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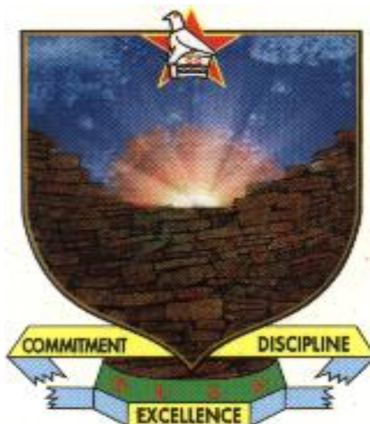

PHYTOCHEMICAL SCREENING AND ANTI-INFLAMATORY ACTIVITY OF AZANZA GARCKEANA LEAVES

BY

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE BACHELOR OF SCIENCE HONOURS DEGREE IN CHEMICAL TECHNOLOGY (HBScCHT)

JUNE 2023

APROVAL FORM

The undersigned certify that they have supervised, read and recommend to the Bindura University of Science Education for acceptance of a research project entitled:

PHYTOCHEMICAL SCREENING AND ANTI-INFLAMATORY ACTIVITY OF AZANZA GARCKEANA LEAVES

Submitted by TARISIRAI-MUNASHE MAGADA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE BACHELOR OF SCIENCE HONOURS DEGREE IN CHEMICAL TECHNOLOGY (HBScCHT)

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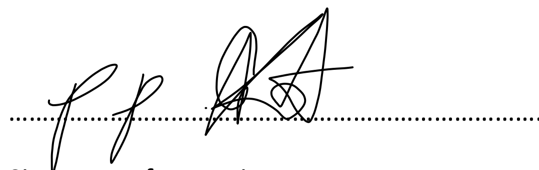

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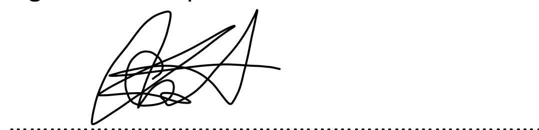

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DECLARATION

I, Tarisirai-Munashe Magada declare to Bindura University of Science Education Senate that the above dissertation is my original work. All other sources of materials used are duly acknowledged. This work has never been submitted to any other university for academic award.

Signed ………………………. Date…………………………………………

DEDICATION

THIS REPORT IS DEDICATED TO MEDICINAL CHEMISTRY, HEALTHY LIFE SCIENCES AND PREVENTING SPREAD OF DISEASES

ACKNOWLEDGEMENTS

Firstly I would like to thank all my colleagues, who guided me to achieve the objective of the report I produced. In particular l would like to thank Dr L Gwatidzo for helping me to produce the copyright material and supervising my work. I would like to express my deepest gratitude to laboratory personels Mrs Zhou, Mr Dzomba and Mr Chibuku for helping me to design and modify my experimental results, and proofreading any errors in the text. In particular, I would like to thank professor Dzomba for access to his lecture notes and Chief laboratory Mr Chayamiti for his help and advice. I wish also to thank the following friends and colleagues for proof-reading chapters and supplying information: Miss Chitare, Miss Benhilda and Miss Kuboya Finally, I would like to thank my mother, and sister for helping me to buy the lab reagents that l used to perform the lab work experiments.

ABSTRACT

Inflammation refers to a series of protective and reparative responses to tissue injury. These are caused by either mechanical and auto immune stimuli or infection. Inflammation is said to be either acute or chronic. Macrophages and dendritic cells are responsible for cytokine production that spreads inflammatory events especially in the first stages of acute phase of inflammation neutrophils. Many diseases are said to have an etiological origin in inflammatory processes e.g arthritis, cancer, ischemic heart disease and atherosclerosis although inflammation has a protective role. Many pathways involve the synthesis and secretion of pro-inflammatory mediators. There are two types of anti-inflammatory drugs namely steroidal anti inflammatory drugs and non steroidal inflammatory drugs. Steroidal reduce inflammation by binding to cortisol receptors and non-steroidal anti-inflammatory drugs decrease damage by inhibition of cyclooxygenase enzymes. There are many risks entailed by anti inflammatory drugs for example gastrointestinal ulceration, bleeding and hepato-toxicity.

In this work phytochemical screening and anti- inflammatory effect of *Azanza garckeana* leaves was carried out.

Qualitative experiments were used to determine the phytochemicals present in *Azanza garckeana* plant leaves. It was found out that this plant is rich in flavonoids, steroids, alkaloids, saponins, tannin, terpenoids, cardiac glycosides, coumarins, and phytosterols. Anti-inflammatory activity was investigated by two assays which are egg albumin denaturation of and nitric oxide radical scavenging assay using Griess reagent. The maximum inhibition of *Azanza gackeana* or *Gorron tulla* leaves and dichlofenac sodium by egg albumin denaturation was found to be 75.51 and 72.19 % respectively. The maximum inhibition of *Azanza gackeana* leaves, dichlofenac sodium and quercetin by nitric oxide radical scavenging assay was 90.63, 76.06 and 92.00% respectively.

List of tables

Table 2.1:anthology of plant under study azanza garckeana leaves 6
Table 2.2 phytochemical constituents and their examples 7
Table 4.1 Results of phytochemical screening........ 18
Table 4.2 inhibition of NO radical by quercetin, absorbance measured at 546nm 19
Table 4.3 Inhibition of NO Radical scavenging activity by dichlofenac sodium drug at 546 nm 19
Table 4.4Inhibition of NO Radical scavenging activity by Azanza garckeana leaves extract. 20
Table 4.5 Inhibition of protein denaturation by azanza garckeana ethanol extract 20
Table 4.6 Inhibition of egg albumin denaturation by dichlofenac sodium drug 21

LIST OF FIGURES

Figure 2.1 image of Azanza garckeana leaves 6
Figure 2.2 of an alkaloid pyrole 7
Figure 2.3 structure of flavonoid quercitin 8
Figure 2.4 structure of a glycoside 9
Figure 2.5 Structure of a volatile oil 9
Figure 2.6 Structure of triterpene 10
Figure 4.1 Extraction yield of ethanol, acetone and hexane 18
Figure 4.2 showing bar graphs of percentage inhibition of NO radical scavenging assay method 20
Figure 4.3Bar graphs of percentage inhibitition of denaturation protein 21

