UNIVERSITY OF EDUCATION, WINNEBA

THE EFFICACY OF DIFFERENT SOLVENT EXTRACTS OF THE DRIED LEAVES OF A MIXTURE OF *CHROMOLAENA ODORATA* L. AND *SIDA ACUTA* BURM. F. ON WOUND HEALING OF ALBINO RATS

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A thesis in the Department of Biology Education, Faculty of Science Education, submitted to the School of Graduate Studies, in partial fulfilment of the requirements for the award of the degree of Master of Philosophy (Biology Education) in the University of Education, Winneba

SEPTEMBER, 2023

DECLARATION

STUDENT'S DECLARATION

I**, Benjamin Sarfo Appau,** hereby declare that, this thesis is the sole effort of the researcher, and that, it has neither in whole nor part been presented elsewhere, with the exception of references made to other people's work which have been cited and acknowledged.

SIGNATURE:.. .....

DATE: .....

SUPERVISOR'S DECLARATION

I, **Professor Yaw Ameyaw,** hereby declare that this thesis has been supervised in accordance with the guidelines on supervision of research as prescribed by the University of Education, Winneba.

SIGNATURE .....

DATE: .....

DEDICATION

I affectionately dedicate this thesis to my parents, Mr. and Mrs. Ampomah for their unfailing love, prayers, financial support and constructive advice throughout my education.

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Had it not been the help of the Almighty God, this thesis would have not been successful. I am highly indebted to God for his favour and abundant mercies that have sustained me throughout the programme from day one to the end.

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ABSTRACT

This study investigated the efficacy of different solvent extracts of the dried leaves of a mixture of C*hromolaena odorata* L. and *Sida acuta* Burm. F. on wound healing of albino rats**.** Convenience sampling was used to select the two herbs from Assin Andoe in the Central Region of Ghana. The study involved thirty albino rats and four solvents (70% ethanol, 30% ethanol, 30% methanol and distil water) were used for the extraction as well as penicillin ointment as the control. The researcher observed the wound surface and the feeding behaviour of the albino rats throughout the fifteen-day experiment. The wound areas and the masses of the albino rats were measured on three days intervals. The phytochemical constituents of the dried leaves of the mixture of C*hromolaena odorata* L. and *Sida acuta* Burm. F. were determined, and statistical analysis was conducted using SPSS version 20. Observation of the wound surface showed improvement throughout the experiment, with all extracts and the control contributing to scar tissue formation and wound healing. The albino rats exhibited normal feeding behaviour and consumed all the provided food. The wound areas were significantly closed in groups PO, SC2, SC3 and SC4 by the twelfth day of the experiment whereas group SC1 achieved complete closure by the fifteenth day of the experiment. There was no significant difference in the means of the wound areas among the five groups, indicating the efficacy of the various extracts on wound healing in albino rats as compared to the control (Penicillin ointment). The average mass of the rats fluctuated throughout the experiment, with a significant increase by the fifteenth day. The eating habits of the rats influenced weight gain. Pearson's correlation coefficient analysis revealed a negative relationship between the average masses and the average wound areas across the five groups, indicating that as the wound areas decreased, the masses of the rats increased. Overall, the study demonstrated the efficacy of different solvent extracts of the dried leaves of the mixture of C*hromolaena odorata* L. and *Sida acuta* Burm. F. in promoting wound healing in albino rats. Further study is recommended for quantitative analysis of the phytochemical constituents of the mixture of *C. odorata* L. and *S. acuta* Burm. F. leaves, and again, other parts of the herbs such as the roots or stems should be investigated for their efficacy on wound healing and acute toxicity test should be done on the albino rats to investigate the effect of the mixture of *C. odorata* L. and *S. acuta* Burm. F. leaves on vital organs such as the kidney and the liver.

CHAPTER ONE

INTRODUCTION

1.0 Overview

This chapter presents the introduction to the study which is made up of the background to the study, statement of the problem, justification of the study, purpose of the study, research objectives, null hypotheses, alternate hypotheses, significance of the study, limitations of the study, delimitations of the study, the organization of the study and terms and abbreviations.

1.1 Background to the Study

Herbal medicines have made significant contributions to humankind's fight against illness and maintenance of health. It is estimated that 2.3 billion people, or 56% of the world's population, still depend on traditional healers and healing methods for the treatment of a broad range of physical and mental ailments. Interest in the use of plant concoctions has grown in recent years (Mukherjee, 2002).

Wound is a damage to the integrity of biological tissue, including skin, mucous membranes and organ tissues (Kujath & Michelsen, 2008). Various types of traumata can cause these and it is critical to ensure wounds are cleaned and appropriately dressed to limit the spread of infection and further injury (Wilkins & Unverdorben, 2013).

There is no standard classification for wounds. On the other hand, wounds can be classified in several ways based on the nature of the injury, the timing whether acute or chronic and the depth of injury to the skin and the underlying tissues. These factors will have a significant effect on the ability of the wound to heal with or without surgical intervention (Percival, 2002).

According to Velnar, Bailey and Smrkolj (2009), the process of wound healing is continuous and it is divided into four phases: coagulation and haemostasis, inflammation, proliferation and lastly, wound remodelling with scar tissue formation. It also involves generation of multiple cell populations, the extracellular matrix and the action of soluble mediators such as growth factors and cytokines. Santos-Buelga, Mateus & De Freitas (2014) asserts that many plants have been used traditionally to treat wounds due to their high efficiency in healing process. The reason is that, plant – based medicines are affordable and cause reduced side effects (Ekor, 2014).

From Nagori and Solanki (2011), extensive research has been done in the field of wound healing and wound management through plant-based medicines recently.
Many plants contain antioxidants which are beneficial and are of therapeutic use in several ailments that are connected with potential pathologic actions of oxidants as well as wound healing (Yeoh, 2000).

Since the introduction of *Chromolaena odorata* into Ghana since 1970 according to Ghanaweb (2020), the herb has been used to stop bleeding, it is efficient in healing wounds, treatment of snake poison when it is immediately administered after a bite. The herb is also used to treat stomach aches, bilharzia and also to preserve dead bodies.

In a typical Ghanaian society, when a person gets a fresh cut with blood oozing non-stop, the local people just rub a leaf or more of *Chromolaena odorata* (Acheampong) in their palms, squeeze and drop the liquid on the cut. The blood then clots few seconds after the treatment (Ghanaweb, 2020).

Sida acuta (Siam weed) extract has been shown to stimulate haemostasis and wound healing management. The phytochemical substances in the leaf of *Chromolaena odorata* were used for antibacterial, antifungal, anti-inflammatory, anticancer,

antidiabetic, antidiarrheal and hepatoprotective activities (Sirinthipaporn & Jiraungkoorskul, 2017).

Sida acuta has been shown to have wound healing potentials. The methanolic extract of *S. acuta* produced significant healing in wounds treated with it. However, *Sida acuta* has been traditionally used for thousands of years and is used for treating various ailments, including malaria, bacterial infections, ulcers (Tcheghebe, Seukep & Tatong, 2017). In the Ghanaian society, a decoction of the leaves of the *Sida acuta* is applied on wounds to stop bleeding (CSIR, 2023).

Several studies have investigated the effect of *Chromolaena odorata* and *Sida acuta* on wound healing. For example, Ezeja, Anaga, and Asuzu (2012) conducted a study to investigate the effect of *Chromolaena odorata* on wound healing in rats. The study showed that *Chromolaena odorata* extracts had a positive effect on wound healing by increasing the rate of wound closure, reducing inflammation, and promoting tissue regeneration. The study also showed that *Chromolaena odorata* extracts increased collagen deposition in the wound tissue, which is an important factor in the healing process.

Similarly, Akilandeswari, Senthamarai,… and Prema (2010) investigated the effect of *Sida acuta* on wound healing in rats. The study showed that *Sida acuta* extracts had a positive effect on wound healing by increasing the rate of wound closure and reducing inflammation. The study also showed that methanolic exctract of *Sida acuta* treated wounds were found to epithelialise faster and the wound contraction rate was higher when compared to the control wounds.

However, other studies have shown no significant effect of these plants on wound healing. For example, a study conducted by Iwu, Duncan,... and Janick (1990) showed that *Chromolaena odorata* extracts had no significant effect on wound healing in rats.

Example of local herbs that are efficacious in healing wounds include; *Arctium lappa, Astragalus propinquus, Rehmannia glutinosa, Ampelopsis japonica, Andrographis paniculata, Caesalpinia sappan, Aloe vera, Prosopis africana, Curcuma longa, Euphorbia hirta* etc.

This research work combined two herbs, *Sida acuta* Burm. F. and *Chromolaena odorata* L., to investigate their efficacy on wound healing in albino rats. There is confidence to explore new knowledge by drawing on the historical empirical experiences of herb-herb combinations as there is growing interest in switching from the one-drug, one-target paradigm to combination therapy or polypharmacy to obtain therapeutic benefits for a multitude of ailments (Che, Wang,… & Lam, 2013).

From previous studies, wound healing in mammals has attracted the attention of many researchers and several herbs have been investigated to solve real life problem. This has led the researcher to gain interest in investigating the efficacy of *Chromolaena odorata* and *Sida acuta* leaves extracts on the wounds of albino rats.

1.2 Statement of the Problem

Assin Andoe is a farming community with 70% of the populace engaged in non-mechanized farming. There is the issue of frequent accidents whereby farmers cut themselves with sharp farm tools when preparing the land for cultivation or performing cultural practices. As it has been rightly said by Kandhare, Dalvi…, and Bodhankar (2016) that wounds are inevitably part of life. Without timely implementation of first aid, most victims lose a lot of blood or the wounds inflicted on the victim becomes

tainted. The problem of wound management greatly affects the livelihood of the people of Assin Andoe in the sense that it prevents them from going about their daily activities. Living with a wound possesses a great effect on one's quality of life. As such, the individual goes through pain, emotional distress due to social distancing, long hospitalization and worse of all mortality (Lindholm & Searle, 2016). And again, wound management is a major clinical concern due to complications that may lead to morbidity and mortality.

Aqueous extract of locally available plants; *Chromolaena odorata*, foliage leaves of plantain etc. are mostly used by these farmers to aid in blood clotting and for wound healing. Since these herbs are cheap to come by, the individuals can manage their wounds before getting worse. Improvement in the extraction of the local herbs and combinations of the herbs are therefore suggested to increase the efficiency of wound healing of the herbs. The researcher gained interest to investigate the efficacy of two local herbs combined together, thus the efficacy of the mixture of the dried leaves of *Chromolaena odorata* and *Sida acuta* on wound healing of albino rats.

1.3 Justification of the Study

In Ghana, it appears herbal medication is a well-known form of healthcare hence herbal practitioners and the local folks typically use it to treat different ailments. It is a general knowledge that, the use of herbal medication is known to at least one member of every family in Ghana. Although some herbalists receive official training, most information of herb use appears to be handed down orally or informally from one generation to the next. Despite the rivalry between orthodox and traditional medicine, the populace continues to engage themselves in traditional medicine for good health (Darko, 2009).

It is recently that, traditional and orthodox medicine practitioners collaborate in Ghana to address the nation's healthcare requirements. The Ghana government established the

Mampong Centre for Plant Medicine Research after realising the significance of traditional medicine (Darko, 2009). It is public knowledge that, Ghanaians in particularly those in the Assin South district, continue to practise the traditional plant medicine in a crude and unrefined manner. To prevent overdosing or underdosing, it is important to measure the dosage of herbal medicines. It is also important to use the right extraction techniques to avoid changing the molecular makeup of the plants. The researcher used different solvents to extract a mixture of *Chromolaena odorata* and *Sida acuta* leaves to determined the efficacy of the solvent extracts on healing wounds in albino Rats.

1.4 Purpose of the Study

The purpose of the study was to determine the efficacy of different solvent extracts of the dried leaves of a mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. on wound healing in albino rats.

1.5 Research Objectives

The objectives of the study were to:

1. determine the healing effect of the different solvent extract of the mixture of dried leaves of *Chromolaena odorata* L. and *Sida acuta* Burm. F. on wounds in albino rats.
2. ascertain whether changes in the masses of the albino rats occur after the application of the different solvent extract of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves.
3. assess the phytochemical compounds present in the solvent extract of the dried leaves of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F.

1.6 Null Hypotheses

1. The solvent extract of the mixture of dried leaves of *Chromolaena odorata* L. and *Sida acuta* Burm. F. has no significant effect on wound healing in albino rats.
2. The different solvent extracts of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves have no significant effect on the masses of the albino rats.

1.7 Alternate Hypotheses

1. The solvent extract of the mixture of dried leaves of *Chromolaena odorata* L. and *Sida acuta* Burm. F. has significant effect on wound healing of albino rats.
2. The different solvent extracts of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves has significant effect on the masses of the albino rats.

1.8 Significance of the Study

The results of this study will provide in-depth knowledge on the best solvent for extraction of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves on healing wounds in albino rats, and also, may lead to discovery of herbal mixture that can heal wounds in animals and as a reference herbal product to heal wounds in humans.

1.9 Limitations of the Study

The following served as limitations of the study:

1. Underlying health conditions of the albino rats may affect the rate of wound healing.

2. Caging the albino rats away from their natural habitat may affect their feeding behavior which may slow down their responses to the treatment of the herbal mixture.

1.10 Delimitations of the Study

The following are delimitations of the study:

1. Leaves of mature *Chromolaena odorata* L. and *Sida acuta* Burm. F. were used.
2. The *C. odorata* and *S. acuta* at Assin Andoe were used.
3. The solvents used are 70% and 30% ethanol, 30% methanol and distil water.

1.11 Terms and Abbreviations

1. Angiogenesis: It is the development of new blood vessels.
2. ECM: Extra-cellular matrix
3. EOs: Essential oils
4. HIF – Iα: Hypoxia-Inducible Factor – Iα
5. IBD: Inflammatory Bowel Disease
6. Menstruum: It is the liquid used to extract herbs.
7. Re – epithelialization: It is a complex process which describes the resurfacing of a wound with new epithelium.
8. TNF – alpha: Tumor Necrosis Factor - alpha
9. VEGF: Vascular Endothelial Growth Factor

1.12 Organization of the Study

The study is divided into six chapters. It starts with chapter one which includes the overview, background to the study, justification, purpose of the study, research objectives, null hypothesis, alternate hypothesis, significance of the study, limitations of the study, delimitations of the study, terms and abbreviations and the organization of the study. Following this chapter is chapter two which deals with the review of related

literature. Chapter three outlines the materials and methods used for the study and chapter four presents the results. Chapter five presents the discussion of the study while the sixth chapter presents the summary, conclusions and recommendations of the study.

CHAPTER TWO

REVIEW OF RELATED LITERATURE

2.0 Overview

This chapter focuses on the review of ideas, facts and thoughts expressed by some authorities in relation to the topic under study.

2.1 The Concept of Wound

Definition of wound from Merriam – Webster dictionary (2022) is "an injury to the body (as from violence, accident, or surgery) that typically involves laceration or breaking of a membrane (such as the skin) and usually damage to underlying tissues".

From the above definition, it can be judged that wound does not only affect the integrity of the skin but also causes damage to the underlying tissue which generally affects the individual's wellbeing.

The various ways by which wound is inflicted on the skin according to Wound Australia (2021) include the following:

- Accidents: for instance, when an individual accidentally cut him/ herself, burns the skin or pierces the skin.
- When an individual undergoes surgical operation.
- Prevalence of a disease such as diabetes and vascular ulcers
- Poor care of the skin may lead to eczema or psoriasis which in turn inflicts wounds on the skin.

2.1.1 Classification of wounds

Wound is categorised on the basis of the cause, site where it is inflicted and the depth. Wound can also range from a simple wound to a severe one. Some classification of wounds from Wound Care Surgeons (2022) include:

1. **Open or closed wounds**

When the underlying tissue or organs are exposed to the outside environment, it is described as an open wound. On the contrary, when the damage to the skin does not expose any underlying tissue or organ it is called a closed wound.

2. **Acute or chronic wounds**

The healing time of the wound explains whether a wound is acute or chronic. Acute wounds are the ones that heal with no complications in a predicted amount of time while chronic wounds are the ones that take a relatively longer time to heal accompanied with complications in a predicted amount of time.

3. **Clean or contaminated wounds**

Clean wounds are the ones that do not have any debris or no prevalence of microorganism whereas contaminated wounds is the ones that might have dirt, microorganism or any foreign material. Contaminated wounds are also described as infected wounds.

4. **Non-penetrating and penetrating wounds**

Non-penetrating wounds are caused by friction with other surfaces. Some examples include abrasions, lacerations, bruises and concussions. On the other hand, penetrating wounds are caused as a result of trauma or a cut through the thick layer of the skin. Examples are stab wounds, cuts, surgical wounds etc.

2.1.2 Factors that affect wound healing in animals

According to Sharma, Khanna,… and Singh (2021), factors that affect wound healing include:

- **Oxygenation**: Almost all wound healing processes require oxygen, which is crucial for cell metabolism and especially for the ATP-based energy production.

It prevents wounds from becoming infected, promotes angiogenesis, boosts keratinocyte differentiation, migration, and re-epithelialization, enhances fibroblast proliferation and collagen production, and makes wounds easier to contract. When an early incision is depleted of oxygen, active cells cause it to become hypoxic.

- **Infections**: When there is a wound, microscopic organisms on the skin's surface can enter the tissues below. Invasive infection is characterized by microorganisms that multiply inside a wound and cause harm to the host as a result. Further tissue deterioration lengthens the healing process.

- **Age:** It has been noted that the effects of ageing cause a brief delay in wound healing. Age-related changes and delays in wound healing have been investigated in clinical and animal studies at the cellular and molecular levels.

- **Poor blood circulation**: Since chemicals for wound healing are poorly transported to damaged tissue for the healing process, decreased blood supply to the injured tissue is another cause.

- **Nutrition:** Food has been acknowledged as having a huge influence on how well wounds heal. Wound healing is slowed down by malnutrition or particular nutritional deficiencies. The healing process will be impacted by how the body uses energy, carbohydrates, proteins, fats, vitamins, and nutrients.

In addition to the above factors, it is empirically proven that lack of hydration or moisture at the surface of the injured tissue impedes cellular migration and declines blood oxygenation. This is to say humans and animals alike need to stay hydrated. Unfortunately, some patients contribute to the delay in wound healing due to lifestyle

choices. Substance abuse, inadequate sleep, smoking, improper cleaning of the wound among others significantly influences the wound healing process.

Joao-De- Masi, Campos, and Joao- De -Masi (2016) posit that growth factors accelerate healing process in rats. Growth factors are proteins that activate and stimulate cell proliferation through the activation of angiogenesis, mitogenesis and gene transcription.

Forty five female Wistar rats were studied and three wounds were made on their back. The first wound was the control wound, the second wound received epithelial growth factor injection and the third received a combination of factors. Macroscopic and microscopic assessments were performed on the third, seventh and the fifteenth days of the experiment.

In the macroscopic assessment, it was concluded that the use of growth factors resulted in faster healing whereas in the microscopic assessment, combination of factors on the wound produced a favourable outcome with a higher number of fibroblasts, angiogenesis and collagen type 1 (Joao -De- Masi *et al.*,2016).

2.1.3 Phases of wound healing

Mohd, Shah,… and Sood (2012) categorised the process of wound healing into four phases. These include:

- **Phase I: Haemostasis**

Haemostasis is the body's natural reaction to an injury that clot and repair damaged tissue.

The activation of Platelets, which starts the coagulation process, leads to haemostasis. Additionally, Platelets produce compounds that start to affect the wound healing process.

