EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES, TOTAL PHENOLIC AND FLAVONOID CONTENT IN SELECTED MEDICINAL PLANTS, NON-EDIBLE MEDICINAL MUSHROOMS AND SEAWEED

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DECLARATION

I hereby declare that this research thesis is my original work and has not been presented for a degree award in any other university.

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DEDICATION

I dedicate this work to my dear grandmother Sofia, my parents Mr. and Mrs. Siangu and siblings. I appreciate them for their encouragements, support, love, care and prayers.

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ABBREVIATIONS AND ACRONYMS

B. micrantha	*Bridelia micrantha*
BW	Body Weight
D/f	Diclofenac
DMSO	Dimethyl Sulfoxide
DPPH*	2, 2-diphenyl-1-picryhydrazyl radical
DPPH-H	2, 2-diphenyl-1-picryhydrazine
E. denticulatum	*Euchema denticulatum*
EDTA	Ethylene DiamineTetraacetic acid
G. applanatum	*Ganoderma applanatum*
G. lucidum	*Ganoderma lucidum*
IL-6	Interleukin 6
N/S	Normal Saline
NSAIDs	Nonsteroidal Anti-inflammatory Drugs
P. africana	*Prunus africana*
PUFAS	Polyunsaturated fatty acids
T. elegans	*Trametus elegans*
U. dioica	*Urtica dioica*
UV – VIS	Ultra Violet visible

ABSTRACT

Medicinal plants play a major role for sources of lead compounds in drug discovery. Studies done have shown that plants, fungi such as mushrooms and seaweeds are a good source of bioactive compounds with anti-inflammatory, antioxidant, anti-cancer and antimicrobial activities. Antioxidants play main role by hindering oxidation by protecting cells from damage by free radicals. This protects the body from cancer and other chronic ailments like heart diseases. Anti-inflammatory effect is also important as it diminishes swelling and pain due to inflammation. A lot of studies have been done on medicinal values of higher plants but less on marine sources and wild non-edible medicinal mushrooms. Phenolics and flavonoids have a wide range of biochemical activities including antioxidant as well as anticarcinogenic. In this study, the anti-inflammatory and antioxidant activities, phenolic content and total flavonoid was evaluated from wild non-edible medicinal mushrooms: *Ganoderma applanatum*, *Ganoderma lucidum* and *Trametus elegan;* medicinal plants*; -Urtica dioica*, *Prunus africana*, *Bridelia micrantha* and brown algae; *Eucheuma denticulutum*. The antioxidant activity was assessed using 2, 2-diphenylpicryl-1-hydrazyl (DPPH) free radical scavenging method with ascorbic acid as reference standard while anti-inflammatory activity was achieved *in vivo* using mice as test animals. The total phenolic analysis was done using Folin-Ciocalteu reagent and expressed as Gallic acid equivalent (GAE/g) while the total flavonoid content was determined by use of aluminium chloride colorimetric method and expressed as Quercetin equivalent (QE/g). Antioxidant activity of all extracts increased with concentration. *Ganoderma applanatum* showed the highest scavenging activity of (95.56%, IC_{50}< 0.025) while *Urtica dioica* leaves had the lowest (11.99%, IC_{50}> 0.03) activity at 0.3mg/ml of the extract. *Ganoderma lucidum* showed the highest total phenolic (156 ± 3.45 GAE/g) and flavonoid (31.16 ± 0.04 QE/g) content. Anti-inflammatory activity was done using formalin induced edema. The plant extracts showed a significant effect ($p<0.05$) in reducing the edema. The results of the study showed that the plants are source of lead compounds with promising antioxidant and anti-inflammatory activity. There is need to separate and identify actual phytochemicals responsible for antioxidant and anti-inflammatory activities. The findings of this study will provide information on potential sources of phytochemicals with anti-inflammatory and antioxidant activity from selected medicinal plants, mushrooms and marine algae.

CHAPTER ONE

INTRODUCTION

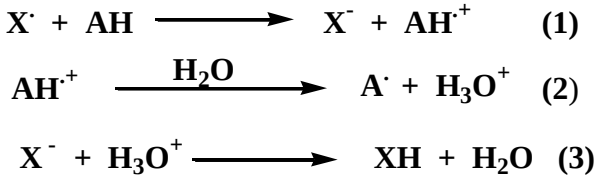
1.1 Background information

Medicines from herbal and natural products were widely used in every culture throughout the world. Medicinal plants which have been known as herbs played significant roles in the development of drugs and this has really shown proper improvement (Wadood, 2013). In developing countries, 80% of the population depends on medicinal plants for their primary health care needs (Evans, 1997; Farnsworth and Soejarto 1998). The use of medicinal plants has been motivated by several factors, including the believe that they are cheaper, are effective and have few side effects as compared to modern synthetic drugs of some plant remedies (Olaokun *et al.*, 2017).

Medicinal plants are used for treatment of human diseases due to presence of bioactive phytochemicals (Wadood, 2013). Oxidation is a very important process in living organisms since it is involved in energy production. During the process, there is production of free radicals. Excess of radicals in the body results in oxidative stress. Oxidation stress is one of the pathways of carcinogenesis associated with inflammation of cells. Antioxidants prevent oxidative stress in human cells, hence useful in the management of many human diseases such as cancer (Soni and Sosa, 2013). Consumption of food rich in antioxidants prevent oxidative damage of the cells (Abugri and Mcelhenney, 2013). Antioxidants can scavenge free radicals through two major mechanisms; hydrogen atom transfer (HAT) and single electron transfer (SET). The end result is the same, regardless of the mechanism (Ismail and Tan, 2002). In HAT process

as shown below the antioxidant (AH) is a hydrogen atom donor that quenches free radicals as shown below.

In the SET process the potential antioxidant (AH) transfers one electron to the radical, reducing it as shown below:



SET and **HAT** mechanisms occurs together most of the time with the balance determined by antioxidant structure and pH of the antioxidant (Wright *et al.,* 2001). Antioxidant activity in sea weeds is high due to high levels of non-enzymatic antioxidant compounds like, phenols, flavonoids and ascorbic acid among others (Farasat *et al.,* 2014). Several anti-inflammatory drugs have shown to have radical scavenging activity (Biosci *et al.,* 2014). Many medicinal plants have shown to be a potential source of natural antioxidants with known active constituents like phenolics. Studies done on *B. micrantha* bark and leaves showed that the bark had a highest level of phenolics (Mburu *et al.,* 2016). Recently, natural antioxidants have been obtained from plants like spices, vegetables, mushrooms, oilseeds, and fruits (Mau *et al.,* 2002). This has created the need to obtain natural antioxidants since they are less toxic (Aqil *et al.,* 2006).

The oceans cover more than 70% 0f earth's surface, remaining to be an outstanding of new discoveries of potential chemotherapeutic agents (Ioannou and Roussis, 2009). Due to wide range of competitive environments marine organisms survive in, they have developed unique defensive strategies of bioactive compounds that in some cases, are unparalleled by their terrestrial counterparts (Barros *et al.,* 2005). Marine organisms have also been incorporated into principally cyanobacteria and soft corals, both of which are rich sources of biologically active natural products (Paul and Puglisi, 2004). Higher plants and animals are not able to synthesize long chain polyunsaturated fatty acids (PUFAs). They must acquire PUFAs from their diets such as fish and fish oil, but their consumption accumulates toxic substances in the body. Microalgae are good alternatives of PUFAs since they are primary producers in marine food chain (Osbourn *et al.,* 2009).

Marine flora is also known for the presence of natural bioactive compounds with anti-inflammatory and antioxidant activities, which protects human diseases like inflammations, melanoma, diabetic mellitus, cardiac disorders and neurodegenerative diseases (Domettila *et al.,* 2013). Brown seaweeds from India have been reported to have high amount of hydrophilic components such as polyphenols and soluble polysaccharides (Delgado *et al.,* 2013). Studies have shown that seaweeds belonging to Genus *sargassum* contain antioxidants (Biosci *et al.,* 2014). Anti-inflammatory effect of *Genus cystoseira* was determined *in vivo* using Wistar rats. It showed that it had the anti-inflammatory effect (Mhadhebi *et al.,* 2014).

Both edible and non-edible mushrooms are considered as good sources of bioactive compounds in human diets. They have secondary metabolites, which include; polyphenols, terpenes, flavonoids, polyketides and steroids with nutritional and pharmacological value (Abugri and Mcelhenney, 2013). Studies have been done on *Ganoderma applanatum* and *Ganoderma lucidum* mushrooms. They have shown to have phenols, polysaccharides, terpenes among others (Pandya *et al.,* 2018). These secondary metabolites could be responsible for anti-inflammatory and antioxidants activities (Rašeta *et al.,* 2016). Several polysaccharide-protein and polysaccharide complexes have been isolated from mushrooms and used as a source of therapeutic agents (Pandya *et al.,* 2018).

Studies have shown that fungus *Geastrum saccatum* has strong anti-inflammatory and antioxidant activity (Dore *et al.,* 2014). The culinary mushrooms, *P. ferulae, P. eryngii, P. citrinopileatus,* and *P. ostreatus of* genus *Pleurotus* have been studied for their therapeutic potential because of their antitumor, antimicrobial, anti-inflammatory activity among others (Nguyen *et al.,* 2016). Though a lot of studies have dealt with the search for antimalarial, antimicrobial drugs, there is less information on antioxidant and anti-inflammatory studies especially from marine sources and mushrooms. There is thus need to search for new sources of phytochemicals with antioxidant and anti-inflammatory activity from plants, fungi and sea algae.

The study was designed to evaluate methanolic extracts of selected medicinal plants; *P. africana* bark and leaves, *B. micrantha* stem bark, *U. dioica* roots and leaves, Mushrooms; *G. applanatum, G. lucidum, T. elegans, F. spatulatus and Coreleopsis sp.*

Seaweeds; *E. denticulatum*, for anti-oxidant and anti-inflammatory activities, total phenolic and flavonoid content due to their ethnobotany.

1.2 Problem statement

Human infections such as malaria and tuberculosis are increasingly becoming resistant to modern treatment. Plants, especially from sea weeds and mushrooms, can be a source of new compounds for drug development to treat various diseases. Though a lot of studies in complementary and alternative medicine have dealt with the search for antimalarial and antimicrobial drugs from plants, there is less information on antioxidant and anti-inflammatory studies especially from marine sources and mushrooms due to their exceptional content of novel bioactive natural products. There is need to search for antioxidants and anti-inflammatory drugs from marine flora, mushrooms and selected plants with known medicinal value.

1.3 Justification

Medicinal plants are very important in the treatment of various diseases related to inflammation as they are cheaper, reliable and have been reported to have few side effects. Synthetic anti-inflammatory drugs like Nonsteroidal anti-inflammatory drugs (NSAIDs) have side effects. Therefore, there is need to search for new sources of phytochemicals from plants, fungi and sea algae with antioxidant and anti-inflammatory activity.

1.4 Hypotheses

i. Extracts of medicinal plants, non-edible medicinal mushrooms and seaweed have antioxidant effect.

ii. There is a significant difference in the levels of total phenolic and flavonoid content present in the plant extracts.

iii. Alkaloids, tannins, flavonoids and terpenoids are present in the methanolic extracts.

iv. Extracts of medicinal plants, seaweed and non-edible medicinal mushrooms show anti-inflammatory activity.

1.5 Objectives

1.5.1 General objective

To evaluate the antioxidant and anti-inflammatory activities, total phenolic and flavonoid content of selected medicinal plants, non-edible medicinal mushrooms and seaweed.

1.5.2 Specific objectives

i. To determine the antioxidant activity of methanolic extacts of mushrooms; *G. applanatum, G. lucidum, Coreleopsis sp., F. spatulatus, T. elegans,* plants; *B. micrantha* bark, *P. africana* bark, *P. africana* leaves, *Urtica dioica* roots and leaves, Seaweed; *E. denticulatum.*

ii. To determine the total phenolic and flavonoid content in the plant extracts.

iii. To test for alkaloids, tannins, flavonoids and terpenoids present in methanolic extracts.

iv. To determine anti-inflammatory activity of the medicinal plants, seaweed and mushrooms methanolic extracts using mice as test animals.

1.6 Significance of the study

The findings of this study will provide information on potential sources of phytochemicals with anti-inflammatory and antioxidant activity from selected medicinal plants, mushrooms and marine algae. The information will be valuable to medical researchers in search of lead compounds to develop new drugs especially in combating inflammation related diseases like arthritis, cancer, cardiovascular diseases, among others.