CONTENTS PAGE

Contents

APROVAL FORM i

ACKNOWLEDGEMENTS iv

ABSTRACT v

List of tables vi

LIST OF FIGURES vi

CHAPTER 1 1

1.1 INTRODUCTION 1

1.2 PROBLEM STATEMENT 2

1.3 AIM 2

1.4 RESEARCH OBJECTIVES 2

CHAPTER 2 3

2.1 LITERATURE REVIEW 3

2.2 CURRENT STATUS OF ANTI-INFLAMMATORY DRUGS 4

2.3 CLASSES OF ANTI-INFLAMMATORY DRUGS 4

2.3.1 IIMMUNE SELECTIVE ANTI-INFLAMMATORY DERIVATIVES 4

2.3.2 NONSTEROIDAL ANTI-INFLAMMATORY DRUGS 5

2.4 AZANZA GARCKEANA LEAVES 5

2.5 PHYTOCOMPOUNDS 6

2.7 Classes of phytochemicals 7

2.7.1 Alkaloids 7

2.7.3 SAPONINS 8

2.7.4 GLYCOSIDE 9

2.12 VOLATILE OILS 9

2.13TERPENOIDS 10

2.15 EXTRACTION OF SOLVENT 10

3.2 REAGENTS 11

3.3 SAMPLE COLLECTION AND PREPARATION 11

3.4 SOLVENT EXTRACTION 11

3.5 PHYTOCHEMICAL SCREENING 12

3.5.1 Testing for flavonoids 12

3.5.2.2 Wagner’s test 12

3.5.3 Testing for terpenoids 12
3.5.3.1 (Salkowaski test) 12
3.5.4 Testing for reducing sugars 12
3.5.5 Testing for tannin content 13
3.5.5.1 Ferric chloride test 13
3.5.6 Testing for quinine 13
3.5.7Testing for glycosides 13
3.5.8 Testing for phytosterols 13
3.5.8.1 Liberdmann Burchad's test 13
3.5.9 Testing for phenol 13
3.5.9.1 Lead acetate test 13
3.5.10 Testing for coumarins 13
3.5.11Testing for cardiac glycosides 14
3.5.12 Testing for acids 14
3.5.12Testing for phlobatannins 14
3.5.13Testing for anthraquinones 14
3.5.14Testing for steroids and phytosterols 14
3.5.15Test for volatile oils 14
3.6 ANTI-INFLAMMATORY ACTIVITY ASSAYS 14
3.6.1 PREPARATION OF STANDARD SOLUTIONS 14
3.6.2 PREPARATION OF STANDARD BUFFER SALINE 15
3.6.3 NITRIC OXIDE RADICAL SCAVENGING ASSAY 15
3.6.4 PREPARATION OF THE GRIESS REAGENT 15
3.6.2 PREPARATION OF STANDARD SOLUTIONS 16
3.6.4 INHIBITION OF EGG ALBUMIN DENATURATION 16
CHAPTER 4 RESULTS 18
4.1 INTRODUCTION 18
4.2 Extracted yield 18
4.4ANTI INFLAMMATORY ASSAY 19
4.5 Nitric oxide radical scavenging assay 19
4.6 Denaturation of protein egg albumin assay results 20
5.2 PERCENTAGE YIELD 22
5.3 PHYTOCHEMICAL SCREENING 22
5.5 INHIBITION OF EGG ALBUMIN DENATURATION ASSAY 24

5.6 CONCLUSION 25

CHAPTER 1

1.1 INTRODUCTION

Azanza garckeana (snot apple) fruit tree is an underutilized indigenous fruit tree (IFT). It is mainly found in warm woodlands of Southern Africa (Maroyi 2017). *Azanza garckeana* fruit tree belongs to the *Malvaceae* family. Its domestication is very important especially to support nutrition. Health and income generation are other benefits of this tree (World Agroforesty Centre 2020). In Zimbabwe, the *Azanza garckeana* is known in as *mutohwe* in Shona and *uxakuxaku* in Ndebele and it ripens from February to September. The ripe fruit is 35 mm in diameter, round, and appears reddish when mature with short dense hairs on the outer surface (Scmidt et al 2002). The ripe fruit is normally eaten raw and this is done by removing seeds and chewing of the fruit. The fruits produce a sweet glutinous slime and sometimes they are dried and crushed to make powder. *Azanza garckeana* leaves are green and form an important part in medicine. The leaves have a smooth margin and are velvet and reddish in colour when they are young whereas the older leaves have rough (star shaped) hairs on the upper surface and softer hairs below. The leaves are simple with 3-5 lobes and 5-7 veins from the base, with a longitudinal slit on the midrib. This tree should not be planted in cotton producing areas. This is because it is a host for cotton strainer a red and black beetle which has detrimental effects on cotton plantations. The ability of this tree to adapt various climatic conditions and soil types makes it a great shade tree in most areas.

Traditionalits use *Azanza garckeana* leaves to relieve pain in swollen legs. Another method used is boiling the leaves in water and left to cool. The leaves are boiled and squeezed on the legs that are swollen. Flavonoids, triterpenoid and saponins inhibits synthesis and release of inflammatory mediators, prostaglandins and polypeptides kinnins according to (Mojeremane and Tshwenyane 2004).

The fruit is also known to have anti-diabetic and fertility. Some phytochemicals such as flavonoids, glycosides and terpenoids present in the fruit are known to be good for sugar level control in the body according to Glew *et al*. (2005) *Azanza garckeana* fruits are consumed for their fertility enhancing activity. According to Van Wyk, (2011). It is also known to increase female lubrication and also improves the body’s immune functions. The fruit also helps to control anti inflammatory problems. *Azanza garckeana* fruit is a good aphrodisiac for men and women who are suffering from low sex drive according to Glew et al. (2005) although there are many health benefits associated with *A. garckeana* fruits such as microbial

contamination there are some unwanted effects such as microbial contamination and adulteration. The high levels of polyphenols can cause issues such as kidney risks and cancer issues. High levels of tannins can cause gastric irritation, nausea and vomiting by Hayat (2020). The crushing of the fruit can cause hydrolysis of cyanogenic glycosides. The end product is cyanides which when consumed can cause growth retardation and other neurological disorders according to Graham (2022).

1.2 PROBLEM STATEMENT

Azanza garckeana leaves can relieve swollen legs and have a strong effect on curing inflammation on the skin. They are also used by traditional healers to cure patients with burns on the skin by boiling the leaves with water and squeezing the wet leaves on the skin of the patient. The juice extracted from the leaves can also be given to patients to take orally. It has been noticed that this cures patients within an average of three days. It is against this background that this project is undertaken to investigate anti-inflammatory activity of *A. garckeana* leaves. This would help provide scientific basis for their use in folk medicine

1.3 AIM

To carry out phytochemical screening and anti-inflammatory activity of *Azanza garckeana* leaves.

1.4 RESEARCH OBJECTIVES

- To extract phytochemicals from *Azanza garckeana* leaves using ethanol, acetone and hexane.
- To determine the percentage yield by the different solvents
- To determine the phytochemical present in *Azanza garckeana* leaves by qualitative analysis
- To determine the anti-inflammatory activity of *Azanza garckeana* leaves using two assays which are nitric oxide radical scavenging assay and egg albumin denaturation assays.

CHAPTER 2

2.1 LITERATURE REVIEW

Inflammation is a reaction of the tissue blood vessels against aggressor agent characterized by access of liquids and of cells to interstice (Lope *et al.,* 1987). The inflammatory reaction is characterized by blush, heat, tumour, and loosing of function (Dassoler *et al*., 2004). The main risk factor of rheumatic immune diseases was also found that it was inflammation. As the incidence rate of this disease keeps increasing in recent years, how to effectively resist inflammation has attracted widespread attraction. Some receptor antagonists have been suggested as treatments for various inflammatory diseases, and they were pursued by several pharmaceutical companies as anti-inflammatory agents the application of anti-inflammatory medical herbs has a complex pharmacological effect. Therefore it has become a research hotspot to search for anti-inflammatory ingredients in medical herbs, among of which alkaloids are of great representativeness (Souto, 2011). Alkaloids are nitrogen containing organic compounds with an alkali like property. Isoquinoline alkaloids have anti-inflammatory, analgesic, spasmolysis, antibacterial and relieving asthma effects (Souto, 2011). Prostaglandins have been commonly linked with the pathology of inflammation and fever (Riccioti, year). They were believed to be synthesized in the prostate gland (hence the name) but are now known to be synthesized throughout the body. Medicinal plants are widely used in folk medicine in many countries to treat different inflammatory conditions and in particular skin inflammations. There are many causes of inflammation but the mechanisms are common to all. The inflammatory agent acts in the cell membranes inducing the activation of phospholipase A2 consequently liberates arachidonic acid and metabolites. There are Inflammatory mediators such as cytokine, histamine, serotonin, leukotrienes and prostaglandin that increase the vascular permeability to all on the migration leukocytes cells to act on the site of inflamed tissue. Any interruption of this sequence of events results in the reduction of the liberation of mediators causing the microcirculation to come back to normal hemodynamic state. Pro inflammatory mediators such as cytokines that are secreted in excess from inflammatory cells leads to development cells leads to development of arteriosclerosis when they chronically affect blood vessels. They cause tissue degeneration and dysfunction to various organs. Chronic inflammation is also involved brings hypo function in the elderly, dementia, osteoporosis, or cancer, several chronic diseases and negatively affects life

