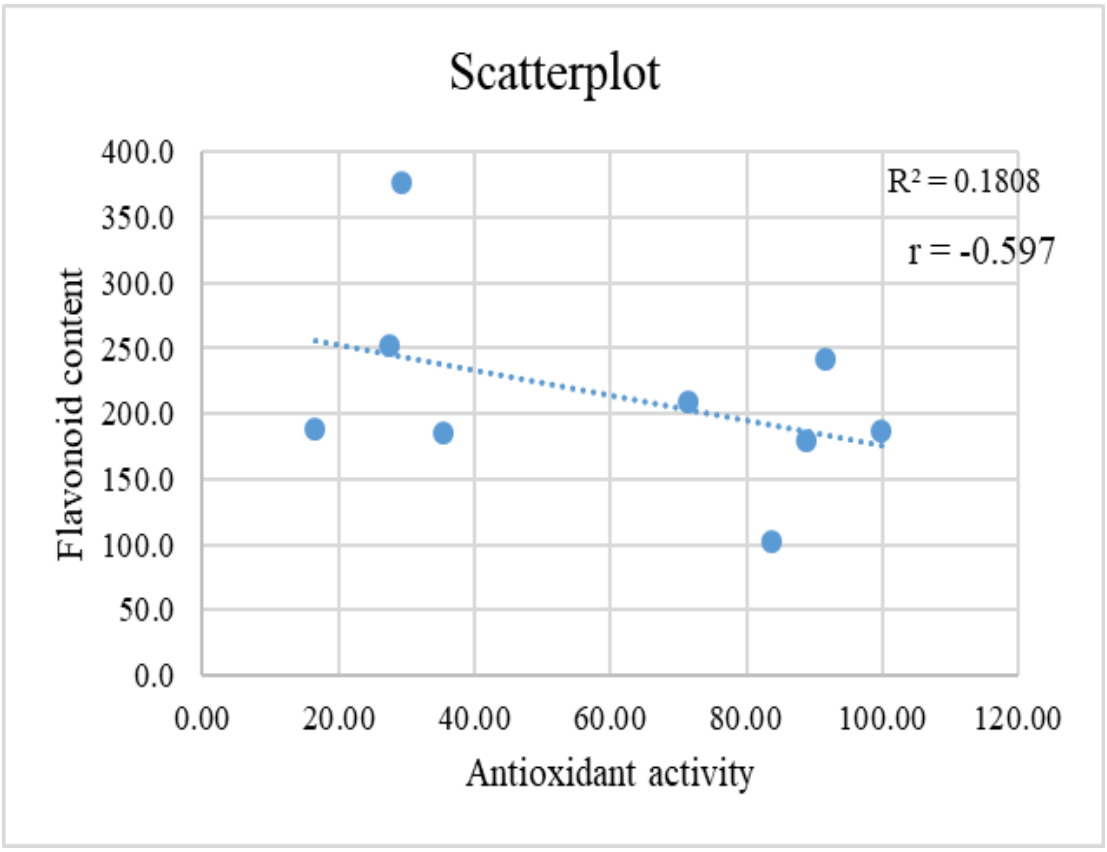

Percentage inhibition versus concentration of *Acacia drepanolobium* to calculate its antioxidant activity.



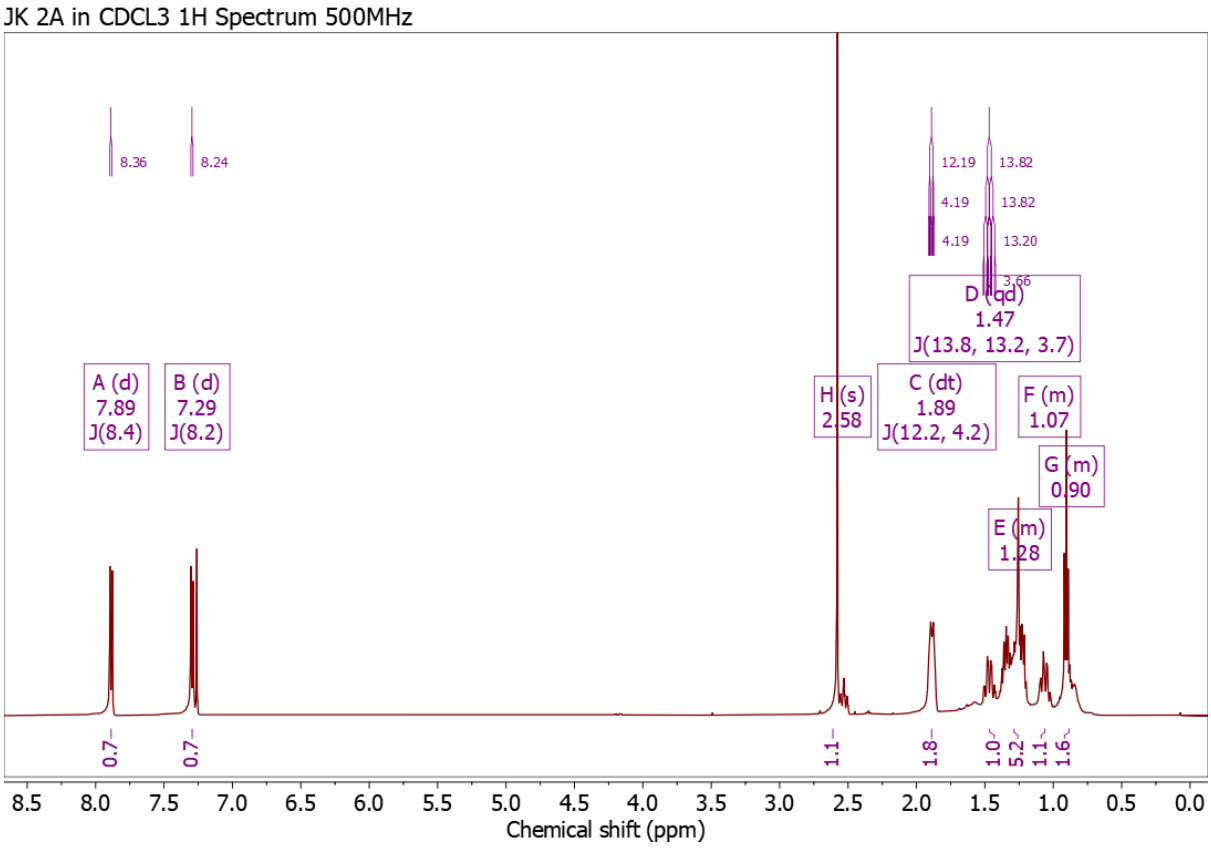

Percentage inhibition versus concentration of *Acacia nilotica* to calculate its antioxidant activity.