1.7 Limitation of the scope

Only a few selected medicinal plants, mushrooms and seaweed were used in this study due to cost and time constraints. Plant extracts were screened for phytochemicals but not on separation and characterization of specific chemical constituents.

CHAPTER TWO

LITERATURE REVIEW

2.1 Oxidative stress

According to Betteridge (2000), oxidative stress is an imbalance between reactive oxygen species produced (free radicals) and antioxidant defense. Aerobic bacteria require oxygen for oxidation of nutrients or respiration to produce energy. Reactive oxygen species are very important in physiological activity but an imbalance in reactive oxygen species causes oxidation stress (Rani *et al.,* 2016). Many radicals are reactive and unstable. They can either accept electrons from other molecules or donate an electron to other molecules, therefore they act as reductants or oxidants (Rani *et al.,* 2016). When stable molecule loses an electron it becomes free radical resulting to a chain of reactions which disrupts various classes of biological molecules such as lipids, proteins and DNA. This alteration increases the oxidative stress (Phaniendra *et al.,* 2015), hence causing a number of diseases as shown in table 2.1 (Preeti *et al.,* 2016).

Table 2.1: Representation of diseases related to oxidative stress

Effect of the diseases	Disease(s)
Erythrocyte disorders	Sickle cell anaemia, malaria, Fanconi'sanaemia
Aging	Disorders of premature aging
Iron overload	Organ transplantation, stroke/ myocardial Idiopathic haemochromatosis, thalassaemia, Bantu iron overload
Inflammatory-immune injury	Autoimmune disease, rheumatoid arthritis Ischaemia-reflow
Lungs	Emphysema, adult respiratory distress syndrome Ocular
Ischaemia-reflow states	Organ transplantation, stroke/ myocardial infarction Sickle
Skin	Solar radiation, porphyria
Brain	Parkinson's disease, Vitamin E deficiency
Heart and cardiovascular system	Artherosclerosis, doxorubicin toxicity Parkinson's
Eye	Ocular hemorrhage, cataractogenesis

Living organs possess a mechanism that protects the body against oxidative stress. Enzymes such as superoxide dismutase (SOD), act upon molecules of superoxide, destroy free radicals and change them into a less reactive state. Also, the self-repair of the body helps in scavenging free radicals. Nutrients such as vitamins and others neutralize free radicals (Preeti *et al.,* 2016).

2.2 Antioxidant activity

Antioxidant or oxidation inhibitor is any compound that inhibits the production of free radicals. It prevents the damage of cellular components that arises due to chemical reactions, which yield free radicals (Seifried and Pilch, 2013). Many herbal plants contain antioxidants. Most of the diseases such as tuberculosis are as a result of oxidative stress, which produces reactive oxygen species such as peroxyl (*OOH, ROO*), Hydroxyl (OH*) and superoxide (O^{*}_{2}) radicals (Ansari *et al.,* 2013). These reactive oxygen species are the ones that cause wide range of diseases. Natural

antioxidants have been shown to possess natural bioactive compounds that prevent these diseases. Natural antioxidants such as; ascorbic acid, carotenoids, and phenolic compounds are known to inhibit lipid peroxidation. Free radicals and active oxygen species reacts through a reaction cycle and chelate heavy metal ions (Lee *et al.*, 2007). There has been growing attention on safety and toxic effects of some synthetic antioxidants such as butylatedhydroxyanisole users (Balakrishnan and Agrawal, 2014). Antioxidant activity is determined by different methods as presented in table 2.2, Preeti *et al.* (2016).

Table 2.2: *In vitro* assays commonly used to screen antioxidant activity in plants and food

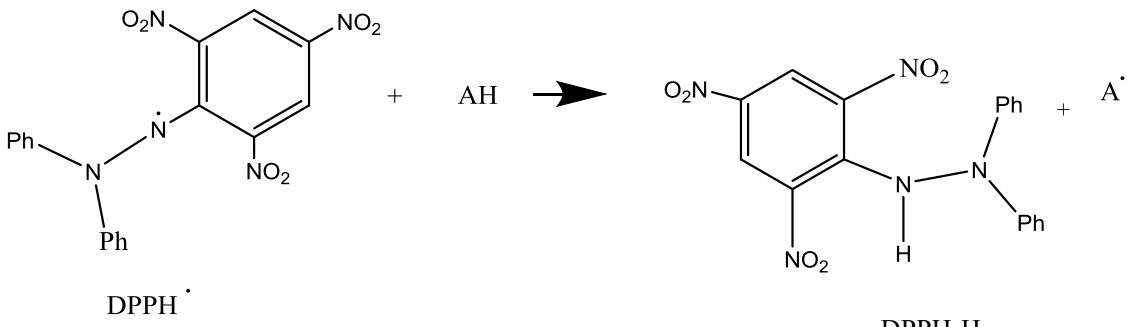
Assay	**Mechanism**	**References**
β-Carotene or crocin-bleaching assay	Hydrogen atom transfer	Karadag *et al.*, 2009
DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay	Both hydrogen atom and single electron transfer	Huang *et al.*, 2005
ORAC (oxygen radical absorbance capacity)	Hydrogen atom transfer	Ou*et al.*, 2001
ABTS ({2,2' - azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid)}) assay	Both hydrogen atom and single electron transfer	Huang *et al.*, 2005
IOU (inhibited oxygen uptake)	Hydrogen atom transfer	Karadag *et al.*, 2009
Total phenolic content assay by Folin-Ciocalteu reagent	Single electron transfer	Karadag *et al.*, 2009
LPIC (lipid peroxidation inhibition capacity) assay	Hydrogen atom transfer	Karadag *et al.*, 2009
FRAP (ferric reducing antioxidant power assay	Single electron transfer	Huang *et al.*, 2005
TRAP (total radical trapping antioxidant parameter)	Hydrogen atom transfer	Karadag *et al.*, 2009
Copper reduction assay	Single electron transfer	Huang *et al.*, 2005

DPPH, ABTS and FRAP assays are commonly used methods as they are faster, easy to use, reliable and do not require special reaction conditions and devices (Ansari *et al.*, 2013).

2.3 Antioxidant activity in medicinal plants

Medicinal plants have shown to have a high level of antioxidant activity and phenolic compounds (Tlili *et al.,* 2013). The method of choice for determining antioxidant activity in medicinal plants is DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay since it is reliable, it gives accurate results, does not require special reactions and device which is illustrated below. Other methods may be limited with those compounds that are soluble in selected solvents. The advantage of DPPH is that it reacts with whole sample and it is stable in methanol.

An illustration on how DPPH reacts with free radical



AH (Antioxidant)

A· (Antioxidant radical)

Study carried out on *Bridelia micrantha* leaves recorded higher antioxidant activity than L-ascorbic acid and high hepatoprotective activity (Chandra, 2012). However, it did not determine the active metabolites that brought about hepatoprotective and antioxidant

activity (Nwaehujor and Udeh, 2011). *U. dioica* leaves were found to have higher level of antioxidant activity than the stem and roots. It also showed the presence of weak antimicrobial activity (Kukrić *et al.,* 2012). Studies have shown that different plants have different antioxidant activity. Some of the plants from West Bengal, India that have been screened for antioxidant activity are listed in table 2.3 (De, Das and Mukhophadhyay, 2018).

Table 2.3: Anti-oxidation activity of aqueous extract of selected medicinal plants

Medicinal plants	**Concentration in µg/ml**	**Antioxidant activity %**
Turmeric (*Curcuma longa*)	100	48.23±0.43
	50	37.64±0.34
Betel leaf (*Piper betle*)	100	20.80 ± 0.81
	50	16.70 ± 1.15
Laalsag (*Amaranthus gangeticus*)	100	67.90 ± 0.73
	50	20.40 ± 1.01
Nisindha (*Vitex negundo*)	100	62.76 ± 0.29
	50	28.57 ± 0.37
Bramhi (*Baco pamonnirie*)	100	91.35 ± 0.23
	50	71.15 ± 0.19
Basaka (*Justicia adhatoda*)	100	67.54 ± 0.85
	50	41.23 ± 0.52
Sajne (*Moringa oleifera*)	100	72.86 ± 0.51
	50	58.54 ± 0.84
Green tea (*Camellia sinensis*)	100	86.31 ± 0.61
	50	64.40 ± 0.30
Black tea (*C. sinensis sinensis*)	100	57.01 ± 0.76
	50	49.20 ± 0.91
Coffee (*Coffe aarabica*)	100	65.11 ± 0.11
	50	49.32 ± 0.74
Licorice (*Glycyrr hizaglabra*)	100	41.17±0.44
	50	36.47 ± 0.26
Pineapple (*Ananas comosus*)	100	52.69 ± 0.57
	50	47.99 ± 0.27

The following medicinal plants used in this study were selected due to their known biochemical and medicinal properties.

2.3.1 *Prunus africana* (African cherry)

Prunus africana known as *muiri* (Kikuyu), *mwiria* (Embu/Meru) and *kumuturu* (Bukusu) belongs to Rosaceae family. It is an evergreen tree ranging in height from medium to large with a spreading crown within 10 to 20 m and a stem diameter of 1 m during maturity as shown in fig. 2.1. In a garden, it is medium sized but in the forest region it is huge (Jeruto *et al.,* 2008). It is used as a traditional medicine by some communities in Eastern African countries (Kokwaro, 2009). It grows mostly in Central Kenya and Elgon regions in Kenya. The tree is also located in Central Africa (Congo, Katanga) and in West Africa (Cameroon). The plant is used to treat diseases like allergies, gonorrhea, stomachache, kidney diseases and prostate glands (Iwu, 2014). Its bark extract also prevents bladder hyperactivity (Nyamai *et al.,* 2015). Extracts from roots and stem contain phytochemicals with anti-inflammatory, anticancer and antiviral activities. The secondary metabolites in *P. africana* are; alkaloids, tannins, flavonoids, phenols, terpenoids and pentacyclictriterpenoids that prevents glucosy-transferase activity with anti-edematous activity (Bii *et al.,*2010).

Figure 2.1: ***Prunus africana***

2.3.2 *Bridelia micrantha* (coast gold leaf)

B. micrantha, mukwego (Meru/Embu), *athuno* (luo) and *Mukoigo* (Kikuyu) shown in fig. 2.2 grows from small to medium size. The plant belongs to Phyllanthaceae family. The genus, *Bridelia* includes 60 to 70 species found throughout subtropical and tropical regions especially in Asia and Africa (Maina *et al.,* 2016). It is used as antibacterial, anti-diabetic, antimalarial, anti-inflammatory, anti-nociceptive, antiviral, anti-diarrhoeal, anti-amebic, anti-convulasant, anthelmintic and in treatment of sexual diseases (Mburu *et al.,* 2016). Secondary metabolites from *B. micrantha* are triterpenes, saponins, flavonoids and lignans which have shown to have medicinal values (Shelembe *et al.,* 2016).

Figure 2.2: *Bridelia micrantha*

2.3.3 *Urtica massaica* (*dioica*) (Stinging nettle)

Urtica massaica (dioica), upupu (Swahili), *thabai* (Kikuyu) shown in fig. 2.3 belongs to Urticaceae family and is native to Asia, Europe, America and Africa. It is a herbaceous

flowering plant which is 50 to 300 cm long and it's hairy leaves sting (Poudel and Khanal, 2011). The plant is used to treat various diseases including hypertension, arthritis, rheumatism and muscular paralysis (Harput *et al.*, 2005). It can also be used as a vegetable and tea (Douglas and Gitonga, 2016). Compounds that have been found in stinging nettle are terpenoids, alkaloids, amino acids, phenols and proteins (Jyoti *et al*., 2016).

Figure 2.3: *Urtica dioica* upper part

2.4 Antioxidant activity in mushrooms

Mushrooms are consumed worldwide by people due to their nutrient content and medicinal value. They are used to treat chronic diseases such as; obesity, heart diseases, cancer and diabetes (Jain *et al.*, 2016). They are a source of bioactive compounds such as polyphenols, polysaccharides, steroids, proteins, terpenoids, glycans and cerebrosides (Kajal *et al.*, 2017). Mushrooms of *genus Basidiomycota* have been studied for cancer treatment. *Trametes elegans* (Fig. 2.4) have been reported to have

antioxidant activity (Blagodatski *et al.*, 2018). Among the Ganoderma species of mushrooms, *Ganoderma lucidum* (Fig. 2.5) is most investigated and best known for medicinal value (Raseta *et al.*, 2016). Species *G. applanatum* has been considerably reported to contain useful antitumor polysaccharide in liquid cultured mycelium (Lee *et al.*, 2007) and has higher antioxidant activity than *G. lucidum* (Soni and Sosa, 2013). Their phytochemicals are responsible for the biological effect such as anti-oxidant, antiviral, antibacterial, cytotoxic, anti-inflammatory and immune stimulatory activities (Alispahić *et al.*, 2015, Zhang *et al.*, 2016).