expectancy. Patients with neuropsychiatric disorders exhibit all cardinal features of inflammation, including increased circulation levels of anti-inflammatory inducers, activated sensors, and inflammatory mediators targeting various tissues. Therefore neuropsychiatric disorders and inflammation are closely intertwined and they possibly induce each other in a bisectional loop. Inflammation also plays an important role in development of several age related diseases such as anthritis. It has been reported that low grade chronic inflammation chronic inflammation can also cause risk of atherosclerosis and insulin resistance which are leading mechanisms in the development of cardiovascular diseases. Inflammation is also related to the progression and development of cancer. The relationship between inflammation and cancer are varied and complex. An important connection between inflammation and cancer is DNA damage. Reactive oxygen and nitrogen species are generated during inflammation to combat pathogens and to stimulate tissue repair as well as regeneration. However these chemicals can also cause DNA damage causing mutations that initiate cancer.

2.2 CURRENT STATUS OF ANTI-INFLAMMATORY DRUGS

The anti-inflammatory drugs currently associated with several side effects at varying degrees including gastrointestinal toxicities, cardiovascular risks, renal injuries, and hepatotoxicity as well as hypertension, sudden cardiac death and other minor disorders. Most of the anti-inflammatory drugs in clinical practice are becoming superseded due to their potential adverse effects. These are found to be highly unsafe for long term use. Therefore it is urgently significance to develop anti-inflammatory drugs with low toxicity and good efficacy.

2.3 CLASSES OF ANTI-INFLAMMATORY DRUGS

2.3.1 IIMMUNE SELECTIVE ANTI-INFLAMMATORY DERIVATIVES

Immune selective anti-inflammatory derivatives (ImSAIDs) are one of the classes of peptides being developed by IMULAN Bio Therapeutics, LLC. These were discovered to have diverse biological properties including anti-inflammatory properties. The working mechanism of ImSAIDs is altering the activation and migration of inflammatory cells which are immune cells responsible for amplifying the inflammatory response. The ImSAIDs represent a new category of anti-inflammatory and are unrelated to steroid hormones and are unrelated to steroid hormones of anti-inflammatory and are unrelated to steroid hormones or nonsteroidal anti-inflammatories.

There are also one lead ImSAIDs, the tripeptide FEG (Phe-Glu-Gly) and its D-isomer feG. They are known to alter leukocyte adhesion involving actions on Amb2 intergrin, and disrupt the binding of CD16b (FCyRlll) antibody to human neutrophils feG. This has also been shown to decrease circulating neutrophil and eosinophil accumulation decrease intracellular oxidative activity. It can also reduce the expression of CD49d after antigen exposure.

2.3.2 NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

Non-steroidal anti-inflammatory drugs (NSAIDS) reduce pain by counteracting the cyclooxygenase (COX) enzyme. On its own COX enzyme synthesizes prostaglandins creating inflammation. In whole the NSAIDs prevent prostaglandins from being synthesized, reducing or eliminating the inflammation and resulting pain.

Aspirin, ibuprofen and naproxen are common examples of non steroidal anti-inflammatory drugs. The newer specific COX-inhibitors are not classified together with traditional NSAIDs, though they presumably share the same mode of action. On some point of view they are analgesics that are commonly associated with anti-inflammatory drugs but have no anti-inflammatory effects. An example is acetaminophen well known by the name paracetamol. Paracetamol is known that it is able to block the reuptake of endocannabinoids which only reduces pain. This explains why it has minimal effect on inflammation. Paracetamol is sometimes combined with NSAID (in place of an opoid) especially in clinical practice. This is done to enhance pain relief of the NSAID, while still the injury/disease (Rossi, 2009).

Long term use of NSAID can cause gastric erosions. This can become stomach ulcers and in extreme cases causes severe haemorrhage resulting in death. The risk of death as a result of Gastro internal bleeding caused by use of NSAIDS is one in 12000 for adults aged 16 to 45. The risk increases almost twentyfold for those over 75. Exacerbating asthma and kidney damage are other dangers of NSAIDS. Apart from aspirin, prescription and over the counter of many NSAIDS also increase risk of heart attack and stroke.

2.4 *AZANZA GARCKEANA* LEAVES

Azanza garckeana has the anthropology shown in table 2.1 its picture is shown in figure 2.1

Table 2.1:anthology of plant under study azanza garckeana leaves

Kingdom	Plantae
subkingdom	Tracheobionta
Division	Spermatophyte
Class	Magnoliospida
Subclass	Dileniide
Order	Malvales
Family	Malvaceae
Genus	Azanza
Specie	Azanza garkeana

Figure 2.1: Image of *Azanza garckeana* leaves

2.5 PHYTOCOMPOUNDS

Phytochemical analysis refers to the extraction, screening and identification of medically active substance found in plants. The name phyto was also derived from the plants.

Some of the bioactive compounds found in plants are flavonoids, alkaloids, saponins, phenolic compounds and caretenoids. Phytochemical analysis of plants includes several aspects that are

- Extraction of the compound to be analysed from a sample
- Separation and isolation of phytoconstituents
- Investigation of biosynthetic routes of a certain molecule

Table 1.2: phytochemical constituents and their examples

PHYTOCHEMICALS	EXAMPLES
Terpernoids	Carotenoids, lactones, diterpenoids, terpenoids
Flavonoids	Quercetin,
Alkaloids	Indole, piperidine,glucosinolates
Phenolic compounds	Ligans, xanthones, quinines
Lipids	Monosaturated fat, fats, fatty acids
Carbohydrates	Sugar alcohols, polysaccharide,monosaccharide
Saponins	Fabaceae, araliaceae

2.7 Classes of phytochemicals

2.7.1 Alkaloids

The term alkaloid refers to natural product that contain a nitrogen atom and is therefore basic in character. There are thousands of alkaloids which has been extracted and identified from various plant sources and examples of alkaloids are shown on the following displayed formulas.