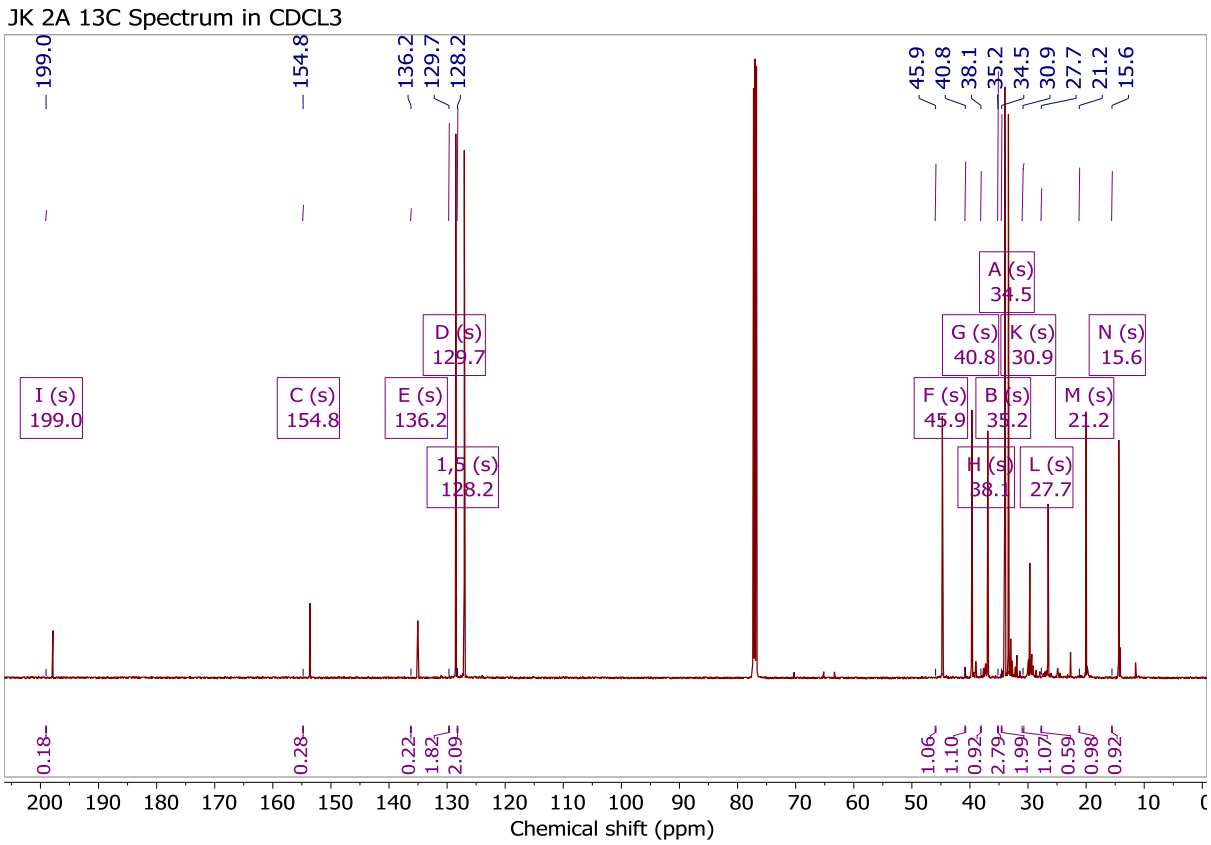

Scatterplot
R² = 0.6669
r = -0.8409
Phenolic content
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
Antioxidant activity


Scatterplot
R² = 0.1808
r = -0.597
Flavonoid content
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
0.00
20.00
40.00
60.00
80.00
100.00
120.00
Antioxidant activity

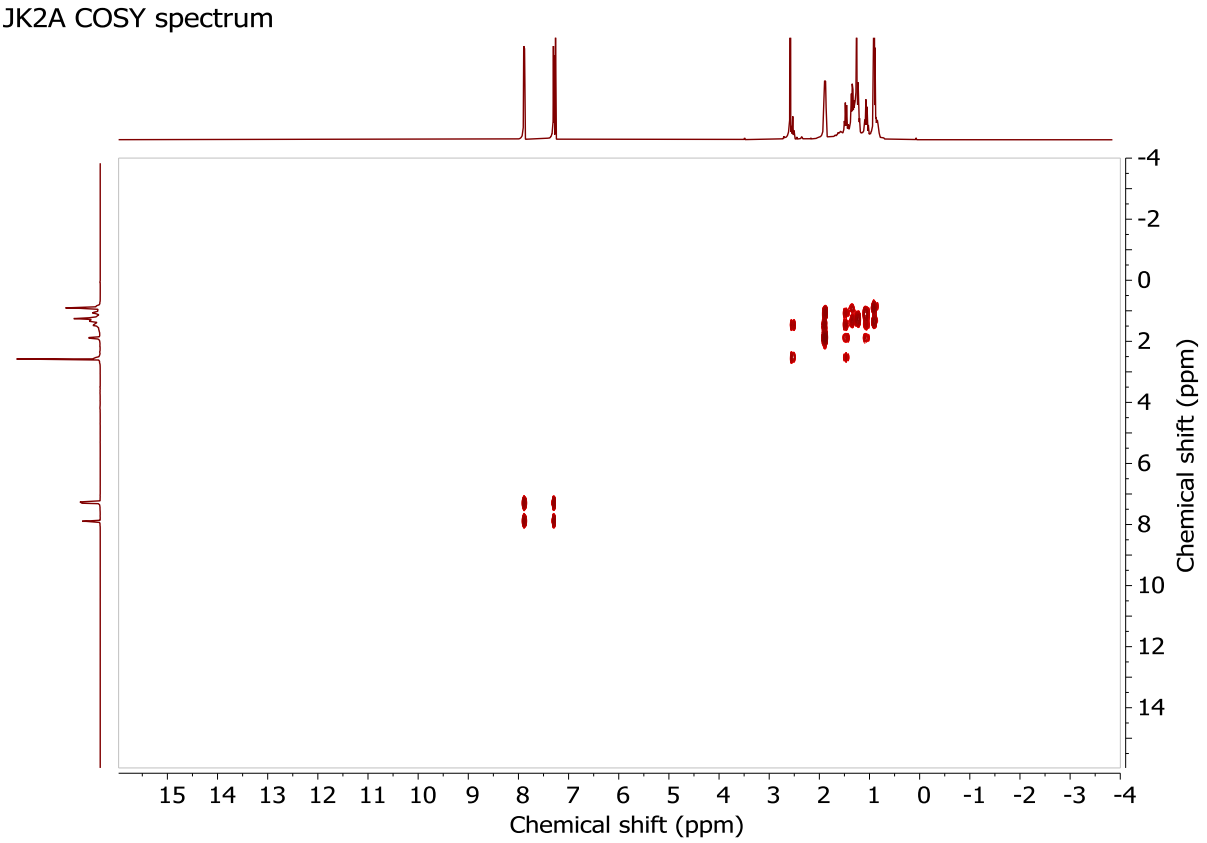
**Appendix 7; $^{1}H$ NMR spectrum for compound JK 2A (500 MHz; $CDCL_3$)**