Figure 2.4: *Trametus elegans* mushroom

Figure 2.5: *Ganoderma lucidum* mushroom

2.5 Seaweeds

Seaweeds belong to a group of marine plants known as algae. They are non-flowering plants with no roots, stem nor leaves. Seaweeds are good sources of medicine and food (Delgado *et al.,* 2013). Phytochemicals present are agar, algin, agarose and carrageenan (used as stabilizing, gelling and thickening agents in food) (Kaliaperumal, 2003). Antioxidant activity has been investigated in brown and green algae (Ismail and Tan, 2002). The antioxidant activity was shown to be higher in dried compared to fresh sample. The antioxidant and anticoagulant activity of phenolic and polysaccharide is high in *padina sp.* (Review, 2013). Research has also been carried out on *E. denticulatum* seaweed (Fig. 2.6) for its antioxidant activity (Kajal *et al.,* 2017). Its anti-diabetic activity was investigated and found to be higher compared to that of *Sargassum polycystum* and *Caulerpa lentillifera,* brown seaweeds (Balasubramaniam *et al.,* 2016).

Figure 2.6: *Eucheuma denticulatum* seaweed

2.6 Anti-inflammatory activity in medicinal plants, non-edible mushrooms and seaweed

Inflammation manifests itself through pain, heat, redness or swelling (El Haouari and Rosado, 2016). Anti-inflammatory activity of *B. micrantha* leaves, *Kigelia africana, Aspilia africana, Codia africana* has also been investigated. They reduced swelling on the hind paw of rat (Nwaehujor *et al.*, 2014). Mushrooms are a group of macrofungi from a family of ascomycetes and basidiomycetes. They are rich in free amino acids, proteins, carbohydrates and vitamins, as well as trace elements and essential minerals. Bioactive compounds present are polyphenols, phenolics, polysaccharides, terpenoids, among others (Elsayed *et al.,* 2014). *G. applanatum* has been investigated for its antinociceptive and anti-inflammatory activity. The extracts gave a positive result on inflammation (Ede *et al.,* 2012).

Studies done on seaweeds have shown a potential anti-inflammatory activity. Red seaweed, *Dichotomari obtusata* was shown to have anti-inflammatory effect (Delgado

et al., 2013). Anti-inflammatory studies done on *sargassum sp.* showed a positive effect with an increase in concentration of the doses (Yuvaraj *et al.,* 2013).

2.7 Total phenolic and flavonoid content

Phenolics and flavonoids naturally occur in plants (Tungmunnithum *et al.,* 2018). Phenolics molecules have high potential of antioxidant activity. These phenolics include stilbenes, coumarins, flavonoids and tannin. Research has been carried out on quantitative determination of total phenolic and total flavonoid content on several medicinal plants from Nigeria. Among the plants selected, *lochner arose*a gave the highest content total phenolic and flavonoid content (Agbo *et al.,* 2015). Total phenolic and flavonoid content has been determined from wild edible and non-edible wild mushrooms including *G. applanatum* and found to contain high content of phenolics and flavonoids (Abugir *et al.,* 2013). Seaweed is also good source of phenolics and flavonoids. The studies done on different species of seaweeds showed that, white seaweeds had the highest phenolic and flavonoid content, followed by purple and green seaweeds (Ling *et al.,* 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials, apparatus and chemicals

3.1.1 Sampling

Samples of various plant parts were collected from their natural habitat. Mushrooms; *G. applanatum*, *T. elegans* and *G. lucidum* species were collected from Kitale, Trans-Nzoia County. The brown sea weed; *E. denticulatum* was collected from the Kenyan South Coast, Kwale County. Medicinal plants; *U. dioica (*roots and leaves), *B. micrantha* stem bark and *P. africana stem bark* were collected from Manyatta constituency, Embu County. Mushroom samples were identified at the National Museums of Kenya (NMK), Nairobi and voucher specimen deposited at the NMK herbarium. Sea algae, *E. denticulatum* specimen was identified at Kenya Marine and Fisheries Research institute (KEMFRI), Mombasa. The selected medicinal plant specimens were identified by taxonomist from the department of plant Sciences and voucher specimen deposited at the department herbarium, Kenyatta University.

3.1.2 Chemicals and reagents

All the chemicals and reagents used in the study were of analytical grade. Folin-Ciocalteu reagent, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical, gallic acid, methanol, sodium carbonate, aluminium chloride, sodium hydroxide, sodium nitrate, nitric acid, chloroform, concentrated sulphuric acid, bismuth nitrate, potassium iodide, concentrated hydrochloric acid, ascorbic acid, quercetin, diclofenac and formalin were procured from Sigma Aldrich (Germany).

3.1.3 Apparatus and instruments

Test tubes, conical flasks, Measuring cylinders, beakers, pipette, Rotary evaporator, Whatman No. 1 filter papers, UV-VIS spectrophotometer (UV-VIS model 6305 Jenway, UK), Grinder (Retsch 200), digital caliper and ultrasonic bath (WUC-A03H) were used in the study.

3.2 Sample preparation

Samples were washed thoroughly 2-3 times with running tap water, shade dried and then ground to fine powder using a grinder. The powder was stored in polythene bags before extraction.

3.2.1 Plant extraction

100 g of each ground plant sample was weighed; 500 ml methanol was added and soaked

Overnight and then sonicated at 60°C on ultrasonic bath for two hours to enhance extraction. The extract was filtered using Whatman filter paper No. 1. The filtrate obtained was concentrated at reduced pressure by rotary evaporator to produce a crude extract.

3.2.2 Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu method (Tibhwa, 2012) with some modification. 0.1 g of the extract was dissolved in 5 ml methanol. 200 µl of the extract solution and 1 ml Folin-Ciocalteu reagent were mixed and 1 ml of 0.1 M of sodium carbonate solution added after 3 min. The mixture was shaken and

allowed to stand for 2 hrs in the dark. The absorbance of the solution was read out in triplicate using UV-Vis spectrophotometer (specord 200 Analytic Jena model, Germany) at a wavelength of 515 nm. A blank solution was prepared and read similarly. A calibration curve of gallic acid was obtained from serial dilutions of various concentrations of gallic acid prepared from its stock solution (Appendix V). The results were expressed as gallic acid equivalents/g of the extract (GAE/g).

3.2.3 Total flavonoid content determination

The total flavonoid content was determined spectrophotometrically (Quettier-Deleu *et al.,* 2000). 4 ml of distilled water was added to 1 ml of the extract in a 10-ml volumetric flask, followed by 1 ml of 5% sodium nitrate. 1 ml of 10% aluminium chloride was added after 5 min. This was left to settle for 5 min where 2 ml of sodium hydroxide was then added and topped up to the mark with distilled water. The absorbance readings were taken at 510 nm against a blank (water). The flavonoid content was determined using quercetin standard curve with sequential concentrations of 10 to 180 mg/ml (Appendix IV). The flavonoid content was expressed as quercetin equivalents (QE mg/g) (Quettier-Deleu *et al.,* 2000).

3.2.4 Test for terpenoids

0.8 g of the extracts was transferred to a test tube and 10 ml of ethanol added. The sample was thoroughly shaken and then filtered. To 5 ml of the filtrate, 2 ml of chloroform was added followed by 3 ml of sulphuric acid. The formation of reddish brown colour was indication of presence of terpenoids in the plant extract (Biosci *et al.,* 2014).

3.2.5 Test for tannins

10 ml of bromine (prepared by decanting of the liquid bromine into a bottle with distilled water in the fume hood. After the airspace above the water is covered with red bromine vapor, both bottles were capped. The mixture was swirled gently) was added to 0.5g of the extract. Decoloration of bromine water was an indication of the presence of tannins (Biosci *et al.*, 2014).

3.2.6 Test for alkaloids

To 2-3ml of the extract solution, a few drops of Drangendorff"s reagent (prepared from 0.5g of bismuth nitrate, 10 ml of concentrated hydrochloric acid and 4 g of potassium iodide) was added. The formation of orange precipitate was an indication of the presence of alkaloids (Biosci *et al.*, 2014).

3.2.7 Test for flavonoids

To 1ml of the extract, a few drops of sodium hydroxide solution was added. An intense yellow color that disappears on addition of dilute acid indicates the presence of flavonoids (Biosci *et al.*, 2014).

3.2.9 Antioxidant activity determination

DPPH free radical scavenging assay was used for determination of antioxidant activity according to the method described by Brand *et al.* (1995) and Jaita *et al.* (2010) with slight modification. Series of dilutions of the methanolic extract (0.025-0.3 mg/ml) was prepared. A measure of 2 ml of the extract was mixed with 3 ml of 0.3 mg/ml of DPPH radical. The mixture was shaken vigorously and allowed to stand for 30 minutes at room temperature. The absorbance of the mixture was read at 517 nm using UV-Vis

spectrophotometer. The absorbance of the resulting solution was converted into a percentage of antioxidant activity (% inhibition) by the use of the following formula:

% Inhibition = $(A_o - A_1) / A_o \times 100$

Where A_o = Absorbance of the control solution containing only DPPH solution; A_1 = Absorbance in the presence of extract in DPPH solution. Ascorbic acid was used as a reference standard. The IC_{50} values were determined from extrapolation curves of the antioxidant activity of extracts on DPPH.

3.2.10 Screening for anti-inflammatory effect

The anti-inflammatory activity was investigated using the formalin-induced hind paw edema model in albino mice employing 5.0% formalin solution as the phlogistic agent (Winter *et al.,* 1962). Albino mice of either sex distributed equally was divided into nine groups of five each. Group 1 served as negative control and received saline at a dose of 0.85 mg/ml. Group 2 served as positive control and treated with 15 mg/kg of diclofenac sodium, 30 minutes prior to formalin injection. Group 3, 4, 5, 6, 7, 8 and 9 received intraperitonial injection of the extract doses. Inflammation was induced by injecting 50 µl of 5% formalin solution in the subplantar region of the left hind paw of the animal. The paw diameter before injection of formalin was measured using digital caliper. One hour after formalin injection, the paw diameter was measured. This was repeated for four hours. The difference between the final diameter and the initial was recorded. Data obtained was expressed as mean and their standard error means (Radhika *et al.,* 2013). The data was further analyzed using one way ANOVA followed by *scheffe* as post-ANOVA. $P < 0.05$ indicated the significant effect. The experiments

were carried out according to the guidelines for care and use of laboratory animals (Wolfensohn and Lloyd, 1998).

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Antioxidant activity

The effect of antioxidant on DPPH radical is due to their hydrogen donating ability. The extracts were allowed to react with DPPH radical in methanol solution. After the crude extract was mixed with DPPH in dark room, color of the DPPH changed from purple to yellow at different intervals. This color change was an indication of the ability of the extract to reduce free radicals. The reducing capability of extract on DPPH radicals, determined by the decrease in its absorbance at 515 nm, a measure of antioxidant activity of extract. The results of % scavenging activity on DPPH are given in figure 4.1.