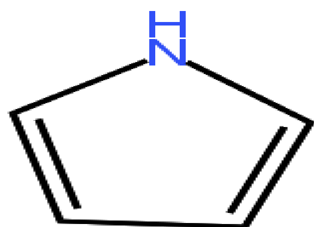

Figure 1.2: structure of an alkaloid pyrole

These compounds are biologically active compounds which can be used as lead compounds into many fields of medicinal chemistry. Opium also contains many alkaloids used in the

medicinal chemistry. Some of the therapeutic value in human which are of benefit from alkaloids include pain killers, muscular relaxation, mind altering drugs and chemotherapy

2.7.2 FLAVONOIDS

Flavonoids are an important class of phenolic compounds found in most components of plants: leaves flowers, fruits. Flavonoids are mostly water soluble because they form hydrogen bond with water. They create flower pigmentation hence serve as signals for pollinators. Some of the important works of flavonoids are as follows

- Reduces the palatability of plants or causes herbivores to avoid the plants.
- Affects interaction of plants with other organisms: Inhibit or encourages bacteria and mychorizzae associations
- Have anti-oxidative, antimicrobial anti-carcinogenic and cardio-protective effects

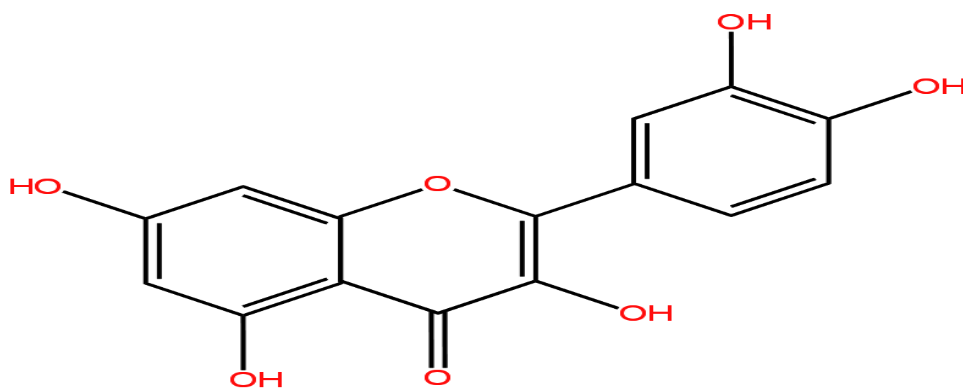

Figure 2.2: structure of flavonoid quercitin

2.7.3 SAPONINS

They are a group of secondary metabolites found widely distributed in the families of monocots. These saponins and are less frequently in dicots. Triterpene saponins are abundant in several dicot families such as Ammaranthaceae (formerly Chenopodiaceae) Caryophyllaceae, Phytolaccaceae. Saponin act upon wounding-induced decompartmentation ,as it stored as bidesmosidic (containing two sugar chains one attached to the C-3 and one at C-22compounds) in the vacuole, which are cleaved to the active monodesmosidic (C-3 position) compounds by a β-glucosidase or an esterase. Saponin are chemically classified as a glycosylated steroids, terpenoids, and steroid alkaloids.

2.7.4 GLYCOSIDE

Glycoside is a class of natural products. It is composed of two molecules a sugar which is primarily D-glucose, sometimes it can be L-rhamnose and L-fructose and A-glycone composed of flavonoid or a terpene. A-glycone is a part of cardiac glycosides which is highly toxic and found in number of plants. A-glycone of cardiac glycosides can be categorized in two chemical groups i.e cardenolide and bufadienolide. Cardenolide plays a important role in cardiac activity. The most important phenomenon of transport activities of cells and neuronal signalling depends on the Na+-, K+- atpase, building up Na+ and K+ gradients which is Inhibited by cardiac glycosides causing death through cardiac and respiratory arrest. Figure 2.4 an example of structure of glycoside

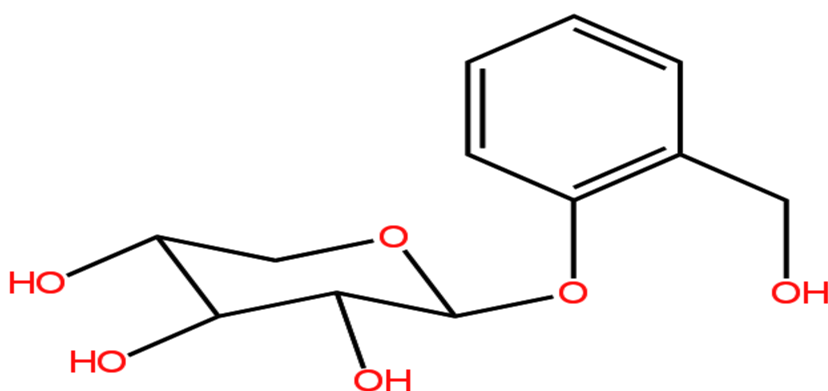

Figure 2.3: structure of a glycoside

2.12 VOLATILE OILS

Volatile oils are hydrophobic liquids that can be extracted from many plants. It is also known as essential oils. Essential oil associated with the plant material from which the oil is extracted, is a complex mixture of volatile plant constituents characterized by low molecular weight components, such as terpenoids and other aromatic and aliphatic chemical compounds.Figure 2.5 an example of structure of volatile oil

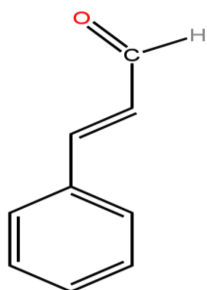

Figure 2.4: Structure of a volatile oil

2.13TERPENOIDS

It consists of six isoprene units e.g. Lanosterol and squalene found in wheat germ, and olives. Steroidal glycosides, the cucurbitacins occurring in members of the Cucurbitaceae and a Few other families' express substantial cytotoxic activities; inhibit tumor growth *in vitro* and *in vivo*.

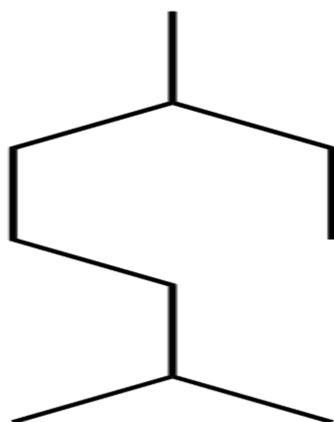

Figure 2.5: Structure of triterpene

2.14 PHENOL

Phenolic compounds are compounds mainly with a benzene ring attached to an oxygen group. The phenolic compounds are present in medicinal plants that are important secondary metabolite. They show a wide range of pharmacological activities like anticancer, anti-inflammatory.

2.15 EXTRACTION OF SOLVENT

Solvent extraction is the first step before characterization or investigation of any components in a sample. Methods of extraction are simple distillation, maceration, pressing and sublimation. In this work maceration was the most appropriate method of extraction of the solvent to be analysed. After harvesting the leaves from a nearby source the leaves were soaked in water and washed. The purpose of washing leaves is to remove any clay and silt and obtain a pure desirable product. After washing the leaves are dried by the sun in order to increase their storage life. Motor and pestle are used to crush the dried leaves in order to get fine powder**.** Different solvents such as ethanol, ethanol, acetone and hexane are used.

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

In this chapter I will be showing the reagents, methods and instruments used to perform experiments with the results in chapter 4. I will also give the study area location and information of the source where l collected the samples.

3.2 REAGENTS

The solvents used for extraction of phytochemicals were ethanol, hexane and acetone. I used acetone in place of ethyl acetate which was unavailable in the laboratory. Distilled water was used throughout the experiments. Quercetin, sodium hydroxide, hydrochloric acid, chloroform, acetic acid, ammonia, concentrated sulphuric acid, ferric chloride, acetic anhydride, lead acetate, glacial acetic acid, sodium bicarbonate were used for phytochemical screening. Egg albumin was used for anti inflammatory analysis. Dichlofenac sodium was used as a standard drug and was purchased from a local pharmacy. Ethanol, sulphanilamide, phosphoric acid, naphthylethylenediamine dihydrochloride (NEDA) and sodium nitroprusside in phosphate buffered saline was used in nitric oxide radical scavenging assay.