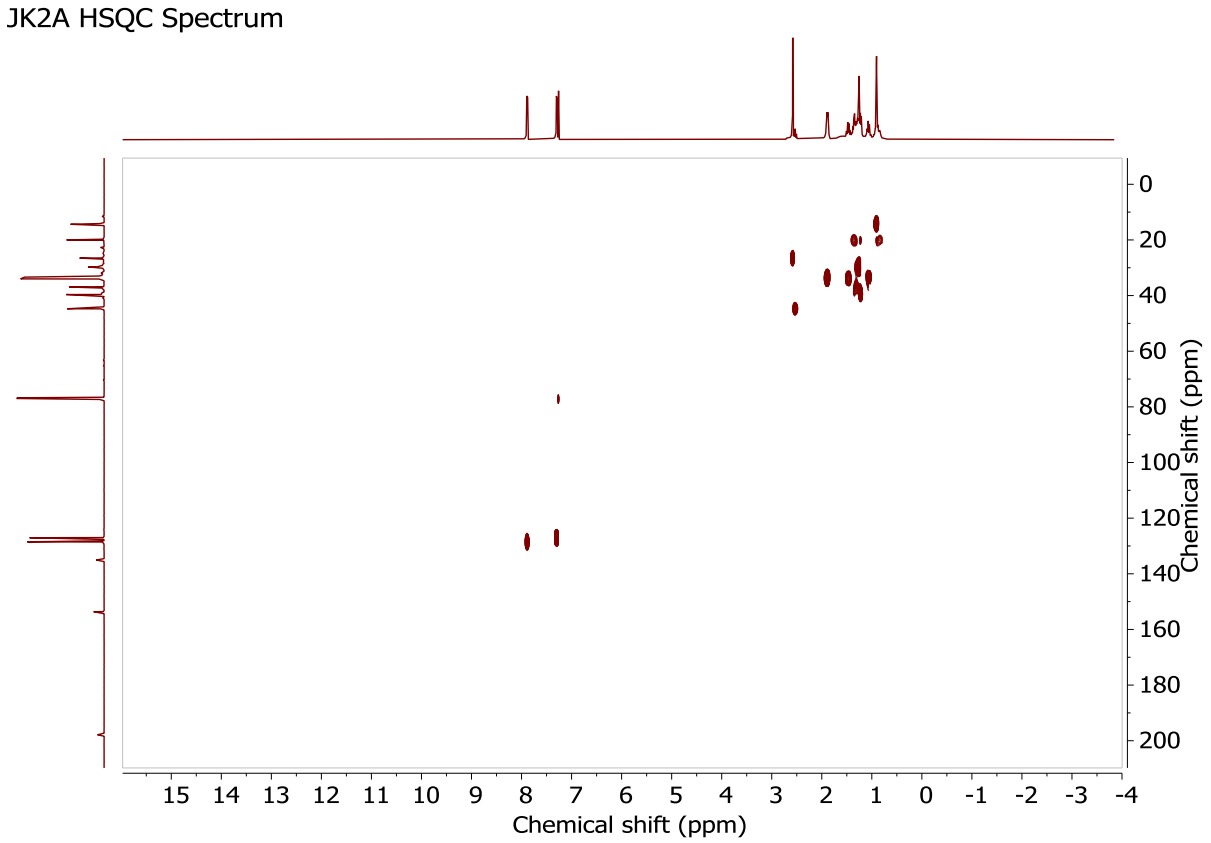
## Appendix 8; $^{13}C$ NMR spectrum for compound JK 2A (125 MHz; $CDCL_3$)



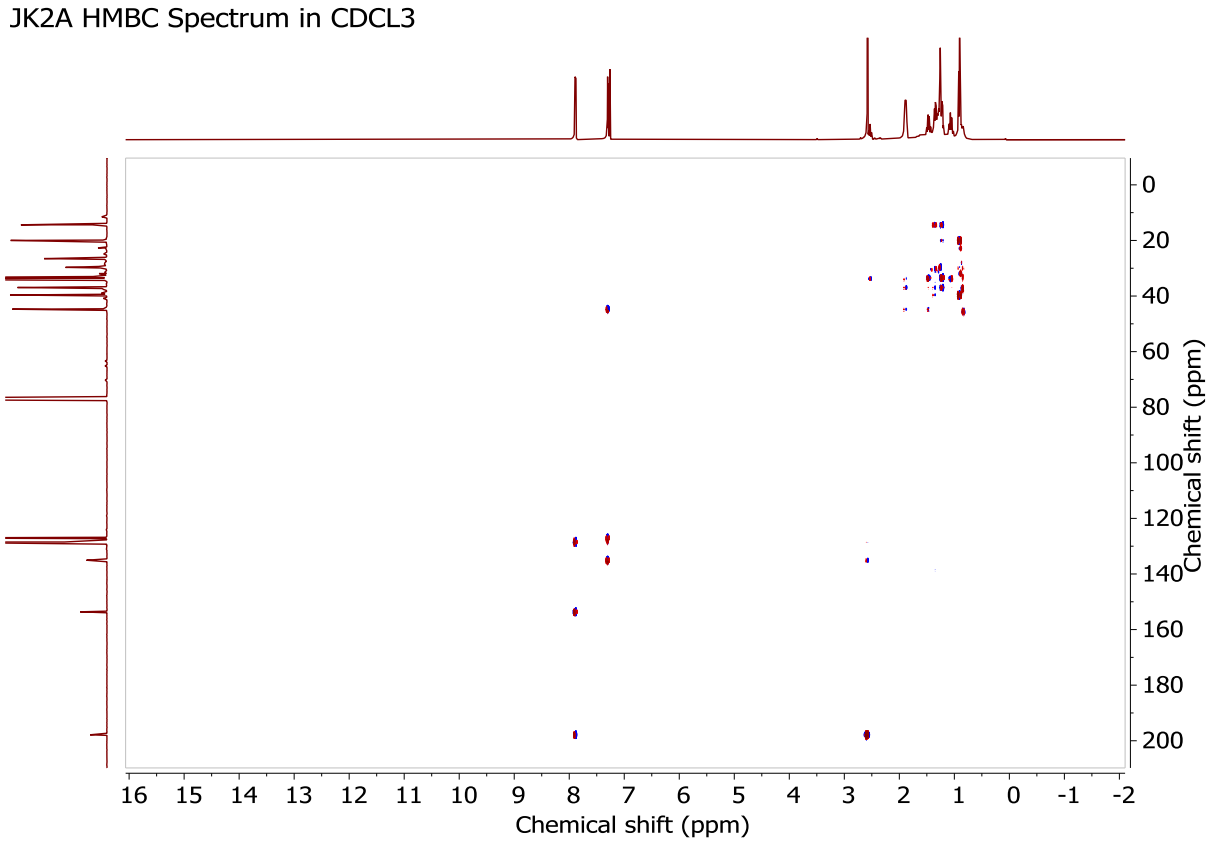
**Appendix 9; COSY spectrum for compound JK 2A (500 MHz; $CDCL_3$)**