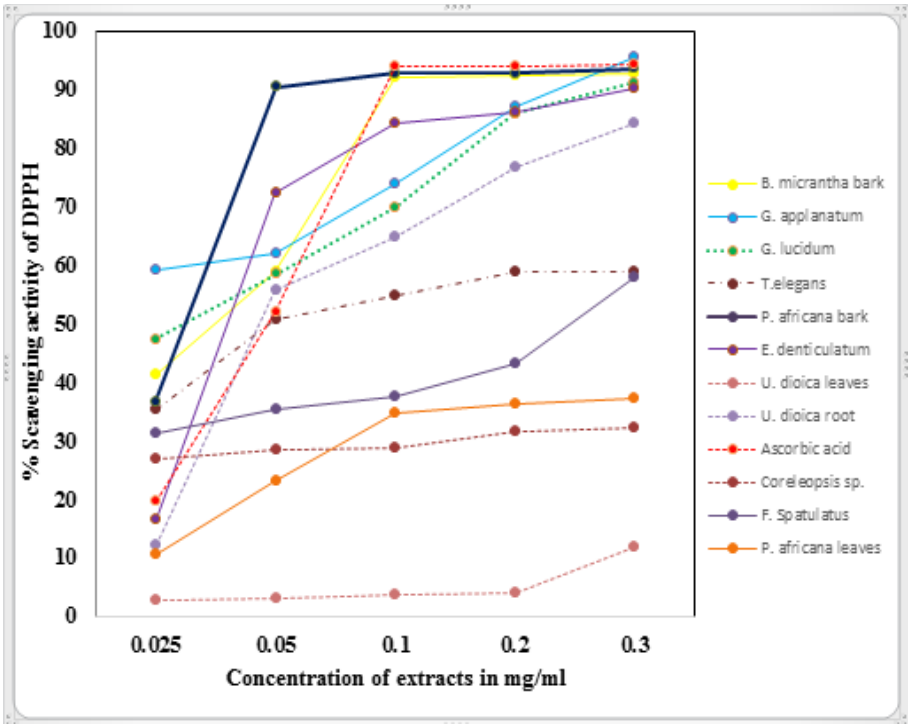


Figure 4.1: Antioxidant activity of the extracts at different concentrations

The plant extracts showed significant radical scavenging activity on DPPH in range 11.99 – 95.56% as compared to 94.32% of standard ascorbic acid at 0.3 mg/ml. The antioxidant activity increased with the concentration of extracts as shown in figure 4.1 and was maximum at 0.3mg/ml of extract. In this study, mushroom showed high scavenging activity. This could be due to the absorption of nutrients from the components they grow from. The scavenging ability of *G. applanatum* was higher than the reported of 71% (Mesías *et al.,* 2014). *T. elegans* had a scavenging activity value of 59.01% which was lower than the one reported of 65% (Batiston *et al.,* 2013). Antioxidant activity of *B. micrantha* (92.6%) was lower than the one reported of 97.70% by Nwaehujor (2014). This variation may be due to the variation in geographical area and the bioactive compounds present. Different parts of the plants showed variation in antioxidant activity due to the composition and presence of different types of biochemical.

Table 4.1: IC_{50} and antioxidant activity of the extracts

Extract	**Antioxidant activity on DPPH at 0.3mg/ml of extract** (%)	**IC_{50} (mg/ml)**
Ascorbic acid	94.32	0.03
G. applanatum mushroom	95.56	< 0.025
P. africana stem bark	93.60	0.033
B Micrantha stem bark	92.67	0.038
G. lucidum mushroom	91.24	0.04
E. denticulatum seaweed	90.21	0.042
U. dioica roots	84.32	0.045
T. elegans mushroom	59.01	0.22
U. dioica leaves	11.99	> 0.3
P. africana leaves	37.31	> 0.3
F. spatulatus mushroom	57.88	0.25
Coreleopsis species mushroom	32.43	> 0.3

The concentration of antioxidant needed to decrease the initial DPPH absorbance by 50% is termed as inhibition concentration or effective concentration (IC_{50} or EC_{50}). This parameter is widely used to measure the antioxidant activity (Sanchez-Moreno *et al.,* 1998). The results of inhibition concentration (IC_{50}) of plant (table 4.1) were obtained from the graph in figure 4.1 at 50%. A lower IC_{50} value corresponds to higher antioxidant ability. *G. applanatum* showed the highest scavenging activity of 95.56% (IC_{50}< 0.025 mg/ml), higher than that of ascorbic acid of 94.32% (IC_{50} = 0.03 mg/ml) followed by *P. africana stem* bark at 93.6% (IC_{50} = 0.033). This was in agreement with the scavenging ability of 84% reported of methanol extract of *P. africana* bark by Soobrattee *et al.* (2005). *U. dioica* leaves showed the lowest scavenging activity of 11.99% (IC_{50}> 0.3 mg/ml). The results of antioxidant activity of other plant extracts were as follows: *B. micrantha* 92.67% (IC_{50} = 0.038 mg/ml), *G. lucidum* 91.24% (IC_{50} = 0.04 mg/ml), *E. denticulatum* 90.21% (IC_{50} = 0.042 mg/ml), *U. dioica* roots 84.32% (IC_{50} = 0.045 mg/ml) and *T. elegans* 59.01% (IC_{50} = 0.22 mg/ml).

4.2 Total phenolic and flavonoid content

All the extracts under the study contained noticeable phenolic and flavonoid content with significant variations (P – value 0.0004) as shown in Table 4.2.

Table 4.2: Total phenolic and flavonoid content

Extract	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
G. applanatum mushroom	127.23 ± 0.64^{d}	14.53 ± 0.28^{d}
P. africana stem bark	148.55 ± 4.05^{b}	9.29 ± 0.06^{e}
B. micrantha stem bark	128.79 ± 1.54^{d}	30.47 ± 0.03^{ab}
G. lucidum mushroom	156.07 ± 3.45^{a}	31.16 ± 0.04^{a}
E. denticulatum seaweed	146.15 ± 1.11^{bc}	9.36 ± 0.12^{e}
U. dioica roots	144.04 ± 3.89^{c}	28.54 ± 0.67^{b}
T. elegans mushroom	103.19 ± 1.23^{f}	9.97 ± 0.32^{e}
U. dioica leaves	43.19 ± 1.15^{g}	3.97 ± 0.06^{f}
P. africana leaves	117.68 ± 1.40^{e}	10.32 ± 0.04^{e}
F. spatulatus mushroom	124.90 ± 1.53^{d}	8.73 ± 0.06^{e}
Coreleopsis species mushroom	101.38 ± 0.94^{f}	17.17 ± 0.03^{c}

The results obtained by one way ANOVA within the column. Values with the same superscript are considered to have no significant difference

The highest phenolic content was observed in *Ganoderma lucidum* (156.07 ± 3.45 mg GAE/g) followed by *Prunus africana* stem bark (148.55 ± 4.04), *Eucheuma denticulatum* (146.15 ± 1.11), *Bridelia micrantha* stem bark (128.79 ± 1.54) and *Ganoderma applanatum* (127.23 ± 0.64 mg GAE/g), respectively. The highest phenolic content in *Ganoderma lucidum* may be due to different polyphenolic compounds and content. This is in line with it having high antioxidant activity. The results obtained for *Ganoderma applanatum* were lower compared to 191.76 mg GAE/g reported by Raseta (2016). The value for *Ganoderma lucidum* obtained (156.07 ± 3.45mg GAE/g) higher than the reported value of 60.41 mg GAE/g (Raseta, 2016). *Trametus elegans* had a moderate value of value, 9.97 ± 0.32 mg GAE/g but higher than reported 4.79 mg GAE/g (Batiston *et al.,* 2013). The total phenolic content was lowest in *Urtica dioica*

leaves (3.97 ± 0.06 mg GAE/g). The results showed a correlation between antioxidant activity and phenolic content (r = 0.9467) as observed from the results. Natural extracts with proven antioxidant activity usually contain compounds with a phenolic moiety such as flavonoids, tocopherols, carotenoids and tannins (Dapkevicius *et al.,* 1998). Phenolic components present in the plant extracts are among the compounds responsible for radical scavenging activity. The total flavonoid content for the different plant extracts are shown in Table 4.2. The highest total flavonoid content was observed in *Ganoderma lucidum* (31.16 ± 0.04 mg QE/g), which was higher than the reported value of 10.82 mg QE/g (Raseta, 2016). *Bridelia micrantha* bark had a value of 30.47 ± 0.03 mg QE/g and *Trametus elegans* with a value of 9.97 ± 0.32 mg QE/g, higher than the reported value of 0.97 mg QE/g (Awala, 2015). *Urtica dioica* leaves had the lowest total flavonoid content (3.97 ± 0.06 mg QE/g). It was observed that extracts with high antioxidant activity had also high total flavonoid content. It was reported that high flavonoid content may provide protection against oxidative stress along with other oxidative defenses such as vitamins and enzymes (Tripathy *et al.,* 2014).

4.3 Phytochemical screening

Phytochemicals are naturally occurring compounds that are found in plants. They protect plants from damage and diseases. Tannins, alkaloids, saponins, alkaloids and terpenoids are known to bioactive principles in plants (Zheng and Wang 2017). The results obtained for phytochemical screening are given in table 4.3.

Table 4.3: Phytochemical constituents of methanolic extracts

Extracts	Tannins	Flavonoids	Terpenoids	Alkaloids
G. applanatum mushroom	+	+	+	+
P. africana stembark	+	+	+	+
B Micrantha stembark	+	+	+	+
G. lucidum mushroom	+	+	+	+
E. denticulatum seaweed	+	+	–	+
U. dioica roots	+	+	+	+
T. elegans mushroom	+	–	+	+
U. dioica leaves	–	–	+	–
P. africana leaves	+	+	–	–
F. spatulatus mushroom	–	+	–	+
Coreleopsis sp. Mushroom	+	+	+	–

Key: (+) present, (-) not present

Tannins, flavonoids, terpenoids and alkaloids were present in *G. applanatum*, *P. africana* stem bark, *B. micrantha* stem bark, *G. lucidum*, *E. denticulatum* and *U. dioica* roots. This was comparative with the high antioxidant activity (84.30 – 95.56%) of the plant extracts. The high antioxidant activity in the plant extracts was probably due to the presence of the four bioactive phytochemicals. Among the four tested phytochemicals, only terpenoids were present in *U. dioica* leaves extract. The plant had the lowest antioxidant activity (11.99%), total flavonoid (3.97 ± 0.06) and phenolic content (43.19 ± 1.15). Terpenoids and alkaloids were absent in *Prunus africana* leaves extract with a moderate antioxidant activity (37.31%). The relationship between antioxidant activity, phytochemicals, total phenolic and flavonoid content is shown in Appendix VII.

4.4 Anti-inflammatory activity

The data obtained for each set of experiment was expressed as a mean and standard errors of the mean (mean ± SEM). It was analyzed using one-way ANOVA with *Scheffé* post *hoc* test. A value of $p < 0.05$ was reviewed as significant as illustrated in appendix I, II and III.

4.4.1 Effect of *B. micrantha* stem bark extract on formalin- induced paw edema in mice

The effect of *B. micrantha* stem bark extract on the mice left hind paw after injection with formalin is given in table 4.4 below. It is also presented in form of bar graph in Appendix VIII.

Table 4.4: **Effect of *B. micrantha* stem bark extract on formalin induced paw edema in mice**

TREATMENT	**TIME IN MINUTES**				
	0	60	120	180	240
Normal saline	0	0.89 ± 0.05	0.87 ± 0.11	1.01 ± 0.07	1.15 ± 0.08
Diclofenac	0	0.94 ± 0.06	0.56 ± 0.04	0.47 ± 0.07	0.94 ± 0.09
25mg/kg	0	0.97 ± 0.05	0.81 ± 0.06[a]	0.70 ± 0.06	0.95 ± 0.06[a]
50mg/kg	0	1.29 ± 0.09[a]	0.73 ± 0.06[a]	0.35 ± 0.09[a]	0.98 ± 0.09[a]
100mg/kg	0	1.04 ± 0.03[a]	0.72 ± 0.05[a]	0.47 ± 0.04[a]	1.01 ± 0.09

Values representing change in paw edema expressed as mean ± SEM relative to vehicle (normal saline). Values with the superscript are considered to have no significant difference relative to normal saline.

The doses of 25, 50 and 100 mg significantly reduced the paw edema diameter ($p < 0.05$) relative to normal saline (negative control). In the first hour, the paw edema was

high in all the three doses as shown in Appendix VIII and table 4.4. The reduction of paw edema by the three doses 25, 50 and 100 mg was minimal in the first hour as compared to the second, third and fourth hour. The dose of 50 mg showed a significant effect ($p < 0.05$) against the normal saline hence higher activity than the dose of 25 and 100mg/kg. The difference in doses between these three doses is due to more active metabolites that increase with concentration. Nevertheless, higher concentration increases enzyme inhibitors, which in return decrease the activity of the drug.

Dose 100mg/kg showed a significant effect ($p < 0.05$) at 60, 120 and 180 minutes, but failed to have an effect ($p >0.05$) at time 240 minutes. This may be due to the effect of the drug in the system that has begun to reduce hence another dose is needed. For dose 25 mg/kg, there was no significant difference of the dose ($p > 0.05$) as compared to normal saline at time 60 and 180 minutes. In all the three doses, dose 50 mg/kg had an effect ($p <0.05$) especially at time 180 minutes and reduced the inflammation better than diclofenac sodium as compared to other two doses. This was followed by dose 100 mg/kg which had a similar effect as diclofenac sodium (Appendix VIII).