- **Phase II: Inflammation**

Enzymes and other mediators secreted by inflammatory cells cause the characteristic signs of the inflammatory phase. These include pain, redness, warmth, and swelling. Although many additional cells are engaged in the process, neutrophils, macrophages, and T-lymphocytes are among the primary factors in wound healing.

- **Phase III: Proliferation**

Fibroplasia, granulation, and epithelialization are the three stages of proliferation phase. It actually starts with the migration of fibroblasts into the wound, which is predominantly triggered by the PDGF that has been generated by Platelets and macrophages.

- **Phase IV: Remodelling**

More covalent cross-linking of collagen molecules causes remodelling to occur.

Inflammatory cells depart, and the number of growth factor-releasing cells decreases. Fibroblasts continue to produce collagen even as their own population starts to decline.

2.2 A Review of Common Medicinal Plants used for Wound Healing

On the basis of the factors that influence wound healing process, some medicinal plants contain microbial inhibiting factors, growth factors e.g., cytokines and also promotes proper blood circulation.

Research by Shedoeva together with other scientists on wound healing and the use of medicinal plants, indicated that several plant medicines have been investigated and are capable of healing wounds. These herbs include; *Arctium lappa, Astragalus propinquus, Rehmannia glutinosa, Ampelopsis japonica, Andrographis paniculata, Caesalpinia sappan and Aloe vera* among others (Shedoeva, Leavesley,... & Fan, 2019).

Again, a review by Payghan, Shrikhande,… and Rushikesh (2023) reviewed twenty common medicinal plants for wound healing. These include; *Phyllanthus emblica, Aloe vera, Musa paradisiaca, Magnifera indica, Datura metal, Cinnamomum cassia, Curcuma longa, Cinnamomum zeylanicum, Allium sativum, Catharanthus roseus, Coriandrum sativum, Daucus carota, Ocimum sanctum, Syzygium aromaticum, Hibiscus rosa- sinensis, Camellia sinensis, Carthamus tinctorius, Eucalyptus deglupta, Cuminum cyminum* and *Lawsonia inermis,*

2.2.1 *Vernonia amygdalina* Delile

Classification of *Vernonia amygdalina* Delile

Kingdom: Plantae Phylum: Tracheophyta Class: Magnoliopsida Order: Asterales Family: Asteraceae Genus: Vernonia Species: *Vernonia amygdalina* (Wikipedia, 2023; The Plant List, 2013).

Description of *Vernonia amygdalina* Delile

Bhattacharjee, Lakshminarasimhan,… and Pathak (2013) and Kaur, D., Kaur, N., and Chopra (2019) give the following description of *Vernonia amygdalina* Delile (Plate 2):

Vernonia amygdalina, commonly known as bitter leaf or 'awonwono' in twi, is a perennial shrub that belongs to the Asteraceae family. It is native to tropical regions of Africa and is widely distributed across countries such as Nigeria, Ghana, Cameroon, and Senegal. This plant is highly valued for its medicinal and culinary uses.

Bitter leaf is a medium-sized shrub that typically grows to a height of 1 to 3 meters (3 to 10 feet). It has a woody stem with numerous branches and dense foliage. The leaves of *Vernonia amygdalina* are the prominent feature of the plant. They are dark green in color, oval-shaped, and have a slightly rough texture. The leaves can measure around 6 to 20 centimeters (2.4 to 7.9 inches) in length.

In many African countries, bitter leaf is commonly used as a culinary ingredient. The leaves have a distinct bitter taste, which adds flavor and depth to various traditional dishes. They are often used in soups, stews, sauces, and herbal teas. Bitter leaf is known to be rich in nutrients and is a good source of vitamins and minerals. The leaves, stem, and roots of the plant are utilized for their therapeutic benefits. Bitter leaf is known for its antibacterial, antifungal, and anti-inflammatory properties. It is used to treat various ailments, including fever, malaria, stomachaches, diabetes, and skin infections. Bitter leaf is also considered a natural detoxifier and is used to cleanse the body.

Bitter leaf is a hardy plant that thrives in tropical and subtropical climates. It prefers well-drained soil and is often found in areas with high rainfall. The plant can tolerate both full sun and partial shade. It is propagated through seeds or stem cuttings. Bitter leaf can be grown in home gardens or on a larger scale for commercial purposes. It has been used for centuries in traditional medicine due to its various health benefits.

Five benefits of *Vernonia amygdalina* Delile:

Vernonia amygdalina is packed with essential nutrients, including vitamins A, C, and E, as well as minerals like calcium, potassium, and iron. These nutrients are important for maintaining overall health and well-being (Yeap, Ho,… & Alitheen, 2010).

According to Yeap *et al.* (2010), the following are few health benefits of *Vernonia amygdalina*:

- **Antioxidant Properties**: Bitter leaf contains potent antioxidants that help protect the body against damage caused by harmful free radicals. Antioxidants play a crucial role in reducing oxidative stress, which is associated with various chronic diseases such as heart disease and certain types of cancer.

- **Anti-inflammatory Effects:** The bioactive compounds found in *Vernonia amygdalina* possess anti-inflammatory properties. Chronic inflammation has been linked to a range of health conditions, including arthritis, diabetes, and cardiovascular diseases. By reducing inflammation, bitter leaf may help alleviate symptoms and promote better overall health.

- **Digestive Health:** Bitter leaf has been traditionally used to support digestive health. It has been found to stimulate the secretion of digestive enzymes, improve bowel movements, and promote better absorption of nutrients.

Additionally, bitter leaf can help relieve digestive issues such as indigestion, bloating, and constipation.

- **Immune System Support:** The immune-boosting properties of *Vernonia amygdalina* are well documented. It contains compounds that enhance the immune response, helping the body fight off infections and diseases. Regular consumption of bitter leaf may strengthen the immune system, leading to a reduced risk of illness and faster recovery from infections.

Vernonia amygdalina also has been traditionally used in African folk medicine for its wound healing properties. Below is an overview of how *Vernonia amygdalina* can aid in wound healing according to Nafiu *et al.* (2016):

Antibacterial and Antimicrobial Effects: Bitter leaf possesses antibacterial and antimicrobial properties, which can help prevent infection in wounds. It contains bioactive compounds such as alkaloids, tannins, and flavonoids that have been shown to inhibit the growth of various bacteria and microorganisms. By reducing the microbial load on the wound, bitter leaf promotes a cleaner and healthier environment for healing (Editorial staff-pharmchoices, 2023).

Anti-inflammatory Activity: Inflammation is a natural part of the wound healing process, but excessive or prolonged inflammation can hinder proper healing. *Vernonia amygdalina* contains anti-inflammatory compounds that help reduce inflammation at the wound site. By modulating the inflammatory response, bitter leaf promotes a more balanced healing process and can potentially accelerate wound closure (Editorial staff-pharmchoices, 2023).

Enhanced Collagen Production: Collagen is a crucial protein that provides structural support to the skin and plays a vital role in wound healing. Studies have shown that bitter leaf extract can stimulate the production of collagen, which helps in the formation

of new tissue and the remodeling of the wound bed. Increased collagen production can contribute to faster wound healing and improved wound strength.

Angiogenesis Promotion: Angiogenesis is essential for delivering oxygen and nutrients to the wound site. *Vernonia amygdalina* has been found to promote angiogenesis, thereby enhancing the blood supply to the wound area. Improved blood circulation facilitates the transport of essential healing factors and aids in the removal of waste products, supporting the healing process.

Antioxidant Activity: Bitter leaf is rich in antioxidants that help protect the wound from oxidative stress. Oxidative stress can impede wound healing by damaging cells and interfering with the normal repair processes. The antioxidants present in Vernonia amygdalina scavenge free radicals and reduce oxidative stress, creating a more favorable environment for wound healing.

Local preparation of *Vernonia amygdalina* Delile by Ghanaians

Ghanaians have various ways of preparing concoctions or herbal remedies using *Vernonia amygdalina* (bitter leaf) for its medicinal benefits. Here are a few common methods:

Bitter Leaf Tea: To make bitter leaf tea, Ghanaians typically use fresh or dried bitter leaves. They boil water in a pot and add a handful of fresh or dried bitter leaves to the boiling water. It is allowed to simmer for 5 to 10 minutes. The liquid is strained and allowed to cool slightly.

Another method employed by Ghanaians is that, sufficient amount of fresh bitter leaves are collected. The leaves are then washed thoroughly to remove any dirt or impurities. The leaves are ground into paste. The paste is then squeezed to get the juice using a cloth or sieve (Editorial staff-pharmchoices, 2023). The resulting juice is the bitter leaf extract, which can be consumed directly or used as an ingredient in other remedies.

2.2.2 *Euphorbia hirta* L.

Classification of *Euphorbia hirta* L.

Euphorbia hirta L. (Plate 3) belongs to kingdom *Plantae*, phylum *Tracheophyta*, class *Magnoliopsida*, order *Malpighiales,* family *Euphorbiaceae,* genus *Euphorbia* and specific epithet *hirta* (GBIF Secretariat, 2022).

Description of *Euphorbia hirta* L.

The thick, hairy stem of this erect or prostrate annual herb, which can reach a length of 60 cm (24 in), yields copious amounts of white latex. Simple, elliptical, hairy leaves with a delicately dentate edge have hairs on both the top and lower surfaces, but especially on the veins on the lower leaf surface. On the stem, leaves are arranged in opposite pairs. The unisexual blooms grow in axillary clusters at each node of the leaf. They often have a stalk and are without petals. The red seeds are small, rectangular, and four-sided, and the fruit is shaped like a capsule with three valves.

Annual medicinal weed *Euphorbia hirta* L. which is also known as the "asthma plant has many medical properties. Along with tropical and temperate regions of the world, India, Bangladesh, Africa, and Australia are also home to these therapeutic herbs. The various plant parts were found to have anti-microbial, anti-diabetic, anti-cancer, anti-tumor, anti-plasmodial, anti-fertility, wound-healing, anti-inflammatory, sedative, and diuretic activities, according to extensive literature investigations. This plant contains polyphenols, flavonoids, steroids, tannins, and alkaloids as its primary phytochemicals (Chandel, Das,… & Chauhan, 2023).

Euphorbia hirta is a herb which its aqueous extract determines analgesic, anti-inflammatory activities and inhibitory action on Platelet aggregation while ethanolic extract of the whole herb has a significant effect on wound healing (Maver, T., Kurecic,… & Maver, 2017).

2.2.3 *Caesalpinia sappan* L.

Description of *Caesalpinia sappan* L.

Caesalpinia sappan L., also known as sappanwood, is a tree species that belongs to the family *Fabaceae*. The tree is native to Southeast Asia and is widely distributed in countries such as Thailand, Indonesia, and Malaysia (Ganapathy *et al.*, 2021; Nava-Tapia *et al.*, 2022). *Caesalpinia sappan* has been valued for its medicinal properties for centuries, and its wood has been used to produce a red dye, which is used in traditional textiles and artwork (Dapson & Bain, 2015). The tree is also grown for its ornamental value, as its leaves and flowers are attractive and aesthetically pleasing.

Taxonomy and Classification

Caesalpinia sappan L. (Plate 4)was first described by Carl Linnaeus in 1753, in his famous work "Species Plantarum" (Alamgir, 2017). The tree is classified under the genus *Caesalpinia,* which is named after the Italian botanist Andrea Cesalpino (Isely, 2002). The tree has been valued for its medicinal properties for centuries, and its wood has been used to produce a red dye, which is used in traditional textiles and artwork.

Phytochemical Constituents of *Caesalpinia sappan* L.

Caesalpinia sappan L. contains a wide range of phytochemical compounds, including flavonoids, phenolics, lignins, and terpenoids. The major active constituents of the plant include brazilin and brazilein, which are red pigments found in the heartwood of the tree (Pawar, Landge & Surana, 2008). These compounds have been shown to possess a variety of biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties. In addition to brazilin and brazilein, other compounds found in *Caesalpinia sappan* include kaempferol, quercetin, and gallic acid, which also exhibit antioxidant and anti-inflammatory activities (Pawar, Landge & Surana, 2008).

Wound Healing Properties

The wound healing properties of *Caesalpinia sappan* L. have been investigated in several studies. In one study, a methanolic extract of the heartwood of *Caesalpinia sappan* L. was found to promote wound healing in rats (Nirmal, Rajput,… & Ahmad, 2015). The extract was administered orally to rats that had undergone excision wounds, and it was found to significantly reduce wound size and increase the rate of wound healing. Histological analysis of the wound tissue revealed that the extract accelerated the formation of granulation tissue and promoted collagen deposition, which are necessary steps in the wound healing process.

The wound healing effects of *Caesalpinia sappan* L. have also been investigated using in vitro models. In one study, the methanolic extract of *Caesalpinia sappan* L. was found to promote wound healing in mouse fibroblast cells (Kim & Bae, 2011). The extract was found to stimulate the proliferation and migration of fibroblast cells, which play a key role in the wound healing process. The extract was also found to increase the production of collagen, which is necessary for the formation of new tissue at the wound site.

Mechanisms of Action

The wound healing effects of *Caesalpinia sappan* L. are thought to be due to its antioxidant and anti-inflammatory properties. The plant's active constituents, including brazilin and brazilein, have been shown to possess antioxidant activity, which can help to protect cells from oxidative damage. Oxidative stress is known to play a role in the pathogenesis of chronic wounds, and antioxidants have been shown to promote wound healing by reducing oxidative stress (Kumar, Sharma & Gupta, 2014).

In addition to its antioxidant activity, *Caesalpinia sappan* L. also possesses anti-inflammatory properties. Inflammation is a key component of the wound healing

process, but chronic inflammation can delay the healing process and lead to the formation of chronic wounds. Anti-inflammatory agents can help to reduce inflammation and promote wound healing. Several studies have demonstrated the anti-inflammatory effects of *Caesalpinia sappan* L. In one study, a methanolic extract of *Caesalpinia sappan* L. was found to inhibit the production of pro-inflammatory cytokines in human monocytes (Jeong, Jin,… & Kim, 2008).

2.2.4 *Arctium lappa* L.

Arctium lappa L., commonly known as burdock, is a biennial plant species that belongs to the family Asteraceae. The plant is native to Europe and Asia and has been introduced to many other parts of the world, including North America (Hartzell & Wilkes-Barre, 2001).

Taxonomy

Arctium lappa L. (Plate 5)was first described by Carl Linnaeus in 1753 in his book Species Plantarum (Eisendrath, 1961). The plant belongs to the family Asteraceae, which is one of the largest families of flowering plants, consisting of over 25,000 species (Rolnik & Olas, 2021).

Morphology

Arctium lappa L. is a biennial plant that can grow up to 2 meters in height. The plant has large, heart-shaped leaves that are arranged in a rosette at the base of the plant during the first year of growth. The leaves can grow up to 50 cm in length and have a wavy margin. During the second year of growth, the plant produces a tall, erect, branched stem that bears numerous purple flowers. The flowers are arranged in small clusters and are surrounded by spiny bracts. The flowers are hermaphroditic, and the plant is pollinated by insects such as bees and butterflies. The fruit of the plant is a small, round, dry achene, which is surrounded by a spiny bract that forms a burr (Salama, 2016; Wikipedia, 2023).

Chemical composition of *Arctium lappa* L.

Arctium lappa L. contains a variety of chemical constituents that are believed to contribute to its wound healing properties. These constituents include phenolic acids, flavonoids, lignans, and polysaccharides.

Phenolic acids, including chlorogenic acid and caffeic acid, have been identified in *Arctium lappa* L. These compounds exhibit antioxidant and anti-inflammatory properties and have been shown to promote wound healing by reducing oxidative stress and inflammation (Da-Silva, Allemand,… & Werner, 2013) .

Flavonoids, such as quercetin, luteolin, and kaempferol, are also present in *Arctium lappa* L. (Alsamarrai, Al-Samarrai & Alsamarrai, 2020). These compounds can promote wound healing by enhancing angiogenesis and reducing oxidative stress and inflammation.

Lignans, including arctigenin and matairesinol, have been isolated from *Arctium lappa* L. These compounds exhibit antioxidant, anti-inflammatory, and antimicrobial properties and have been shown to promote wound healing by reducing inflammation and preventing infection (De-Souza, De-Oliveira,… & Bavia, 2022).

Polysaccharides, such as inulin and fructooligosaccharides, are also present in *Arctium lappa* L. These compounds are believed to promote wound healing by enhancing immune function and stimulating the growth of beneficial gut bacteria.

Effects of *Arctium lappa* L. on wound healing

Arctium lappa L. has been shown to exhibit a variety of effects on wound healing. These effects include accelerating wound closure, reducing inflammation, promoting angiogenesis, and preventing infection.

In a study conducted by Kim and Bae (2011), *Arctium lappa* extracts were applied topically to full-thickness wounds in rats. The results of the study showed that the *Arctium lappa* extracts significantly accelerated wound closure and increased the breaking strength of the wounds compared to the control group.

In one study, *Arctium lappa* L. was shown to exhibit anti-inflammatory effects that can promote wound healing. The study found that *Arctium lappa* L. extracts reduced the

levels of inflammatory markers, including tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6), in animal models of inflammation (Gao, Yang & Zuo, 2018).

In addition to its effects on wound healing, *Arctium lappa* L. has also been shown to exhibit antibacterial properties that can prevent infection.

Medicinal Properties

Arctium lappa L. has been used for centuries in traditional medicine for its numerous therapeutic properties. The plant contains a variety of bioactive compounds, including inulin, mucilage, tannins, and volatile oils (Smet, Keller,… & Chandler, 1993). The root of the plant is the most commonly used part in traditional medicine and is known for its diuretic, diaphoretic, and depurative properties (Miraldi & Mostaghimi, 2001). It is also believed to have anti-inflammatory and anti-cancer properties. *Arctium lappa* L. has been used to treat various ailments, including skin conditions, diabetes, and arthritis.

Skin Conditions

Arctium lappa L. has been used for centuries in traditional medicine to treat various skin conditions such as eczema, psoriasis, and acne. The plant has been found to have anti-inflammatory and antimicrobial properties, which make it effective in treating skin infections (Zhang, Wang,… & Jin, 2019). The root of the plant can be consumed in the form of tea or taken in capsule form. The leaves of the plant can also be used topically in the form of a poultice to treat skin conditions (Salama & Salama, 2016).

Diabetes

Arctium lappa L. has been used in traditional medicine to treat diabetes. The plant is believed to have hypoglycemic properties, which can help lower blood sugar levels. The root of the plant can be consumed in the form of tea or taken in capsule form. *Arctium lappa* L. can also be used in combination with other medicinal plants such as

Gymnema sylvestre, which has been found to enhance the hypoglycemic activity of *Arctium lappa* L. (Gupta, Mishra… & Tambuwala, 2022).

Arthritis

Arctium lappa L. has been found to have anti-inflammatory properties, which make it effective in treating arthritis (Zhang *et al.*, 2019). The plant can help reduce pain and swelling associated with arthritis. The root of the plant can be consumed in the form of tea or taken in capsule form.

Economic Value

Arctium lappa L. has both medicinal and economic value. The plant is used in traditional medicine in many parts of the world and is also used in the cosmetic industry. The root of the plant is used to produce a natural hair conditioner, which is believed to promote hair growth and prevent hair loss. *Arctium lappa* L. is also used in the food industry as a source of inulin, which is a type of dietary fiber that is used as a food additive and a sugar substitute (Bhanja, Sutar, & Mishra, 2022).

The plant is also used to produce a natural dye, which is used in textiles and art (Basak, Samanta,… & Pandit, 2018).