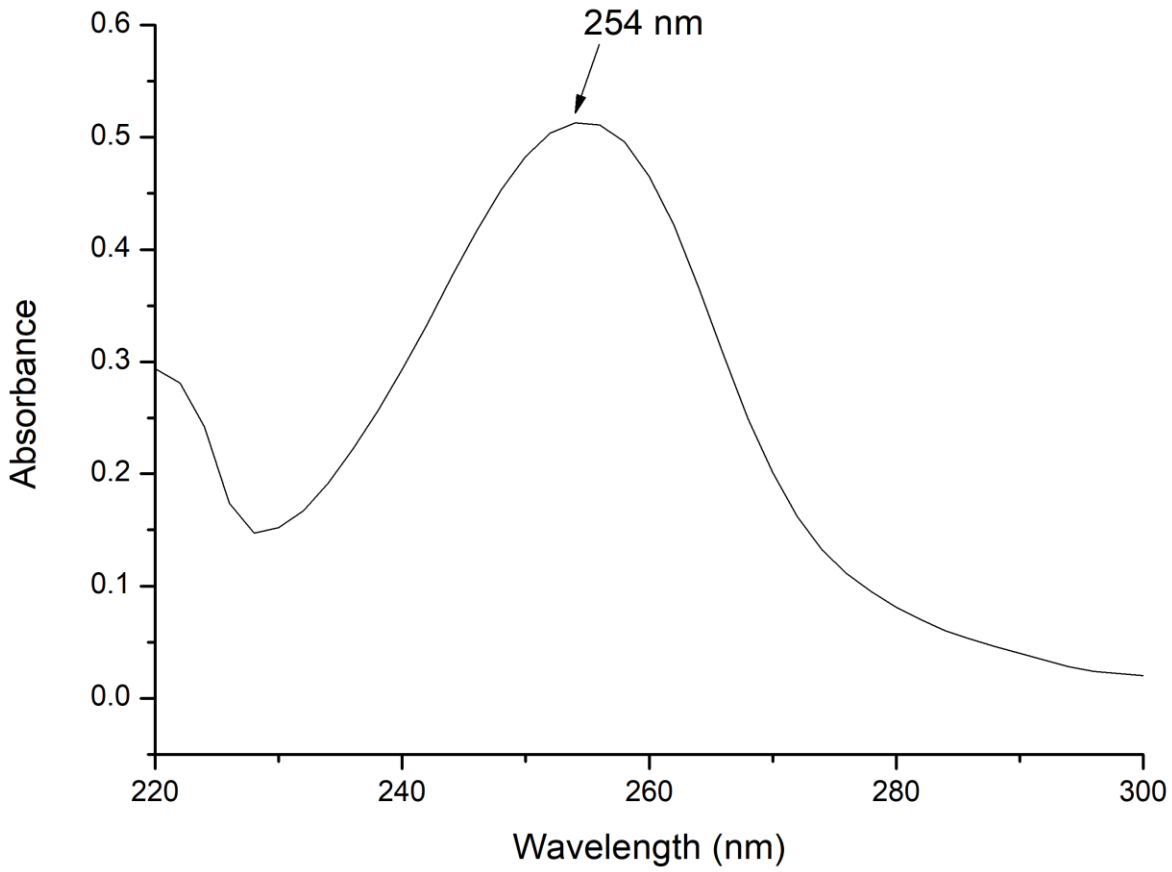
**Appendix 10; HSQC spectrum for compound JK 2A ($CDCL_3$)**



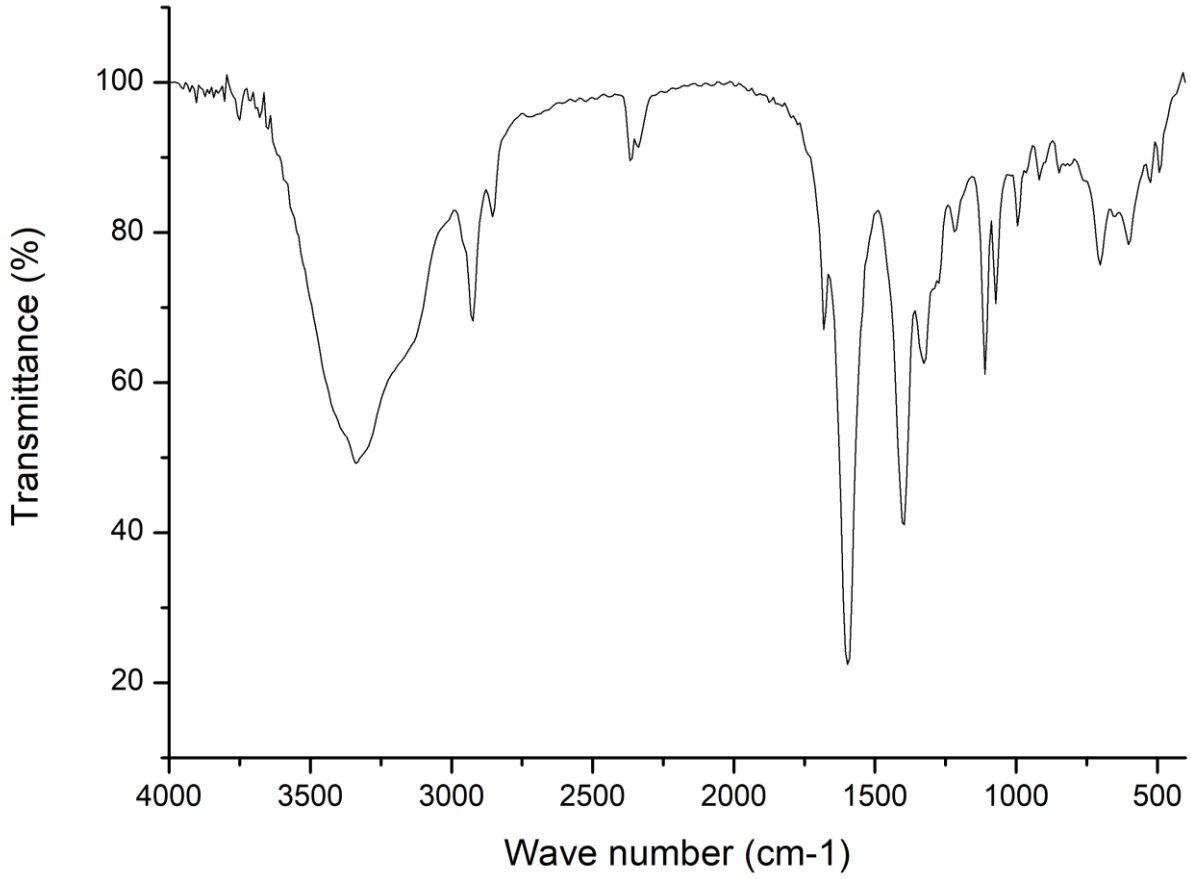
**Appendix 11; HMBC spectrum for compound JK 2A ($CDCL_3$)**