Anti-inflammatory activity of *B. micrantha* bark extract has been carried out in Nigeria, using carrageenan-induced, histamine-induced and formaldehyde-induced rat paw edema. They all showed a significant reduction in paw edema. Reduction in formaldehyde-induced paw edema lasted for 24 hours with dose 400 mg/kg having a potential effect as compared to dose 200 mg/kg (Nwaehujor *et al.,* 2014). The results obtained in this study shows that *B. micrantha* stem bark may be a potential source of

anti-inflammatory drugs that reduces edema that is related to diseases such as arthritis that causes pain in human.

4.4.2 Effect of *G. applanatum* extract on formalin- induced paw edema in mice

The results in table 4.5 show the effect of *G. applanatum* on formalin-induced paw edema in mice. The results are also presented in form of bar chart in appendix IX.

Table 4.5: Effect of *G. applanatum* extract on formalin induced paw edema in mice

TREATMENT	**TIME IN MINUTES**				
	0	60	120	180	240
Normal saline	0	0.89 ± 0.05	0.87 ± 0.11	1.01 ± 0.07	1.15 ± 0.08
Diclofenac	0	0.94 ± 0.06	0.56 ± 0.04	0.47 ± 0.07	0.94 ± 0.09
50mg/kg	0	1.21 ± 0.06[a]	0.75 ± 0.28	0.42 ± 0.14[a]	0.95 ±0.20
100mg/kg	0	1.21 ± 0.07[a]	0.81 ± 0.05	0.72 ± 0.04[a]	1.06 ± 0.12
200mg/kg	0	1.08 ± 0.08[a]	0.70 ± 0.04	0.44 ± 0.07[a]	1.08 ± 0.12

Values representing change in paw edema expressed as mean ± SEM relative to vehicle (normal saline). Values with the superscript are considered to have no significant difference relative to normal saline.

Doses 50, 100 and 200 mg/kg of *G. applanatum* extract exhibited a significant effect ($p < 0.05$) on induced edema on mice at 60 and 180 minutes. These doses were selected based on trials. There was no effect ($p > 0.05$) against normal saline in all the doses at 120 and 240 minutes. At 180 minutes, the 50 mg/kg dose reduced the edema even more significantly than diclofenac. Dose of 200 mg/kg also reduced the inflammation more than diclofenac sodium. However, a dose of 100 mg did not have an effect on edema.

Research carried out in Indonesia on anti-inflammatory effect of brown algae, *Sargassum polycystum* using carrageenan-induced hind paw edema in mice on different

fractions. Hexane fraction decreased the edema in mice. The paw edema reduced in correspondent with an increase in concentration of the hexane extract. High concentration increases the active metabolites such as flavonoids and alkaloids results to higher activity (Buwono *et al.,* 2018). This was in line with the results obtained in this study at time 60, 120 and 180 minutes where the extract exhibited significant dose and time dependent anti-inflammatory activity in the induced edema. The presence of secondary metabolites such as alkaloids suggests the potential anti-inflammatory in this drug.

4.4.3 Effect of *E. denticulatum* extract on formalin-induced paw edema in mice

Methanolic extract of *E. denticulatum* reduced the paw edema, where the 12.5 and 50 mg/kg doses showed significant effect ($p < 0.05$) on edema reduction relative to normal saline (Table 4.6; Appendix X).

Table 4.6: Effect of *E. denticulatum* extract on formalin induced paw edema in mice

TREATMENT	**TIME IN MINUTES**				
	0	60	120	180	240
Normal saline	0	0.89 ± 0.05	0.87 ± 0.11	1.01 ± 0.07	1.15 ± 0.08
Diclofenac	0	0.94 ± 0.06	0.56 ± 0.09	0.47 ± 0.03	0.94 ± 0.09
12.5mg/kg	0	1.03 ± 0.05[a]	0.69 ± 0.06[a]	0.54 ± 0.06[a]	0.96 ± 0.11[a]
25mg/kg	0	1.11 ± 0.09[a]	0.68 ± 0.09[a]	0.49 ± 0.05[a]	1.02 ± 0.08
50mg/kg	0	1.03 ± 0.02[a]	0.58 ± 0.04[a]	0.45 ± 0.07[a]	0.89 ± 0.04[a]

Values representing change in paw edema expressed as mean ± SEM relative to vehicle (normal saline). Values with the superscript are considered to have no significant difference relative to normal saline.

The dose 25 mg/kg exhibited significant effect on edema ($p < 0.05$) at 60, 120 and 180 minutes. Reduction in paw edema at time 120 and 180 minutes was more effective 60 and 240 minutes. Dose 50 mg/kg exhibited a significant effect at time 60, 120, 180 and 240 minutes. Dose 50 mg/kg showed maximum suppression of the paw edema as compared to other two doses. At time 180 minutes dose 50 mg/ml had exhibited reduction more than diclofenac sodium at time. The effect is attributed by the presence of phytochemicals such as alkaloids and flavonoids which inhibits the production of cyclooxygenase.

In vitro anti-inflammatory activity effect of *E. denticulatum* studies using Alpha-amylase, showed untreated cells used as control released small amount of IL-6, but the extract showed a reduction in production of IL-6. Seaweeds have previously been noted for their abundance of metabolites and diversity, including a diverse type of chemical constituents, namely from polyphenols, polysaccharides, vitamins and which may contribute to the important biological effects such as antidiabetic, antioxidant, and anti-inflammatory (Ahmad *et al.*, 2015).

4.4.4 Effect of *U. dioica* roots extract on formalin- induced paw edema in mice

All the three doses of *U. dioica* roots exhibited a reduction in paw oedema (Appendix XI, Table 4.7).

Table 4.7: Effect of *U. dioica* roots extract on formalin induced paw edema in mice

TREATMENT	TIME IN MINUTES				
	0	60	120	180	240
Normal saline	0	0.89 ± 0.05	0.87 ± 0.11	1.01 ± 0.07	1.15 ± 0.08
Diclofenac	0	0.94 ± 0.06	0.56 ± 0.04	0.47 ± 0.07	0.94 ± 0.09
12.5mg/kg	0	1.06 ± 0.06[a]	0.68 ± 0.01[a]	0.62 ± 0.02[a]	0.88 ± 0.05[a]
25mg/kg	0	1.01 ± 0.05[a]	0.59 ± 0.06[a]	0.50 ± 0.06[a]	0.82 ± 0.07[a]
50mg/kg	0	1.09 ± 0.02[a]	0.77 ± 0.03	0.46 ± 0.03[a]	1.03 ± 0.06

Values representing change in paw edema expressed as mean ± SEM relative to vehicle (normal saline). Values with the superscript are considered to have no significant difference relative to normal saline.

The 12.5 and 25 mg/kg doses showed a significant effect ($p < 0.05$) relative to normal saline at 60, 120, 180 and 240 minutes, while the dose of 50 mg/kg showed a significant effect ($p < 0.05$) at 60 and 180 minutes. Nevertheless, dose 50 mg/kg failed to reduce the edema at 120 and 240 minutes. There was a significant reduction in paw edema at 120 and 180 minutes. Dose 50 mg/kg reduced the inflammation better than diclofenac sodium at time 180 minutes.

4.4.5 Effect of *U. dioica* leaves extract on formalin- induced paw edema in mice

All the three doses had a significant effect ($p < 0.05$) as compared to normal saline at 60 and 180 minutes (Table 4.8; Appendix XII).

Table 4.8: Effect of *U. dioica* leaves extract on formalin induced paw edema in mice

TREATMENT	TIME IN MINUTES				
	0	60	120	180	240
Normal saline	0	0.89 ± 0.05	0.87 ± 0.11	1.01 ± 0.07	1.15 ± 0.08
Diclofenac	0	0.94 ± 0.06	0.56 ± 0.04	0.47 ± 0.07	0.94 ± 0.09
25mg/kg	0	1.23 ± 0.08[a]	0.97 ± 0.06	0.76 ± 0.05[a]	1.18 ± 0.07
50mg/kg	0	1.43 ± 0.09[a]	0.89 ± 0.08	0.64 ± 0.06[a]	1.08 ± 0.12
100mg/kg		1.14 ± 0.05[a]	0.76 ± 0.06	0.62 ± 0.05[a]	1.17 ± 0.08

Values representing change in paw edema expressed as mean ± SEM relative to vehicle (normal saline). Values with the superscript are considered to have no significant difference relative to normal saline.

The doses failed to show a significant effect on the mice edema at 120 and 240 minutes. At 180 minutes, the reduction on induced edema was high compared to 60, 120 and 240 minutes. From 60 minutes to 180 minutes, there is a reduction in paw edema. At 240 minutes, the paw edema increased due to reduction in effectiveness of the drug. Study has been done in Iran on *U. dioica* leaf using carrageenan-induced edema using mice. Doses of 100, 200 and 400mg/kg body weight (BW) were used. Extract dose at 400mg/kg BW significantly reduced the inflammation ($p < 0.05$) (Hajhashemi and Klooshani, 2013).

4.4.6 Effect of *P. africana* stem bark extract on formalin- induced paw edema in mice

The results of anti-inflammatory activity of *P. africana* extract are given in appendix XIII and in form of a bar chart in table 4.9.

Table 4.9: Effect of *P. africana* stem bark extract on formalin- induced paw edema in mice

TREATMENT	TIME IN MINUTES				
	0	60	120	180	240
Normal saline	0	0.89 ± 0.05	0.87 ± 0.11	1.01 ± 0.07	1.15 ± 0.08
Diclofenac	0	0.94 ± 0.06	0.56 ± 0.04	0.47 ± 0.07	0.94 ± 0.09
25mg/kg	0	1.39 ± 0.05[a]	0.44 ± 0.07	0.24 ± 0.06[a]	0.79 ± 0.08
50mg/kg	0	1.10 ± 0.08[a]	0.75 ± 0.05	0.59 ± 0.08[a]	0.99 ± 0.11
100mg/kg	0	1.35 ± 0.05[a]	0.79 ± 0.03	0.57 ± 0.05[a]	0.99 ± 0.07

Values representing change in paw edema expressed as mean ± SEM relative to vehicle (normal saline). Values with the same superscript are considered to have no significant difference relative to normal saline.

At 60 and 180 minutes, *P. africana* stem bark showed a significant effect ($p < 0.05$) relative to normal saline. The paw edema was large at 60 minutes but at 120 and 180, there was a reduction in paw edema. The edema increased again at 240 minutes. The extract was effective at 120 and 180 minutes where it reduced the paw edema more than 60 and 240 minutes. Dose 25 mg/kg worked very well at 120 and 180 minutes more than diclofenac sodium. Dose 50 mg/kg reduced the edema than 100 mg/kg and it reduced edema at 180 minutes more than diclofenac sodium.

4.4.7 Effect of *G. lucidum* extract on formalin- induced paw edema in mice

G. lucidum appeared to be more potent in reducing the inflammation than the other six extracts (Table 4.10; Appendix XIV).

Table 4.10: Effect of *G. lucidum* extract on formalin induced paw edema in mice

Treatment	**Time in minutes**				
	0	60	120	180	240
Normal saline	0	0.89 ± 0.05	0.87 ± 0.11	1.01 ± 0.07	1.15 ± 0.08
Diclofenac	0	0.94 ± 0.06	0.56 ± 0.09	0.47 ± 0.03	0.94 ± 0.09
12.5mg/kg	0	0.92 ± 0.07	0.94 ± 0.09	0.79 ± 0.08[a]	0.88 ± 0,05[a]
25mg/kg	0	0.86 ± 0.03	0.64 ± 0.06[a]	0.51 ± 0.08[a]	0.65 ± 0.07[a]
50mg/kg	0	0.73 ± 0.07[a]	0.44 ± 0.09[a]	0.28 ± 0.08[a]	0.40 ± 0.09[a]

Values representing change in paw edema expressed as mean ± SEM relative to vehicle (normal saline). Values with the superscript are considered to have no significant difference.

