TOTAL FLAVONOID CONTEN AND ANTIOXIDANT ACTIVITY OF *Launaea cornuta* LEAF METHANOL AND AQUEOUS EXTRACTS

FOUZIA IBRAHIM ISMAIL

BPHARM/2016/57256

A RESEARCH PROJECT SUBMITTED TO THE SCHOOL OF PHARMACY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF PHARMACY DEGREE OF MOUNT KENYA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF PHARMACY

DEPARTMENT OF PHARMACOGNOSY

AUGUST 2021

DECLARATION

I declare that this is my original work and has not been submitted in any institution for awarded of degree.

Signature………………………………… Date ……………………

FOUZIA IBRAHIM ISMAIL

BPHARM/2016/57265

Supervisor's approval

I confirm that this research project has been conducted and submitted with my approval as the student supervisor.

Signature………………………………… Date ……………………

Dr. JARED ONYANCHA

School of pharmacy

Mount Kenya University

DEDICATION

I would like to dedicate this work and give special thanks to my parent and guardian; Mr. Adan Ismail and Mrs. Hawa Hassan, my elder brother and Sister Abdullahi Ibrahim and Fatuma Kuno who supported me throughout my entire education. Their affection, unconditional love, encouragement and dedication for the upliftment in my life is incredible, I owe them a lot for their selfless support towards my education.

ACKNOWLEDGEMENT

First and foremost, all the praises and thanks be to Allah, the Almighty, for His showers of blessings throughout my research work to complete the research successfully.

I would like to express my deep and sincere gratitude to my research supervisor, Dr. Jared Onyancha for giving me the opportunity to do research and providing invaluable guidance throughout this research. He has taught me the methodology to carry out the research and to present the research works as clearly as possible. It was a great privilege and honor to work and study under his guidance. I am extremely grateful for what he has offered me. I would also like to thank him for his friendship, empathy, and great sense of humor.

I also acknowledge Mr. Elias Nelson who worked with me tirelessly in the laboratory to ensure that I achieve my objectives.

I am extremely grateful to my parents for their love, prayers, caring and sacrifices for educating and preparing me for my future. I am very much thankful to my brothers and my sisters for their love, understanding, prayers and continuing support to complete this research work.

ABSTRACT

Plants contain chemical constituents identified as phytochemicals which are vital in their therapeutic properties. These bioactive compounds have seen a wide application of medicinal plants in managing various conditions both chronic and acute. Phenolic compounds such as flavonoids constitutes the huge percentage of phytochemicals present in plants. These compounds are characterized by presence of hydroxyl groups. This characteristic feature has seen them possess the antioxidant activity elicited through free radical scavenging mechanism. The use of plants as alternatives to the conventional medication has seen growth in the percentage of research basing on pharmacological properties of plant. This study aimed at evaluating the total flavonoid content and the antioxidant activity of methanol and aqueous leaf extract of *Launaea cornuta*. The total flavonoid content was evaluated using the aluminum chloride calorimetric method while DPPH free radical scavenging method was used to evaluate the antioxidant activity. Catechin was used as the standard flavonoid while L-ascorbic acid was used as the standard antioxidant. From the catechin standard calibration curve the total flavonoid content in methanol and aqueous extract was 8.004±0.005 mg CE/g dw 7.986±0.028 mg CE/g dw respectively. The total flavonoid content of both extracts was not significantly different ($p>0.05$). The DPPH free radical scavenging activity results showed that L-ascorbic acid significantly scavenged the free radicals more as compared to both methanol and aqueous leaf extract from 1 µg/ml to 1000 µg/ml ($p<0.05$). Aqueous leaf extracted showed higher antioxidant activity as compared to methanol leaf extract between concentration levels 0.01 µg/ml to 10 µg/ml. However, methanol leaf extract recorded significantly higher antioxidant as compared to aqueous extract between concentration levels 100 µg/ml and 100 µg/ml. L-ascorbic acid, methanol and aqueous leaf extract recorded IC_{50} values of 4.0800 µg/ml, 4.620 µg/ml and 5.140 µg/ml respectively. In conclusion, this study revealed that *Launaea cornuta* has free radical scavenging activity.

TABLE OF CONTENT

DECLARATION......i

DEDICATION......ii

ACKNOWLEDGEMENT...... iii

ABSTRACT......iv

TABLE OF CONTENT......v

LIST OF TABLES...... viii

TABLE OF FIGURES......ix

LIST OF APPENDICES......x

LIST OF ABBREVIATIONS......xi

CHAPTER ONE: INTRODUCTION......1

1.1 Introduction......1

1.2 Problem statement and justification......3

1.3 Objective......4

1.3.1 General objective......4

1.3.2 Specific objectives......4

1.4 Research questions......5

CHAPTER TWO: LITERATURE REVIEW......6

2.1 Oxidative stress......6

2.2 Antioxidants......6

2.2.1 Endogenous antioxidants......7

2.2.1.1 Enzymatic antioxidants......7

2.2.1.2 Non-Enzymatic antioxidants 7

2.2.2 Exogenous antioxidants 8

2.2.2.1 Vitamin E 8

2.2.2.2 Vitamin C 9

2.3 Use of plants management of oxidative stress 9

2.4 *Launaea cornuta* 10

2.4.1 Plant Morphological Description 10

2.4.2 Plant distribution 11

2.4.3 Biological Activity of *Launaea cornuta* 11

2.4.4 Traditional uses of *Launaea cornuta* 11

2.4.5 Photochemistry of *Launaea cornuta* 12

CHAPTER THREE: MATERIALS AND METHODS 13

3.1Source of plant extract 13

3.2 Preparation of the plant extract dilution. 13

3.3 Total flavonoid content of methanol and aqueous leaf extract of *Launaea cornuta* 13

3.4 DPPH free radical scavenging activity of methanolic and aqueous leaf extract of *Launaea cornuta* 14

3.6 Data management and statistical analysis 15

CHAPTER FOUR: RESULTS AND DISCUSSION 16

4.1 Total flavonoid content of methanol and aqueous leaf extract of *Launaea cornuta* 16

4.2 Antioxidant activity of methanol and aqueous leaf extract of *Launaea cornuta*. 16

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION 22

5.1 Conclusion 22

5.2 Recommendation 22

REFERENCES 23

LIST OF TABLES

Table 4. 1 Total flavonoid content of aqueous and methanol leaf extract of Launaea cornuta 16

Table 4. 2 DPPH free radical scavenging activity of aqueous and methanol leaf extract of Launaea cornuata 18

TABLE OF FIGURES

Figure 2. 1A flowering herb of Launaea cornuta 10

Figure 4. 1 Total flavonoid content of aqueous and methanol leaf extract of Launaea cornuta 16

Figure 4. 2 DPPH free radical scavenging activity of Launaea cornuta leaf methanol and aqueous extracts 19

Figure 4. 3 DPPH free radical scavenging activity of Launaea cornuta leaf methanol and aqueous extracts 19

Figure 4. 4 IC_{50} of aqueous and methanol leaf extract of Launaea cornuata 20

LIST OF APPENDICES

Appendix 1. 1 Catechin standard calibration curve 27

Appendix 1. 2 Absorbance of Catechin and Launaea cornuta leaf methanol and aqueous extracts 27

Appendix 1. 3 Antioxidant raw data 27

LIST OF ABBREVIATIONS

% RSA	Percentage radical scavenging activity
BHA	Butylatedhydroxyl toluene
BHT	Butylatedhydroxyl anisole
CE	Catechin
DNA	Deoxy nucleic acid
DPPH	2,2 diphenyl-1-picrylhydrazyl
SOD	Superioxide dismutase
TFC	Total flavonoid content

CHAPTER ONE: INTRODUCTION

1.1 Introduction

Oxidative stress is a state of imbalance between the free radicals and the antioxidants in the body (Dela Torre et al., 2017). In this state usually the free radicals are higher in concentration than the endogenous antioxidants that act as body defense system (Rahman et al., 2015). This is usually as a result of overproduction of these reactive oxygen species or the decrease in the antioxidant defense system as compared to the available reactive oxygen species (Hardman et al., 2016).The damage due to oxidative stress can be triggered by various physiological processes such as the exposure to environmental factors such as heat and light or oxidizing chemical agents and UV radiations (Bhat et al., 2015). These free reactive species that includes both the nitrogen (RNS) based and oxygen (ROS) based reactive species attack the biomolecules (nucleic acids, proteins and lipids) as they try to gain stability (Moriasi et al., 2021). In the process this results into oxidative damage via the various chemical reactions such as peroxidation, carboxylation, nitration and nitrosylation (Weidinger & Kozlov, 2015). These effects of free reactive species results into oxidative stress that is among the triggers of genesis of disorders such as obesity, aging, inflammation, cognitive impairment, cardiovascular diseases, cancer and Alzheimer's (Guidi et al., 2006).