3.3 SAMPLE COLLECTION AND PREPARATION

Azanza garckeana leaves were collected from a local homestead in Mashonaland central province, Bindura in Zimbabwe with the help of an environmentalist and immediately put in polyethene bag and transported to Bindura University of Science Education Chemistry laboratory. The plant material was thoroughly washed individually under running tap water to remove any traces of soil particles, dust and other dirt then dried in the shade. The dried plant material is stored in the laboratory at room temperature before the extraction. The main purpose of drying is to remove the water content from plants so that the plants can be stored.

3.4 SOLVENT EXTRACTION

The dried A. garckeana leaves were powdered using motor and pestle 100 g of each powdered sample was soaked in 100-150 mL ethanol, acetone, hexane in a conical flask, shaken occasionally to mix, and macerated for 72 hours at room temperature. Maceration intends to soften and break the plant's cell wall to release the soluble phytoconstituents (Handa *et al.*, 2008). All the laboratory activities were performed in the laboratory of the

Department of Chemistry, Bindura University Science Education. Electronic analytical balance was used for weighing the powdered sample and chemicals in the experiments. Then the solution was percolated through cotton. Filtrate and marc were obtained.

3.5 PHYTOCHEMICAL SCREENING

3.5.1 Testing for flavonoids

To a portion of 4ml of the extract, 5ml of the dilute ammonia was added followed by the addition of concentrated sulphuric acid. A red brown precipitate indicated the presence of flavonoids.

3.5.2 Testing for alkaloids

3.5.2.1 Dragenndorff's test

To a portion of the extract a few drops of acetic acid was added followed by Draggendorff's reagent. Orange red precipitate indicated presence of alkaloids

3.5.2.2 Wagner's test

To a portion of the extract a few drops of Wagner's reagent was added in the sides of the test tube. A red brown precipitate indicated presence of flavonoids

3.5.3 Testing for terpenoids

3.5.3.1 Salkowaski test

To a portion of the extract 1 ml of chloroform was added followed by few drops of concentrated sulphuric acid. A red brown precipitate indicated the presence of terpenoids

3.5.4 Testing for reducing sugars

To a portion of plant extract, 1mL of water, and 5-8 drops of Fehling's solution were added and heated. The presence of reducing sugar was indicated by the appearance of brick red precipitate.

3.5.5 Testing for tannin content

3.5.5.1 Ferric chloride test

To a portion of plant extract 5% ferric chloride solution was added. The formation of blue, blue-black or brownish green colour indicated the presence of tannin.

3.5.6 Testing for quinine

To a portion of the extract, freshly prepared 1ml $FeSO_4$ solution and ammonium thiocyanate were added then concentrated H_2SO_4 was added drop by drop. The deep red colour indicated the presence of quinine.

3.5.7Testing for glycosides

To a portion of the plant extract 3ml of chloroform and 10% of ammonia solution were added to 2ml plant extract. Formation of pink colour indicated the presence of glycosides

3.5.8 Testing for phytosterols

3.5.8.1 Liberdmann Burchad's test

5ml of the extract were added in 2 ml acetic anhydride and one to two drops of concentrated sulphuric acid was added slowly along the sides of the test tube. The formation of blue green color indicated the presence of phytosteroids.

3.5.9 Testing for phenol

3.5.9.1 Lead acetate test

5mg of the extract was dissolved in distilled water and 3 ml of 10% lead acetate solution was added. A bulky white precipitates indicated the presence of phenols.

3.5.10 Testing for coumarins

To 1ml of the plant extract add 1ml of 10% NaOH . Formation of yellow color indicated presence of coumarins

3.5.11Testing for cardiac glycosides

Add 2ml of Glacial acetic acid and few drops of 5% ferric chloride to 0.5ml of the extract. This was under layered with 1ml of concentrated sulphuric acid. Formation of brown ring at the interface indicated presence of cardiac glycosides

3.5.12 Testing for acids

A portion of 0.5ml of the plant extract was treated with sodium bicarbonate solution. Formation of effervescence indicated presence of acids.

3.5.12Testing for phlobatannins

Few drops of 10% ammonia solution were added to 0.5 ml of plant ectract. A pink colour precipitate indicated the presence of phlobatannins.

3.5.13Testing for anthraquinones

Few drops of 2% HCl were added to 0.5 ml of root extract. Appearance of red color precipitate indicated presence of anthraquinones.

3.5.14Testing for steroids and phytosterols

To 0.5 ml of the plant extract equal volume of chloroform was added and subjected with few drops of concentrated sulphuric acid. Appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicated the presence of phytosteroids

3.5.15Test for volatile oils

2mL of extract was shaken with 0.1 mL of NaOH and a small quantity of dilute HCl. White precipitate indicated the presence of volatile oil.

3.6 ANTI-INFLAMMATORY ACTIVITY ASSAYS

3.6.1 PREPARATION OF STANDARD SOLUTIONS

AA mass of 0.2 g quercetin was dissolved in 25 cm^3 of ethanol to make 8000 mg/l solution. The 8000 mg/l solution was serially diluted to give solutions of (125, 250, 500 and 1000 mg/l).

Similarly, 0.2 g of dichlofenac was also dissolved in 25 cm^3 of ethanol making 8000 mg/l stock solution. The stock solution was then serially diluted to give solutions of concentrations of (125, 250, 500 and 1000 mg/l).

3.6.2 PREPARATION OF STANDARD BUFFER SALINE

Anhydrous sodium dihydrogen orthophosphate (2.7250 g), disodium hydrogen orthophosphate (0.8000 g) and sodium chloride (22.5000 g) were weighed on a balance and dissolved in distilled water. The solution was put in a 250 ml volumetric flask and filled to the mark. Sodium hydroxide and hydrochloric acid were used to adjust the pH to 7.4.

3.6.3 NITRIC OXIDE RADICAL SCAVENGING ASSAY

200 mg of extract was dissolved in 25 ml of ethanol. Immediately serial dilutions of concentrations of (125,250,500and 1000 mg/l) were made. The standard solutions of quercetin and dichlofenac sodium were serially diluted to make concentrations of (1000, 500, 250, 125 mg/l). The freshly prepared solutions were refrigerated at 4 ^{0}C for later use

3.6.4 PREPARATION OF THE GRIESS REAGENT

Equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1%4naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid were mixed.

A volume of 1.5 ml of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 2 ml of the different concentrations of the plant extract, standard quercetin and dichlofenac and incubated at 25 ^{0}C for 180 minutes.

The extracts as well as the standards were mixed with an equal volume of freshly prepared Griess reagent.

Control samples without the extract but with an equal volume of buffer were prepared in a similar manner as was done for the test samples.

The absorbance was measured at 546 nm using a UV/Vis spectrophotometer (Genesys 10S) by using triple distilled water as blank. The percentage inhibition of the extract and standard was calculated and recorded. The percentage nitric radical scavenging activity of the ethanol extract, dichlofenac and quercetin were calculated using the following formula:

$$\% \text{ Nitric Oxide scavenged} = \frac{Absorbance\ of\ control - Absorbance\ of\ test\ sample}{Absorbance\ of\ control} \times 100\%$$

3.6.2 PREPARATION OF STANDARD SOLUTIONS

A mass of 0.2 g quercetin was dissolved in 25 cm^3 of ethanol to make 8000 mg/l solution. The 8000 mg/l solution was serially diluted to give solutions of (125, 250, 500 and 1000 mg/l).