It was followed by *P. africana* stem bark. The extract at dose 50 mg/kg was effective ($p < 0.05$) relative to normal saline. Both 25 and 50 mg/kg at 60, 120, 180 and 240 reduced the inflammation more than diclofenac sodium

In a study done In South India on anti-inflammatory activity using *G. lucidum*, the mice treated with 500 and 1000 mg/kg BW of both ethyl acetate and methanol extract of *G. lucidum,* methanol extract of dose 1000 mg/kg exhibited a reduction in paw edema compared to ethyl acetate extract (Sheena *et al.,* 2003). Flavonoids were shown to hinder inflammation by inhibiting lipoxygenase and cyclooxygenase pathway (Avula *et al.,* 2010). The alkaloids, which comprise one of the largest class of secondary plant metabolite, possess a wide range of pharmacological activity such as anti-inflammatory activity (Souto *et al.*, 2011). It is possible that these phytochemicals are responsible for the anti-inflammatory activity.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

i. The medicinal plants, mushrooms and seaweeds in this study had an effect on the scavenging of free radicals. There was an increase in antioxidant activity with concentration. *G. applanatum* mushroom showed the highest antioxidant activity of 95.56% (IC_{50} < 0.025mg/ml). This was followed by *P. africana* stem bark (93.60%, IC_{50} = 0.033 mg/ml), *G. lucidum* (91.24, IC50 = 0.04). *U. dioica* leaves had the lowest antioxidant activity 11.99% with IC_{50}> 0.03. The study revealed that these Kenyan plants and mushroom species can be potential sources of new natural antioxidants.

ii. Medicinal plants, non-edible medicinal mushrooms and seaweed showed different levels of total phenolic and flavonoid content. *G. lucidum* extract had the highest total flavonoid content (31.16 ± 0.04 mg QE/g) and total phenolic content (156.07 ± 3.45 mg GAE/g).

iii. Phytochemical screening on the methanolic extracts showed positive results for presence of tannins, flavonoids, terpenoids and alkaloids in *G. applanatum*, *G. lucidum, B. micrantha, P. africana* and *U. dioica* roots. *U. dioica* leaves extract tested positive for presence of only terpenoids.

iv. Based on anti-inflammatory effect, the treatment of the inflammation induced by formalin on the hind paw of the Swiss albino mice using *P. africana* stem bark, *B. Micrantha* stem bark, *U. dioica* roots, *U. dioica* leaves, *G. applanatum, G. lucidum* and *E. Denticulatum* showed a significant effect with *G. lucidum*

reducing the edema more effectively compared to other six extracts. The results for anti-inflammatory activity from this validate the efficacy of the medicinal plants in the management and treatment of inflammation.

5.2 Recommendations

5.2.1 Recommendations from the study

i. *G. applanatum* mushroom species showed the highest antioxidant activity therefore, it can be a potential source of new natural antioxidant.

ii. *G. lucidum* mushroom species had the best anti-inflammatory activity hence, can be a source of lead compounds towards the development of anti-inflammatory drugs.

5.2.2 Recommendations for further research

i. Separation and identification of actual phytochemicals in plant extracts responsible for antioxidant and anti-inflammatory activities

ii. Evaluation of pain and fever properties of the medicinal plants, non-edible medicinal mushrooms and seaweed.

iii. Elucidation of cytotoxic effect of the medicinal plants, non-edible medicinal mushrooms and seaweed.

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APPENDICES

Appendix I: Analysis of variance (ANOVA) of *G. lucidum* anti-inflammation at 120 minutes

G. lucidum at 120 minutes

Independent Group Analysis
Summary Data

Group Means and Standard Deviations

12.5mg:	mean = .938	s.d. = .192	n = 5
25mg:	mean = .64	s.d. = .139	n = 5
50mg:	mean = .44	s.d. = .24	n = 5
N/S:	mean = .872	s.d. = .241	n = 5
D/f:	mean = .463	s.d. = .243	n = 5

Analysis of Variance Table

Source	S.S.	DF	MS	F	Appx P
Total	1.97	24			
Treatment	1.05	4	.26159	5.66	0.0032
Error	.92366	20	.04618		

Error term used for comparisons = .05 with 20 d.f.

Scheffe Multiple Comp.	Difference	Critical S (.05)	
Mean(12.5mg)-Mean(50mg) =	0.498	3.664	3.388 *
Mean(12.5mg)-Mean(D/f) =	0.475	3.495	3.388 *
Mean(12.5mg)-Mean(25mg) =	0.298	2.193	3.388
Mean(12.5mg)-Mean(N/S) =	0.066	(Do not reject - within range)	
Mean(N/S)-Mean(50mg) =	0.432	3.178	3.388
Mean(N/S)-Mean(D/f) =	0.409	(Do not reject - within range)	
Mean(N/S)-Mean(25mg) =	0.232	(Do not reject - within range)	
Mean(25mg)-Mean(50mg) =	0.2	(Do not reject - within range)	
Mean(25mg)-Mean(D/f) =	0.177	(Do not reject - within range)	
Mean(D/f)-Mean(50mg) =	0.023	(Do not reject - within	

range)

Note: (Do not reject - within range) indicates that a comparison is within the range of a non-significant comparison, and so must also be non-significant.

Homogeneous Populations, groups ranked

Gp 1 refers to GROUP=12.5mg
Gp 2 refers to GROUP=25mg
Gp 3 refers to GROUP=50mg
Gp 4 refers to GROUP=N/S
Gp 5 refers to GROUP=D/f

Gp Gp Gp Gp Gp
3 5 2 4 1 ----------

This is a graphical representation of the Scheffe's multiple comparisons test. At the 0.05 significance level, the means of any two groups underscored by the same line are not significantly different.

Simultaneous 95% Confidence Limits

Significant comparisons based on Conf. Limits indicated by ***.
CI uses Tukey-Kramer procedure. P-values reflect a Bonferroni adjustment.
Error term used = .05 with 20 d.f.

Group Comparison	Difference	p-value	Simultaneous 95% Confidence Limits	
Mean(12.5mg)-Mean(50mg) =	0.498	0.015	(0.0913, 0.9047)	***
Mean(12.5mg)-Mean(D/f) =	0.475	0.023	(0.0946, 0.8554)	***
Mean(12.5mg)-Mean(25mg) =	0.298	0.403	(-0.0459, 0.6419)	
Mean(12.5mg)-Mean(N/S) =	0.066	1.000	(-0.2175, 0.3495)	
Mean(N/S)-Mean(50mg) =	0.432	0.047	(0.0516, 0.8124)	***
Mean(N/S)-Mean(D/f) =	0.409	0.069	(0.0651, 0.7529)	***
Mean(N/S)-Mean(25mg) =	0.232	1.000	(-0.0515, 0.5155)	
Mean(25mg)-Mean(50mg) =	0.2	1.000	(-0.1439, 0.5439)	
Mean(25mg)-Mean(D/f) =	0.177	1.000	(-0.1065, 0.4605)	
Mean(D/f)-Mean(50mg) =	0.023	1.000	(-0.2605, 0.3065)	

Note: Because different multiple comparisons procedures are based on different methods, they may not completely agree for marginally significant comparisons.

Appendix II: Analysis of variance (ANOVA) of *G. applanatum* anti-inflammation at 60 minutes

```
G. lucidum at 60minutes
   ------------------------------------------------------------------------
   Independent Group Analysis
Summary Data
   ------------------------------------------------------------------------

   Group Means and Standard Deviations
   ----------------------------------
   12.5mg:  mean = .916          s.d. = .385             n =  5
   25mg:  mean = .86             s.d. = .066             n =  5
   50mg:  mean = .733            s.d. = .414             n =  5
   N/S:  mean = .886             s.d. = .109             n =  5
   D/f:  mean = .785             s.d. = .369             n =  5


Analysis of Variance Table

Source              S.S.        DF             MS      F          Appx
P
   ------------------------------------------------------------------------
Total                 2.        24
     Treatment       .11343         4         .02836      .3
0.8742
     Error             1.89        20          .0944

   Error term used for comparisons = .09 with 20 d.f.


Critical S
Scheffe Multiple Comp.           Difference         S          (.05)
   ------------------------------------------------------------------------
Mean(12.5mg)-Mean(50mg) =              0.183          .942       3.388
Mean(12.5mg)-Mean(D/f) =               0.131  (Do not reject - within
range)
Mean(12.5mg)-Mean(25mg) =              0.056  (Do not reject - within
range)
Mean(12.5mg)-Mean(N/S) =                0.03  (Do not reject - within
range)
Mean(N/S)-Mean(50mg) =                 0.153  (Do not reject - within
range)
Mean(N/S)-Mean(D/f) =                  0.101  (Do not reject - within
range)
Mean(N/S)-Mean(25mg) =                 0.026  (Do not reject - within
range)
Mean(25mg)-Mean(50mg) =                0.127  (Do not reject - within
range)
Mean(25mg)-Mean(D/f) =                 0.075  (Do not reject - within
range)
Mean(D/f)-Mean(50mg) =                 0.052  (Do not reject - within
range)
```

Note: (Do not reject - within range) indicates that a comparison is within the range of a non-significant comparison, and so must also be non-significant.

Homogeneous Populations, groups ranked

Gp 1 refers to GROUP=12.5mg
Gp 2 refers to GROUP=25mg
Gp 3 refers to GROUP=50mg
Gp 4 refers to GROUP=N/S
Gp 5 refers to GROUP=D/f

Gp Gp Gp Gp Gp
3 5 2 4 1 ----------

This is a graphical representation of the Scheffe's multiple comparisons test. At the 0.05 significance level, the means of any two groups underscored by the same line are not significantly different.

Simultaneous 95% Confidence Limits

Significant comparisons based on Conf. Limits indicated by ***.
CI uses Tukey-Kramer procedure. P-values reflect a Bonferroni adjustment.
Error term used = .09 with 20 d.f.

Group Comparison	Difference	p-value	Simultaneous 95% Confidence Limits
Mean(12.5mg)-Mean(50mg) =	0.183	1.000	(-0.3985, 0.7645)
Mean(12.5mg)-Mean(D/f) =	0.131	1.000	(-0.4129, 0.6749)
Mean(12.5mg)-Mean(25mg) =	0.056	1.000	(-0.4356, 0.5476)
Mean(12.5mg)-Mean(N/S) =	0.03	1.000	(-0.3754, 0.4354)
Mean(N/S)-Mean(50mg) =	0.153	1.000	(-0.3909, 0.6969)
Mean(N/S)-Mean(D/f) =	0.101	1.000	(-0.3906, 0.5926)
Mean(N/S)-Mean(25mg) =	0.026	1.000	(-0.3794, 0.4314)
Mean(25mg)-Mean(50mg) =	0.127	1.000	(-0.3646, 0.6186)
Mean(25mg)-Mean(D/f) =	0.075	1.000	(-0.3304, 0.4804)
Mean(D/f)-Mean(50mg) =	0.052	1.000	(-0.3534, 0.4574)

Note: Because different multiple comparisons procedures are based on different methods, they may not completely agree for marginally significant comparisons.

Appendix III: Analysis of variance (ANOVA) of *E. denticulatum* anti-inflammation at 240 minutes

E. denticulatum at 240 minutes

Independent Group Analysis

Summary Data

Group Means and Standard Deviations

12.5mg:	mean = .962	s.d. = .25	n = 5
25mg:	mean = .822	s.d. = .162	n = 5
50mg:	mean = 1.034	s.d. = .067	n = 5
N/S:	mean = 1.146	s.d. = .184	n = 5
D/f:	mean = .783	s.d. = .383	n = 5

Analysis of Variance Table

Source	S.S.	DF	MS	F	Appx P
Total	1.54	24			
Treatment	.44944	4	.11236	2.05	0.1255
Error	1.1	20	.05476		

Error term used for comparisons = .05 with 20 d.f.

Critical S

Scheffe Multiple Comp.	Difference	S	(.05)
Mean(N/S)-Mean(D/f) =	0.363	2.453	3.388
Mean(N/S)-Mean(25mg) =	0.324	(Do not reject - within range)	
Mean(N/S)-Mean(12.5mg) =	0.184	(Do not reject - within range)	
Mean(N/S)-Mean(50mg) =	0.112	(Do not reject - within range)	
Mean(50mg)-Mean(D/f) =	0.251	(Do not reject - within range)	
Mean(50mg)-Mean(25mg) =	0.212	(Do not reject - within range)	
Mean(50mg)-Mean(12.5mg) =	0.072	(Do not reject - within range)	
Mean(12.5mg)-Mean(D/f) =	0.179	(Do not reject - within range)	
Mean(12.5mg)-Mean(25mg) =	0.14	(Do not reject - within range)	
Mean(25mg)-Mean(D/f) =	0.039	(Do not reject - within range)	

Note: (Do not reject - within range) indicates that a comparison is within the range of a non-significant comparison, and so must also be non-significant.

Homogeneous Populations, groups ranked

Gp 1 refers to GROUP=12.5mg
Gp 2 refers to GROUP=25mg

Gp 3 refers to GROUP=50mg
Gp 4 refers to GROUP=N/S
Gp 5 refers to GROUP=D/f

Gp Gp Gp Gp Gp
5 2 1 3 4 ----------

This is a graphical representation of the Scheffe's multiple comparisons test. At the 0.05 significance level, the means of any two groups underscored by the same line are not significantly different.