2.2.5 *Aloe vera* (L.) Burm. F.

Description of *Aloe vera* (L.) Burm. F.

Aloe vera (L.) Burm. F. (Plate 6) is a succulent plant species that belongs to the family *Asphodelaceae* (Joseph & Raj, 2010). The plant is commonly grown for its medicinal and agricultural values and is indigenous to the Arabian Peninsula, although it has been extensively cultivated in different parts of the world (Kumar, Yadav,… & Yadav, 2017). The plant is characterized by thick, fleshy leaves that contain a gel-like substance that is rich in bioactive compounds, including vitamins, minerals, and antioxidants. *Aloe vera* is renowned for its numerous therapeutic properties and has been used for centuries in traditional medicine to treat various ailments, including skin conditions, digestive disorders, and inflammation (Saleem, Naureen,… & Tasleem, 2022).

***Aloe vera* (L.) Burm. F. Gel Composition and Nutritional Value**

Aloe vera (L.) Burm. F. gel is a rich source of bioactive compounds that have been shown to have numerous therapeutic properties. The gel is composed of over 75 different constituents, including sugars, vitamins, enzymes, amino acids, and minerals whereas the main polysaccharides found in *Aloe vera* gel are glucomannans (Vithalkar, Kaiwartya & Patel, 2022). Glucomannans are long-chain sugars that have been shown to have immunomodulatory, anti-inflammatory, and wound-healing properties (Vithalkar *et al.*, 2022). The gel also contains numerous vitamins and minerals, including vitamins A, C, and E, and calcium, magnesium, and potassium.

Aloe vera (L.) Burm. F. gel has been shown to have a range of health benefits, including the ability to improve skin health, promote wound healing, and reduce inflammation and pain (Saleem *et al.*, 2022). The nutritional content and the therapeutic properties of

the gel make it an ideal ingredient in various skincare products and other personal care products.

Skin Health Benefits

Aloe vera (L.) Burm. F. gel is commonly used topically to improve skin health and treat various skin conditions, including acne, eczema, and psoriasis. The gel's anti-inflammatory and antibacterial properties can soothe and reduce skin inflammation, reducing redness and promoting healing. *Aloe vera* gel has also been shown to have moisturizing and emollient properties, which can help to hydrate and soften the skin (Saleem *et al.*, 2022).

One of the primary active ingredients in *Aloe vera* gel according to Sadgrove and Simmonds (2021) is acemannan, a polysaccharide that has immunomodulatory effects and can improve wound healing. Acemannan has been shown to enhance the activity of immune cells, which can speed up the healing process and reduce the risk of infection (Sadgrove & Simmonds, 2021). *Aloe vera* gel also contains other bioactive compounds, such as vitamins and antioxidants, which can protect the skin from damage caused by free radicals and UV radiation.

Digestive Health Benefits

Aloe vera (L.) Burm. F. gel has been used for centuries in traditional medicine to treat various digestive disorders, including constipation, diarrhea, and ulcers. The gel's laxative properties are attributed to the presence of anthraquinones, a group of compounds that stimulate bowel movements by increasing intestinal contractions (Riaz, Hussain,… & Anwar, 2021).

Studies have also shown that *Aloe vera* gel can help to reduce inflammation in the digestive tract, particularly in people with inflammatory bowel disease (IBD). *Aloe vera*

gel has been shown to have anti-inflammatory effects that can help to reduce the symptoms of IBD, such as abdominal pain and diarrhoea (Hassanshahi *et al.*, 2020). The gel's immunomodulatory effects can also boost the activity of immune cells in the gut, which can promote healing and reduce the risk of infection (Sadgrove & Simmonds, 2021).

Effects on Inflammation

Inflammation is a critical component of the wound healing process, as it plays a crucial role in the recruitment of immune cells to the site of injury. However, excessive inflammation can delay the healing process and can lead to the development of chronic wounds (Eming *et al*., 2007; Raziyeva *et al*., 2021). *Aloe vera* (L.) Burm. F. has been shown to exert anti-inflammatory effects, both in vitro and in vivo. The anti-inflammatory effects of *Aloe vera* have been attributed to its ability to inhibit the production of pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) (Bałan, Niemcewicz,… & Skopinski, 2014).

Effects on Angiogenesis

Angiogenesis is the process of new blood vessel formation, which is crucial for the delivery of nutrients and oxygen to the site of injury. *Aloe vera* has been shown to promote angiogenesis, both in vitro and in vivo. The angiogenic effects of aloe vera have been attributed to its ability to stimulate the production of growth factors, such as vascular endothelial growth factor (VEGF) (Majewska, & Gendaszewska-Darmach, 2011)

Effects on Collagen Synthesis

Collagen is a crucial component of the extracellular matrix (ECM) that provides structural support to tissues. *Aloe vera* has been shown to promote collagen synthesis. The collagen-promoting effects of *Aloe vera* have been attributed to its ability to

stimulate the production of collagen by fibroblasts, which are the primary cells responsible for ECM synthesis (Steele, 2022)

Aloe vera promotes collagen synthesis by stimulating the activity of fibroblasts, which are the primary cells responsible for ECM synthesis. *Aloe vera* has been shown to regulate the expression of collagen I and III, which are the main types of collagen found in the skin (Steele, 2022).

Mechanisms of Wound Healing in *Aloe vera* (L.) Burm. F.

The wound healing effects of *Aloe vera* (L.) Burm. F. have been attributed to its ability to modulate several cellular and molecular pathways involved in the wound healing process. The mechanisms by which *Aloe vera* promotes wound healing are complex and involve multiple pathways, such as inflammation, angiogenesis, and collagen synthesis.

Aloe vera has been used to treat various wounds, including burns, cuts, and abrasions. The therapeutic properties of *Aloe vera* (L.) Burm. F. have been attributed to its bioactive compounds, which include vitamins, minerals, and antioxidants (Sharrif-Moghaddasi & Res, 2011). Several studies have investigated the wound healing properties of aloe vera, both in vitro and in vivo.

2.2.6 *Curcuma longa* L.

Classification of *Curcuma longa* L.

Kingdom*: Plantae*, Subkingdom: *Tracheophyta,* Superdivision: S*permatophyta,* Division: *Angiospermophyta,* Class: *monocotyledonae,* Subclass: *Zingiberidae,* Order: *Zingiberales,* Family: *Zingiberaceae*, Genus: *Curcuma*, Specific epithet: *longa* (Chanda & Ramachandra, 2019).

Description of *Curcuma longa* L.

The tuberous rhizomes of turmeric (*Curcuma longa*), a perennial herbaceous plant of the ginger family (Zingiberaceae), have been used as a spice, a textile dye, and a medical stimulant since ancient times. Turmeric, which originates in southern India and Indonesia, is widely grown on both the continent and the Indian Ocean islands. It was used as a spice and a perfume in the past. The rhizome (Plate 8) is strongly stained orange-yellow in colour and has a pepper-like scent and a warm, slightly bitter taste. It is the substance that gives prepared mustard its colour and flavour. The powdered form of turmeric (Plate 7) component of curry powder, relishes, pickles, spicy butters for vegetables, fish and egg meals, poultry, rice, and hog dishes. Turmeric water is used as a cosmetic in some parts of Asia to give the skin a golden glow. Turmeric is commonly used as a tea or in pill form to treat a range of conditions, such as arthritis and gastrointestinal problems, and is thought to have anti-inflammatory qualities (Britannica, 2023). *Curcuma longa* L. possess anti-bacterial, anti-fungal, analgesic and anti-inflammatory activities. The presence of vitamin A as well as proteins stimulate formation of collagen fibres which increases fibroblastic activity. Fresh juice from turmeric is used to treat fresh wounds, bruises and also leech bites (Maver *et al.*, 2017). According to Chanda and Ramachandra (2019), Turmeric purifies the brain and heart, purifies the blood, and is used to cure a variety of conditions including leucoderma.

2.2.7 *Prosopis africana* (Guill., Perrott, & Rich.) Taub.

Classification of *Prosopis africana* (Guill., Perrott, & Rich.) Taub.

The tree belongs to kingdom: *Plantae,* phylum: *Angiospermophyta,* class:*Dicotyledonae* order: *Fabales* family: *Fabaceae* genus: *Prosopis* specific epithet: *africana*

Description of *Prosopis africana* (Guill., Perrott, & Rich.) Taub.

Prosopis africana (Guill., Perrott, & Rich.) Taub. (Plate 9) is a perennial leguminous tree found in the savanna regions of Senegal and Nigeria. It belongs to the subfamily Mimosaidae. The *Prosopis* family contains multiple species, but only *P. africana* is a native to that continent. The tree produces 10–20 cm long and 2-3 cm wide indehiscent dark brown pods. The seeds are loose and rattling when the pods are grown but are fleshy when they are immature. About 10 seeds are present in each pod (Nwokocha, 2021). Its common names include African mesquite, iron tree, gele (Malinke), traditional djembe wood or somb tree (iNaturalist, n. d.). The roots are used in making chewing sticks to prevent tooth decay, as well as the hard wood is employed in the production of furniture, wooden farm tools, charcoal and mortar and pestle (Obode, Adebayo & Chunyang, 2020). According to Obode *et al.* (2020), ethnobotanical surveys in Nigeria, Ghana and Mali indicates the usage of the leaves of *Prosopis africana* in the treatment of migraines, hypertension, dysentery as well as rheumatism. Rwang, Fabiyi,… & Mercy (2016) cited in Obode *et al.* (2020) confirmed the presence of saponins, terpenes, tannins, steroids, anthraquinones and cardiac glycosides in the leaves of *P. africana.*

Invasive *Prosopis* species are seriously threatening Australia's economy and ecosystem. Cattle cannot access watering holes or other obstructions because *Prosopis* bushes develop impenetrable thickets with their thorns and numerous low branches. They take up scarce water resources and engulf pastoral meadows. *Prosopis* species cause land

erosion due to the loss of grassland which provides habitat for local plants and animals. Wild animals like pigs and cats can find shelter in prosopis thickets (Wikipedia, 2023).

Ezike, Akah, & Iloani, (2010) worked on *Prosopis africana* to determine its efficiency in wound healing. The methanol extract of the stem bark of the *P. africana* on bleeding and coagulation time, excision and dead space wounds were studied in albino rats. It was concluded that the extract significantly reduced bleeding and coagulatoin time in rats. Epithelialization period of wound excisions was reduced as well as growth of some bacteria like *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi* etc.

Phytochemicals present in *Prosopis africana* (Guill., Perrott, & Rich.) Taub.

Table 1 summarizes the phytochemical constituents present in the ethanolic extract of *Prosopis africana* as indicated by Obode *et al.* (2020).

Table 1 : List of phytochemicals present in *Prosopis africana* (Guill., Perrott, & Rich.) Taub

Phytochemicals	Results
Tannins	++
Saponins	++
Flavonoids	++
Alkaloids	++
Anthocyanin	++
Steroids	++
Terpenoids	++
Quinones	++
Glycosides	−
Cardiac glycosides	−
Triterpenoids	−
Phenols	−
iBetacyanin	−
Coumarins	−
Acids	−

2.2.8 *Allium sativum* L.

Classification of *Allium sativum* L.

Kingdom: Plantae, phylum: *Angiospermophyta*, class*: monocotyledonae*: order: *Asparagales*, family: *Amaryllidaceae*, genus: *Allium*, specific epithet: *sativum.*

Description *Allium sativum* L.

Allium sativum L. (Plate 10) is an herbaceous plant whose bulbs are frequently used to flavour various foods because of its distinctive flavour and aroma. It is widely consumed around the world for food, and it is also used medicinally as a standardised extract, a crude medication, and a food supplement. It is used extensively over worldwide and is well-known in popular medicine for its medicinal properties as a dietary supplement, standardised extracts, and as a crude medication. To facilitate storage, the bulbs are collected in the late spring and early summer and then dried in the shade at 40 degrees Celsius (Froldi, 2023).

Garlic is a perennial plant with flowers that emerges from a bulb (Plate 11). It grows about 1 m tall flowering stem which is upright. The leaf blade is firm, flat, linear, and ranges in width from 1.25 to 2.5 cm. Usually composed of 10 to 20 cloves, the bulb has an intense smell. Cloves at the centre are symmetrical, but cloves on the outside can be asymmetrical. Layers of outer sheath surround each clove, which is protected by a leaf sheath on all sides. Bees, butterflies, moths, and other insects pollinate its hermaphroditic flowers (Wikipedia, 2023).

Other species of garlic include *Allium tuncelianum*, *Allium macrochaetum*, and *Allium truncatum* (Zohary & Hopf, 2000).

According to Wikipedia (2023), there are two *A. sativum* subspecies. There are hundreds of varieties or cultivars, divided into ten main groupings.

A. sativum var. sativum, or softneck garlic comprises artichoke garlic, silverskin garlic, and creole garlic.

A. sativum var. ophioscorodon or hardneck garlic comprises of porcelain garlics, rocambole garlic, and purple stripe garlics.

Phytochemicals present in *Allium sativum* L.

Allicin, ajoene, diallyl polysulfides, vinyldithiins, and S-allylcysteine are sulfur-containing compounds that come from raw or crushed garlic. When cooked, however, garlic also produces enzymes, saponins, flavonoids, and Maillard reaction products that are not sulfur derivatives (Wikipedia, 2023).

According to research by Alhashim and Lombardo (2020) on the effect of topical garlic on surgical wound healing and scarring compared with Vaseline by analysis of visual analog scales and digital photograph analysis, seventeen patients that were given two skin excisions were used. 30% garlic ointment was applied to one surgical wound and Vaseline as a control remedy was used on the other surgical wound twice daily. After the operation, they were checked on at second and fourth weeks. In addition to taking digital pictures of the locations, the patient and the doctor both completed wound visual analogue scales.

It was observed that, the garlic site healed better in 59% and 65% of the wounds respectively, on the second week. On the fourth week, the patients and the onsite physician reported garlic site healed better in 76% and 88% of wounds, respectively.

The research concluded that, surgical wounds treated with 30% garlic ointment had more cosmetically pleasing scars as compared to locations treated with Vaseline (Alhashim & Lombardo, 2020).

2.3 A Review of the Two Herbs Used for the Experiment

2.3.1 *Chromolaena odorata* L.

Description of *Chromolaena odorata* L.

Eupatorium odoratum or *Chromolaena odorata* (Plate 12) is an herb originated from Central and South America and has spread throughout tropical climates. It was initially introduced to Southeast Asia in the 1920s and Africa in the 1940s as a plantation cover crop, and it has since spread around the world. It is known in English as devil weed or Siam weed, and in Akan as "Acheampong". *Chromolaena odorata* L. lives for at least ten years. It is a scrambling perennial shrub that reaches a height of 2-3 metres and has straight, brittle stems that branches easily. The leaves are 6-12 cm long and 3-7 cm wide, with three veins that run through it. Along the stems, the leaves grow in opposing pairs and several colors of the leaves have been identified. The colour of seeds is brown to black and is 4-5mm long. The roots are narrow and fibrous which are 0.3 km deep. *C. odorata* peculiar features include the colour of the flowers, shape of the leaves and scent of the crushed leaves (Sirinthipaporn & Jiraungkoorskul, 2017).

Classification of *Chromolaena odorata* L.

C. odorata L. is in Kingdom Plantae, Subkingdom Viridiplantae, Infrakingdom Streptophyta, Superdivision Embryophyta, Division Tracheophyta, Subdivision Spermatophyta, Class Magnoliopsida, Superorder Asteranae, Order Asterales, Family Asteraceae, Genus *Chromolaena,* Specific epithet *odorata* (Omokhua, McGaw,… & Van-Staden, 2016).

Health benefits of *Chromolaena odorata* L.

According to Ikuenobe and Analiefo (2003) cited in Tiamiyu and Okunlade (2020), *Chromolaena odorata* L. is a perfect fallow plant as it met the expected properties of

species for fallow plant which include easy establishment, large biomass, fast rate of decomposition and weed suppression.

Research done in Nigeria by Ikuenobe and Analiefo showed that infestation of weeds was lower in plots which have been cultivated by *C. odorata* than plots that are modified by natural bush fallow. This is to say *C. odorata* is a very good weed suppressor (Tiamiyu & Okunlade, 2020).

Research done by Kanmegne *et al.* (1999) in central-southern Cameroon indicated that, *C. odorata* significantly improved the soil quality by exchanging potassium with a sandy developed on granites and a sandy – clayey soil developed on gneiss.

Approximate constituent analysis of *C. odorata* by Tiamiyu and Okunlade (2020) are; 9.26% moisture, 15.28% fiber, 3.56% fat, 18.86% protein and 41.28% Carbohydrate. It was concluded that, the possession of these secondary metabolites could be ascribed to its medicinal and nutritional usefulness.

In Hill (1952), listed some important phytochemicals of plants such as alkaloids, tannins, flavonoids and phenolic. It is believed that medicinal values of every plant are contributed by its phytochemical components.

Chromolaena odorata L. is also traditionally used for the treatment of various ailments in humans and animals. (Vijayaraghavan & Ashokkumar, 2017).

The herb possesses insecticidal properties and is used as a green manure. It is also used for the preservation of dead bodies (Ukwueze & Shorinwa, 2013). The fresh leaves of *C. odorata* or the decoction has been used by practitioners of traditional medicine for the treatment of human burns, soft tissue wounds, ulcerated wounds, burn wounds, post – natal wounds and also for the treatment of leech bites, indigestion and skin infections (Panyaphu, Van-On,… & Nathakarnkitkul, 2011).

Tiamiyu and Okunlade (2020) reported different scientific findings and health benefits from other authorities on *Chromolaena odorata*. The investigation done by other researchers, form and part of the *C. odorata* used for the trial and the authors' submission are summarized in Table 2.

Table 2: Some health benefits of *Chromolaena odorata*

Objective of investigation	Form and part of the plant used for the trial	Author's submission/ conclusion
Antioxidant, antibacterial, wound healing, haemostatic activities	Aqueous and ethanolic leaf extracts	Prove effective as antioxidant, antibacterial
Haemostatic activity	Ethanolic leaf extracts	Possess haemostatic activity
Antibacterial activity	Ethanolic leaf extracts	Effective antibacterial agent
Antioxidant	Ethanolic leaf extracts	Natural antioxidant comparableto synthetic antioxidant
Antimicrobial	Water and ethanol extracts of leaf	Antimicrobial effect on *Escherichea coli* and *Staphyloccocus aureus*
Antifungal effects	Water and ethanol extracts of leaf	Potential antifungal effect on *Candida albicans*
Larvicidal activity	Methanolic leaf extract	Potential source of herbal larvicide for vector control
Antifungal effects	Water and ethanol extracts of leaf	Potential antifungal effect on *Candida albicans*
Larvicidal effect	Methanolic leaf extract	Herbal herbicide for vector control
Anti – cholesterolemic effect	Aqueous extract of leaf	Protection against development of atherosclerosis and coronary heart diseases.
Immunoptentiating activities	Crude extract of leaf	Immunopotentiating activities on the innate immunity.
Molluscicidal activities	Aqueous and ethanolic leaf extracts	Potential molluscicide
Anti – insecticidal property	Crude aqueous leaf extract	Herbal insecticide

Negative effects of *Chromolaena odorata*

On the other hand, *Chromolaena odorata* is an aggressive herb species which possess a serious threat to agriculture and the environment at large. However, the weed spreads rapidly due to the extensive seed production which is estimated to be 93000 – 160 000 seeds/plants (Tiamiyu & Okunlade, 2020).

Matthew and Brand (2004) considered *C. odorata* a problem in commercial tree plantations as it hampers the growth of young pine and eucalypt trees and easy catches fire when it dries which leads to the burning of the whole plantation.