Similarly, 0.2 g of dichlofenac was also dissolved in 25 cm^3 of ethanol making 8000 mg/l stock solution. The stock solution was then serially diluted to give solutions of concentrations of (125, 250, 500 and 1000 mg/l).

3.6.4 INHIBITION OF EGG ALBUMIN DENATURATION

The anti-inflammatory activity of extracts of Azanza garckeana leaves was determined *in-vitro* against denaturation of egg albumin protein. the method used was described by Mizushima and Kobayashi (1968) and some modifications were made. 0.2 ml of 1% egg albumin solution, 2 ml of different concentrations (125, 250, 500 and 1000 mg/l) of extracts and 2.8 ml of phosphate buffered saline (PBS at pH 7.4) were mixed. Control used was 5 ml made from a solution of 2 ml of triple distilled water, 0.2 ml of 1% egg albumin solution and 2.8 ml of phosphate buffered saline solution. Standard samples of dichlofenac sodium used were 2 ml standard solution, 2.8 ml phosphate buffer saline, 0.2 ml 1 % egg albumin solution. Concentrations of standard samples were (125, 250, 500 and 1000 mg/l).

The mixtures were then incubated at (25 ^{0}C) for 15 minutes and heated in a water bath at (70±2) ^{0}C for 5 minutes. After cooling, the absorbance was measured at 660 nm by UV/Vis spectrophotometer (Genesys 10S) using triple distilled water as blank.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100\%$$

CHAPTER 4

RESULTS

4.1 INTRODUCTION

This chapter is mainly based on analysis of the results pertaining the research project. Graphs and tables have been employed in this chapter to display results

4.2 Extracted yield

The graph in figure 4.1 shows the extraction yield by three different solvents employed

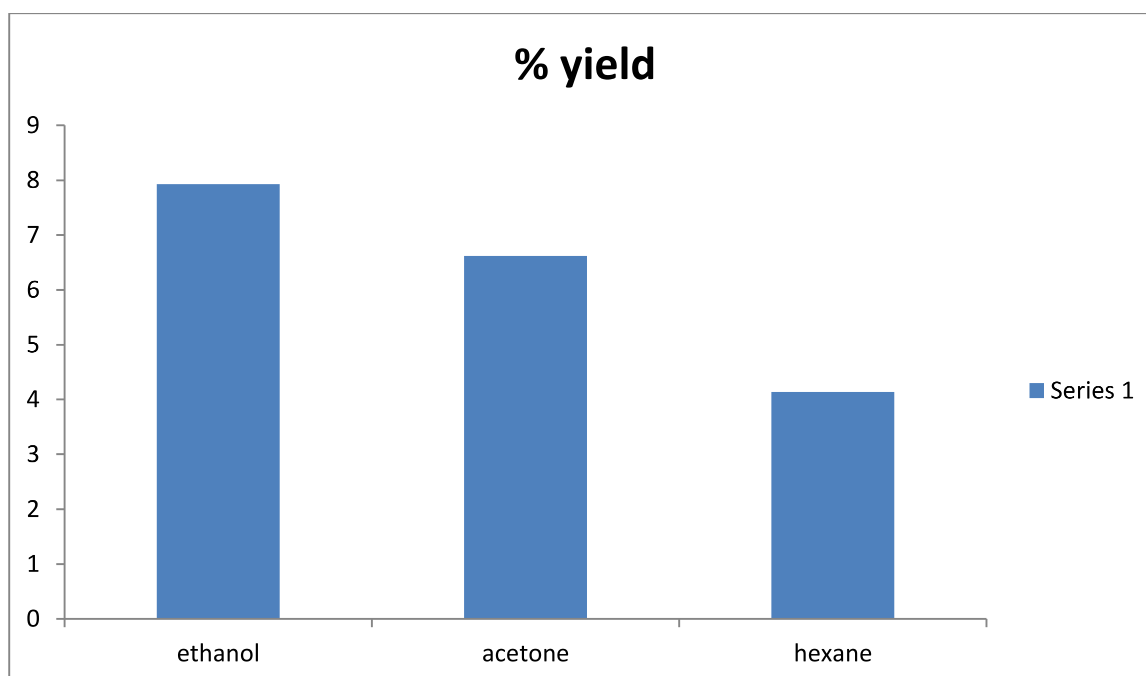

Figure 4.1: Extraction yield of ethanol, acetone and hexane

The percentage yield was calculated using the formula

$$\text{Yield} = \frac{\text{mass of dried extract}}{\text{Mass of sample extracted}}$$

4.3 PHYTOCHEMICAL SCREENING

Table 4.1 Results of phytochemical screening

PHYTOCHEMICAL TEST	ETHANOL	ACETONE	HEXANE
ALKALOIDS	++	+	+

TERPERNOIDS	++	+	-
REDUCING SUGARS	+	+	-
TANNIN	++	++	+
QUININE	+	++	-
GLYCOSIDES	-	+	-
PHYTOSTEROLS	+	++	+
PHENOL	--	+	+
COUMARINS	++	++	+
CARDIAC GLYCOSIDES	+	++	-
ACIDS	+	+	+
PHLABOTANNINS	+	+	+
ANTHRAQUINONES	-	-	-
STEROIDS AND PHYTOSTEROIDS	++	++	+
FLAVONOIDS	++	++	-
VOLATILE OILS	-	-	--

Key

+ indicate the presence of the phytochemical

++ indicate high degree of abundance of the chemical

- -indicate the absence of the phytochemical

4.4ANTI INFLAMMATORY ASSAY

4.5 Nitric oxide radical scavenging assay

Table 2.2 :inhibition of NO radical by quercetin, absorbance measured at 546nm

concentration mg/L	first absorbance	second absorbance	third absorbance	Mean	standard deviation	%inhibition
125	1.236	1.234	1.228	1.232	0.003399	35.19
250	1.084	1.083	1.081	1.083	0.001247	43.03
500	0.637	0.636	0.632	0.635	0.00216	66.75
1000	0.153	0.152	0.15	0.152	0.001247	92.00
control	1.849	1.925	1.929	1.901	0.036806	0

Table 4.3: Inhibition of NO Radical scavenging activity by dichlofenac sodium drug at 546 nm

concentration mg/L	first absorbance	second absorbance	third absorbance	mean	standard deviation	% Inhibition
125	0.109	0.108	0.105	0.107333	0.0017	43.09

250	0.092	0.09	0.087	0.089667	0.002055	52.30
500	0.066	0.063	0.06	0.063	0.002449	66.49
1000	0.048	0.045	0.042	0.045	0.002449	76.06
control	0.186	0.187	0.192	0.188333	0.002625	0

Table 4.4: Inhibition of NO Radical scavenging activity by *Azanza garckeana* leaves extract

concentration mg/L	first absorbance	second absorbance	third absorbance	mean	standard deviation	% Inhibition
125	0.502	0.497	0.492	0.497	0.004082	66.23
250	0.399	0.396	0.392	0.395667	0.002867	73.12
500	0.225	0.223	0.217	0.221667	0.003399	84.99
1000	0.145	0.137	0.133	0.138333	0.004989	90.63
control	1.462	1.473	1.481	1.472	0.007789	0