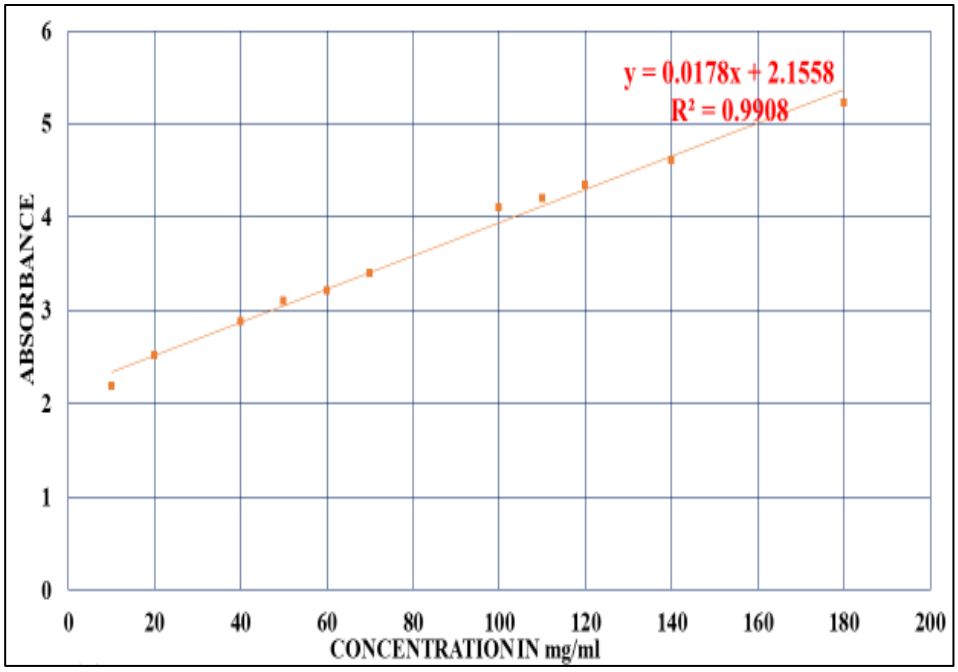
Simultaneous 95% Confidence Limits

Significant comparisons based on Conf. Limits indicated by ***.
CI uses Tukey-Kramer procedure. P-values reflect a Bonferroni adjustment.
Error term used = .05 with 20 d.f.

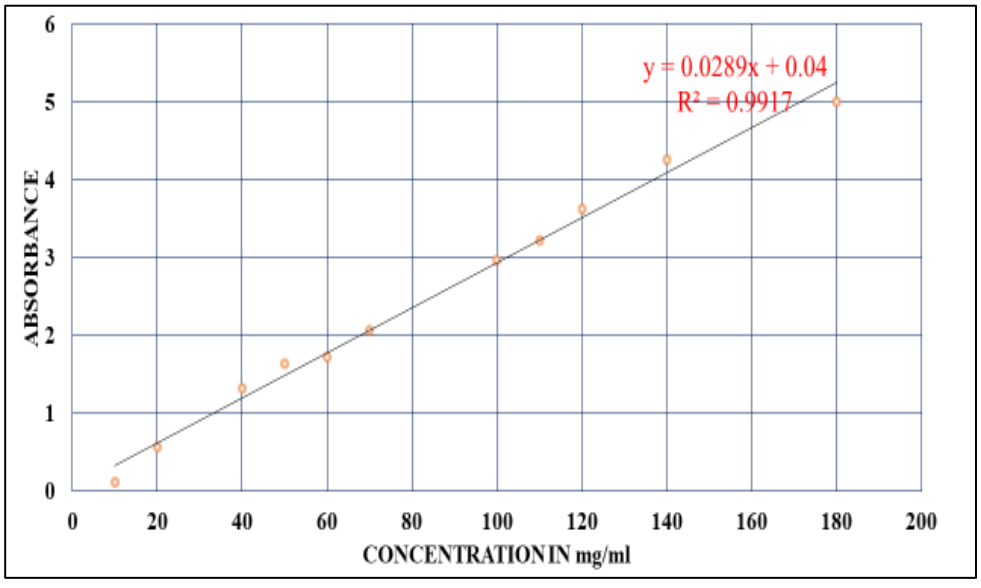
Group Comparison	Difference	p-value	Simultaneous 95% Confidence Limits
Mean(N/S)-Mean(D/f) =	0.363	0.235	(-0.0799, 0.8059)
Mean(N/S)-Mean(25mg) =	0.324	0.406	(-0.0902, 0.7382)
Mean(N/S)-Mean(12.5mg) =	0.184	1.000	(-0.1904, 0.5584)
Mean(N/S)-Mean(50mg) =	0.112	1.000	(-0.1967, 0.4207)
Mean(50mg)-Mean(D/f) =	0.251	1.000	(-0.1632, 0.6652)
Mean(50mg)-Mean(25mg) =	0.212	1.000	(-0.1624, 0.5864)
Mean(50mg)-Mean(12.5mg) =	0.072	1.000	(-0.2367, 0.3807)
Mean(12.5mg)-Mean(D/f) =	0.179	1.000	(-0.1954, 0.5534)
Mean(12.5mg)-Mean(25mg) =	0.14	1.000	(-0.1687, 0.4487)
Mean(25mg)-Mean(D/f) =	0.039	1.000	(-0.2697, 0.3477)

Note: Because different multiple comparisons procedures are based on different methods, they may not completely agree for marginally significant comparisons.

Appendix IV: Calibration curves for quercetin standard



Appendix V: Calibration curve for Gallic acid standard



Appendix VI: %Scavenging activity of extracts on DPPH

Conc. in mg/ml	**% SCAVENGING ACTIVITY ON DPPH**											
	B. micrantha bark	*G. applanatum*	*G. lucidum*	*T.elegans*	*E. denticulatum*	*U. dioica root*	*U. dioica leaves*	*P. africana* bark	*P. africana* leaves	*F. spatulatus*	*Corelepsis sp.*	Ascorbic acid
0.025	41.56	59.1	47.43	35.52	16.76	12.27	2.93	36.86	10.62	31.53	35.52	19.69
0.05	58.94	61.96	58.6	50.72	72.25	55.87	3.11	90.41	23.29	35.6	51.93	51.93
0.1	92.04	73.83	69.93	54.88	84.43	64.82	4.0	92.78	34.7	37.77	54.88	93.95
0.2	92.28	87.05	85.88	58.95	86.18	76.71	4.28	92.84	36.5	43.44	58.95	94.08
0.3	92.67	95.56	91.24	59.01	90.21	84.32	11.99	93.6	37.31	57.88	59.01	94.32

Appendix VII: Relationship between the antioxidant activity, phytochemicals, total phenolic and flavonoid content of the extracts

Extract	**Total phenolic (mg GAE/g)**	**Total flavonoid (mg QE/g)**	**Antioxidant activity on DPPH at 0.3mg/ml of extract %**	**IC_{50} (mg/ml)**	**Tannins**	**Flavonoids**	**Alkaloids**	**Terpenoids**
Ascorbic acid			94.32	0.03				
G. applanatum	127.23 ± 0.64	14.53 ± 0.28	95.56	< 0.025	+	+	+	+
P. africana stem bark	148.55 ± 4.05	9.29 ± 0.06	93.60	0.033	+	+	+	+
B Micrantha stem bark	128.79 ± 1.54	30.47 ± 0.03	92.67	0.038	+	+	+	+
G. lucidum	156.07 ± 3.45	31.16 ± 0.04	91.24	0.04	+	+	+	+
E. denticulatum	146.15 ± 1.11	9.36 ± 0.12	90.21	0.042	+	+	+	+
U. dioica roots	144.04 ± 3.89	28.54 ± 0.67	84.32	0.045	+	+	+	+
T. elegans	103.19 ± 1.23	9.97 ± 0.32	59.01	0.22	+	-	+	+
U. dioica leaves	43.19 ± 1.15	3.97 ± 0.06	11.99	0.3	-	-	-	+
P. africana leaves	117.68 ± 1.40	10.32 ± 0.04	37.31	0.3	+	+	-	-
F. spatulatus	124.90 ± 1.53	8.73 ± 0.06	57.88	0.25	-	+	+	-
Coreleopsis	101.38 ± 0.94	17.17 ± 0.03	59.01	0.3	+	+	-	+