People allergic to Siam weed may experience skin problems and mild asthma due to the strong smell of the herb.

Siam weed contains high levels of nitrate. In some parts of the tropical countries, livestock that are fed on fodder contaminated with the weed die or experience abortions (Biosecurity Act, 2020).

A research done by Ayodeji and Olorunsola (2010) indicates that Siam weed harvested within the vicinity of the highways in Nigeria are not suitable for therapeutic purposes as a result of high amount of metals in them. According to the research, levels of five metals namely; cadmium, mercury, manganese, nickel and lead were determined in the herb by applying acid digestion and an atomic absorption spectrophotometer. It was found out that cadmium concentration was higher than the maximum permissible concentrations (MPCs) of metals safe for human health.

Prevention and control of *Chromolaena odorata* (Siam weed)

Chromolaena odorata is a category three restricted invasive plant under the Biosecurity Act 2014. It must not be sold, gifted or released into the environment.

Queensland Government Biosecurity (2014) suggested methods to control this herb so that it does not pose threat to the community. The methods include:

- Livestock should be restricted from contacting Siam weed by fencing them.
- Underparts of vehicles or machines should be cleaned.
- Livestock that have been infected with Siam weed should be quarantined for at least week.
- Mechanical control is advised for smaller infestations. The basal ball of the weed is removed from the soil by hand picking. Slashing the weed is not effective since the weed can re-sprout from the basal ball.
- The use of correct rates of herbicide on the weed before flowing.
- *Cecidochares connexa* (stem galling fly) causes galls along the stems of the weed which in turn deprive the weed from nutrients leading into withering and death of the weed.

2.3.2 *Sida acuta* Burm. F.

Classification of *Sida acuta* Burm. F.

This perennial shrub belongs to Kingdom: Plantae, Division: Angiospermophyta, Class: Dicotyledonae, Order: Malvales, Family: Malvaceae, Genus: *Sida*, Specific epithet: *acuta* (Senthilkumar, Bhuvaneshwari,… & Sathiyavimal, 2018).

Description of *Sida acuta* Burm. F.

Sida acuta Burm. F. (Plate 13) is a perennial shrub which originated from central America. It has successfully invaded the tropics worldwide since it can tolerate wide range of growing conditions. It infests various crops and habitats and serves a contaminant in pastures and rangelands. It is usually 30 – 150 cm in height but under favourable conditions it can grow to 3m in Northern Australia (Lonsdale, Farrell & Wilson, 1995). It has fibrous to woody stem with a tough bark. It has deep and tough

taproot which makes it difficult to uproot. The leaves are alternate, acute and tapers towards both ends. The leaves are serrated. It bears yellow flowers which are solitary with a diameter of 1 – 2 cm on a short stalk. There are five petals joined at the base with a shallow notch at the apex. It has hard fruit enclosed in a green to brown capsule. The capsule has 5 – 8 segments each containing one seed and has a pair of sharp awns which sticks to animal fur or clothing for dispersal. The seeds are small, reddish-brown to black, wedge-shaped, deeply indented on both sides, rounded on the back and a length about 1.5 mm. The weed thrives well on most soil types with the exception of clayey soils that are flooded seasonally and soils with limestone (Rojas-Sandoval, Acevedo-Rodriguez & Clements, 2022).

Flanagan, Hills and Wilson (1999) posits that the taproot of the weed can withstand drought, mowing and shallow tillage. Again, it is a weed that affects pastures, plantation farms, cereals, vegetables, planted forests, lawns etc.

The weed infests tea in Taiwan and Sri Lanka, in Ghana it infests groundnut, cassava and maize while in Nigeria, it infests cassava, cowpeas, sweet potatoes and maize. Foliage-eating chrysomelid beetle, *Calligrapha pantherine,* has been introduced into many areas as a biological control agent to control *Sida sp.* (Rojas-Sandoval *et al.*, 2022).

Health benefits of *Sida acuta* Burm. F.

Senthilkumar *et al.* (2018) highlighted some usefullness of wire weed. The whole plant contains anthelmintic, antiemetic, diuretic, aphrodisiac, stomachic and antipyretic properties. The plant was observed inhibiting the activities of *Bacillus subtilis* and *Escherichia coli*. The aqueous extraction of the leaves also showed moderate anti-bacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

The secondary metabolites present in the plant is useful in treating diseases such as headache, cold, fever, skin diseases, urinary diseases, ulcer, snake bite, facial paralysis. A research work done in Nigeria by treating methanolic extracts of the leaves of *Sida acuta* Burm. F. of different dosages (3000mg, 4000mg and 5000 mg mixed in 1 Kg paraffin oil each) on the wounds excision on guinea pigs confirmed that methanolic extracts of the leaves of *Sida acuta* is efficient in healing wounds (Oduwegwu, Mgbenka,… & Eyo, 2017).

Toxicity of *Sida acuta* Burm. F.

Research by Anjos, Peixoto,… and Armien, (2015) indicated that nine out of fifty – five sambar deer were poisoned with swainsonine as a result of ingesting *Sida sp.* Symptoms of the poisoning included abnormal consciousness, posterior paresis, musculoskeletal weakness. In histological studies of the poisoned animals, there was vacuolation of neurons and epithelial cells of the pancreatic acines and thyroid follicules. There was axonal degeneration, necrosis and loss of neurons and ultimately led to the death of some of the animals.

Prevention and control of *Sida acuta* Burm. F.

According to Rojas-Sandoval *et al.* (2022), the various methods that can be employed to control the weed include:

- **Cultural method**

Some pasture grasses are able to compete with *Sida acuta* Burm. F. and these include *Urochloa mosambicensis* or *Calopogonium mucunoides.*

Strict monitoring of overgrazing should be employed since overgrazing reintroduce the weed into the area.

Larger farmlands can be controlled by repeated cultivation until there is not enough seeds of the weed left in the soil. Mostly, this method is costly and impractical.

- **Biological control**

According to Rojas-Sandoval *et al.* (2022), a survey in Mexico in April 1984 to November 1986 identified natural enemies of *S. acuta.* About thirty one arthropods were known to feed on *S. acuta* among them are eight arthropods considered to have potential control on *S. acuta.*

Two species of chrysomelid beetle *Calligrapha pantherina* have been released in Autralia. Landowners have reported considerable return of their lands to native pasture. Other phytophagous species that control *S. acuta* include: *Acanthoscelides brevipes, Brachycoryna pumila, Calligrapha felina, Asphondylia sp., Stegasta albocapitella, Pyrgus adepta* and *Bucculatrix species.*

- **Mechanical control**

Tillage is the best method to mechanically control the weed. The weed has a strong tap root which is buried deep in the soil. Slashing the weed does not prevent it from growing again. Tillage ensures the removal of the root in the soil.

- **Chemical control**

S. acuta grown into maturity are hard to control with chemicals such as herbicides. Mature weeds can be slashed and two weeks later sprayed with amine 2,4 – D.

Other herbicides such as glyphosate and dicamba are also efficacious in controlling the weed.

2.4 Phytochemical Screening of *C. odorata* L. and *S. acuta* Burm. F. Leaves

Primary metabolites play a direct role in the growth, development, and reproduction of plants. They are known as vital or important compounds. Cell components such as carbohydrates, polysaccharides, amino acids, sugars, proteins, and lipids as well as fermentation byproducts such as ethanol, acetic acid, citric acid, and lactic acid are

examples of primary metabolites that are primarily utilized by organisms during their growth and developmental phases (Stephane, Jules,… & Bruno, 2021).

Secondary metabolites typically serve a purpose, but they are not crucial for the plant because they are not actively involved in metabolic processes. Examples include phenolic, steroids, lignans, etc. They only appear in a particular organism or sets of organisms and demonstrate the species' uniqueness (Stephane *et al.*, 2021).

They are not always made, and often neither their purpose nor the benefits they confer on the organism are fully understood. It is logical to assume that all do not play some vital role for the well-being of the producer (Cooper & Nicola, 2014). Some are definitely produced for easily understood reasons, e.g., as toxic material providing defense against predators, as volatile attractants towards the same or other species. After the growing period, secondary metabolites are produced, and they help plants survive and overcome environmental obstacles. Terpenoids, alkaloids, nitrogen-containing compounds, organosulfur compounds, and phenolic compounds are different categories of bioactive substances (Omeroglu, Acoglu,… & Copur, 2019).

According to reports, bioactive substances have a variety of bioactivities, including antioxidant, anticancer, antimalarial antiulcer, antimicrobial, anti-inflammatory activity (Shang, 2018).

2.4.1 Phytochemicals present in Chromolaena odorata

The phytochemical components of dried leaf powder of *Chromolaena odorata* was screened by Tiamiyu and Okunlade (2020) and the results are summarized in the Table 3.

Table 3: Phytochemical analysis of dried leaf powder of *Chromolaena odorata*

Bioactive compounds	Relative abundance
Alkaloids	+ + +
Tannin	+ ++
Phlobatannin	+ +
Saponin	+ + +
Flavonoids	+
Anthraquinones	-
Steroids	+
Terpenes	+
Cardenolides	-
Phenol	+ + +
Chalcones	-
Cardiac glycoside	+ +

Key: + + + = appreciable amount present, ++ = moderate amount present, + = trace amount present, - = completely absent.

Similar research done by Anyasor, Aina,…& Aniyikaye (2011) shows that the method of extraction of the *C. odorata* leaf has a significant effect on the phytochemical components of the herb.

In the Table 4, two methods of extraction were employed and, in each case, the bioactive compounds were shown with '+' or '- 'sign to indicate presence or absence of a particular bioactive compound.

Table 4: Phytochemical analysis of aqueous and ethanolic extracts of *Chromolaena odorata* leaf

Bioactive Compounds	Relative Abundance	
	Aqueous	Ethanolic
Terpenoids	-	+ +
Tannin	+	+ +
Saponin	+ +	+
Phlobatannin	+ +	-
Cardiac glycoside	-	+ +
Flavonoids	-	-
Cardenolides	-	-
Anthraquinones	-	+
Phenol	+ +	+
Alkaloid	+ +	-
Volatile oil	-	-

Key: + + = abundant, + = trace, - = absent. Source: Anyasor *et al.*, (2011).

2.4.2 Phytochemicals present in aqueous and ethanolic extraction of *Sida acuta*

In the preliminary photochemical screening of the aqueous extraction of the leaves, it was found that, alkaloids, steroids, flavonoids, phenols, terpenoids and cardiac glycosides were present. On the other hand, tannins, saponins, anthroquinones and phlobatannins were absent (Senthilkumar *et al.*, 2018).

In the ethanolic extraction of 80% ethanol concentration, *Sida acuta* is found to contain alkaloids, flavonoids, saponins, steroids, tannins, phlobatannins, terpenoids and cardiac glycosides (Adeniyi, Orjiekwe,… & Arimah, 2010).

2.5 Effect of the Phytochemicals in *C. odorata* and *S. acuta* Leaves on Wounds

1. **Flavonoids (Fig. 7):** these phytochemicals are used in traditional medicine as anti – inflammatory, pain relieving, promoting healing and anti – allergens among others. they also react with free radicals, can chelate metals, increases enzymatic reactions and have an action on adenosine receptors and influences

biological membranes. The main molecular structure of these chemicals is two aromatic rings connected by a three carbon bridges. All flavonoid compounds contain phenol groups which in general stimulates an antioxidant activity (Maver *et al.*, 2017). Flavonoids are mainly water-soluble compounds but can be extracted by 70% ethanol. They are mostly present in plants as mixtures and it is scarcely found in isolation. Flavonoids are present in all vascular plants (Harborne, 1998).

2. **Alkaloids (Fig. 8):** they are heterocyclic compounds that has nitrogen atom in at least one of the heterocyclic compounds. They have bitter tastes; this accounts for why *C. odorata* leaf tastes bitter. Alkaloids have potential effects on wound healing. They stimulate the bone marrow leucocytes which modulate the inflammation phase of wound healing (Maver *et al.*, 2017). Harborne (1998) reports that alkaloids are either absent or infrequently occur in lower plants.

3. **Monoterpenes (Fig. 9):** these are chemicals compounds with a core of ten carbons. Many of these compounds exist in the form of essential oils because of their low molecular weight. Monoterpenes have wound – healing ability, antibiotic activity and anti-inflammatory activity by limiting leukocyte migration etc. (Barreto, Albuquerque-Junior & Quintans-Junior, 2014).

4. **Saponins (Fig. 10):** they are phytochemicals that can accelerate numerous biological activities involving hemolytic, anti-bacterial, anti-viral and anti-oxidative functions. They do not only promote re-epithelization of wound but also efficiently prevents inflammatory reactions. They also promote matrix synthesis throughout the wound healing process. On the basis of this, saponins are effective in healing incisional skin wounds (Kim *et al.*, 2011).

5. **Steroids (Fig. 11):** Corticosteroids augments risk of wound infection and delay healing of wounds. Steroids do that by interfering with inflammation, collagen synthesis and degradation, angiogenesis, wound contraction and re – epithelialization (Anstead, 1998). In effect, steroids are used on doctor's advice and they are used for skin conditions but areas on the skin that have cuts, scrapes and burns should be exempted.

6. **Tannins (Fig. 12):** According to Harborne (1998), tannins mostly occur in vascular plants and are usually associated with proteins that helps in converting hide into leather in leather industry. Tannins are detastable hence animals tend to avoid eating tanning – containing plants.

   Advantages of these phytochemicals are: relief of pain, inhibition of secondary infection, prevention of loss of plasma and enhancement of epithelialization. Tannins also improve wound healing and reduction in scar tissue formation by preventing the formation and elimination of reactive oxygen substances (Chokotho & Hasselt, 2005).

7. **Anthraquinone (Fig. 14):** This compound is a phenolic compound which is a special type of quinone in plants. Other groups of quinones includes benzoquinones, naphthaquinones and isoprenoid (Harborne, 1998).

   Anthraquinones have been found to exhibit anti-inflammatory properties by suppressing the release of inflammatory mediators and reducing inflammation at the wound site (Kshirsagar, Panchal,… & Shaikh, 2014). This can help in the control of excessive inflammation during wound healing and again, it has been found to increase angiogenesis and collagen synthesis (Zhao, Wang,… & Zhang, 2020).

8. Gum and mucilage:

Gum is a viscous substance that is derived from the sap or resin of certain plant species. In wound healing, gum acts as a protective barrier to prevent the wound from becoming infected. Gum provides a moist environment for the wound to heal, which helps to promote cell growth and tissue regeneration. Gum also contains natural antimicrobial properties that can help to prevent infection (Gutierrez-Reyes, Caldera-Villalobos,… & Herrera-Guerrero, 2023).

Gum acts as a protective barrier to prevent the wound from becoming infected. It provides a moist environment for the wound to heal, which helps to promote cell growth and tissue regeneration. Gum also contains natural antimicrobial properties that can help to prevent infection. Mucilage (Fig. 15), on the other hand, promotes wound healing by its wound-healing, moisturizing, soothing, and anti-inflammatory effects. Mucilage is also known to promote granulation tissue formation, which is an essential step in the healing process (Valizadeh, Hemmati,… & Bahadoram, 2015).

Glycosides (Fig. 16):

Glycosides are a type of organic compound that consist of a sugar molecule (glycone) attached to a non-sugar moiety (aglycone or genin) through a glycosidic bond (Nandi, Ghosh,…& Saha, 2021). The sugar molecule in a glycoside is typically a monosaccharide, such as glucose, fructose, or galactose, while the non-sugar component can vary and may include phenols, flavonoids, alkaloids, or terpenoids (Alamgir, 2018).

Glycosides are abundant in nature and can be found in various plants, fungi, and microorganisms (Rafiei, Velez & Tzelepis, 2021). They serve important biological functions, such as storage of carbohydrates, defense against

pathogens and herbivores, and attraction of pollinators. In addition, many glycosides have medicinal properties and are used in traditional medicine and pharmaceutical applications (Sayed, 1980).

2.6 Herbal Combination

Herbal combinations refer to the use of multiple herbs together in a single formula or treatment regimen to achieve a therapeutic effect. The use of herbal combinations is a common practice in traditional medicine systems, such as Ayurveda, Chinese Medicine, and Unani Medicine (Jangale, 2022). The combination of herbs has been shown to enhance the therapeutic efficacy of individual herbs by working synergistically to provide a broad range of therapeutic actions.

According to Okaiyeto and Oguntibeju (2021), the concentration of phytochemicals differ from one plant to another. Again the geographical distribution and abiotic factors affect the distribution of these phytochemicals in the same plant species. In order to increase the efficacy of herbal medicine, it is necessary to combine different plants nonetheless, herbs used together with other herbs for therapeutic purposes will have complex effect.

Herbal combinations have been studied for their therapeutic efficacy in several health conditions, such as diabetes, cardiovascular diseases, and cancer etc. For instance:

- ***Diabetes mellitus***

One such combination is the mixture of *Coccinia indica* and *Cassia auriculata*. A study conducted on diabetic rats showed that this herbal combination was effective in reducing blood glucose levels (Kumar, Arya,…& Gupta, 2010). Another study conducted on diabetic patients showed that a combination of *Gymnema sylvestre*, *Momordica charantia*, and *Trigonella foenum-graecum* reduced blood glucose levels (Mohan *et al.*, 2011).

- **Cardiovascular Diseases**

Herbal combinations have also been studied for their cardio-protective effects. A combination of *Allium sativum* (garlic), *Crataegus oxyacantha* (hawthorn), and *Vaccinium myrtillus* (bilberry) has been shown to improve lipid profiles and reduce blood pressure in patients with metabolic syndrome (Helmy, Mohamed & Omar, 2020). Another study conducted on patients with coronary artery disease showed that herbal combination of *Terminalia arjuna*, *Withania somnifera*, and *Commiphora mukul* improved cardiac function and reduced inflammation (Kumar *et al.*, 2014).

- **Cancer**

Herbal combinations have also been studied for their anticancer effects. A combination of *Curcuma longa* (turmeric), *Emblica officinalis* (Indian gooseberry), and *Tinospora cordifolia* has been shown to have potent anticancer properties (Sharma, Parmar,… & Sharma, 2013). Another study conducted on breast cancer cells showed that herbal combination of *Withania somnifera* and *Withaferin* had a cytotoxic effect on cancer cells (Khan, Malik,… & Singh, 2011).

A review done by Che, Wang…, & Lam (2013) identified five basic modes of herb - herb interactions. These include:

1. **Reinforcement:** this type of interaction occurs when the herbs that are used for the combination possess similar medicinal properties. Some examples are the rhizomes of *Corydalis yanhusuo* and *Curcuma phaeocaulis* which were used by the Chinese to improve blood circulation and relieving pain again *Lonicera japonica* flower and *Forsythia suspense* fruit for the treatment of influenza.

   Reinforcement of herbs can either produce additive effect which means the combined effect of two herbs equals the sum of the effect of the individual herb

or synergistic effect which means the combined effect of the herbs will exceed the sum of the effects of the individual herbs.

2. **Potentiation:** this occurs when one of the herbs serves as the principal herb and the other herb serves as an auxiliary herb. The auxiliary herb strengthens the effect of the principal herb. Example ginseng and aconite (*Aconitum carmicahelii*) where ginseng is the principal herb and aconite the auxiliary herb for restoring vitality and resuscitation during collapse and shock.