The body usually has an internal defense system that consist of endogenous antioxidant system responsible for protection of the body cells and tissues from the deleterious effects of free reactive species (Amit et al., 2017). These antioxidant defense system is well organized and consist of both enzymatic and non-enzymatic antioxidants (Ishino et al., 2010). They usually quench the free radicals maintaining the equilibrium between the free reactive species and antioxidants hence the protecting both the cells and the organ systems from damages due to oxidative stress (Umar et al., 2012). The non-enzymatic antioxidants include the dietary antioxidants such as vitamins E, vitamin C, beta-carotene, glutathione and uric acid while

enzymatic antioxidants comprise the superoxide dismutase (SOD), glutathione reductase, catalase and the glutathione peroxidase (Ishino et al., 2010). In certain circumstances the endogenous antioxidants are overwhelmed as a result of increased free reactive species. This results into introduction of the exogenous antioxidants that comprises of the synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone. This is through various foods and cosmetics in which these agents are added as preservatives. Even though they are efficient as antioxidants, they have been faced with many challenges. Adverse side effects such as hepatic damage, malignancies, allergy reactions and as well as reduced potency in some of the animal models (Arika et al., 2019) has been witnessed. As a result an increase in search for alternative antioxidant from natural sources such as plants has been witnessed worldwide (Salazar et al., 2008).

Medicinal plants have been in use for many years with many parts of the world such as in the sub Saharan Africa plants have entirely been the means of health care. This is as a result of the less toxic effects being witnessed upon the use of medicinal plants and less cost of obtaining them. Plants hub many phytochemicals that are responsible for the therapeutic effects associated medicinal plants. Worldwide many medicinal plants are available and are distributed all over. These include *Lonchocarpus eriocalyx*, *Hypericum revolutum* subspecies *kenienses* and *Launaea cornuta.* These plants are used to manage different ailments for instance *Hypericum perforatum* is used to treat depression related conditions. *Launaea cornuta* is a perennial herb that is erect and grows to about 1.30 M tall characterized with hollow stem. It's taxonomically placed in the family asteraceae and is commonly known as the bitter lettuce(Khan et al., 2016). The herb produces white milky substance when it's cut through the stems or leaves. Traditionally, in some communities in African countries utilize leaves of *Launaea cornuta* as wild vegetables and as source of vitamin C (Misonge et al., 2015). These communities located on the coastal side of Kenya, kilifi and those in Nigeria. Traditinaly.

Launaea cornuta is used as a remedy for various conditions. The decoction is used in East Africa as a remedy for disease such as typhoid, the hypoglycemic activity and safety of the ethyl acetate extract (Machocho et al., 2014). leaves juice is used as analgesic agent to relive the ear pain and the boiled herbs are used as remedy for of measles (Khan et al., 2016). The leave decoction is used as a remedy for gonorrhea, ascariasis, and stomach pains while fresh roots are chewed to treat swollen testicles in Tanzania (Khan et al., 2016). In Kenya, the *Launaea cornuta* is browsed on by goats and rabbits. The roots are to relieve chronic joint pains (Misonge et al., 2015). Currently biological activity reported about *Launaea cornuta* include. Despite the many uses of *Launaea cornuta*, its antioxidant activity has not been clearly elaborated. Hence this study was designed to determine the total flavonoid content and evaluate the antioxidant activity of the methanol and aqueous leaf extract of *Lauanaea cornuata*.

1.2 Problem statement and justification

Oxidative stress, the state of imbalance between the free radicals (reactive oxygen species and nitrogen species) and the antioxidant with the free radicals being in more concentrations. This oxidative stress has played a major role in the manifestation of the various diseases such as the liver disease, cancer, aging, autoimmune disorders and cardiovascular as well as the neurodegenerative diseases (Bhat et al., 2015). This is due to the continued oxidative reactions of the free radicals that involves reacting with the biomolecules such as DNA, protein and lipids causing oxidative damage. For instance, the oxidation of the lipids triggers cellular and tissue damage via the covalent bonds resulting into lipid peroxidation, damage to the DNA, inflammation and eventually cell death (Chang & Kim, 2018). To prevent all this, synthetic antioxidants are used to complement the internal antioxidant defense system that is usually overwhelmed by the increased reactive oxygen and nitrogen species. These antioxidants agents have been efficient in quenching the free radicals. However, their use is on the decline due to side effects incurred both on human health and the environment (Weidinger & Kozlov, 2015).

This has seen an increase in interest towards alternatives that are safer and potent as well. Many of these alternatives are from natural sources such as plants

Plants of medicinal values dominated the list of man's health needs since time in memorial. They were the only choice of remedy for the many ailments that affected man. Due to their safety, less cost involved in obtaining them and high potency, medicinal plants have been regarded as the alternatives to the conventional medicine. Medicinal plants have various phytochemicals that elicit different biological activities such as antioxidant, analgesic, antimicrobial and anti-inflammation. Even though plants are the after sought as the reservoirs for the natural antioxidants, very few studies have been conducted to a certain this claims. Many plants have not been screened for their phytochemicals and the various pharmacological properties. For those test, the studies have not gone beyond investigation of the mechanism of action of the specific pharmacological property. This study was designed to determine the quantity of total flavonoid and the free radical scavenging activity of both methanol and aqueous extract of *Launaea cornuta*.

1.3 Objective

1.3.1 General objective

To evaluated the total flavonoid and antioxidant activity of methanol and aqueous leaf extract of launaea cornuta.

1.3.2 Specific objectives

I. To determine the total flavonoid content in methanol and aqueous leaf extract of *Launaea cornuta*.

II. To evaluate the DPPH free radical scavenging activity of methanol and aqueous leaf extract of *Launaea cornuta*.

1.4 Research questions

I. What is the total flavonoid content of methanol and aqueous leaf extract of *Launaea cornuta?*

II. Does the methanol and aqueous leaf extract of *Launaea cornuta* have free radical scavenging properties?

CHAPTER TWO: LITERATURE REVIEW

2.1 Oxidative stress

The oxidation process is a natural and important process of the body. However, at times it's destructive as it can result into noxious damages. The body cells usually produce in a continuous manner, the free radicals both reactive oxygen species and nitrogen species as part of the metabolic process (Lushchak, 2014). These free radicals are unpaired molecules generated through the normal biochemical processes in the body as it utilizes the oxygen for metabolism of nutrients. Additionally, these molecules can be generated as a result of stress, radiation, infections and even smoking (G et al., 2017; Muthumalage et al., 2017). These free radicals are known to be extremely harmful to the body and may favor the development of many damages to the biomolecules such as nucleic acids, lipids and proteins. Similarly they may induce DNA changes that interferes with the organic homeostasis of the body and eventually leads to various oxidative related disorders such as cardiovascular and other degenerative disorders, and even cancer (Incalza et al., 2018);(Prasad et al., 2017). Moreover, oxidative stress reflects loss of balance between the antioxidant system and oxidants in the body (Thanan et al., 2014). This loss of balance is usually in favor of the oxidants that includes the free radicals. Oxidative stress can as well cause impairment of the cell membranes function and in turn induce irreversible damages, which culminates with cell death and/or triggering of age-related chronic diseases, such as Alzheimer, Parkinson, arthritis, atherosclerosis, osteoporosis, dementia, cardiovascular diseases and cancer (Liguori et al., 2018).