Appendix VIII,

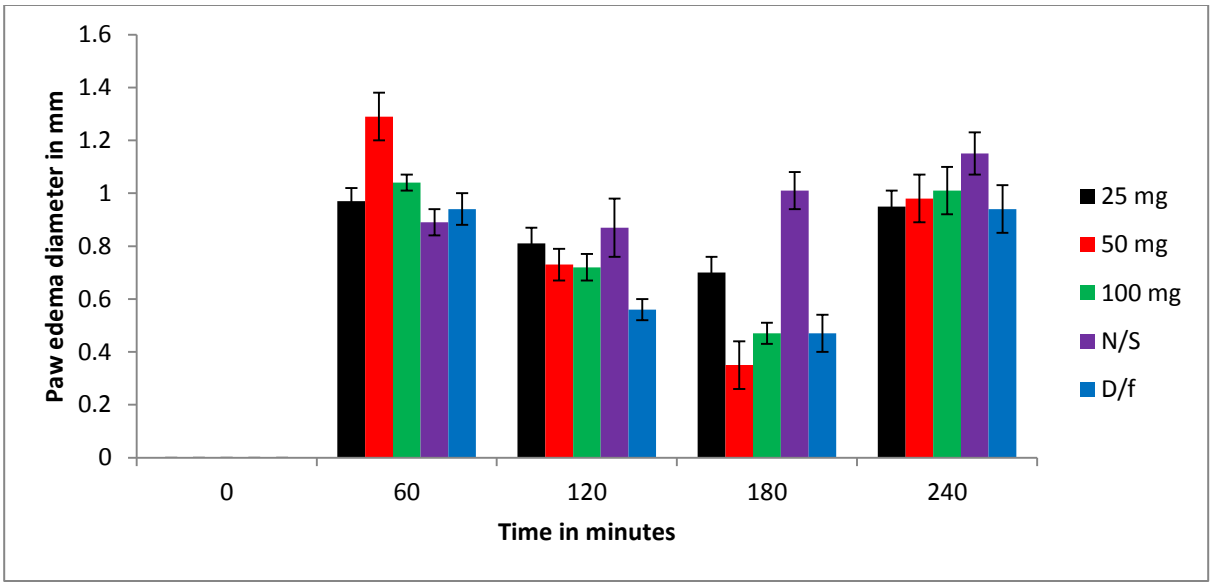


Figure 4.2: Anti-inflammatory effects of *B. micrantha* stem bark

Appendix IX

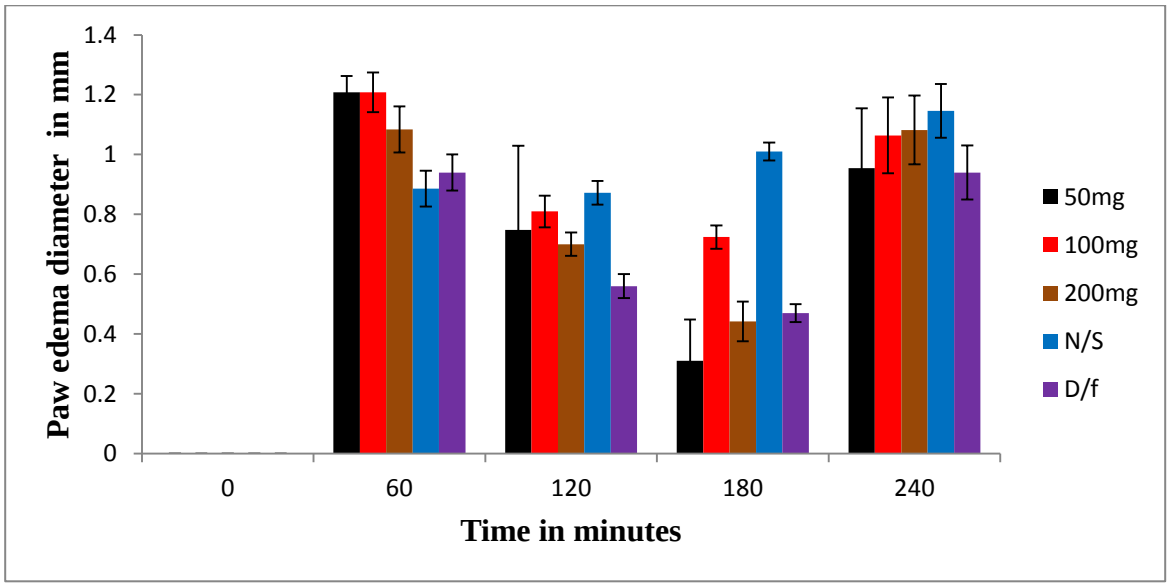


Figure 4.3: Anti-inflammatory effect of *G. applanatum* extract

Appendix X

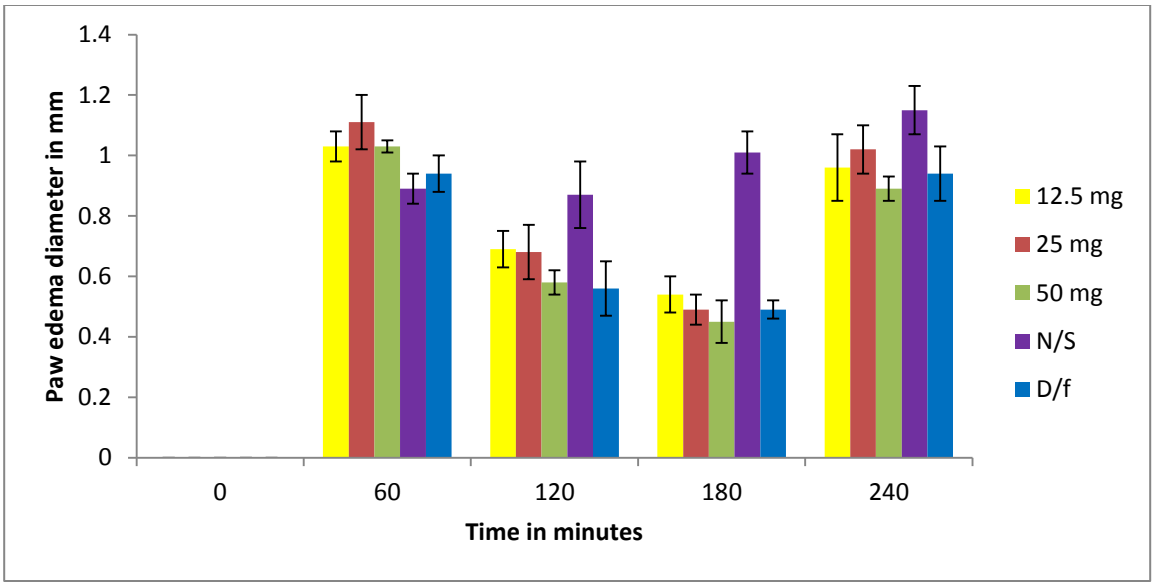


Figure 4.4: Anti-inflammatory effects of *E. denticulatum* extract

Appendix XI

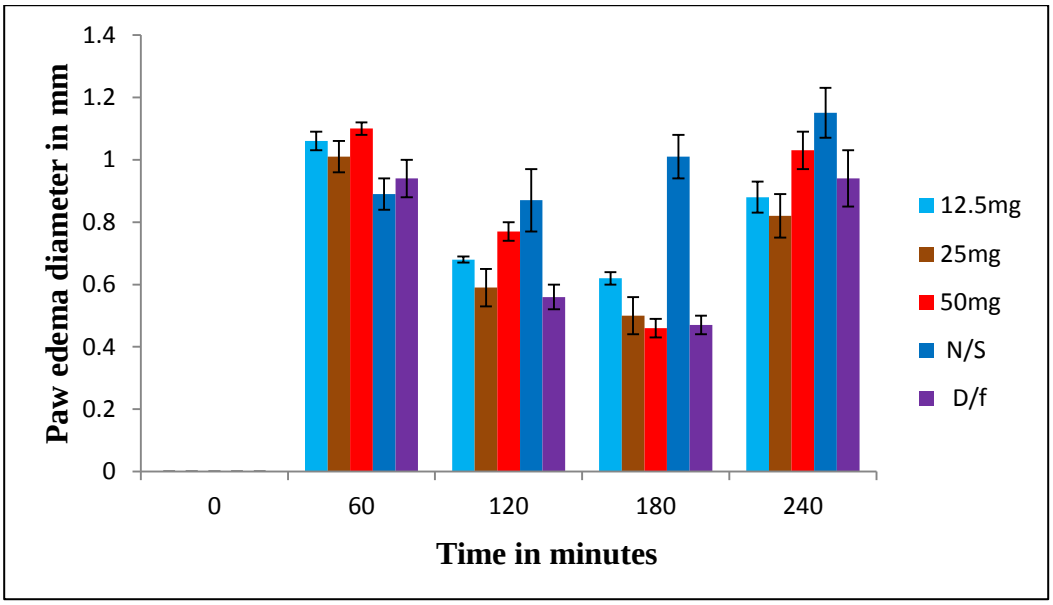


Figure 4.5: Anti-inflammatory effects of *U. dioica* roots extract

Appendix XII

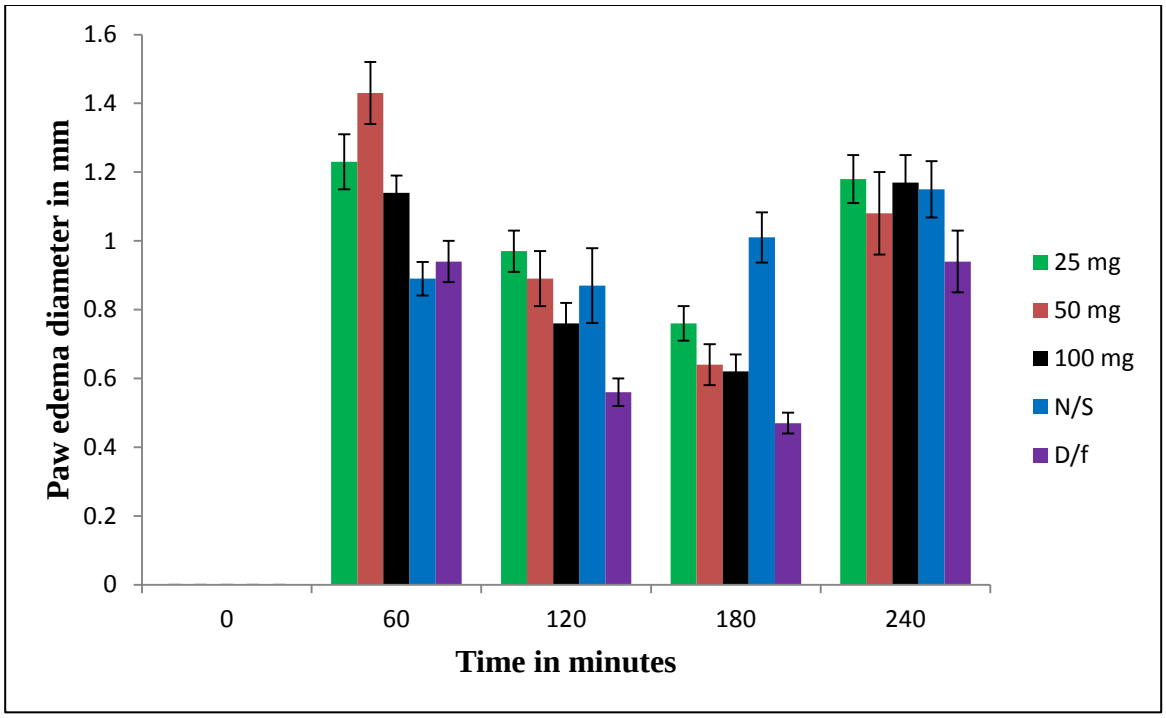


Figure 4.6: Anti-inflammatory effects of *U. dioica* leaves extract

Appendix XIII

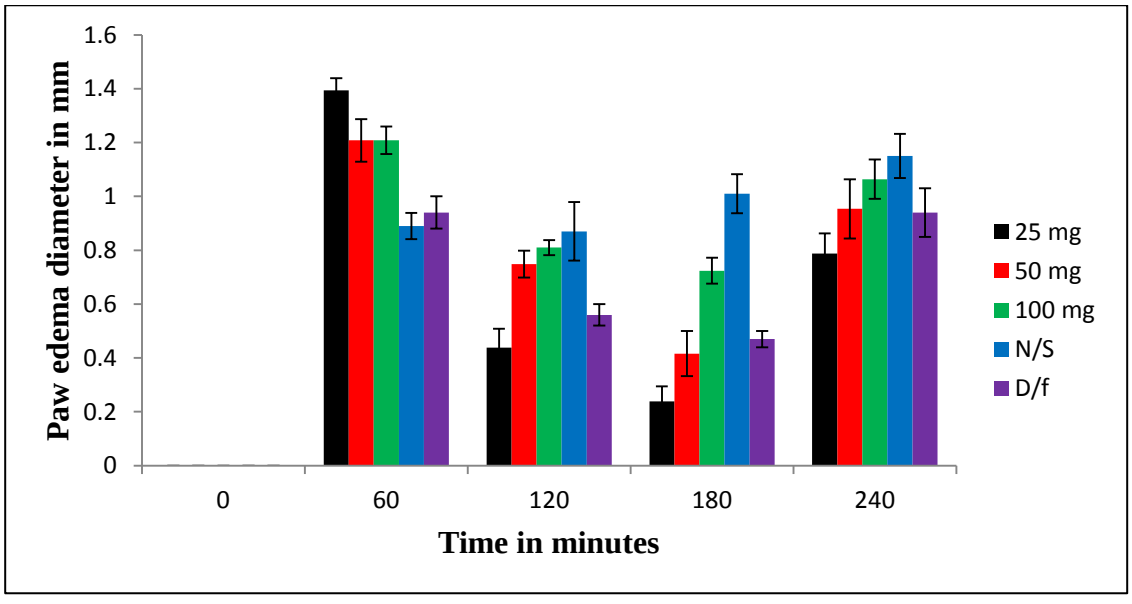


Figure 4.7: Anti-inflammatory effects of *P. africana* stem bark extract

Appendix XIV

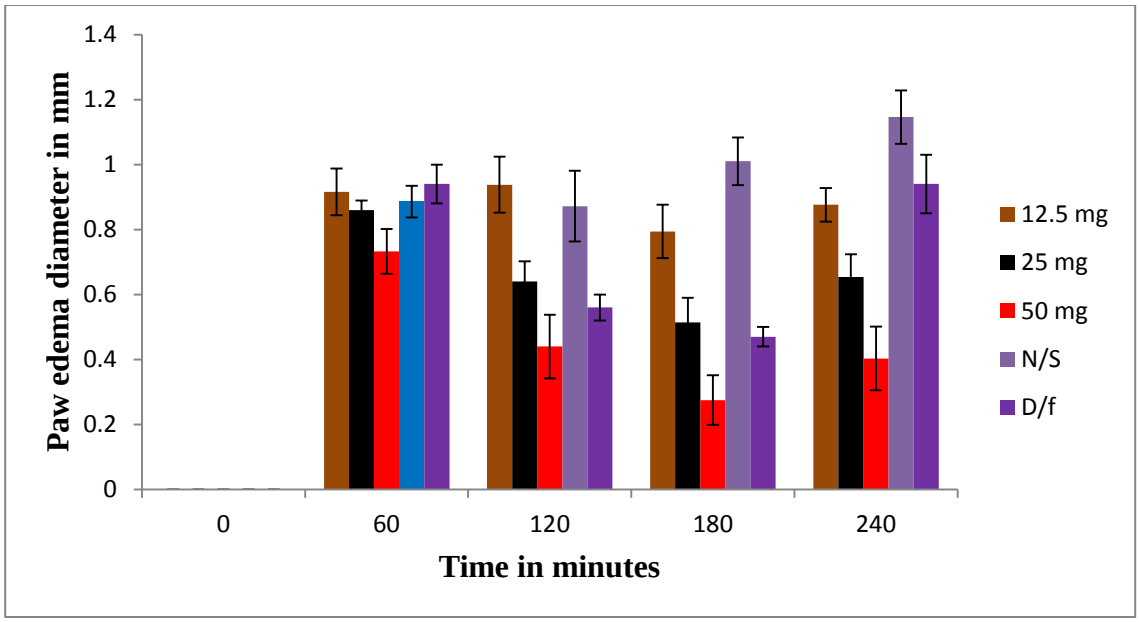


Figure 4.8: Anti-inflammatory effects of *G. lucidum* extract