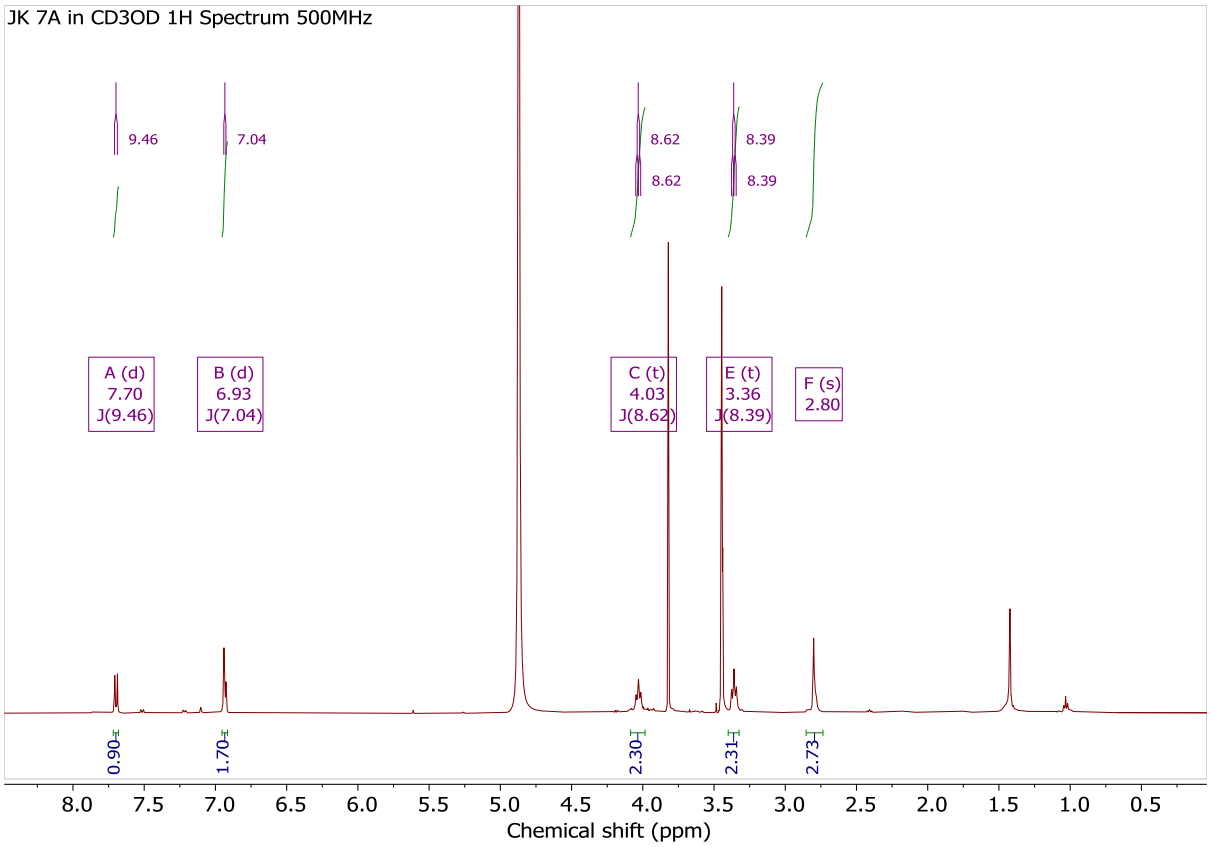
**Appendix 12; Ultraviolet-visible spectrum of compound JK2A in dichloromethane**