2.2 Antioxidants

Antioxidants are molecules that scavenge for excess free radical in the body (Carocho & Ferreira, 2013). These molecules elicit their action by interacting with free radical species preventing or terminating their oxidation reactions(A. A. Hamid1, O. O. Aiyelaagbe, L. A. Usman, 2010). Through this mechanism, the deleterious effects caused by free radicals as well as the oxidative stress is prevented. The antioxidants are of either endogenous origin in which

the body produces its own and exogenous origin that are introduced into the body from external sources such as from diet.

2.2.1 Endogenous antioxidants

The endogenous antioxidants are naturally produced by the body as its defense system against the deleterious effect of free radicals. These antioxidants play a crucial role in maintaining the hemostasis balance between the antioxidants and the free radicals in the body. The human antioxidant defense system comprises of two major groups; the enzymatic and non-enzymatic antioxidants.

2.2.1.1 Enzymatic antioxidants

The enzymatic antioxidants are further divided into two groups; primary and secondary enzymatic antioxidants. The primary enzymatic antioxidant defense system comprises of the three enzymes; catalase, superoxide dismutase and glutathione peroxidase (Medpilwar et al., 2015). These enzymes either prevent the formation or neutralize free radicals. Glutathione peroxidase, donates two electrons to reduce peroxides as potential substrates for Fenton reaction. Catalase on the other hand, is responsible for the conversion of hydrogen peroxide into water and oxygen molecules. Lastly superoxide dismutase converts superoxide anions into hydrogen peroxide, the substrate for catalase. The secondary enzymatic defense system is made up of glutathione reductase and glucose-6-phosphate dehydrogenase. Glutathione reductase reduces the glutathione from its oxidized form to reduced form so that it can continue with its neutralizing property.

2.2.1.2 Non-Enzymatic antioxidants

The non-enzymatic endogenous antioxidants compromises of molecules such as vitamins (A), enzyme cofactors (Q10), nitrogen compounds such as uric acid and glutathione. Vitamin A also known as retinal is a carotenoid produced in the liver. Its antioxidant activity is due to its ability to combine with peroxyl radicals before they initiate lipid peroxidation. The coenzyme Q10 which is present in all cells and membranes has a vital role in the respiratory chain and in

other cellular metabolism. It elicits antioxidant property by preventing lipid peroxidation that results in the formation of the lipid peroxyl radicals. Additionally, coenzyme Q10 is able to regenerate vitamin E. Uric acid is the final product of purine nucleotide metabolism in humans. It has important value in the body that is shown by its higher reabsorption percentage into the body. It's reported to prevent the overproduction of oxo-hem oxidants that is as a result of the reaction with hemoglobin with peroxides. Similarly, it is also able to prevent lysis of erythrocytes by peroxidation. It is as well a potent scavenger of singlet oxygen and hydroxyl radicals. Glutathione is atripeptide that protects the body cells from the deleterious effects of the free radicals. Glutathione achieves this by either donating a hydrogen or an electron to the free radicals. It is also responsible for regeneration of other antioxidants such as ascorbate.

2.2.2 Exogenous antioxidants

On the other hand, the body also relies on the exogenous antioxidants mostly from the diet to maintain the level of free reactive radicals at lower concentrations. These are commonly known as dietary antioxidants and include the Vitamins C and E from the fruits and vegetables respectively. This is the case since at times the body's antioxidant defense mechanisms is overwhelmed resulting in overproduction of free radicals that cause oxidative stress (Sofna and Nina, 2014).

2.2.2.1 Vitamin E

Vitamin E is the fat-soluble vitamins that is made up of tocopherols and tocotrienols forms with the antioxidant properties. Tocopherol form is the principal form of vitamin E as it prevents membranes from oxidation by reacting with free radicals produced as result of lipid peroxidation (McDowell, 2000). This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidized α-tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants (Carocho & Ferreira, 2013).

2.2.2.2 Vitamin C

Vitamin C also identified as ascorbic acid is water soluble vitamin required by the body as it's very important in many biological functions. Ascorbic acid is made of two components; L-ascorbic acid and L-dehydroascorbic acid that have antioxidant properties. These two compounds can are absorbed through the gastrointestinal tract and as well can be interchanged enzymatically in in vivo. Vitamin C is one of the potent reducing agents and scavenger of free radicals in biological systems. It scavenges oxidizing free radicals and harmful oxygen-derived species, such as hydroxyl radical, hydrogen peroxide (H2O2),superoxide radical and singlet oxygen as well as reactive nitrogen oxide (Carocho & Ferreira, 2013).

2.3 Use of plants management of oxidative stress

Natural medicine from medicinal plants is regarded as an ancient form of healthcare that has been in use for many years (Petrovska, 2012). The use of herbal medicine and their ability to cure majority of the emerging diseases has been boosted by support from the WHO. This has been majorly due to their affordability, potency and safety. This characteristics have made them be the most preferred as compared to the conventional medicine (S. Antwi-Baffour, 2014). The usage of these medicinal plants has greatly increased with about 80 % of the global population relying on them as the primary source of healthcare (WHO 2015). In countries such as Kenya and other countries in the sub-Saharan Africa herbal medicine is part of their culture hence they are more accepted than the modern medicine(Obakiro et al., 2020).

Through various studies, medicinal plants have shown promising pharmacological properties that is effective against various ailments. This can be attribute to the many phytocompounds that plants contain. These bioactive compounds are reported to confer various properties such as antioxidant, anti-inflammatory, anticancer and ant nociceptive activities (Klayman, 1985). Various medicinal plants that are potent antioxidant are available and used in compacting oxidative stress and its related disorders. These include phenolic, flavonoids, steroids, tannins, saponins and alkaloids. The potential antioxidant activities of these plants have been shown

through the various studies (Cai et al., 2004). The pure compounds or crude extracts from these plants have shown to be effective antioxidants as compared to even vitamin E and BHT via the invitro antioxidant assays (Gu & Weng, 2001). Additionally, the medicinal plants have shown to exhibit much higher antioxidant activity that is directly proportional to the higher levels of phenolic compound as compared to some of the common vegetables and fruits (Cai et al., 2004)

2.4 *Launaea cornuta*

2.4.1 Plant Morphological Description

Lauanea cornuta is a perennial herb that is erected and characterized by a hollow stem. This herb produces milky juice from the stems and leaves. It grows to a height of about 150 cm tall. It has a creeping rhizome as well. The leaves are deeply divided and form a rosette at the base and they arranged in an alternating manner on the stem. The leaves are also sessile, with width of up to 2.5 cm long by 3 cm, entire or with two to three pairs of lobes acute-pointed near the base. Inflorescence large, diffuse with numerous yellow flower heads on peduncle about 2.5 cm long involucres up to 10 cm long by 4 mm cross, glabrous or shortly pubescent, phyllares in two to three rows, 2-4 mm long outside, up to 10 mm long inside. Florets 10-25, yellow up to 15 mm long, ligules often reddish outside seeds pale brown, elliptical, ribbed 2-4 mm long with white pappus 5 mm long (Misonge et al., 2015).

Figure 2. 1A flowering herb of *Launaea cornuta*

2.4.2 Plant distribution

Launea cornuta is native to Africa and is commonly known as wild or bitter lettuce moleita and merlot (Sudan), muthunga (Kikuyu) muthunga (Meru and Embu) mchunga (Swahili), Mnyinya (Taita), and Achak (Luo). It grows on alluvial soils in cultivated areas, including irrigated crops, along roadsides, near rivers and bush vegetation. A single plant has ability to cover a larger area because of its spreading rhizomes. *Launaea cornuta* is the commonest species of *Launaea* around Nairobi, Kenya (Misonge et al., 2015)

2.4.3 Biological Activity of *Launaea cornuta*

The *Launaea cornuta* reported pharmacological activities in previous studies include hypoglycaemic activity and safety studies of the ethyl acetate extract of Launaea cornuta shrub (Machocho et al., 2014) and the cytotoxicity against brine shrimp with $LC_{50}< 1000$ μg/ml (Musila et al., 2013).