3. **Restraint and Detoxification:** Some of the herbs have varying undesirable effects when taking. These herbs are termed as toxic herbs. Example of a toxic herb used for healing is *Tripterygium wilfordii.* The toxicity of these herbs can be restrained or become more tolerable when used in combination with other non-toxic herbs. Example of this interaction is the use of *Pinellia ternata* and *Zingiber officinale.* The later reduces the toxicity of the former thereby making the former more tolerable for consumption.

4. **Counteraction:** This is an antagonistic interaction where by the effect of one herb is reduced in the presence of another herb. Counteraction is a serious problem in herbal medicine and herbs should not be used together anyhow since the combined effect of the herbs maybe less than the effect of the individual herbs unless the herbs in question have been subjected to experiment to know their combined effect.

5. **Incompatibility**: In this type of interaction, the herbs are mutually incompatible therefore their combined effect is toxic. Example is the root of *Veratrum nigrum* and *Panax ginseng.*

The researcher's interest in investigating the combined effect of *C. odorata* and *S. acuta* in healing wound was elated since there is not enough work done on herb-herb interaction (Che *et al.*, 2013).

Though it is difficult to investigate the therapeutic influence of such a combination but this kind of combination offer promising treatments to hypertension, cancer, wound healing etc. with lower adverse effects (Wang, Du & Zhou, 2021).

2.7 Common Solvents Used for Extracting Medicinal Plants and their Properties

Abdullahi and his colleague Haque outlined some common solvents for medicinal plants extraction. These solvents are called menstruum, these include polar solvents such as water, methanol, ethanol as well as non-polar solvents such as hexane and dichoromethane which are used to extract polar and non-polar compounds respectively. The choice of the menstruum is dependent on factors such as the type of the plant, the part of the herb that will be used for the extraction, nature or behaviour of the active ingredient present in the herbs and finally the availability of the menstruum.

Properties of these solvents are illustrated below:

1. **Water:**
    - It contains two hydrogen atoms and one oxygen atom. It is therefore polar which is appropriate for extracting polar compounds.
    - It has the ability to dissolve wide range of compounds.
    - It is non-flammable, non-poisonous and inexpensive to obtain.
    - On the other hand, it promotes growth of fungi and bacteria.
    - It may induce hydrolysis of compounds which will require enough heat to concentrate the extract.

2. Chloroform:

- It is non-polar solvent which is efficient for the extraction of terpenoids, flavonoids, fats, oils etc.
- It is non-polar and also dissolves in alcohols.
- It posesses sedative properties and also carcinogenic agent.

3. Ether:

- It is non-polar and suitable for extracting compounds such as terpenoids, coumarins, alkaloids and fatty acids.
- It has low boiling point and also miscible in water.
- It is stable and unreactive to acids, bases and metals.
- It is highly volatile hence catches fire easily.

4. Alcohol:

- It is polar hence miscible with water.
- It is self-preservative when it has a concentration above 20%.
- It is non-poisonous in low concentrations.
- Small amount of heat is needed to concentrate the extract.
- It is highly volatile hence readily catches fire.
- It does not dissolve wax, gums and fats (Abdullahi & Haque, 2020).

2.8 Common Methods of Extraction of Medicinal Plants

The choice of the method of extraction is important since it has significant effect on the chemical components of the plant extract.

Some common methods of extraction of medicinal plants reviewed by (Azwanida, 2015) include:

1. Maceration:

In order to obtain plant extracts, this procedure involves soaking the plant materials in a powdered form in a solvent for a period of two to three days at room temperature while stirring frequently. To prevent solvent evaporation at ambient pressure, an extractor is sealed. In order to liberate the soluble phytoconstituents, the method aims to soften and break down the plant's cell walls. After a predetermined period of time, the mixture is then squeezed, strained, or decanted. The simplest and most popular method is maceration. The extraction process in this stationary method relies on the labor-intensive molecular diffusion principle. Maceration makes sure that the concentrated solution that has accumulated around the surface of the particles is dispersed and brings fresh solvent to the surface of particles for further extraction.

2. Digestion:

This is another type of maceration. The extraction stage of the maceration is heated gently. Menstruum is used more effectively because the active components of plant material are not affected by temperature. Despite being limited to 50°C, temperatures between 35 and 40°C are the most common. The plant portion that has to be extracted is put in a container with the liquid that has been pre-heated to the appropriate temperatures, and is kept there for a duration that can last anywhere from 30 minutes to 24 hours while being periodically shaken. The herbal material or plant pieces that include insoluble chemicals or polyphenolic compounds are treated using this method.

3. Infusion:

A simple chemical procedure known as infusion is used to extract plant material from volatile plants that rapidly dissolve or release their active components in organic solvents. Similar to maceration, infusion and decoction entails soaking the plant material in hot or cold water and letting it steep in the liquid. However, the infusion

maceration time is shorter. A rotary evaporator can then be used to separate and concentrate the liquid while it is under vacuum. Infusion is used in the preparation of tea and is recommended for consumption in conditions such as psychophysical asthenia, diarrhoea, bronchitis, and asthma, etc.

4. Lixiviation (elution):

The word "lixiviation" is derived from the Latin word "lixivium," which means "lessive". Always use fresh, new solvent for extracting, whether it's cold or heated. Water is used as the solvent during component extraction.

5. Decoction:

To obtain plant extracts, the present procedure includes boiling plant material in water. Convection and conduction are two ways that heat is transported, and the kind of substance that can be recovered from plant material depends on the solvent choice. The sample is brought to a boil in a predetermined amount of water for between 15 and 60 minutes. After cooling, straining, and filtering, it is given the desired volume by adding just enough water through the medication. This process typically yielded more oil-soluble chemicals than maceration and is appropriate for extracting thermostable (that is, not affected by temperature) and water-soluble compounds, hard plant materials.

6. Tincture:

Plant material is extracted using alcohol in this process. Typically, 1:5 ratios of fresh plant material and ethyl alcohol are used. The tinctures can be kept at room temperature without going bad because of the alcohol concentration.

7. Percolation:

It involves circulating the heated solvent through the plant material at a controlled and moderate rate (for example, 5-7 drops every minute) until the extraction is finished before evaporation is done. The concentrated plant extracts are often gathered at the

vessel's bottom. Adding new solvent to the percolator between each percolation and pooling all the extracts together will provide a substantial amount of extract. This process is mostly used to extract active ingredients for making tinctures. The main limitations of this method include the need for huge quantities of solvents, time - consuming and the potential need for experienced personnel.

8. Steam distillation and hydrodistillation:

Typically, volatile chemicals, such as essential oil, which are insoluble in water, are extracted from a variety of aromatic and medicinal plants using steam and hydrodistillation techniques. After vapour condensation, the plant materials are boiled in water to produce EOs. Steam distillation takes place at a temperature below the materials' boiling points. The technique works well with thermosensitive bioactive substances, such as naturally occurring aromatic compounds. The target compound can then be released from a matrix as a result of the heat causing pore rupture in the sample. According to Raoult's law, the boiling point will decrease when two immiscible liquids are combined. As a result, when water has a boiling point of approximately 150°C and volatile chemicals have a boiling temperature between 150 and 300°C. There are similarities between the hydrodistillation and the steam distillation principles.

9. Sohxlet extraction/ soxhletation:

The Soxhlet apparatus is used in this approach, which involves placing a porous bag or thimble containing finely powdered sample in the thimble chamber. The porous bag is produced from cellulose or a sturdy filter paper. Extraction solvents are warmed in a round bottom flask, vaporised into a sample thimble, condensed in a condenser, and then dripped back. The process is resumed when the liquid content is dumped back into the bottom flask when it reaches the siphon arm. Some disadvantages include the inability to stir and the need for a significant volume of solvent. Because extended heat

exposure could cause thermolabile chemicals to degrade, this approach is not appropriate for them.

10. Serial exhaustive extraction:

It is a standard extraction technique that entails a series of extractions using different solvents of increasing polarity from non-polar to polar ones. The goal is to make it possible to extract chemicals with a wide variety of polarities.

11. Fermentation (aqueous-alcoholic extraction):

Some pharmaceutical treatments use fermentation as a method of obtaining the active ingredients. The crude medication, which can be either a powder or a decoction, is soaked for a predetermined amount of time during the extraction process. After fermentation, alcohol is produced in-situ, making it easier to extract the plant material's active ingredients. As a result, alcohol is produced and acts as a preservative. If the fermentation is going to be done in an earthen jar, the water needs to be heated to a boil first. In large-scale manufacturing, wooden vats, porcelain jars, or metal containers are utilized in place of earthen vessels. This procedure is still not standardized.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Overview

This chapter presents the research design, the study area, sample and sampling techniques, plant materials collection and processing, extraction procedure, treatment of the albino rats, wound excision and treatment of the wounds, phytochemical screening and finally the data analysis employed for this study.

3.1 Research Design

According to Sileyew (2019), research design is intended to provide an appropriate framework for a study. This study is quantitative research and the design is experimental research design.

Experimental research is a type of research design in which the study is carried out by utilizing a scientific approach and two sets of variables (Sileyew, 2019). The effectiveness of the experimental investigations is dependent on the researcher verifying that a variable change is due only to modification of the constant variable.

In this case, five groups were assigned to the albino rats. Group 1 rats were used as the control whereas the groups 2 – 3 were used as the experimental groups. Quantitative data was measured thus the wound areas and the masses of the albino rats. Statistical analysis was performed on the data obtained and finally, conclusions were drawn from the analyzed data.

3.2 The Study Area

Through unstructured observation by the researcher, the researcher found out that Assin Andoe is one of the many towns with largest *Chromolaena odorata* and *Sida acuta* infestation in the Assin South District.

Assin Andoe is in the Southern part of Ghana, which lies in the rain forest belt along Cape Coast to Kumasi highway (Fig. 1).

The town is rich in biodiversity by different forms of plant species, ranging from perennating trees to annual crops and rich in biodiversity. Among the herbs in the town are *Sida acuta, Chromolaena odorata, Phyllantus amarus, Tuja nana, Sporobolus pyramidalis* etc. The quality of the soil supports agriculture as well as weeds, hence the abundance of *Chromolaena odorata* L. and *Sida acuta* Burm. F.

Assin Andoe was selected due to the abundance of the *Chromolaena odorata* L. and *Sida acuta* Burm. F. which provided easy access to the herbs and also a convenient place close to the place where the albino rats were caged.

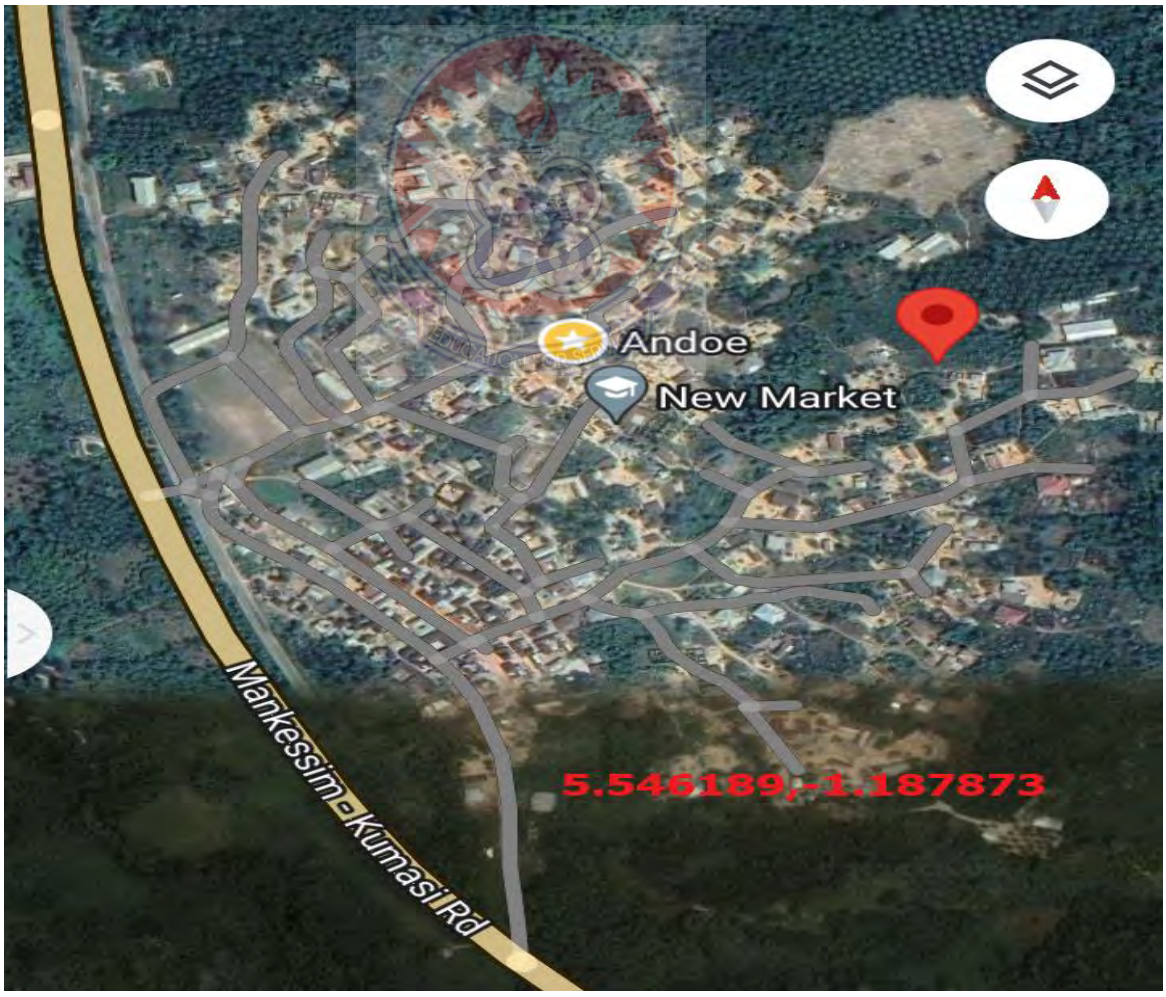


Fig. 1: A map to Assin Andoe
Source: Google map

3.3 Sample and Sampling Techniques

Sampling is the process of choosing a portion of a group or aggregate also called sample in order to learn more about the entire population (Guest, 2014).

With the sorting of the thirty albino rats into groups, simple random sampling technique was employed to sort the rats into five groups. This sampling technique was necessary since the rats form a homogeneous group and no specific qualities in the rats are of interest to the researcher.

A simple random sampling is a random method of selecting a subset from a population. In this sampling method, each member of the population has an exactly equal chance of being selected. Given that it only requires one random selection and minimal prior population knowledge, this approach is commonly use of all the probability sampling techniques (Banerjee & Chaudhury, 2010). A non-probability sampling technique, convenience sampling technique was used for adopting maceration method of extraction.

Again, convenience sampling was used for the research instrument used which was unstructured observation, which was used to determine the area where mature and healthy *C. odorata* and *S. acuta* are abundant. In convenience sampling, units are chosen for the sample based on how convenient they are for the researcher to access (Pace, 2021).

3.4 Plant Materials Collection and Processing

Fully mature dark green leaves of *C. odorata* and *S. acuta* were collected in Assin Andoe near the vicinity of the Andoe new market. The plant materials were collected in the morning. The plant species were identified by verifying the pictures of the species on google lens as well as by following the description and characters of the species by eye inspection.

Two large black polythene bags were used to collect the leaves of *C. odorata* and *S. acuta* separately. This was to prevent direct effect of the harmful rays from the sun on the leaves in order not to alter the chemical compositions of the leaves.

The leaves were thoroughly washed with water. The leaves were dried under shade separately for four weeks and the temperature of the room was approximately 25 degrees celsius. The dried leaves were powdered by using electric blender. A sieve with size 1.0 mm was used to sieve the pulverized leaves. The sieved leaves were kept in two plastic containers separately.

3.4.1 Extraction procedure

The extraction method adopted was maceration.

The researcher picked 15g each of the powdered leaves of *C. odorata* and *S. acuta* making 30g, and mixed them in a beaker. The mixture was introduced into one plastic container. Using measuring cylinder, 500mL of distil water was added to the mixture. The mixture was then stirred followed by vigorous shaking to form a uniform solution.

70% ethanol was prepared by picking 350mL of absolute ethanol and diluted with 150mL of distil water. 15g powdered leaves of *S. acuta* and 15g powdered leaves of *C. odorata* leaves were dissolved in the 70% ethanol in another plastic container. The same procedure was repeated for 30% ethanol and 30% methanol. The four solutions were left for three days with intermittent agitation to aid in the extraction procedure.

Whatman no. 1 filter paper was used to filter the solution after three days. On the other hand, the aqueous extract was filtered after two days and was kept in a freezer to prevent it from going bad. The ethanolic and methanolic extracts were concentrated by using a vaporizer followed by heating over water bath. The aqueous extract was concentrated by heating it over water bath.

3.5 Treatment of the Albino Rats, Wound Excision and Treatment of the Wounds

Thirty albino rats were used for the study. These albino rats were procured from the University of Cape Coast animal farm. The rats were fed with the same food for a week to ensure they are all provided with the same nutrients. The meal provided to the albino rats include; milled corn and wheat, dried fish, bread crumps and vegetables i.e., cabbage. Maintenance of hygiene and proper care of the rats were taken to ensure the rats are healthy. Guide for the care and use of lab animals, 8th edition, was duly followed. The eating behaviour of the rats were sternly observed and noted.

The rats were randomly divided into five groups. A circular wound of a specific diameter (2cm) was inflicted on the side of each rat by using a sterilized scalpel.

Rats in group 1 were treated with penicillin ointment (synthetic ointment) and this group was used as the control group.

Rats in group 2 were treated with 70% ethanolic extract, group 3 with 30% ethanolic extract, group 4 with 30% methanol extract and group 5 with aqueous extract once a day for fifteen days. The groups of the rats were labelled PO, SC1, SC2, SC3 and SC4 for groups 1,2,3,4 and 5 respectively.

The areas of the topical wound inflicted on the sides of the albino rats were calculated by multiplying the longest vertical dimension by the longest horizontal dimension i.e. length x breath.

The mass of the rats and the area of the wounds and also, the surfaces of the wounds were observed to monitor scar formation at three days interval until the fifteenth day. The results were tabulated. The average mass of each group of rats were compared to the different extracts of the leaves as well as the penicillin ointment to determine whether there is a linear correlation. Thus, the progress of the healing of the wounds (wound areas) was compared to the average mass for each treatment group.

3.6 Phytochemical Screening

The researcher ran a qualitative test to assess the phytochemical compounds present in the various extracts of the dried leaves. The methods used were adopted from Dhawan and Gupta (2017) and other authorities.

- **Test for saponins**

10 mL extract of each of the solvents was transferred into four different test tubes labelled SC1, SC2, SC3 and SC4 thus ;70% ethanolic extract, 30% ethanolic extract, 30% methanol extract and aqueous extract respectively. The extracts were diluted with water and was shaken for 15 mins. The extracts that formed foam on the supernatant indicated the presence of saponins.

- **Test for flavonoids**

10 mL of each of the different extracts was transferred from the stock into four different test tubes; SC1, SC2, SC3 and SC4. Few drops of dil. NaOH were added to each extract. There was the appearance of yellow colour. The yellow colour changes to colourless upon addition of few drops of dil. H_2SO_4. The disappearance of the yellow colour confirms the presence of flavonoids.

- **Test for alkaloids**

10 mL of each of the different extracts was transferred into four different test tubes. 2 mL of the Wagner's reagent was added to each test tube. The appearance of reddish – brown precipitate confirmed the presence of alkaloids.

- **Test for tannins**

10 mL of each of the different extracts was taken and dissolved in 45% of the ethanol in four different test tubes. The test tubes and their content were boiled for 5 mins and 1

mL of 15% ferric chloride solution was added to each. The formation of greenish to black colour confirmed the presence of tannins in the leaf extracts.