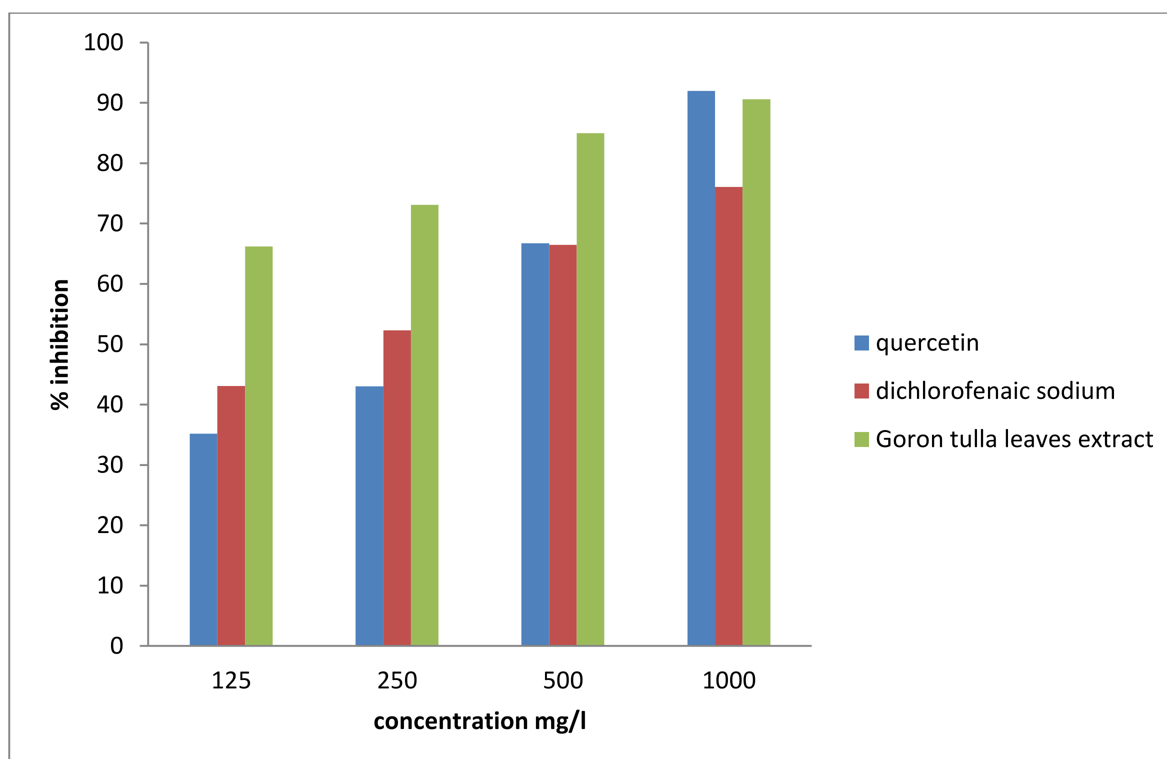

Figure 4.3: bar graphs of percentage inhibition of NO radical scavenging assay method

4.6 DENATURATION OF PROTEIN EGG ALBUMIN ASSAY RESULTS

Table 3: Inhibition of protein denaturation by *Azanza garckeana* ethanol extract

Concentration mg/L	First absorbance	Second absorbance	Third absorbance	Mean	Standard deviation	% Inhibition

125	0.433	0.431	0.428	0.430667	0.002055	37.31
250	0.357	0.354	0.35	0.353667	0.002867	48.40
500	0.228	0.225	0.221	0.224667	0.002867	67.34
1000	0.172	0.168	0.165	0.168333	0.002867	75.51
Control	0.683	0.685	0.69	0.686	0.002944	0

Table 4: Inhibition of egg albumin denaturation by dichlofenac sodium drug

Concentration mg/L	First absorbance	Second absorbance	Third absorbance	Mean	Standard deviation	% Inhibition
125	0.114	0.118	0.122	0.118	0.003266	36.31
250	0.091	0.095	0.096	0.094	0.00216	46.29
500	0.07	0.065	0.064	0.066333	0.002625	62.10
1000	0.047	0.049	0.05	0.048667	0.001247	72.19
Control	0.173	0.175	0.177	0.175	0.001633	0

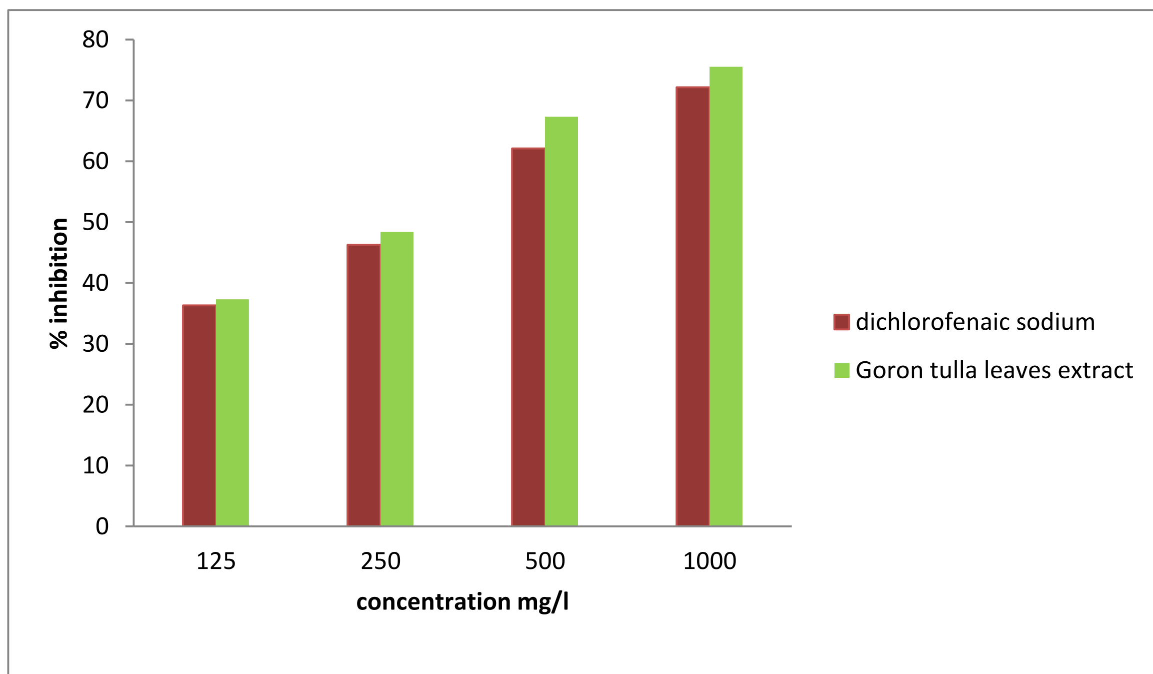

Figure 4.4: Bar graphs of percentage inhibition of denaturation protein

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 INTRODUCTION

In this chapter I shall be discussing the results in chapter four.