2.4.4 Traditional uses of *Launaea cornuta*

Launaea cornuta is used as a wild vegetable in African communities such as in Kilifi, of the coastal region of Kenya and Nigeria as a source of vitamin C (Misonge et al., 2015). In East Africa the plant is used in the treatment of typhoid. The leaves juice is used as a pain reliever in the ear where it's applied as a drip in the ear. The entire plant is used as a remedy of measles where the plant is boiled and the infected individual baths with the extract. In Tanzania the leave decoction is used in the management and treatment of various diseases such as of gonorrhea, ascariasis, and stomach pains. Additionally, fresh roots are used to treat swollen testicles when chewed raw (Misonge et al., 2015).

In Kenya, it is used as animal fodder such as goats and rabbits. Roots are used in the treatment of warts and also as analgesic for the management of chronic joint pains when its administered orally(Misonge et al., 2015). The Launea cornuta concoction used as an anticancer agent for the breast cancer and benign prostate hyperplasia diabetes (Kareru et al., 2007).

Among the Kamba communities of Makueni and Machakos counties in Kenya use *Launaea cornuta* as a remedy for chronic joint pain. They use shoots or the whole obtained from the wild plant *Launaea cornuta* herbs which they regard as a weed. This plant is macerated in water and the resultant infusion take orally a glass 2 to 3 times a day for complete two weeks (Wambugu et al., 2011).

2.4.5 Photochemistry of *Launaea cornuta*

From previous studies on the phytochemical screening of *Launaea cornuta* Revealed the presences of alkaloids, saponins, flavonoids and sesquiterpene lactones (Musila et al., 2013). In the study of Misonge et al.(2015) all these phytochemical constituents were reported however, saponins were lacking. Gas chromatography profiling of the ethyl acetate extracts of *L. cornuta* shrub revealed compounds such as benzimidazol (2,1-a) isoquinolone (an alkaloid), 2-propenoic acid, 6- methylheptyl ester and 1-Decanol, 2-hexyl (fatty acid derivatives), n-Hexadecanoic acid, 9,12 Octadecanoic acid, 9, Octadecenoic acid, Octadecanoic acid and Heinecosane (fatty acids), Lanosterol, Sigmasterol and Cholest-5-en-3-ol (3-beta),-carbonochlorinate (Phytosterols), 2H-1-Benzopyran-2-one, 6- acetyl-7-(acetyloxy)-4-methyl(Coumarin), β-amyrin, Lup-20(29)-en-3-one and Fern-7-en-3.beta.-ol(Pentacyclic triterpenoids) (Machocho et al., 2014). In other study have reported the presence of essential oils extracted by hydrodistilation, but only at very lower percentage that was below the detectable limit (Bandeira Reidel et al., 2018). These essential oils of *Launaea cornuta* have been reported to consist of predominantly oxygenated monoterpenes which make up 79.2 % in the gas chromatography-mass spectrophotometer analysis of the essential oils (Bandeira Reidel et al., 2018).

CHAPTER THREE: MATERIALS AND METHODS

3.1Source of plant extract

The methanolic and aqueous leaf extract of *Launaea cornuta* was provided by my project supervisor; Dr. Jared Onyancha of school of pharmacy Mount Kenya University. all extracts were in their respective glass sample bottles and kept in the refrigerator at 4 ^{0} C until the experiment day.

3.2 Preparation of the plant extract dilution.

The methanolic and aqueous leaf extract of *Launaea cornuta* were prepared accordily and serially diluted prior to use in this study. Exactly 10 mg of both methanolic and aqueous extract were weighed by difference using the analytical balance (shimadzu). The 10 mg were dissolved in 10 ml of analytical grade methanol (Lobachemie) and sonicated in the ultrasonic sonicator for 10 minutes. The resultant concentration of the plant extract was 1mg/ml and this was serial diluted by a factor of 10 to obtain the working concentrations of 100, 10, 1, 0.1 and 0.01 that were used in the DPPH antioxidant assay. For the total flavonoid the plant extract was used at stock concentration of 1 mg/ml. the L-ascorbic acid (sigma Aldrich) used as a standard antioxidant was prepared in the similar manner as the plant concentration. The standard flavonoid; catechin (Lobchemie) was prepared in methanol to obtain the working concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml 50 mg/ml, 25 mg/ml and 12.5 mg/ml.

3.3 Total flavonoid content of methanol and aqueous leaf extract of *Launaea cornuta*

The total flavonoid content present in both methanolic and aqueous leaf extract of *Launaea cornuta* was evaluated by the aluminum chloride method. The assay was performed by following the procedure of Bouguerra et al.(2019) with minimum modifications. the plant extracts were prepared by dissolving 10 mg of the dried extract in 10 ml of pure methanol to obtain the concentration of 1mg/ml. out of this stock solution of the respective extracts, 125 ul was pipetted in a clean test-tube while in the other test tubes 125 ul of standard (catechin)at various concentrations was added. Into this 75 ul of sodium nitrate (5%) was added in all the

test tubes mixed by swirling and incubated at room temperature for 6 minutes. Aluminum chloride (10%) was then added in all the tubes at a volume of 150 ul followed by 750 ul of 1 M sodium hydroxide and then carefully mixed. The reaction mixture content was then topped to 2500 ml with distilled water and incubated for 15 minutes under room temperature conditions. Absorbance of all the reaction mixtures (samples and standard) were measured at 510 nm wavelength using double beam spectrophotometer (labtech). The standard curve was drawn using the absorbances and the concentrations of catechin from which the straight-line equation (y=mx+c) was obtained. Using this equation, the concentration of flavonoid in the samples was calculated.

3.4 DPPH free radical scavenging activity of methanolic and aqueous leaf extract of *Launaea cornuta*

The antioxidant activity of methanolic and aqueous leaf extract of *Launaea cornuta* was evaluated by determining its ability to scavenge the DPPH free radicals (Bouguerra et al., 2019). The leaf extracts (methanol and aqueous) and L-ascorbic acid were first dissolved in methanol to prepare a stock solution of 1 mg/ml. this stock solution was then serially diluted to obtain six concentrations; 1000, 100, 10, 1, 0.1 and 0.01. the reaction mixture was then reconstituted by pipetting 1600 ul of the respective concentration of extract/L-ascorbic acid into clean test-tubes. Into this 2400 ul of the freshly prepared 0.3 mM DPPH methanolic solution, mixed by swirling and then incubated in the dark for 15 minutes. The negative control was reconstituted by adding 1600 ul of pure methanol and 2400 ul of 0.3mM DPPH solution. The absorbances

of both standard and extract were monitored spectrophotometrically at 517 nm using double beam spectrophotometer (Labtech). The percentage free radical scavenging activity of both the extract and standard were computed following the equation.

From the curve of percentage DPPH free radical scavenging activity against concentration the IC_{50} of both extracts and L-ascorbic acid were determined.

3.6 Data management and statistical analysis

The antioxidant absorbances were first calculated to get the percentage radical scavenging activity while the total phenolic content was calculated from the standard calibration curve. The percentage free radical scavenging activity data and total phenolic content data were then tabulated in an excel spread sheet. This data was then imported in the GraphPad prism statistical analysis software version 9.0 and descriptive statistics conducted and data presented as Mean±SEM. Further analysis of the data using two- way anova was conducted followed by tukeys post hoc test to establish the level of significance between the means at various concentration. The results were then presented in form of tables and graphs.