- **Test for steroids**

10 mL each of the extract were transferred to test tubes SC1, SC2, SC3 and SC4 and 1 mL of conc. H_2SO_4 was added to each of the test tube. Appearance of dark reddish – green colour confirmed the presence of steroids.

- **Test for Terpenoids**

This method was adopted from Prabhavathi, Prasad & Jayaramu, (2016). 10 mL of each extract was transferred to test tubes SC1, SC2, SC3 and SC4; 0.5 ml of chloroform was added to each followed by few drops of concentrated sulphuric acid. There was formation of reddish – brown precipitate, indicating the presence of terpenoids.

- **Glycoside**

3 mL of chloroform was added to all the extracts followed by 10% ammonia. Formation of pink colour indicated the presence of glycoside (Roghini & Vijayalakshmi, 2018).

- **Phenol**

Few drops of distil water was added to each of the extracts followed by few drops of 10% ferric chloride. Blue or green colour indicated presence of phenol (Roghini & Vijayalakshmi, 2018).

- **Anthraquinone**

5 ml of chloroform was added to 0.5 g of the extracts. The resulting mixture was shaken for 5 mins after which it was filtered. The filtrate was then shaken with equal volume of 10 % ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of free anthraquinones (Ajayi, Ajibade & Oderinde, 2011).

- **Phlobatannin**

Few drops of 2% hydrochloric acid were added to 1ml of each of the extracts. Appearance of red colour precipitate indicated the presence of phlobatannins (Roghini & Vijayalakshmi, 2018).

- **Gum and mucilage**

10 mL of distil water was added to each of the extracts followed by 2 mL of absolute alcohol. White or cloud precipitate formed indicated the presence of gum and mucilage (Banu & Cathrine, 2015).

3.7 Data Analysis

Data was analysed using SPSS version 20. Line graphs were drawn to compare the average wound areas as well as the average masses among the five groups of the albino rats after treatment with the herbal extracts and the penicillin ointment (control).

Percentage reduction in the wound areas in the albino rats was also calculated and represented on a line graph. One – way ANOVA was calculated to compare the means of the average wound area among the five groups of the albino rats at a 0.05 level of significance.

Pearson correlation coefficient was also calculated to determine the linear relationship between the different solvent extracts of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves on the average wound areas and the average masses of the albino rats.

CHAPTER FOUR

RESULTS OF THE STUDY

4.0 Overview

This chapter presents the presentation and analysis of data obtained in the study.

4.1 Observation of the Eating Habit of the Rats

The albino rats were fed one week before the fifteen days experiment began. The rats were selective in eating some of the meal provided in the first three days. They preferred wheat, corn, and dried fish to bread crumps and vegetables i.e., cabbage until the end of the first week. The rats fed well throughout the fifteen days of the experiment. The rats eating habit contributed to weight gain in that, their masses increased by the end of the second week of the experiment.

Objective 1: To determine the healing effect of the solvent extract of the mixture of dried leaves of *Chromolaena odorata* L. and *Sida acuta* Burm. F. on wounds in albino rats.

H_0: The solvent extract of the mixture of dried leaves of *Chromolaena odorata* L. and *Sida acuta* Burm. F. has no significant effect on wound healing in albino rats.

4.2 Observation of the Wound Surface

The following observations were made about the wound surface after the wounds were inflicted on the sides of the albino rats (Plate 1).

- On day 1 (Plate 1), when the wounds were inflicted, the researcher used cotton to clean blood from the wound site and applied the various extracts as well as the penicillin ointment on the wounds. In a few minutes time, there was blood clotting. This characterizes the first and second stages of wound healing thus haemostasis and inflammation (Wikipedia, 2023). A day after the infliction of

the wounds on the sides of the rats and application of the extracts, it was observed that the surfaces of the wounds were dry and hardened, besides, there was no significant decrease in the wound area among the albino rats. On the subsequent days, it was observed that some of the rats licked the extract applied on the wounds. This made some groups of the rats having their wound surfaces to be wet, as a result, healing was delayed in these groups of the rats.

- On the third day, most rats had hardened wound surfaces thus re-epithelialization with the epithelial tissue being light pink in colour as well as a significant decrease in the wound area. Angiogenesis (formation of new vascular tissues), granulation and re-epithelialization characterized the third stage of wound healing which is the proliferative stage (Wikipedia, 2023).
- By the end of the first week, specifically on the sixth day, there was formation of scar tissue and the disappearance of the light pink colour from the wound area and also a significant decrease in the wound area. The tissue remodelling and scar formation continued into the second week of the experiment. According to Wikipedia (2023) on wound healing, tissue remodelling is the last phase of wound healing and can last for a short time or long time depending on the type and depth of the wound.
- On day 9, the wound area continued to decrease across the five groups of the rats and by day 12, there was significant reduction in the wound areas as compared to the previous days (Plate 2). The wound area in PO, SC2, SC3 and SC4 were closed except in SC1.
- By day 15, the wound area was closed in all the groups including SC1. The wounds were replaced by linear scar tissue.

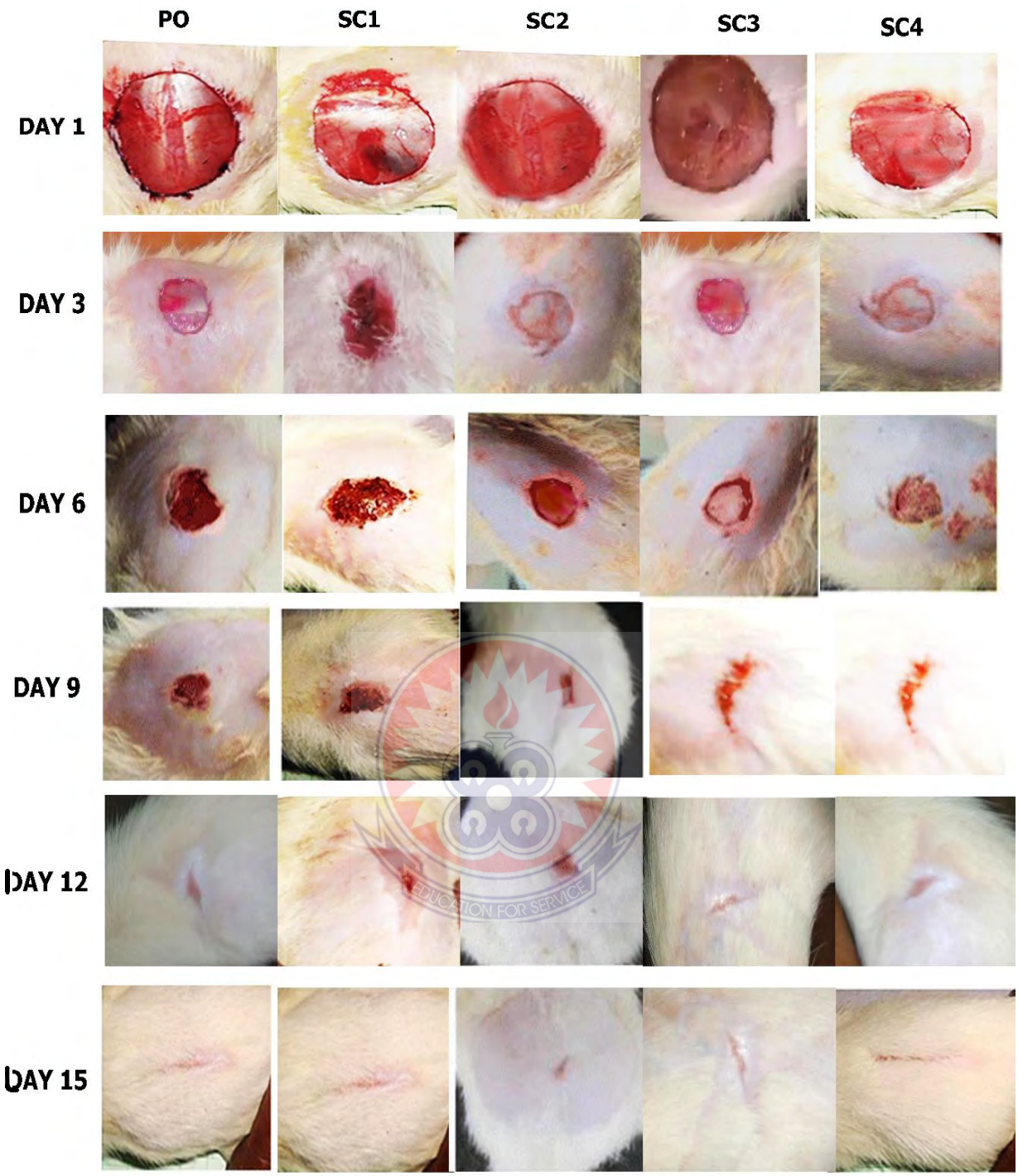


Plate 1: Images of the wounds of the five groups of the albino rats

4.3 The Average Wound Areas for the Five Groups of Rats

From the beginning of the experiment, the area of the wounds were approximately the same for all the albino rats. Figs 2 and 3 are the summaries of the average wound areas in the rats for the first and second weeks of the experiment respectively. From Fig. 2, it

can be deduced that all groups, including the control group (PO) and the experimental groups (SC1, SC2, SC3, SC4), had similar wound areas of 4.00 cm^2 on the first day.

On day 3, the control group (PO) showed a slight reduction in wound area to 3.60 cm^2. Among the experimental groups, SC1 had the highest reduction to 3.28 cm^2, followed by SC2 (3.07 cm^2), SC4 (2.48 cm^2) and SC3 (2.25 cm^2).

On day 6, the control group (PO) experienced a further decrease in wound area to 2.09 cm^2.

SC2 showed the highest reduction in wound area among the experimental groups with a value of 2.14 cm^2. The other experimental groups had slightly higher values: SC4 (2.33 cm^2), SC1 (2.30 cm^2) and SC3 (1.56 cm^2).

In the second week, from Fig. 3, on day 9, the control group (PO) continued to exhibit a decrease in wound area, reaching 0.85 cm^2.

Among the experimental groups, SC3 had the lowest wound area (0.55 cm^2), followed by SC2 (0.73 cm^2), SC4 (0.81 cm^2), and SC1 (0.92 cm^2).

On day 12 and day 15, both the control group (PO) and the experimental groups (SC1, SC2, SC3, SC4) achieved complete reduction in the wound area by reaching a wound area of 0.00 cm^2.

It can be judged from the graphs (Fig. 2 and Fig. 3) that, the extracts of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves had potential wound-healing effects on the wounds of albino rats. The groups treated with the extracts consistently showed smaller wound areas compared to the control group, indicating their efficacy in promoting wound closure.

Again, the groups treated with the extracts showed faster wound closure compared to the control group treated with penicillin ointment and the group treated with 30% methanol extract (SC3) showed the most pronounced effects in reducing wound area.

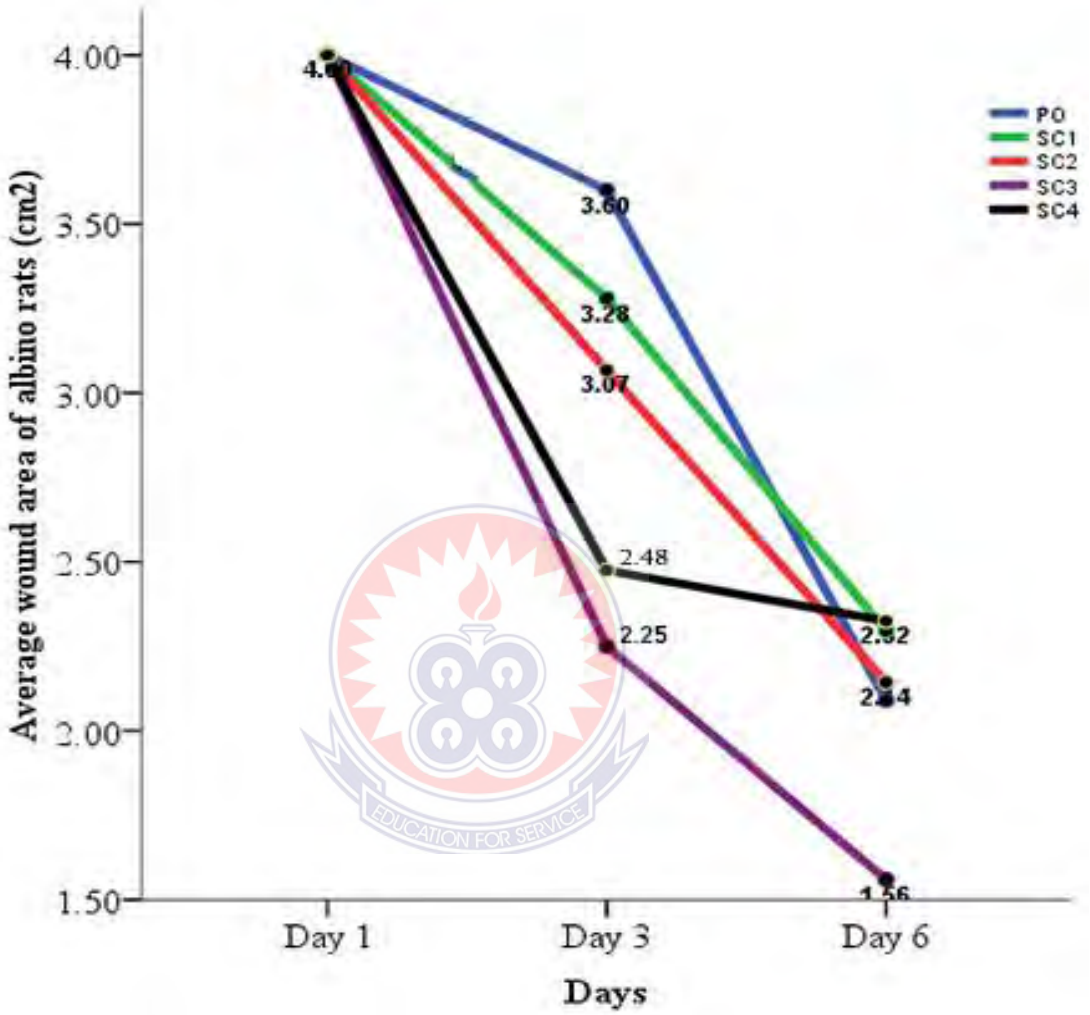


Fig. 2: Average wound area in Albino rats for the first week of the experiment

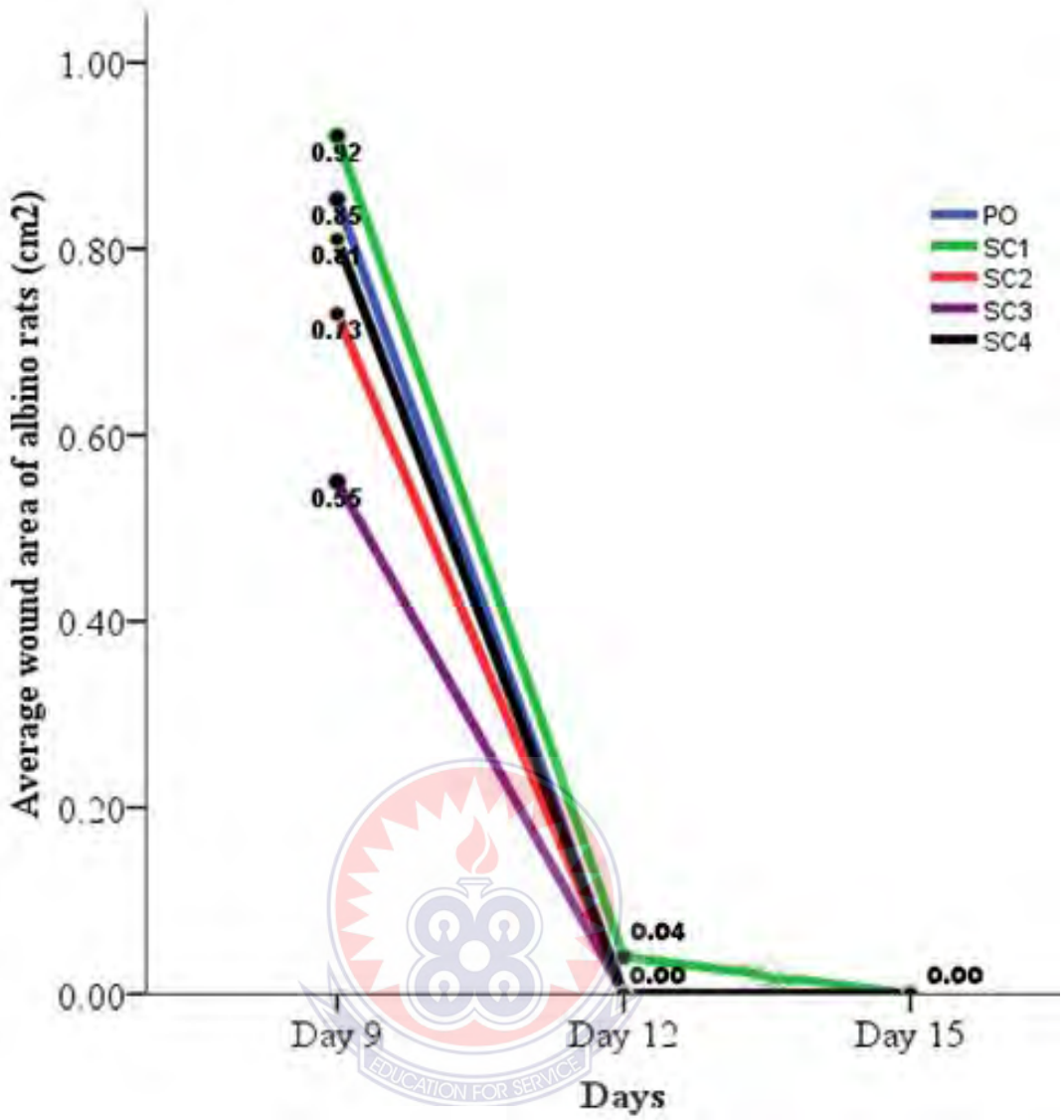


Fig. 3: Average wound area in Albino rats for the second week of the experiment

4.3.1 Effectiveness of the different extracts of the herbal mixture on wound closure of the albino rats

H_0: There is no significant difference among the means of the wound areas of the five groups of the rats

Table 5 is the comparison of the means of the average wound areas of the five groups of the albino rats. This comparison showed how efficacious the various extracts of the mixture of *C. odorata* and *S. acuta* leaves were on the wounds as compared to the control.

Table 5: One - way ANOVA Table on the wound area among the five groups of the rats

Source of Variation	Sum of Squares	df	Mean Square	F-ratio
Extracts	.538	4	.134	.049
Error	68.915	25	2.757	
Total	69.452	29		

From Table 5, the calculated F-value is 0.049. from the ANOVA Table (Appendix III), using the degree of freedom of the various extracts = 4 and the degree of freedom of error = 25, at the level of significance = 0.05, the critical value is 2.76

Since the calculated F-value is less than the critical F-value, there is no significant difference therefore the researcher fails to reject the null hypothesis.

Thus, there is no significant difference among the means of the wound areas of the five groups of the rats. This means that the different solvent extracts of the mixture of the dried leaves of *C. odorata* and *S. acuta* were effective in closing the wound areas in the albino rats in the same way as the penicillin ointment which was used as the control.

4.4 Percentage Reduction of Wound Areas

Percentage reduction of the wound areas were calculated for the five groups of the albino rats.