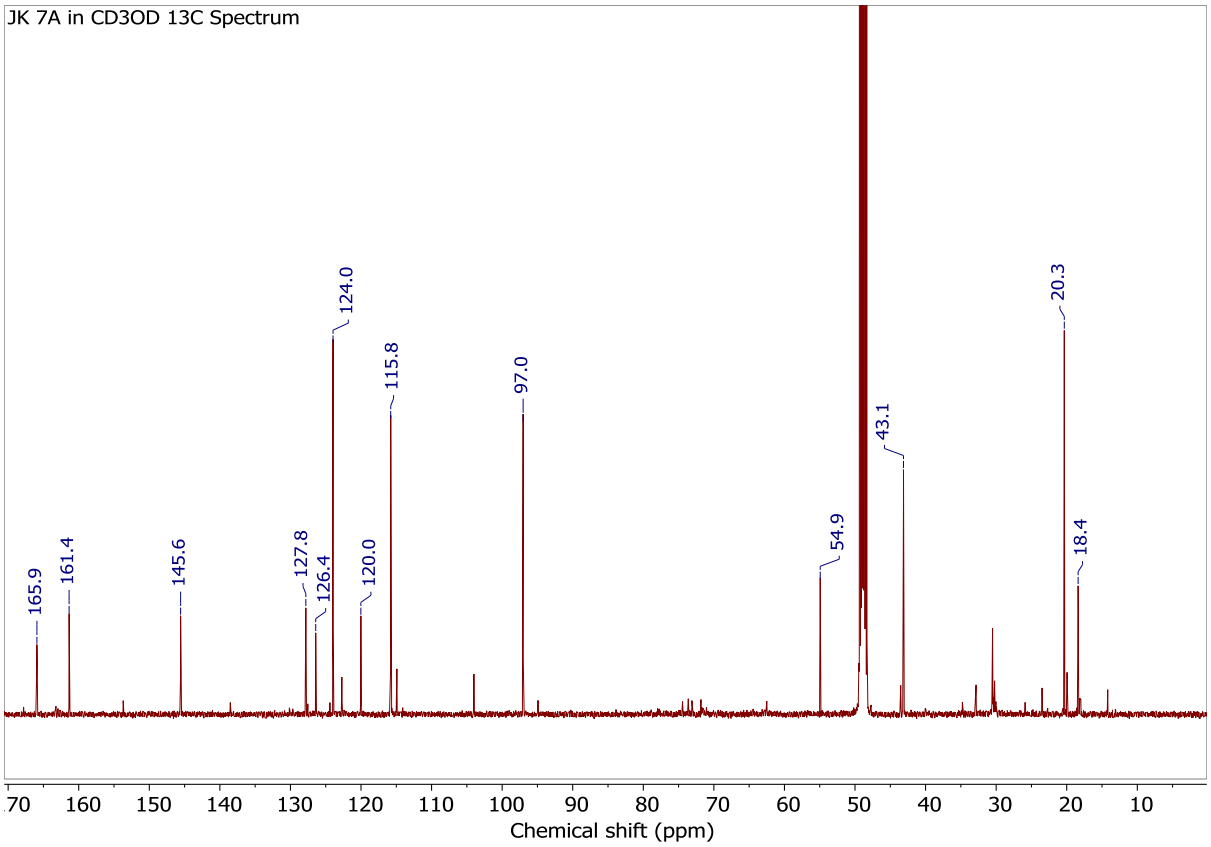
**Appendix 13; Infrared spectrum of compound JK2A**



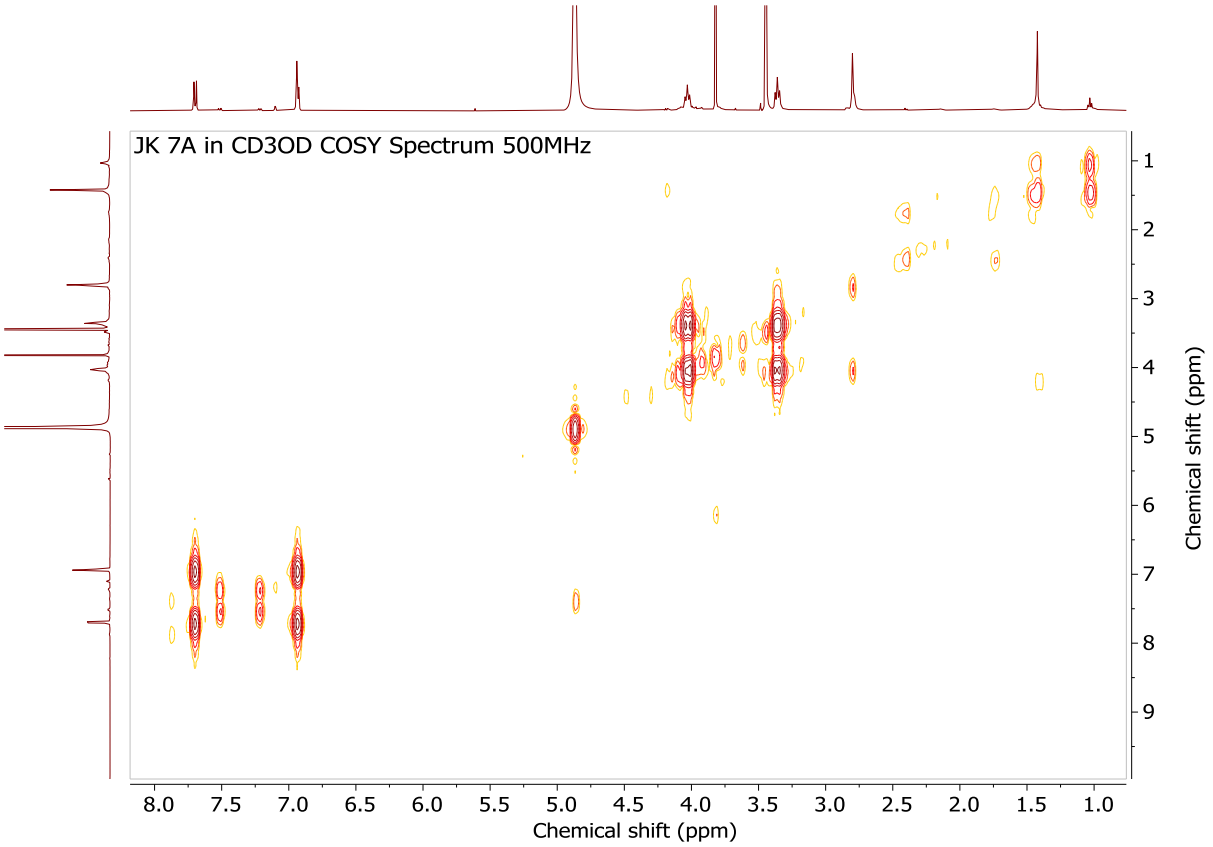
## Appendix 14; $^{1}$H NMR spectrum for compound JK 7A (500 MHz; CD3OD)