CHAPTER FOUR: RESULTS AND DISCUSSION

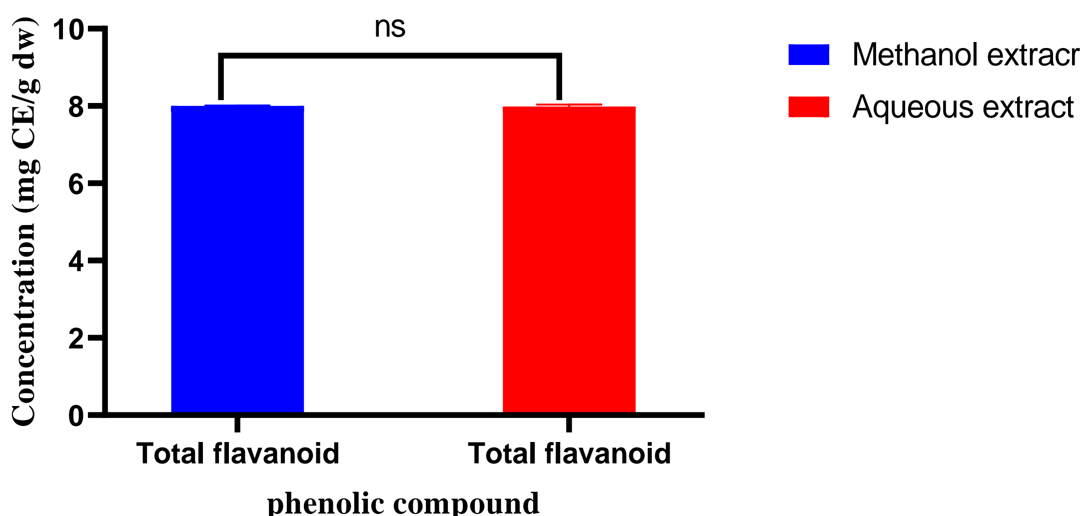
4.1 Total flavonoid content of methanol and aqueous leaf extract of *Launaea cornuta*

The results for the total Flavonoid content in methanol and aqueous leaf extract of *Launaea cornuta* are presented in table 4.1 and fig 4.1. Methanol extract recorded 8.004±0.005 mg CE/g dw while in aqueous extract 7.986±0.028 mg CE/g dw of total flavonoid content was recorded. From these results no significant difference was noted in the total flavonoid content recorded between the two extracts (p>0.05; fig 4.1).

Table 4. 1 Total flavonoid content of aqueous and methanol leaf extract of *Launaea cornuta*

Phytochemical compound	Methanol extract	Aqueous extract
Total flavonoid content	8.004±0.005	7.986±0.028

Figure 4. 1 Total flavonoid content of aqueous and methanol leaf extract of *Launaea cornuta*



4.2 Antioxidant activity of methanol and aqueous leaf extract of *Launaea cornuta*.

The results for the DPPH free radical scavenging activity of the aqueous and methanolic leaf extract of *Launaea cornuta* are presented in table 4.2 and figures 4.2, 4.3 and 4.4. the results showed a dose dependent free radical scavenging activity for both the methanolic, aqueous and L-ascorbic acid. The percentage free radical scavenging activity recorded at all concentration

of aqueous leaf extract of *Launaea cornuta* showed significant difference between them. However, the percentage free radical scavenging activity recorded at concentrations 0.1 µg/ml was no significantly different from 1 µg/ml.

The methanolic leaf extract significantly recorded lower percentage free radical scavenging activity at concentration 0.01 µg/ml and significantly higher percentage free radical scavenging activity at concentration 1000 µg/ml (table 4.2). The percentage free radical scavenging activity recorded at concentration levels 0.01 µg/ml and 0.1 µg/ml, 0.1 µg/ml and 1 µg/ml and concentration level 0.01 µg/ml and 1 µg/ml was not significantly difference from each other ($p<0.05$; table 4.2; fig 4.2). However, between the other all concentration levels significant difference was noted between them.

The L-ascorbic acid recorded no significant difference in the percentage free radical scavenging activity recorded between concentration level 1000 and 100 and concentration levels 0.01 µg/ml and 0.1 µg/ml ($p>0.05$; fig 4.2). However, between the all other concentration levels, significant difference was noted in the percentage free radical scavenging activity recorded ($p<0.05$; fig 4.2).

At concentration level 0.01 the aqueous extract recorded no significant difference in the percentage free radical scavenging activity from m that recorded by L-ascorbic acid. However, methanolic leaf extract recorded significantly lower percentage free radical scavenging activity as compared to aqueous and L-ascorbic acid ($p<0.05$; table 4.2; fig 4.3). at concentration 0.1 both methanolic extract, aqueous extract and L-ascorbic acid recorded significant difference in the percentage free radical scavenging activity. Aqueous leaf extract recorded significantly higher percentage free radical scavenging activity followed by L-ascorbic acid and methanolic leaf extract recorded significantly lower percentage free radical scavenging activity. At concentration 1 and 10 of both aqueous, methanolic leaf extract and L-ascorbic acid recorded

significant difference in the percentage free radical scavenging activity recorded. At both the two concentration levels L-ascorbic acid recorded significantly higher percentage free radical scavenging activity followed by aqueous leaf extract and methanolic leaf extract recorded the least percentage free radical scavenging activity. Similarly, at concentration level 100 and 1000 significant difference was noted in the percentage free radical scavenging activity recorded. However, at both concentration methanolic leaf extract recorded significantly higher percentage radical scavenging activity as compared to the aqueous extract while L-ascorbic acid recorded the highest percentage radical scavenging activity.

Aqueous and methanol leaf extracts of *Launaea cornuta* recorded IC_{50} values of 5.140 ug.ml and 4.620 ug/ml respectively while L-ascorbic acid recorded IC_{50} value of4.0800 ug/ml (figure 4.4).

Table 4. 2 DPPH free radical scavenging activity of aqueous and methanol leaf extract of *Launaea cornuata*

CONCENTRATION µg/ml	% DPPH FREE RADICAL SCAVENGING ACTIVITY.		
	Aqueous extract	Methanolic extract	L-Ascorbic acid
1000	73.36±0.105	81.863±0.317	95.966±0.970
100	46.546±0.154	68.53±0.216	93.313±0.887
10	31.73±2.438	14.956±0.943	43.693±3.365
1	20.963±0.300	3.57±0.225	36.466±1.082
0.1	17.656±0.314	1.606±0.114	9.68±0.397
0.01	7.196±0.288	1.213±0.089	7.436±0.296

Figure 4. 2 DPPH free radical scavenging activity of *Launaea cornuta* leaf methanol and aqueous extracts

DPPH free radical scavenging activity of aqueous and methanol leaf extract of *Launaea cornuta* and L-ascorbic acid.

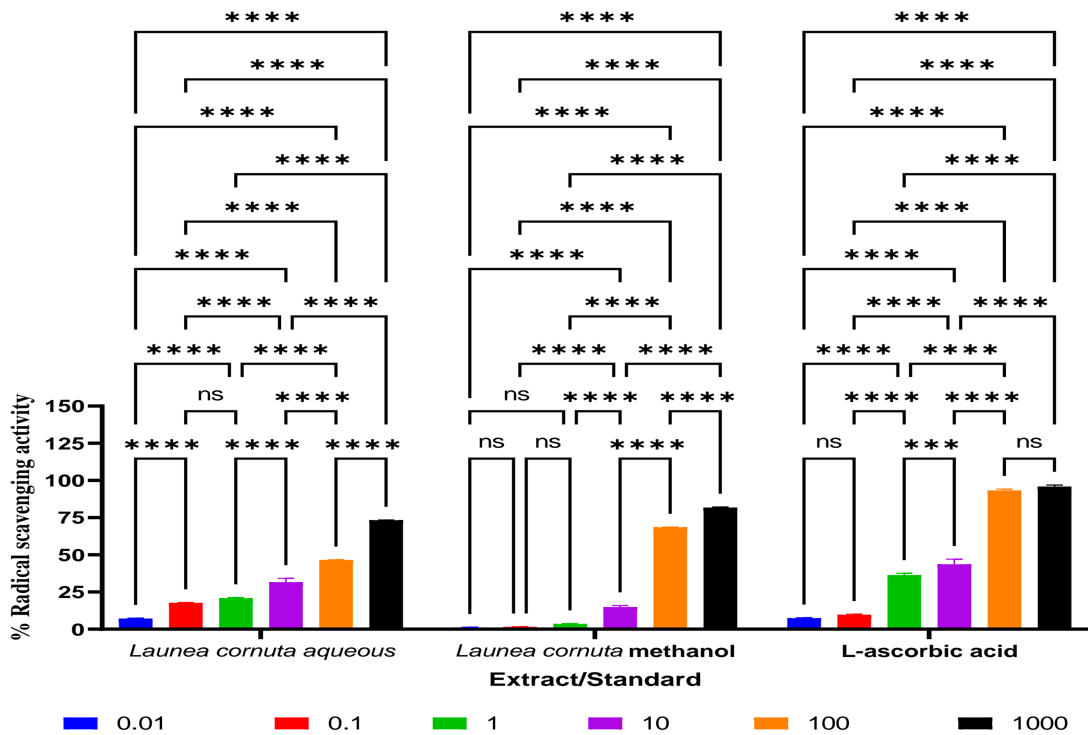


Figure 4. 3 DPPH free radical scavenging activity of *Launaea cornuta* leaf methanol and aqueous extracts