Percentage reduction is the percent change in the wound area compared to the initial wound area. Comparison of the percentage reduction of the wound areas after the treatment of the extracts on the wounds of albino is summarized in Fig. 4.

From Fig. 4, on day 1, there is 0.00% reduction in the wound area for all the groups of the albino rats. This means, there was no significant decrease in the wound areas.

On day 3, the control (PO) had the least percentage reduction in the wound area as compared to the other groups of the albino rats. There was 10.00% reduction in the

wound area of PO (group 1 rats), 18.00%, 23.33%, 43.75% and 38.13% wound area reduction in SC1, SC2, SC3 and SC4 respectively (Fig. 4).

On day 6, SC3 had the highest reduction of 61.00% of wound area reduction, while PO, SC1, SC2 and SC4 had 47.75%, 42.58%, 46.42% and 41.88% respectively (Fig. 4).

On day 9, while the control (PO) had a reduction of 78.67%, SC3, SC2 and SC4 had a reduction of 86.25%, 81.75% and 79.75% respectively. But SC1 had the least percentage reduction of 76.98% (Fig. 4).

On day 12, there was 100.00% of wound area reduction in PO, SC2, SC3 and SC4 while in SC1 there was 99.00% reduction of the wound area. The wound areas were completely closed in all the groups with the exception of SC1 (Fig. 4).

By day 15, there was 100.00% wound closure in all the groups of the albino rats (Fig. 4).

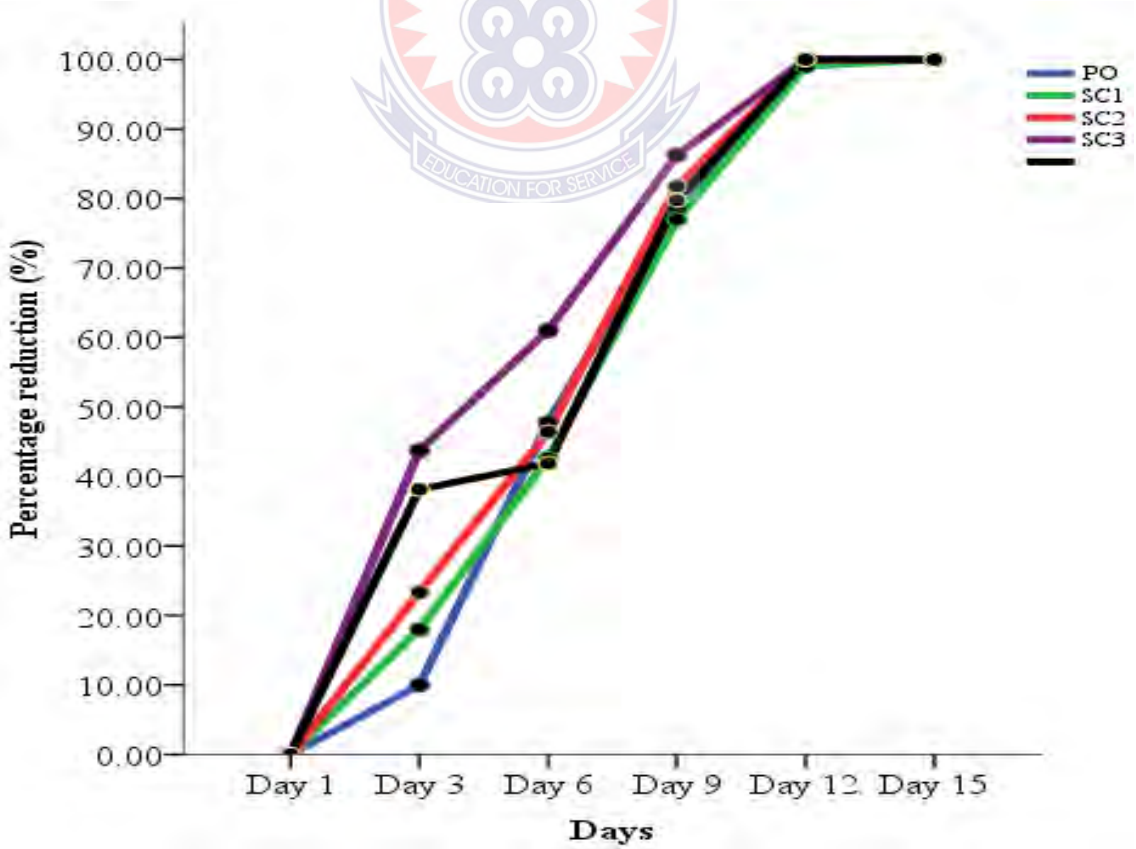


Fig. 4: Percentage reduction (%) of the wound area of the five groups of the albino rats

4.5 The Average Mass of the Groups of the Rats

The graphs of the average masses for the first and second weeks are shown in Figs. 5 and 6.

From Fig. 5, the initial masses of the rats in all the groups varied, with the control group (PO) having the lowest mass of 131.20g and the SC2 group having the highest mass of 166.10g.

On day 3, there was increment in the masses of the rats except for SC2 which decreased to 162.83g (Fig. 5).

On the sixth day, the mass of SC1 declined to 134.97g as compared to the initial mass of 139.37g. The mass of SC3 declined to 137.50g as compared to the mass on the third day (Fig. 5).

In the second week of the experiment, all the masses increased except for SC3 which had average mass of 137.33g on the ninth day (Fig. 6).

On day 12, there were slight variations in the masses of the rats. The masses of the rats remained relatively stable, with minimal changes compared to day 9 (Fig. 6).

By the fifteenth day of the experiment, there were slight variations in the masses among the groups but in comparison to the initial masses, all the groups showed increment in the average masses (Fig. 6).

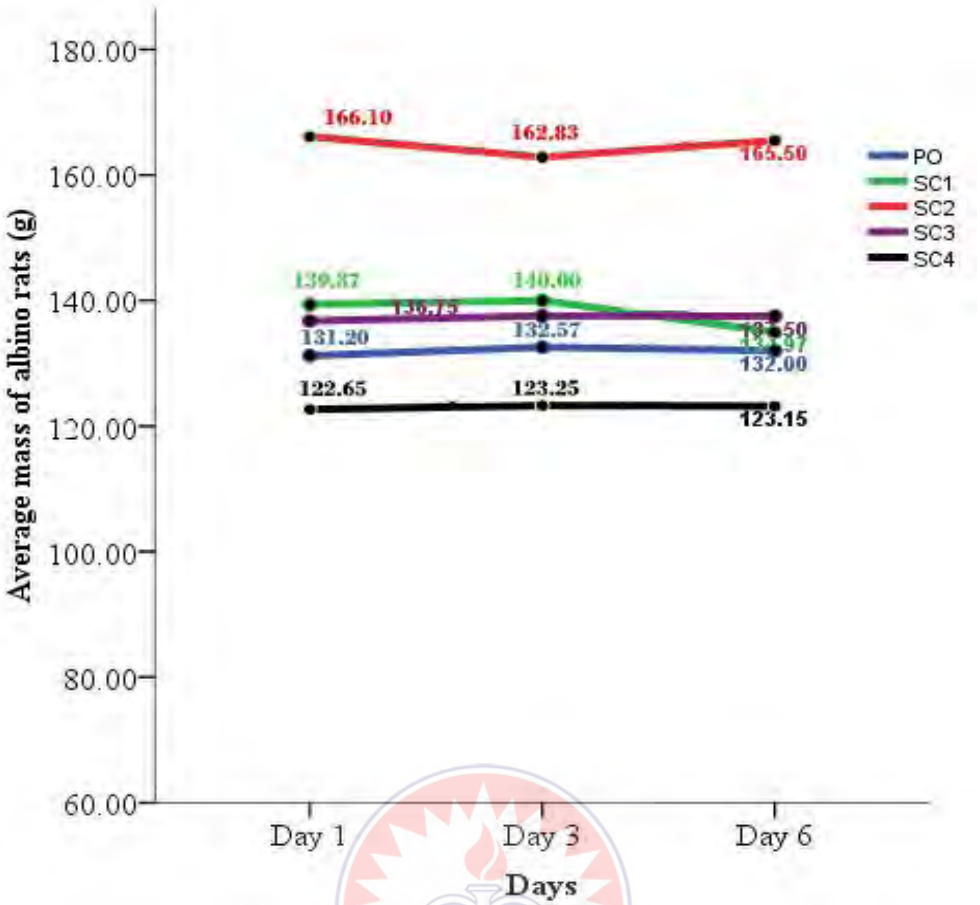


Fig. 5: Average mass of the albino rats for the first week of the experiment

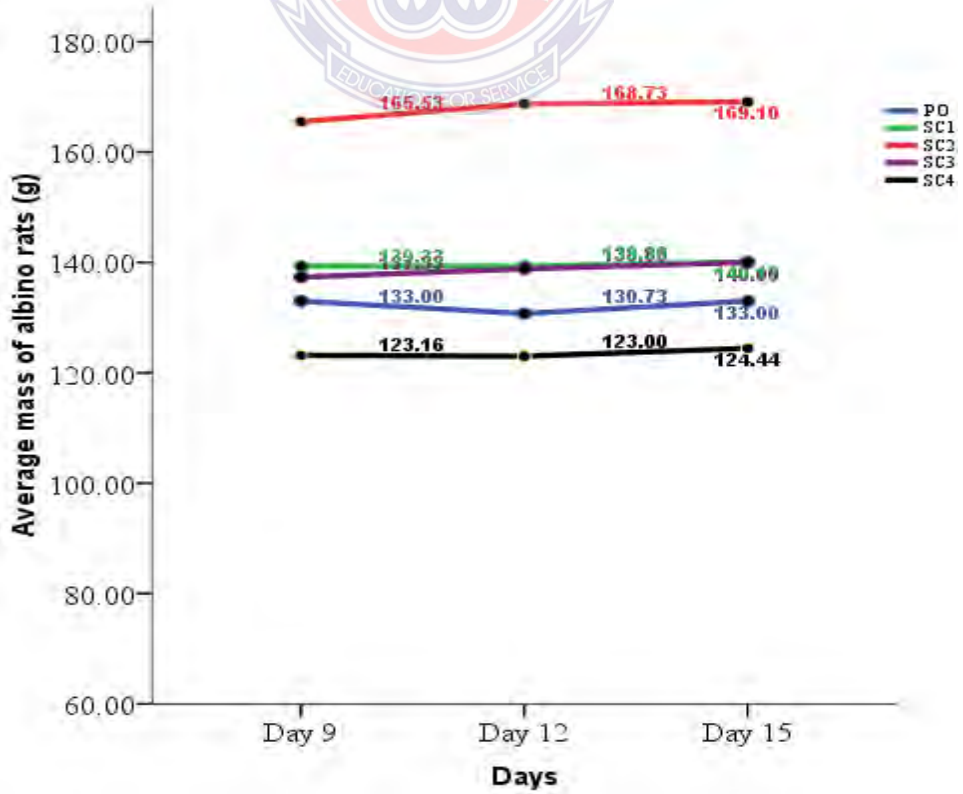


Fig. 6: Average mass of the albino rats for the second week of the experiment

***4.5.1 Objective 2*: To ascertain changes in the masses of the albino rats after the application of the different solvent extracts of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves.**

H_0: There is no linear correlation between the different solvent extracts of the mixture of *Chromolaena odorata* and *Sida acuta* leaves and the masses of the rats.

Table 6 is a summary of Pearson's correlation coefficients between the average masses and the average wound areas for the five groups of the albino rats. This was necessary to determine if there is a relationship between the application of the different solvent extracts of the mixture of Chromolaena odorata L. and Sida acuta Burm. F. leaves on the wounds and the masses of the albino rats.

Table 6: Pearson's correlation coefficients between the average mass and the average wound area for the five groups of rats

	MPO	WPO	MSC1	WSC1	MSC2	WSC2	MSC3	WSC3	MSC4	WSC4
MPO	1									
WPO	-.141	1								
MSC1	.154	-.086	1							
WSC1	-.167	.996**	-.158	1						
MSC2	-.258	-.761	.147	-.741	1					
WSC2	-.196	.993**	-.145	.998**	-.703	1				
MSC3	.176	-.777	.292	-.805	.745	-.787	1			
WSC3	-.279	.957**	-.074	.968**	-.566	.979**	-.764	1		
MSC4	.651	-.550	.244	-.579	.423	-.574	.830*	-.593	1	
WSC4	-.235	.963**	-.239	.983**	-.638	.987**	-.813*	.983**	-.605	1

Pearson's correlation coefficient was determined by using SPSS v. 20. **Key: M = average mass of rats, W = average wound area, PO = group 1, SC1 = group 2, SC2 = group 3, SC3 = group 4, SC4 = group 5**

From Table 6, the following deductions were made:

- In Group 1 (WPO and MPO), the correlation coefficient of -0.141 indicates a weak negative correlation between the masses and the wound areas.
- In group 2 (WSC1 and MSC1), The correlation coefficient of -0.158 also indicates a weak negative correlation between the masses and wound areas. Similar to Group 1, the negative association exists, but not strong.
- In Group 3 (WSC2 and MSC2), the correlation coefficient of -0.703 indicates a moderate to strong negative correlation between the masses and wound areas. This suggests that as the mass increases, the wound area tends to decrease, or vice versa, with a relatively stronger relationship compared to the first two groups.
- In group 4 (WSC3 and MSC3), the correlation coefficient of -0.764 indicates a strong negative correlation between the masses and wound areas. The negative correlation is stronger in group 4 than in Group 3.
- In group 5 (WSC4 and MSC4), the correlation coefficient of -0.605 indicates a moderate negative correlation between the masses and wound areas. While the negative association is present, it is not as strong as in Groups 3 and 4 but still stronger than in Groups 1 and 2.

From Table 6, the correlation coefficients suggest that there is a negative correlation between the masses and wound areas in all five groups of albino rats. This means as the wound area in the rats decrease, the masses increase and vice versa. However, the strength of the correlation varies across the groups. Groups 3 and 4 exhibit stronger negative correlations, while Groups 1, 2, and 5 show weaker negative correlations.

4.5.2 Determination of the significance of the coefficient of correlation

Further analysis was performed to determine whether the relation is significant.

From Pearson's significance table (Appendix IV), with the degree of freedom, df = 6, two tailed, and level of significance = 0.05, the Pearson's critical value = 0.707. Comparison of the Pearson's calculated r-value to the critical r - value is summarized in Table 7.

Table 7: Comparison of the Pearson's calculated r-value to the critical r – value

Groups	Comparison	Conclusion
WPO and MPO	0.141 < 0.707	Not significant
WSC1 and MSC1	0.158 < 0.707	Not significant
WSC2 and MSC2	0.703 < 0.707	Not significant
WSC3 and MSC3	0.764 > 0.707	significant
WSC4 and MSC4	0.605 < 0.707	Not significant

From Table 7, there is no significant linear relation between the average mass and the average wound area in groups 1, 2, 3, and 5. Therefore the researcher fails to reject the null hypothesis. Thus, there is no linear correlation between the different solvent extracts of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves and the masses of the rats.

On the other hand, there is a significant linear relation between the mass and the wound area in group 4. Therefore, the researcher rejects the null hypothesis. Thus, there is a linear correlation between the different solvent extracts of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves and the masses of the rats.

4.6 Results from the Qualitative Analysis of the Extracts

Objective 3: Assess the phytochemical compounds present in the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves extracts

During the qualitative analysis of the chemical constituents of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves, the following observations were made:

- **Saponins:**

All the extract in the test tubes SC1, SC2, SC3 and SC4 containing 70% ethanolic extract, 30% ethanolic extract, 30% methanol extract and aqueous extract respectively, foamed on the supernatant indicating the presence of saponins in all the extracts.

- **Flavonoids:**

There was formation of yellow precipitate in all the extracts, which disappeared upon addition of few drops of dil.H_2SO_4, the yellow precipitate disappeared in all the extracts which confirmed the presence of flavonoids in all the extract.

- **Alkaloids:**

The Wagner methodological procedure confirmed the presence of alkaloids in test tubes SC3 and SC4 but not in SC1 and SC2.

- **Tannins:**

The methodological procedure used resulted in the confirmation of the presence of tannins in all the extracts.

- **Steroids:**

The methodological procedure used resulted in the confirmation of the presence of steroids in all the extracts.

- **Terpenoids:**

The methodological procedure used resulted in the confirmation of the presence of terpenoids in all the extracts.

- **Glycoside:**

The methodological procedure used resulted in the confirmation of the presence of Glycoside in all the extracts.

- **Phenol:**

The procedure followed resulted in the confirmation of the presence of phenol in SC1, SC3 and SC4.

- **Anthraquinone:**

The cloudy precipitate formed did not change colour to pink which confirmed the absence of anthraquinone in all the extracts.

- **Phlobatannin:**

Brownish-red precipitate formed in SC2, SC3 and SC4 indicated the presence of phlobatannin.

- **Gum and mucilage:**

The formation of cloudy precipitate confirmed the presence of gum and mucilage in all the extracts.

Table 8 is the summary of the phytochemical constituents in the various extracts.

Table 8: Phytochemical compounds present in the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves extract

Chemical compound	SC1	SC2	SC3	SC4
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	-	-	+	+
Tannins	+	+	+	+
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Glycoside	-	-	-	-
Phenol	+	-	+	+
Anthraquinone	-	-	-	-
Phlobatannin	-	+	+	+
Gum and mucilage	+	+	+	+

Key: + presence of the phytochemical, – absence of phytochemical

CHAPTER FIVE

DISCUSSION OF RESULTS

5.0 Overview

This chapter presents the discussion of results in chapter four.

5.1 Observation

The preference for mature leaves to young leaves in herbal medicine can vary depending on the specific plant and its medicinal properties. Both types of leaves can contain beneficial compounds, but the composition and concentration of these compounds may differ based on the plant's growth stage.

Mature leaves generally have a higher concentration of certain compounds, such as essential oils, flavonoids, and alkaloids (Ghosh, Chowdhury & Chandra, 2008). These compounds are often responsible for the medicinal properties of the plant.

5.1.1 Wound surface and the wound area

Miriam-Webster dictionary (2022) posits that wound breaks the surface membrane of the skin and causes damage to the integrity of skin as well as damage to the underlying tissues.

Wound healing is important to prevent invasion of microorganisms into the skin therefore the body produces Platelets, neutrophils, macrophages and fibroblasts at the wound site which produce cytokines and growth factors to restore the integrity of the skin (Mohd *et al.*, 2012).

Some of the phytochemicals present in the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves contribute and enhance the production of the cells and chemicals that aid in the wound healing process.

On day 1, after the treatment of the extracts, there was blood clotting in all the groups of the rats. Haemostasis is initiated whereby Platelets become activated and produce Platelet-derived growth factor (PDGF), transforming growth factors (TGFs), fibroblast growth factors (FGFs) and vascular endothelial growth factors (VEGFs) to help in blood clotting. Haemostasis overlaps with inflammation phase whereby enzymes are produced by inflammatory cells to aid in healing (Mohd *et al.*, 2012; Wikipedia, 2023).

On day 3, the wound area was reduced in all the groups and the wound appeared light pink in colour. From observation of the wound surface there was re-epithelialization, thus, formation of new epithelial tissue.

Mohd *et al.* (2012) reiterates that epithelialization, fibroplasia and granulation constitutes the proliferation phase which is the third phase of the wound healing process. Fibroblasts produce collagen at the wound site as well as formation of blood vessels to replace damaged blood vessels. Re-epithelialization is initiated after the proliferative phase where epithelial cells interact with keratinocyte growth factors and transforming growth factors (TGF-α) to aid in the reduction of the wound area (Mohd *et al.*, 2012; Wikipedia, 2023).