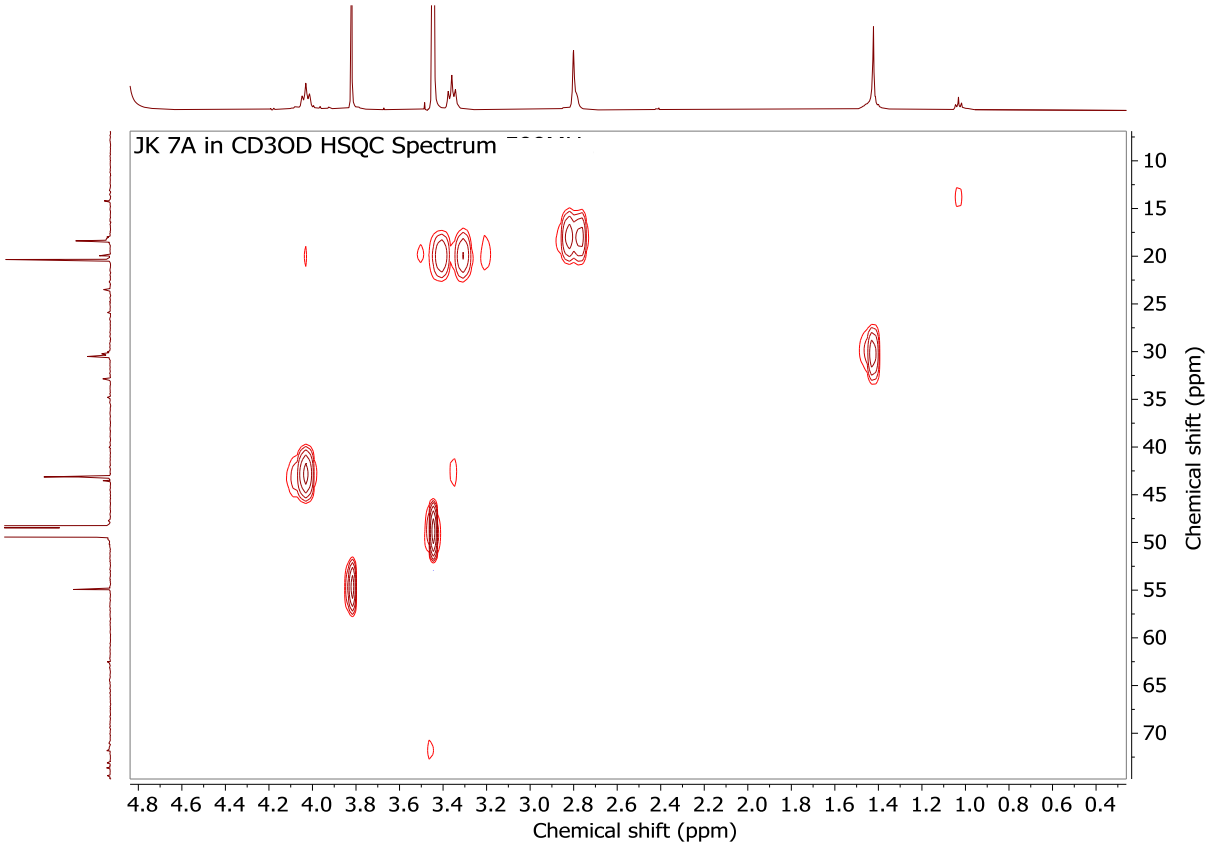
## Appendix 15; $^{13}$C NMR spectrum for compound JK 7A (125 MHz; CD3OD)



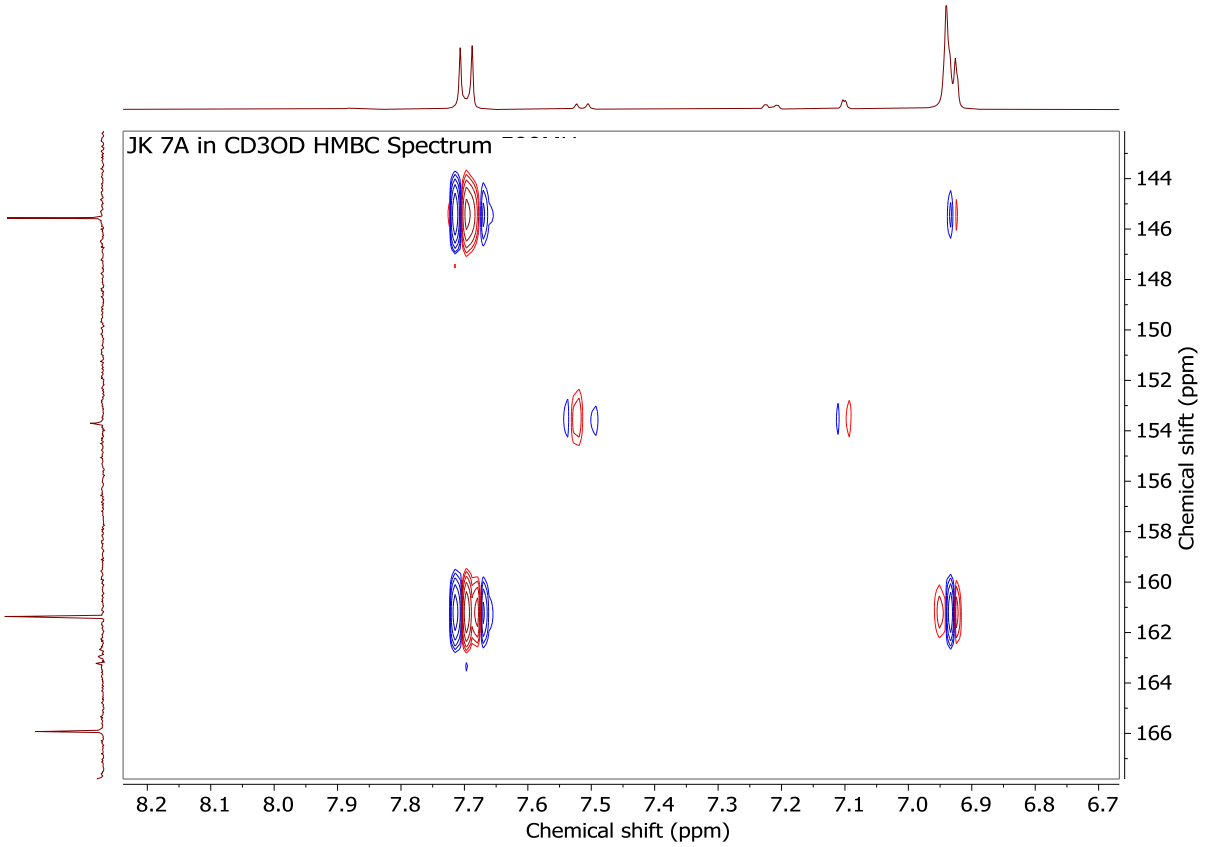
**Appendix 16; COSY spectrum for compound JK 7A (500 MHz; CD3OD)**