DPPH free radical scavenging activity of L-ascorbic acid, methanol and aqueous leafr extract of a Launaea cornuta

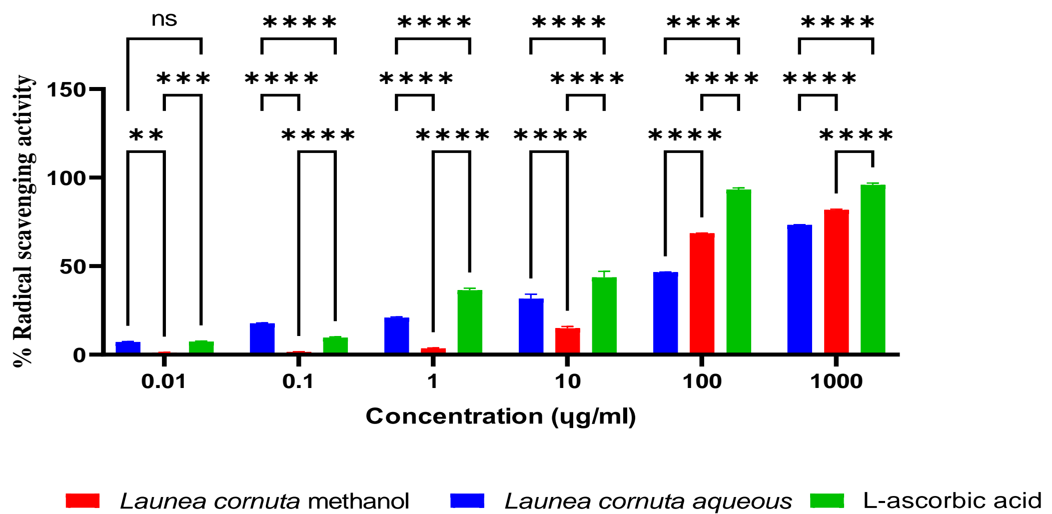
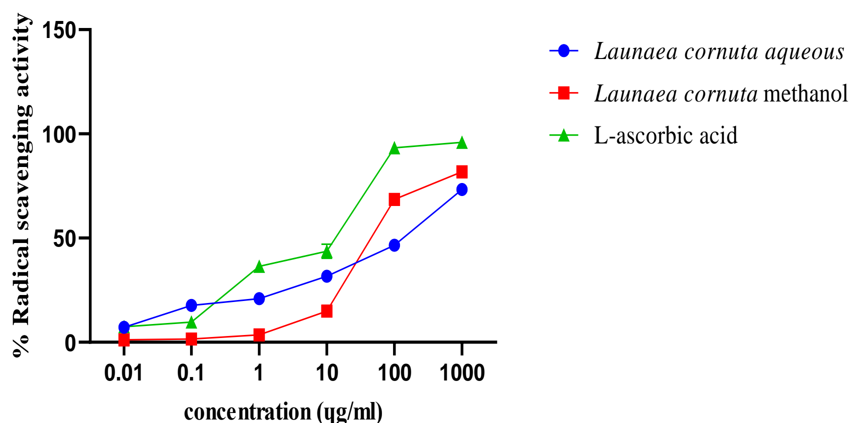


Figure 4. 4 IC_{50} of aqueous and methanol leaf extract of *Launaea cornuata*



Antioxidant compounds have the ability to either scavenge, inhibit or terminate the chain reactions of the free radicals. Through this mechanisms, the biomolecules such as lipids, proteins and nucleic acid are protected from oxidative damage as result of oxidation reaction of the reactive oxygen species (Pisoschi & Negulescu, 2012). The in vitro assays for determining the antioxidant activity of natural products has been achieved via various mechanisms including assay such as 2.2-diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging assay. The effects of natural products with antioxidants properties on the 2.2-diphenyl-2-picryl-hydrazil (DPPH) is usually due to their ability to donate hydrogen to the free radical (Rahman et al., 2015). The 2.2-diphenyl-2-picryl-hydrazil (DPPH) free radical assay is widely accepted mechanism of determining the radical scavenging activity of many substance mainly of plant origin. This has been attributed to the less time and simplicity of the technique. 2.2-diphenyl-2-picryl-hydrazil (DPPH) is a free radical that is organic in nature and form a purple solution when dissolved in either methanol or ethanol. When this 2.2-diphenyl-2-picryl-hydrazil (DPPH is mixed with the antioxidant agent is reduced to form diphenylpicryl hydrazine (DPPH-H) that is yellow in colour. In the current study the total flavonoid content and antioxidant activity of methanol and aqueous leaf extract of *Launaea cornuta* was evaluated. The methanol and aqueous leaf extract of *Launaea cornuta* recorded flavonoid

content of 8.004±0.005 mg CE/g dw and 7.986±0.028 mg CE/g dw respectively. The flavonoid content present in both methanol and aqueous extract was similar. The methanol and aqueous leaf extract significantly scavenged the 2.2-diphenyl-2-picryl-hydrazil (DPPH) radical in a dose depended manner, in which the activity increased with increase in the concentration of the extract. The aqueous root extract significantly scavenged the free radical (DPPH) more than the methanol extract between concentrations 0.01 µg/ml, to 10 µg/ml. However, at concentrations 100 µg/ml, and 1000 µg/ml, the methanol extract had a higher free scavenging ability as compared to the aqueous extract. IC_{50}, the minimum concentration that is required to scavenge 50 % of the free radical was in calculated from the percentage radical scavenging activity versus the concentration curve. The antioxidant agents with lower IC_{50} values are regarded to be more potent as antioxidants. The methanol extract recorded IC_{50} of 4.620 µg/ml and aqueous extract recorded 5.140 µg/ml while L-ascorbic acid recorded IC_{50} of 4.0800 µg/ml. From these values the antioxidant activity was in the order of L-ascorbic acid > methanol extract > aqueous extract.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, the findings from the current study shows that *Launaea cornuta* is a potential antioxidant agent. Its ability to scavenge the free DPPH radicals it's an evidence that it has the ability to quench the free reactive species in the body. Similarly, the considerable quantity of the total flavonoid content present in the two extracts validates its free radicals scavenging property. Therefore this plant can be helpful in clearing the excess free radicals such as reactive oxygen species reducing the chances of occurrence of oxidative stress which is an etiology in many disorders such as the cognitive impairment, aging, and other neurodegenerative diseases.

5.2 Recommendation

From this study, various recommendation were made:

I. The guided isolation of the active components of the Leaf extract to be done and the resultant fraction evaluated for the antioxidant activity.

II. In vivo models of evaluating the antioxidant activity be done to determine the in vivo antioxidant potential of *Launaea cornuta*.

III. Determination of other phytochemicals such as tannins quantitatively to be done.

REFERENCES

A. A. Hamid1, O. O. Aiyelaagbe, L. A. Usman, O. M. A. and A. L. (2010). Antioxidants: Its medicinal and pharmacological applications. *African Journal of Pure and Applied Chemistry*, *4*(August), 1–4.

Amit, N., Vishal, S., & Sufiyan, C. A. B. U. (2017). Roles of Antioxidants in Biological System : a Review. *Mintage Journal of Pharmaceutical & Medical Sciences*, *6*(1), 7–12.