5.2 PERCENTAGE YIELD

The results shown on percentage yield reflected that different solvents had different yield of extractable solid of *Azanza garckeana* leaves (Figure 4.1). Ethanol recorded the highest yield of 7.93% followed by acetone which had 6.62% and hexane had 4.14%. These results were also proved by Zotek *et al*. (2016). The chemistry behind the results were that the polarity of the solvent played an important role and this explains that polar substances would dissolve in polar solvents and non-polar substances will dissolve non-polar solvents

5.3 PHYTOCHEMICAL SCREENING

Table 4.1 shows phytochemicals present in *Azanza garckeana* leaves extracted by the different solvents. Phytochemical screening of extracts of *Azanza garckeana* extracted by different solvents showed the presence of the most important phytoconstituents. The medicinal value of the plant can be shown by the presence of various bioactive chemical constituents as shown on Table 4.1. Hexane extracts showed the presence of alkaloids, tannin, phytostrols, coumarins, phlabotannis, phenol, steroids, and anthraquinones. The other phytochemicals gave a negative test. Ethanol extract gave positive results for most phytochemicals. These are alkaloids, tannins, glycosides, flavonoids, coumarins, saponins, cardiac glycosides, terpenoids, acids, phlobatannins, steroid, phytosteroids. Phytochemicals such as glycoside, anthraquinones and volatile oils gave a negative test in ethanol extract. Acetone extract gave a positive test for all phytocompounds tested except volatile oils and anthraquinones

Hexane extract gave least positive tests compared to the other extracts. Alkaloids, flavonoids, phenol, alkaloids, quinine, tannin, and carbohydrates are known to show the medicinal activity as well as physiological activity (Sunitha, 2012). The presence phytochemicals such as alkaloid, quinones, terpenoids, tannins, phytosterols, coumarine, quinones and steroids may have good anti-inflammatory activity (Bouic, 2001; De Sousa, 2012; Miguel, 2010). The

results for phytochemical screening (Table 4.2) expose the presence of the above named phytochemicals hence the plant can be said to have anti-inflammatory activity. Moreover the results (Table 4.2) indicate that the plant has quite a number of chemical constituents, which may be responsible for the many pharmacological actions.

5.4 NITRIC OXIDE RADICAL SCAVENGING ASSAY

In the inflammatory response NO is an important molecule that play an important role. In this study I determined the viability of NO inhibition of azanza gackeana leaves at different concentrations of (125, 250, 500, and 1000g/L The percentage inhibition of NO radical by quercetin a typical flavonoid increased from (35.19%) at 125 mg/l to (92.00%) at 1000 mg/l (Table 4.3). Dichlofenac drug had its percentage inhibition increasing from (43.09%) at 125 mg/l to (76.06%) at 1000 mg/l. (Table 4.4). Ethanolic extract of *Azanza gackeana* exhibited good nitric oxide scavenging activity.

The aqueous extract inhibited nitrite formation in concentration dependent manner (Table 4.5), this may be as a result of the presence of antioxidant principles in the extract, which compete with oxygen to react with nitric oxide. There was increase in percentage inhibition with the increase in concentration of the extract (Table 4.5). According to Figure 4.2, the inhibition of NO radical by ethanol extract (66.23%) is superior to that of the standard quercetin (35.19%) and dichlofenac sodium (43.09 %) at125 mg/l. This gives an account that suggests that a mixture of phytocompounds in the extract has powerful NO scavenging activity compared to the quercetin and dichlofenac sodium standards. Also high concentration of 1000 mg/l quercetin showed superior percentage NO scavenging activity of 92.00% which was comparable to that of *Azanza gackeana* (90.63%) as shown in Figure 4.2. It is known that inflamtion rise can be caused by the conversion to peroxynitrite (ONOO-), from high nitric oxide concentration produced by nitric oxide synthase (Nijveldt et al., 2001).

According to Hazra (2008), continual production and accumulation of peroxynitrite radical directly participate in the vascular break down which is associated with inflammation conditions such as arthritis, sclerosis and ulcerative colitis among a host of others. The results (Figure 4.2) show that the crude extracts of *Azanza gackeana*can be a valuable source of lead compounds for production of anti-inflammatory drugs. Inhibition of NO may be beneficial for treatment of other diseases like Alzheimer's disease and cardiovascular disorders including atherosclerosis, as well as cancer to mention a few.

5.5 INHIBITION OF EGG ALBUMIN DENATURATION ASSAY

Ethanol extracts of *Azanza garckeana* leaves were studied for in vitro anti-inflammatory activity by protein denaturation. Qualitative analysis revealed that *Azanza garckeana* leaves contained alkaloids, flavonoid, tannins, glycosides, steroids, terpernoids and saponins in ethanol extract. Denaturation of tissue proteins is one of the dominant cause of inflammatory diseases. From biological perspective protein denaturation occurs when proteins lose their secondary or tertiary structure. There are certain causes of protein denaturation that includes, high temperatures, oxidative stress, high concentration of acid or base, organic solvent or concentrated inorganic salt. According to Umapathy *et al*. (2010) denaturation causes loss of function of protein. Denatured protein due to heat treatment expresses antigens related to type (III) hyper sensitive reaction. It is suggested that denatured protein are equally effective as native protein and play a key role to provoke delayed hypersensitivity. Also alteration of electrostatic, hydrogen and hydrophobic and disulphide bonding can be related to denaturation of protein (Bharti and Bhushan, 2015).

5.6 CONCLUSION

The results showed that *Azanza gackeana* leaves possess marked *in-vitro* anti-inflammatory activity. Crude *Azanza gackeana* extract possessed the highest efficacy of inhibition of egg albumin and this was further confirmed by NO scavenging assay. In this investigation it was shown that phytochemicals from *Azanza gackeana leaves* are a potential source of anti-inflammatory agents of natural origin. They may be also suitable candidates for treating pathologies related inflammation and free radical oxidation due to their overall anti-inflammatory effect in protein inhibition and scavenging of free radicals and active oxygen species. *Azanza gackeana* leaves have been used to cure swollen legs and some drank to cure pains. The plant is a good candidate for evaluating the nitric oxide scavenging as well as anti-inflammatory potentials due to its proven positive pharmacological activities. In conclusion, it can be justified beyond reasonable doubt that *Azanza gackeana* possessed marked *in vitro* anti-inflammatory effect against the denaturation of protein. The effect was due to the polyphenols contents such as flavonoids, steroids, phenols and alkaloids. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. Ability of the phytochemicals to inhibit thermal denaturation of protein is possibly a contributing factor for its anti-inflammatory activity.

Table 4.5 and 4.6 indicated the stabilization of protein due to the presence of *Azanza garckeana* samples that were tested with respect to the control sample which theoretically shows that heat-induced denaturation was averted. Table 4.5 shows that the percentage inhibition of *Azanza garckeana* leaves at 125 mg/l was 37.31% and that of dichlofenac sodium on table 4.6 was 36.31%. This shows that *Azanza garckeana* leaves are more effective in inhibiting protein denaturation than dichlofenaic sodium standard drug.

The minimum and maximum percentage inhibition at 125 and 1000 mg/l, with diclorofenac sodium was 37.31 % and 75.51 % respectively, whereas, for *Azanza gackeana* it was 44.54%

and 80.87%, respectively (Figure 4.3) this shows that *Azanza garckeana* was more effective than dichlofenac sodium, the standard drug prescribed against inflammation. *Azanza gackeana* leaves have been drank as tea since time immemorial with no side effects. The results of this study may suggest its use as a nutraceutical with lead compounds that could further be developed into anti-inflammatory agents.

5.7 RECOMMENDATIONS

The extract of Azanza garckeana leaves contained significant amounts of alkaloid, flavonoids and phytosterols which have anti-inflammatory activity. It was also proven by Aeri (2013) that agents that prevent the protein denaturation are worthwhile to be considered for anti-inflammatory drug development.

There is need of more scientific attention in order to actualize the potentialities of all the parts of the *Azanza garckeana* leaves in the fields of medicine and health sciences.

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