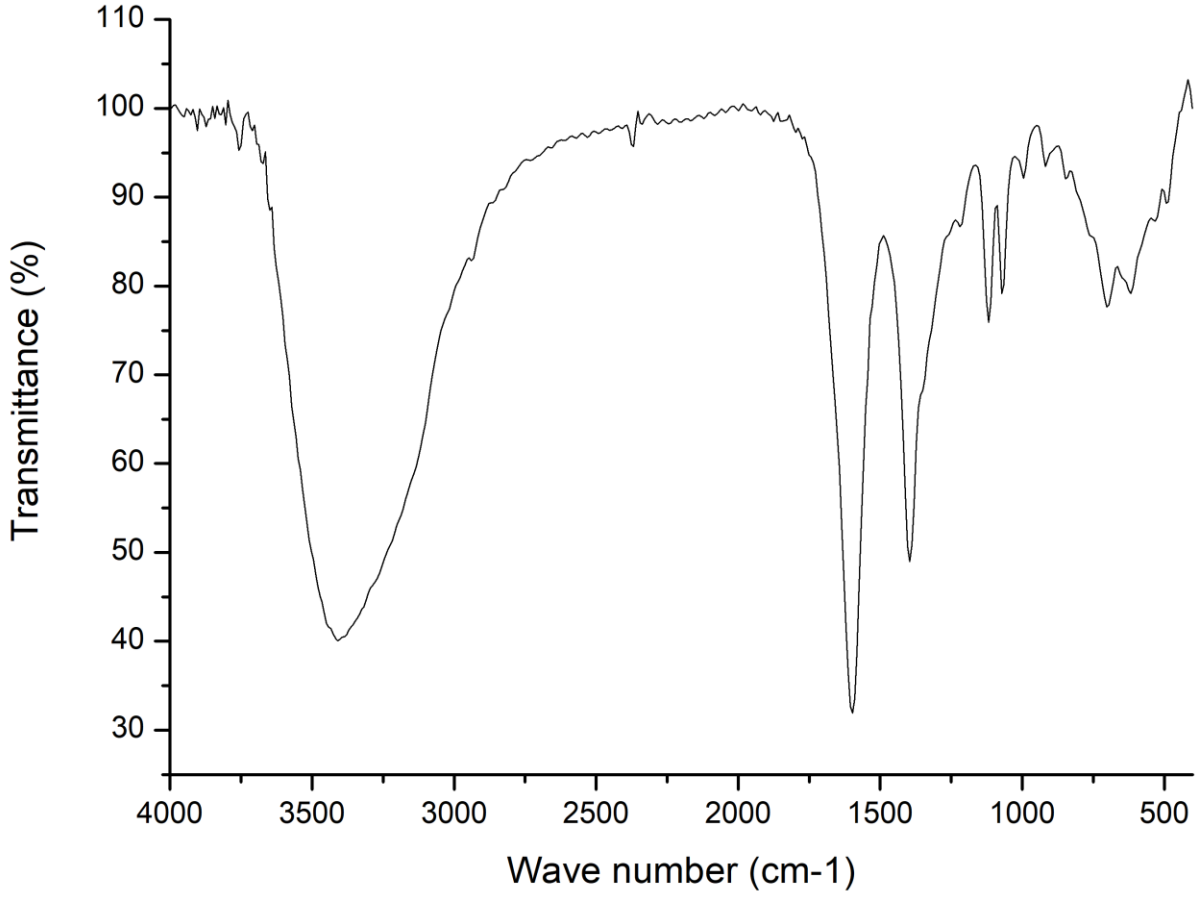
**Appendix 17; HSQC spectrum for compound JK 7A (CD3OD)**



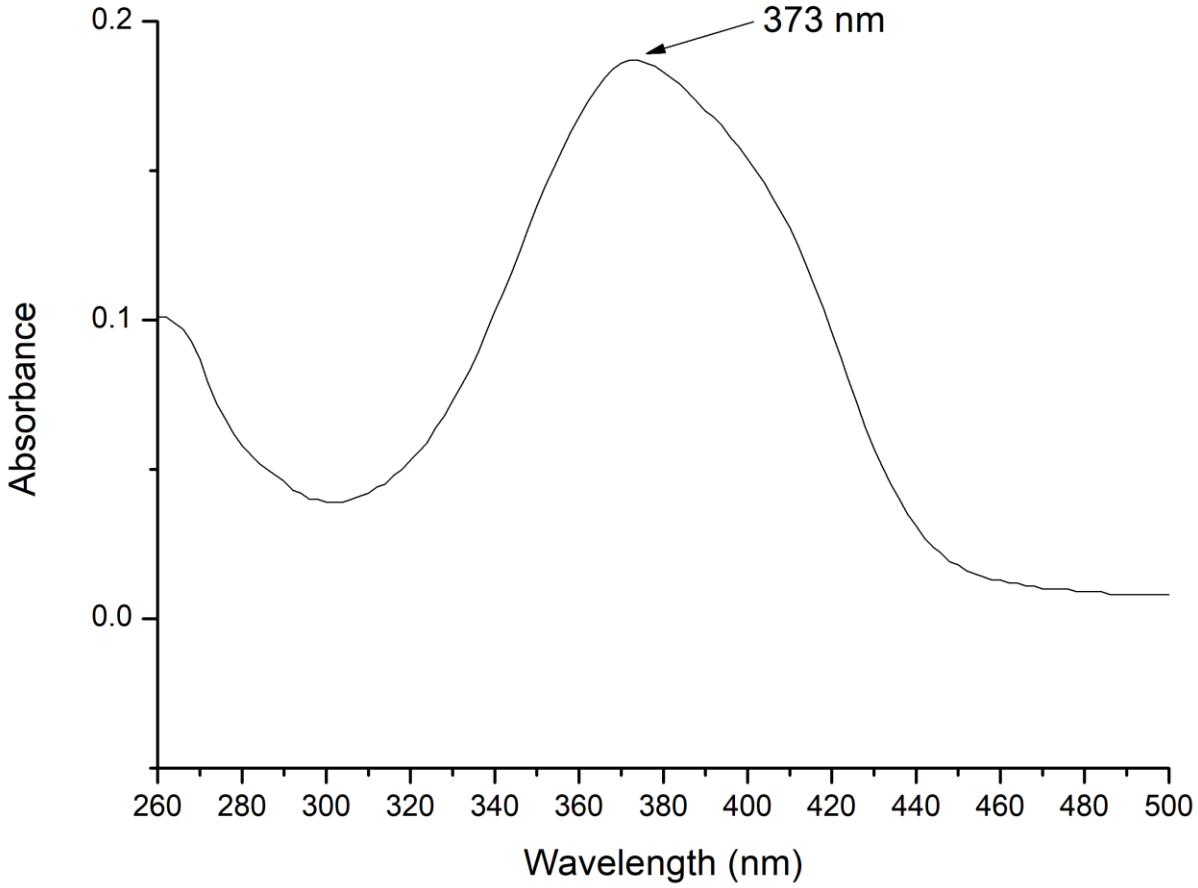
**Appendix 18; HMBC spectrum for compound JK 7A (CD3OD)**



**Appendix 19; Infrared spectrum of compound JK7A**



**Appendix 20; Ultraviolet-visible spectrum of compound JK7A in methanol**



**Appendix 21; Mass spectrum of compound JK7A**