Arika, W., Kibiti, C. M., Njagi, J. M., & Ngugi, M. P. (2019). In Vitro Antioxidant Properties of Dichloromethanolic Leaf Extract of Gnidia glauca (Fresen) as a Promising Antiobesity Drug. *Journal of Evidence-Based Integrative Medicine*, *24*, 1–17. https://doi.org/10.1177/2515690X19883258

Bandeira Reidel, R. V., Nardoni, S., Mancianti, F., Anedda, C., El Gendy, A. E. N. G., Omer, E. A., & Pistelli, L. (2018). Chemical composition and antifungal activity of essential oils from four Asteraceae plants grown in Egypt. *Zeitschrift Fur Naturforschung - Section C Journal of Biosciences*, *73*(7–8), 313–318. https://doi.org/10.1515/znc-2017-0219

Bhat, A. H., Dar, K. B., Anees, S., Zargar, M. A., Masood, A., Sofi, M. A., & Ganie, S. A. (2015). Oxidative stress, mitochondrial dysfunction and neurodegenerative diseases; a mechanistic insight. *Biomedicine and Pharmacotherapy*, *74*, 101–110. https://doi.org/10.1016/j.biopha.2015.07.025

Bouguerra, A., Hadjadj, M., Dekmouche, M., Rahmani, Z., & Dendougui, H. (2019). Determination of phenolic content and antioxidant capacity of Launaea resedifolia from Algerian Sahara. *Journal of Applied Biology and Biotechnology*, *7*(4), 63–69. https://doi.org/10.7324/JABB.2019.70410

Cai, Y., Luo, Q., Sun, M., & Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences*, *74*(17), 2157–2184. https://doi.org/10.1016/j.lfs.2003.09.047

Carocho, M., & Ferreira, I. C. F. R. (2013). *A review on antioxidants , prooxidants and related controversy : Natural and synthetic compounds , screening and analysis methodologies and future perspectives*. *51*, 15–25. https://doi.org/10.1016/j.fct.2012.09.021

Chang, E., & Kim, C. Y. (2018). Lipid Peroxidation and Antioxidant Activities of the Aqueous Rhizome Extract of Rheum officinale Baillon. *Journal of Food Quality*, *2018*. https://doi.org/10.1155/2018/5258276

Dela Torre, G. L. T., Arollado, E. C., Atienza, A. A., & Manalo, R. A. M. (2017). Evaluation of antioxidant capacity and identification of bioactive compounds of crude methanol extracts of Caesalpinia pulcherrima (L.) Swartz. *Indian Journal of Pharmaceutical Sciences*, *79*(1), 113–123. https://doi.org/10.4172/pharmaceutical-sciences.1000207

G, S. B. A., Choi, S., Krishnan, J., & K, R. (2017). Cigarette smoke and related risk factors in neurological disorders: An update. *Biomedicine and Pharmacotherapy*, *85*, 79–86. https://doi.org/10.1016/j.biopha.2016.11.118

Gu, L., & Weng, X. (2001). Antioxidant activity and components of Salvia plebeia R.Br. - A Chinese herb. *Food Chemistry*, *73*(3), 299–305. https://doi.org/10.1016/S0308-8146(00)00300-9

Guidi, I., Galimberti, D., Lonati, S., Novembrino, C., Bamonti, F., Tiriticco, M., Fenoglio, C., Venturelli, E., Baron, P., Bresolin, N., & Scarpini, E. (2006). Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiology of Aging*, *27*(2), 262–269. https://doi.org/10.1016/j.neurobiolaging.2005.01.001

Hardman, R. J., Kennedy, G., Macpherson, H., Scholey, A. B., & Pipingas, A. (2016). Adherence to a Mediterranean-Style Diet and Effects on Cognition in Adults: A Qualitative Evaluation and Systematic Review of Longitudinal and Prospective Trials. *Frontiers in Nutrition*, *3*(July). https://doi.org/10.3389/fnut.2016.00022

Incalza, M. A., D'Oria, R., Natalicchio, A., Perrini, S., Laviola, L., & Giorgino, F. (2018). Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. *Vascular Pharmacology*, *100*, 1–19. https://doi.org/10.1016/j.vph.2017.05.005

Ishino, K., Wakita, C., Shibata, T., Toyokuni, S., Machida, S., Matsuda, S., Matsuda, T., & Uchida, K. (2010). Lipid peroxidation generates body odor component trans-2-nonenal covalently bound to protein in vivo. *Journal of Biological Chemistry*, *285*(20), 15302–15313. https://doi.org/10.1074/jbc.M109.068023

Kareru, P. G., Kenji, G. M., Gachanja, A. N., Keriko, J. M., & Mungai, G. (2007). *Kareru et al. (Traditional medicines among Embu, Solanum).pdf* (pp. 4(1), 75–86).

Khan, Z., Iqbal, M., Ahmed, W., Khayam, M. U., Kiran, S., Subhan, A., & Yasmine, R. (2016). In vitro haemolytic and thrombolytic activities of Launaea cornuta. *Asian Journal of Chemistry*, *28*(5), 1084–1086. https://doi.org/10.14233/ajchem.2016.19591

Klayman, D. L. (1985). Qinghaosu (artemisinin): An antimalarial drug from China. *Science*, *228*(4703), 1049–1055. https://doi.org/10.1126/science.3887571

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., & Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, *13*, 757–772. https://doi.org/10.2147/CIA.S158513

Lushchak, V. I. (2014). Free radicals, reactive oxygen species, oxidative stress and its classification. *Chemico-Biological Interactions*, *224*(October), 164–175. https://doi.org/10.1016/j.cbi.2014.10.016

Machocho, A., Karau, G., Njagi, E., Koech, L., & Wangai, L. (2014). *Profiling of the chemical compounds in ethyl acetate extracts of launaea cornuta asteraceae by gc-ms*. *1*(5), 296–300. https://doi.org/10.13040/IJPSR.0975-8232.1(5).296-00

Medpilwar, M., Maru, D., Upadhyay, M., Lavania, N., Vernekar, M., & Harmalkar, M. (2015). In-vitro antioxidant and anti-lipid peroxidation activity of ethanolic extracts of bougainvillea shubhra, bougainvillea peruviana and bougainvillea bhuttiana golden glow: A comparative study. *Journal of Natural Remedies*, *15*(1), 43–48. https://doi.org/10.18311/jnr/2015/475

Misonge, J. O., Kinyanjui, J. G., Kingori, W. M., & Mwalukumbi, J. M. (2015). Phytochemical screening and cytotoxicity evaluation of Launaea Cornuta H . (Asteraceae) using brine shrimp. *Merit Research Journal of Medicine and Medical Sciences*, *3*(4), 116–120. https://doi.org/10.3945/ajcn.110.007336

Moriasi, G., Nelson, E., & Twahirwa, E. (2021). In Vitro Anti-Inflammatory , Antioxidant,

and Qualitative Phytochemical Evaluation of the Phytexponent Preparation of Selected Plants Advanced Techniques in Biology & Medicine. *Advanced Techniques in Biology & Medicine*, *9*(1 (277)), 1–9. https://doi.org/10.21203/rs.3.rs-124749/v2

Musila, M. F., Dossaji, S. F., Nguta, J. M., Lukhoba, C. W., & Munyao, J. M. (2013). In vivo antimalarial activity, toxicity and phytochemical screening of selected antimalarial plants. *Journal of Ethnopharmacology*, *146*(2), 557–561. https://doi.org/10.1016/j.jep.2013.01.023

Muthumalage, T., Pritsos, K., Hunter, K., & Pritsos, C. (2017). Commonly used air filters fail to eliminate secondhand smoke induced oxidative stress and inflammatory responses. *Toxicology Mechanisms and Methods*, *27*(6), 458–466. https://doi.org/10.1080/15376516.2017.1320694

Obakiro, S. B., Kiprop, A., Kowino, I., Kigondu, E., Odero, M. P., Omara, T., & Bunalema, L. (2020). Ethnobotany, ethnopharmacology, and phytochemistry of traditional medicinal plants used in the management of symptoms of tuberculosis in East Africa: A systematic review. *Tropical Medicine and Health*, *48*(1), 1–21. https://doi.org/10.1186/s41182-020-00256-1

Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy Reviews*, *6*(11), 1–5. https://doi.org/10.4103/0973-7847.95849

Pisoschi, A. M., & Negulescu, G. P. (2012). Methods for Total Antioxidant Activity Determination: A Review. *Biochemistry & Analytical Biochemistry*, *01*(01), 1–10. https://doi.org/10.4172/2161-1009.1000106

Prasad, S., Gupta, S. C., & Tyagi, A. K. (2017). Reactive oxygen species (ROS) and cancer: Role of antioxidative nutraceuticals. *Cancer Letters*, *387*, 95–105. https://doi.org/10.1016/j.canlet.2016.03.042

Rahman, M. M., Islam, M. B., Biswas, M., & Khurshid Alam, A. H. M. (2015). In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. *BMC Research Notes*, *8*(1), 1–9. https://doi.org/10.1186/s13104-015-1618-6

S. Antwi-Baffour, S. (2014). The Place of Traditional Medicine in the African Society: The Science, Acceptance and Support. *American Journal of Health Research*, *2*(2), 49. https://doi.org/10.11648/j.ajhr.20140202.13

Salazar, R., Pozos, M. E., Cordero, P., Perez, J., Salinas, M. C., & Waksman, N. (2008). Determination of the antioxidant activity of plants from northeast Mexico. *Pharmaceutical Biology*, *46*(3), 166–170. https://doi.org/10.1080/13880200701498952

Thanan, R., Oikawa, S., Hiraku, Y., Ohnishi, S., Ma, N., Pinlaor, S., Yongvanit, P., Kawanishi, S., & Murata, M. (2014). Oxidative stress and its significant roles in neurodegenerative diseases and cancer. *International Journal of Molecular Sciences*, *16*(1), 193–217. https://doi.org/10.3390/ijms16010193

Umar, S., Asif, M., Sajad, M., Ansari, M., Hussain, U., Ahmad, W., Siddiqui, S. A., Ahmad, S., & Khan, H. A. (2012). Anti-inflammatory and antioxidant activity of Trachyspermum ammi seeds in collagen induced arthritis in rats. *International Journal of Drug Development and Research*, *4*(1), 210–219.

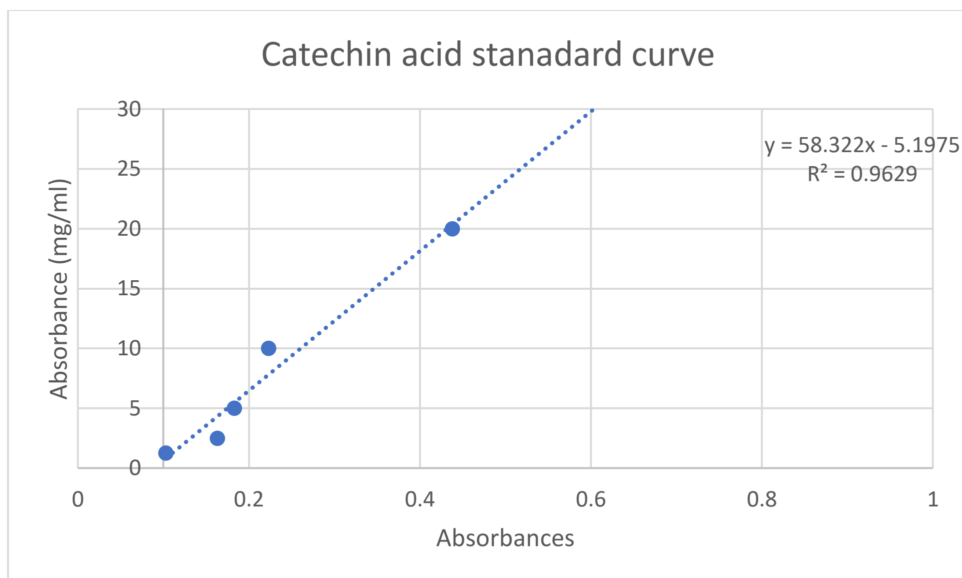
Wambugu, S. N., Mathiu, P. M., Gakuya, D. W., Kanui, T. I., Kabasa, J. D., & Kiama, S. G.

(2011). Medicinal plants used in the management of chronic joint pains in Machakos and Makueni counties, Kenya. *Journal of Ethnopharmacology*, *137*(2), 945–955. https://doi.org/10.1016/j.jep.2011.06.038

Weidinger, A., & Kozlov, A. V. (2015). Biological activities of reactive oxygen and nitrogen species: Oxidative stress versus signal transduction. *Biomolecules*, *5*(2), 472–484. https://doi.org/10.3390/biom5020472

APPENDICES

Appendix 1. 1 Catechin standard calibration curve



Appendix 1. 2 Absorbance of Catechin and *Launaea cornuta* leaf methanol and aqueous extracts

Concentration mg/ml	Absorbance at 510 nm		
20	0.438	0.553	0.274
10	0.223	0.240	0.188
5	0.183	0.176	0.179
2.5	0.163	0.224	0.150
1.25	0.103	0.113	0.113
LCLW	0.079	0.085	0.080
LCLM	0.067	0.063	0.060

Appendix 1. 3 Antioxidant raw data

Launaea cornuta leaf methanol extract

Concentration	Absorbance at 517 nm		
	T1	T2	T3
1000ug/ml	0.138	0.143	0.147
100ug/ml	0.248	0.245	0.250
10ug/ml	0.666	0.659	0.680
1ug/ml	0.754	0.764	0.759
0.1ug/ml	0.772	0.775	0.773
0.01ug/ml	0.776	0.778	0.776
0.00	0.785	0.789	0.787

Launaea cornuta leaf methanol extract percentage radical scavenging activity

Concentration	% RSA		
	T1	T2	T3

1000ug/ml	82.42%	81.85%	81.32%
100ug/ml	68.41%	68.95%	68.23%
10ug/ml	15.16%	16.48%	13.23%
1ug/ml	3.95%	3.17%	3.59%
0.1ug/ml	1.65%	1.39%	1.78%
0.01ug/ml	1.15%	1.1%	1.39%

Launaea cornuta leaf water extract

Concentration	Absorbance at 517 nm		
	T1	T2	T3
0.00	0.785	0.789	0.787
0.01ug/ml	0.733	0.730	0.728
0.1ug/ml	0.650	0.645	0.649
1ug/ml	0.624	0.619	0.623
10ug/ml	0.574	0.577	0.521
100ug/ml	0.419	0.420	0.423
1000ug/ml	0.210	0.211	0.208

Launaea cornuta leaf water extract percentage radical scavenging activity

Concentration	% RSA		
	T1	T2	T3
1000ug/ml	73.25%	73.26%	73.57%
100ug/ml	46.62%	46.77%	46.25%
10ug/ml	34.52%	26.87%	33.80%
1ug/ml	20.51%	21.55%	20.83%
0.1ug/ml	17.18%	18.25%	17.54%
0.01ug/ml	6.62%	7.48%	7.49%

L-Ascorbic acid

Concentration	Absorbance at 517 nm		
	T1	T2	T3
1000ug/ml	0.046	0.025	0.022
100ug/ml	0.041	0.043	0.065
10ug/ml	0.418	0.395	0.484
1ug/ml	0.472	0.495	0.496
0.1ug/ml	0.689	0.695	0.696
0.01ug/ml	0.711	0.704	0.717
0.00	0.769	0.764	0.770

L-Ascorbic acid

Concentration	% RSA		
	T1	T2	T3
1000ug/ml	94.04%	96.72%	97.14%

100ug/ml	94.02%	94.37%	91.55%
10ug/ml	45.64%	48.30%	37.14%
1ug/ml	38.62%	35.20%	35.58%
0.1ug/ml	10.40%	9.03%	9.61%
0.01ug/ml	7.54%	7.89%	6.88%