On day 6, scar tissue was prominent and a significant reduction in the wound area. Formation of scar tissue and disappearance of the pink colour of the wound site indicate the on-set of phase IV of the wound healing process. Tissue remodeling (phase IV) can last for a long time depending on the type of wound (Wikipedia, 2023).

After the sixth day till the fifteenth day, tissue remodeling continued until the wound was closed. At this stage, cells involved in the production of growth factors are reduced at the site as well as the inflammatory cells.

Although fibroblasts also decrease in number, they continue to produce collagen (Mohd *et al.*, 2012). Collagen interact with each other to form scar during the remodeling phase.

On day 12, the wound areas for all the groups of the rats were reduced and replaced with a linear scar with the exception of group 2 rats.

On the fifteenth day, it was observed that the wound area was reduced and replaced with a linear scar.

5.2 Wound Area

Objective 1: To determine the healing effect of the solvent extracts of the mixture of dried leaves of *Chromolaena odorata* L. and *Sida acuta* Burm. F. on wounds in albino rats.

H_0: The solvent extract of the mixture of dried leaves of *Chromolaena odorata* L. and *Sida acuta* Burm. F. has no significant effect on wound healing in albino rats.

The wound area was the same for all the groups of the rats on day 1. But on days 3 and 6, there was reduction in the wound area for all the five groups. The four extracts were effective in reducing the wound area as compared to the control (PO).

It is observed that, the wound areas further decreased on day 9 with SC3 showing the shortest peak.

From the phytochemical analysis (Table 8), SC3 and SC4 were most effective in extracting *Chromolaena odorata* and *Sida acuta* leaves. Two groups; SC3 and SC4 showed high reduction in the wound area as compared to the control.

By day 12, the wound area in all the groups were closed and it was difficult calculating the area except SC1 which had the average wound area equals 0.04 cm^2. By day 15, the wound area in all the groups were closed.

According to Vijayaraghavan and Ashokkumar (2017), a wound is considered healed when the damaged surface is held firmly together by collagen.

It can be judged that there was wound healing in the groups PO, SC2, SC3 and SC4 by day 12 whereas by day 15, there was wound healing in SC1.

From Table 5, the calculated F-value is 0.049. from the ANOVA table, using the degree of freedom of the various extracts = 4 and the degree of freedom of error = 25, at the level of significance = 0.05, the critical value is 2.76. Since calculated F – value is less than tabulated F- value, I failed to reject the null hypothesis. Thus, there is no significant difference among the means of the wound area for the five groups.

In other words, the extracts as well as the penicillin ointment were equally effective in healing wounds in the rats with confidence level of 95%.

5.2.1 Percentage reduction of wound areas in the rat groups

The efficacy of the solvent extract of the mixture of dried leaves of *Chromolaena odorata* L. and *Sida acuta* Burm. F. was assessed by measuring the percentage reduction of wound area in the five groups of the rats.

On day 1, there was no significant reduction in the wound area observed across all groups. This indicated that the wounds were in the early stages of healing.

By day 3, a discernible difference in the percentage reduction of the wound area emerged between the control group (PO) and the treated groups. The control group had the least reduction, with only a 10.00% decrease in wound area, while the treated

groups, SC1, SC2, SC3, and SC4 displayed higher percentage reductions of 18.00%, 23.33%, 43.75% and 38.13% respectively.

On day 6, SC3 had a remarkable reduction of 61.00% in wound area, exhibiting the highest progress among all the groups. The control group (PO) and the other treated groups showed moderate reductions ranging from 41.88% to 47.75%. These findings suggest that, 30% methanolic extract of the dried leaves (SC3) contributes to accelerated wound healing compared to the control group on the sixth day.

On day 9, increment of percentage reduction of the wound areas was observed. While the control group showed a reduction of 78.67%, the treated groups, SC3, SC2 and SC4 demonstrated higher reductions of 86.25%, 81.75% and 79.75% respectively. On the other hand, SC1 had the least percentage reduction of 76.98%.

On day 12, notable progress was observed, with most groups, PO, SC2, SC3, and SC4 achieving 100.00% wound area reduction, signifying a complete wound closure. SC1 closely follows with a 99.00% reduction, indicating almost complete closure but with a slight difference compared to the other groups.

By day 15, all groups achieved complete wound closure, demonstrating 100.00% wound closure in each case. This further supports the effectiveness of the solvent extracts of the dried leaves of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. as the wounds successfully healed in all the groups.

The groups treated with the solvent extracts of the dried leaves of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. consistently outperformed the control group in terms of wound area reduction.

5.3 Average Masses of the Rats

The masses were different on day 1 and remained so throughout the fifteen days experiment.

The masses of the rats for the first week were not stable. The mass of PO increased to 132.00g, SC1 reduced to 134.97g, SC2 reduced to 165.50g, SC3 increased to 137.50 and SC4 increased to 123.15g on the sixth day.

The masses of the rats increased significantly on day 15 when compared to the initial masses on day 1. The mass of PO increased to 133.00g, SC1 to 140.12g, SC2 to 169.10g, SC3 to 140.00g and SC4 to 124.44g.

The fluctuations in the masses of the rats for both the first and second weeks of the experiment may be due to the feeding habits or application of the extracts on the wounds which might be toxic to the rats. From observation, the rats fed well and ate almost everything given them.

The average masses and average wound areas were negatively correlated in all the five groups of albino rats, according to the correlation coefficients.

Accordingly, the masses increased as the rat wound areas decreased and vice versa. The linear correlations between the groups varied in strength, though negative relationships were stronger between the average mass and the wound area in Groups 3 and 4, while in Groups 1, 2, and 5, correlations were weak.

5.4 Objective 2: To ascertain changes in the masses of the albino rats after the application of the different solvent extracts of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves.

H_0: The different solvent extracts of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves has no significant effect on the masses of the albino rats.

In groups 1, 2, 3, and 5, there is no statistically significant linear relationship between the average mass and the average wound area. As a result of this, I failed to reject the null hypothesis. Thus, there is no linear correlation between the different extracts of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves and the masses of the rats.

In group 4, however, there is a significant linear relationship between the mass and the area of the wound. The null hypothesis is thus rejected. As a result, there is a linear relationship between the masses of the rats and the solvent extracts of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F.

This means the application of the herbal extracts on the wounds had effect on weight gain in albino rats in group 4. However, weight gain in group 4 rats was associated with the herbal treatment and this agrees to the research by Igwe and his colleague, when they treated broiler chicken with extract of *Chromolaena odorata* and recorded weight gain (Igwe, Udo,… & Nwose, 2020).

5.5 Discussion of Results from the Qualitative Analysis of the Extracts

Objective 3: Assess the phytochemical compounds present in the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves extracts

Saponins, flavonoids, alkaloids, tannins, steroids, terpenoids, phenol, phlobatannin, gum and mucilage are the phytochemicals present in the various extracts of the mixture

of *Chromolaena odorata* and *Sida acuta* leaves. Glycoside and anthraquinone on the other hand were absent in all the extracts. And this agrees to the work done by Tiamiyu and Okunlade (2020).

A phytochemical screening of *Chromolaena odorata* L. leaves extract by Tiamiyu and Okunlade (2020) showed that alkaloids, tannin, phlobatannin, saponin, flavonoids, steroids, terpenes, phenol and cardiac glycosides are present whereas anthraquinone, cardenolides are absent.

According to Anyasor *et al.* (2011), the following phytochemicals are present in the ethanolic extract of the *Chromolaena odorata* leaves; terpenoid, tannin, saponin, cardiac glycoside, anthraquinone, phenol whereas the following were absent; phlobatannin, flavonoids, cardenolides, alkaloid and volatile oil.

Again, research by Adeniyi *et al.* (2010) on the phytochemical analysis of 80% ethanolic extract of *Sida acuta* leaves showed that, alkaloids, flavonoids, saponins, steroids, tannins, phlobatannins, terpenoids and cardiac glycosides are present.

When the two research works were married, the following phytochemicals were expected to be present in the ethanolic extract of the mixture of *Chromolaena odorata* and *Sida acuta* leaves; terpenes, tannins, saponin, phlobatannin, cardiac glycoside, flavonoids, anthraquinone, phenol, alkaloids and steroids.

SC1 and SC2 form ethanolic extracts of the mixture of *Chromolaena odorata* and *Sida acuta* leaves. Thus, the results from the two extracts are include; saponins, flavonoids, tannins, steroids, terpenoids, phenol, phlobatannin and gum and mucilage. The results do not agree to the results from Adeniyi *et al.* (2010) and Anyasor *et al.* (2011). This is because, alkaloids and anthraquinones are absent. This may be as a result of the

difference in concentrations of the ethanol used in the extraction method by the researcher.

Again, according to Anyasor *et al.* (2011), the following phytochemicals are present in the aqueous extract of *Chromolaena odorata* leaves; tannin, saponin, phlobatannin, phenol and alkaloid whereas terpenoid, cardiac glycoside, flavonoids, cardenolides, anthraquinone and volatile oil were absent.

The aqueous extract of *Sida acuta* leaves according to Senthilkumar *et al.* (2018) showed the following chemical constituents; alkaloids, steroids, flavonoids, phenols, terpenoids and cardiac glycosides.

When the two works were married, the following phytochemicals were expected; terpenoids, tannin, saponins, phlobatannin, cardiac glycosides, flavonoids, phenols and alkaloids.

The aqueous extract showed the following chemical constituents; saponins, flavonoids, alkaloids, tannins, steroids, terpenoids, phenol, phlobatannin, and gum and mucilage.

The results of the aqueous extracts agree with the work by Senthilkumar *et al.* (2018) and Anyasor *et al.* (2011

Nine out of eleven phytochemicals were successfully extracted from the mixture of *Chromolaena odorata* and *Sida acuta* leaves in SC3 and SC4 while seven out of eleven phytochemicals were successfully extracted in SC1 and SC2. This means 30% methanol and distil water are most effective in extracting *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves, which agree to Truong *et chal.* (2019) that methanol was most effective in extracting *Severinia buxifolia.* Abdullahi and Haque (2020) confirmed that water is capable of dissolving wide range of chemicals.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.0 Overview

This section of the study provides the summary, conclusions and recommendations for the study.

6.1 Summary of Findings

The study was conducted on the wounds of thirty albino rats by using mature leaves of *Chromolaena odorata* L. and *Sida acuta* Burm. F. that are located at Assin Andoe in the Central Region of Ghana. Convenience sampling was employed to select the two herbs for the study.

The purpose of the study was to determine the efficacy of different solvent extracts of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves on wound healing of albino rats. Four menstrua were used to extract the powdered form of the mixture; which are 70% ethanol, 30% ethanol, 30% methanol and distil water. Penicillin ointment was used as the control. The efficacy of the various extracts was determined by the rate of reduction of the wound area in the albino rats for a period of fifteen days.

Measurement of the wound areas as well as the masses of the rats were taken on three days intervals for the fifteen days and were as well represented on line graphs. Percentage wound reduction for the five groups of the albino rats was also calculated and represented on a line graph. The feeding behaviour of the albino rats and the surface of the wounds were observed as well. Phytochemical constituents of the mixture of the *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves were determined. One-way ANOVA was conducted to determine the significant difference among the average

wound areas for the five groups of the rats at a level of significance 0.05, whereas Pearson's correlation coefficients were determined to ascertain the linear relationship between the average mass and the average wound area for each of the groups of the rats. All data was analyzed using SPSS version 20.

6.1.1 Observation of the wound surface and eating habit of the albino rats

From the observation of the wound surface, there was improvement in the wound surface from day 1 of the experiment through to day fifteen. All the extracts together with the control enhanced the formation of scar tissue which aided the wound healing process. The rats fed well throughout the fifteen days. They ate all the food provided them.

6.1.2 Results on the observation of the wound areas and percentage wound reduction in the albino rats

The wound areas of the albino rats on day 1 was approximately equal for all the groups of the rats. There was significant reduction of the wound area in all the groups by the fifteenth day of the experiment.

On day 1, there was 0.00% reduction of wound area for all the five groups of the albino which signifies the initial stages of the wound healing process on the first day.

By day 12, the wound area was closed in the following groups; PO, SC2, SC3 and SC4 whereas the wound area of SC1 was completely closed by day fifteen.

100.00% reduction of wound area was observed in PO, SC2, SC3 and SC4 on the twelfth day while SC1 achieved 100.00% reduction on day 15.

The closure of the wound areas indicated the efficacy of the various extract of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. Again, there was no

significant difference among the means of the wound areas of the five groups of the rats.

6.1.3 Results on the average mass of the rats

The rats had different masses on day 1 and remained so throughout the fifteen days of the experiment. The average masses of the five groups were not stable and showed fluctuations on different days through to day 15.

By the fifteenth day, the masses of the rats increased significantly. The eating habits of the rats influenced the weight gain in the rats, thus the increment in the masses of the rats.

6.1.4 Correlation coefficient between the average mass and the average wound area of the rats

The Pearson's correlation coefficient between the average mass and the average wound area was determined for the five groups of the rats.

The masses and wound areas were negatively correlated, according to the correlation coefficients. Accordingly, the masses increase as the rat wound area decreases and vice versa. The linear correlations between the groups vary in strength, though negative relationships are stronger in Groups 3 and 4, while in Groups 1, 2, and 5, correlations are weak.

There is no significant linear relation between the average mass and the average wound area in groups 1, 2, 3, and 5. Therefore the researcher fails to reject the null hypothesis. Thus, there is no linear correlation between the different extracts of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves and the masses of the rats.

On the other hand, there is a significant linear relation between the mass and the wound area in group 4. Therefore, the researcher rejects the null hypothesis. Thus, there is a

linear correlation between the different extracts of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves and the masses of the rats.

6.1.5 The phytochemical constituents of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves extracts

The following chemicals were screened for; Saponins, flavonoids, tannins, alkaloids, phlobatannin, steroids, terpenoids, phenol, glycosides, anthraquinone and gum and mucilage. Saponins, flavonoids, tannins, steroids, terpenoids, phenol and gum and mucilage were present in SC1. Saponins, flavonoids, tannins, steroids, terpenoids, phlobatannin and gum and mucilage were present in SC2. Saponins, flavonoids, alkaloids, tannins, steroids, terpenoids, phenol, phlobatannin and gum and mucilage were present in SC3. In SC4, saponins, flavonoids, alkaloids, tannins, steroids, terpenoids, phenol, phlobatannin and gum and mucilage were present.

Nine out of eleven phytochemicals were successfully extracted from the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves in SC3 and SC4 while seven out of eleven phytochemicals were successfully extracted in SC1 and SC2. This proves that, 30% methanol and distil water are most effective in extracting *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves.

6.2 Conclusions

From the study, it is concluded that:

- The four solvent extracts of the mixture of the dried leaves of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves had significant effect in healing wounds in albino rats.
- Application of the extracts on the wounds of the albino rats had no significant effect on the eating behaviour of the rats.

- There was no significant linear relationship between the average mass and the average wound area of the groups of the rats except in group 4 that were treated with 30% methanolic extract.
- Distil water and 30% methanol were most effective in the extraction of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves.

6.3 Recommendations

On the basis of the findings of the study, the following recommendations were made:

- Quantitative analysis of the phytochemical constituents of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves should be done to ascertain the amount of each chemical present in the mixture.
- Other parts of *Chromolaena odorata* L. and *Sida acuta* Burm. F. such as the roots or stems should be worked on to investigate their efficacy on wound healing.
- Acute toxicity test should be done on the albino rats to investigate the effect of the mixture of *Chromolaena odorata* L. and *Sida acuta* Burm. F. leaves on vital organs such as the kidney and the liver.

REFERENCES

Abdullahi, R. A. & Haque, M. (2020). Preparation of Medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy & Bioallied Sciences,* 12(1), 1-10.

Adeniyi, S. A., Orjiekwe, C. L., Ehiagbonare, J. E. & Arimah, B. D. (2010). Preliminary phytochemical analysis and insecticidal activity of ethanolic extracts of four tropical plants(*Vernonia amygdalina, Sida acuta, Ocimum gratissimum* and *Telfaria occidentalis*) against beans weevil. *Academic journals*. Retrieved from https://academicjournals.org/journal/IJPS/article-full-text-pdf/CA0348A26825.pdf

Ajayi, I. A., Ajibade, O., & Oderinde, R. A. (2011). Preliminary phytochemical analysis of some plant seeds. *Res. J. Chem. Sci.*, 1, 58-62.

Akilandeswari, S., Senthamarai, R., Valarmathi, R., & Prema, S. (2010). Wound healing actvity of *Sida acuta* in rats. *International Journal of PharmTech Research*, 2(1), 585-587.

Alamgir, A. N. (2017). Pharmacognostical Botany: Classification of medicinal and aromatic plants (MAPs), botanical taxonomy, morphology, and anatomy of drug plants. *Therapeutic Use of Medicinal Plants and Their Extracts*, 1, 177-293. doi:https://doi.org/10.1007/978-3-319-63862-1_6

Alamgir, A. N. (2018). Secondary metabolites: Secondary metabolic products consisting of C and H; C, H, and O; N, S, and P elements; and O/N heterocycles. *Therapeutic Use of Medicinal Plants and their Extracts,* 2, 165 - 309.

Alhashim, M. & Lombardo, J. (2020). Effect of Topical Garlic on Wound Healing and Scarring. A clinical trial, 46(5), 618-627 . doi:10.1097/DSS.0000000000002123

Alsamarrai, A. Z., Al-Samarrai, R. R., & Alsamarrai, A. (2020). Isolation and identification of flavonoids from Arctium lappa stem and study the hepato protective effect on acetaminophen induced liver damage. *International Journal of Psychosocial Rehabilitation*, 24(5), 1009 - 1018.

Anjos, B. L., Peixoto, P. V., Caldas, S. A., Bhaltazar, D., França, T. N., & Armién, A. G. (2016). Poisoning by the swainsonine - containing plant Sida carpinifolia in captive Sambar deer (Cervus unicolor). *National Library ofmedicine*, 47(3), 1048-1052.

Anstead, G. M. (1998). Steroids, retinoids, and wound healing. *Advances in Wound Care: The Journal for Prevention and Healing*, *11*(6), 277-285.

Anyasor, G. N., Aina, D. A., Olushola, M. & Aniyikaye, A. F. (2011). Phytochemical constituent, proximate analysis, antioxidant, antibacterial and wound healing properties of leaf extracts of *Chromolaena odorata*. In *Annals of Biological Research, 2, 441-451.*

Austrialia Wound aware (2021). Wound aware. Retrieved from Wounds Austrialia healing wounds together: http://www.woundaware.com/au/what-is-a-wound/

Ayodeji, F. B., & Olorunsola, O. E. (2010). Siam weed along highways, herbal medicine or poison? *Toxicological and Environmental Chemistry*, 93, 487-493. doi:10.1080/02772248.2010.540243

Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal and Aromatic Plants*, 4(196), 2167-2412.

Bałan, B. J., Niemcewicz, M., Kocik, J., Jung, L., Skopinska-Rozewska, E., & Skopinski, P. (2014). Oral administration of Aloe vera gel, anti-microbial and anti-inflammatory herbal remedy, stimulates cell-mediated immunity and antibody production in a mouse model. *Central-European journal of immunology,* 39(2), 125-130.

Banerjee, A., & Chaudhury, S. (2010). Statistics without tears: Populations and samples. *Industrial psychiatry journal,* 19(1), 60-65.

Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International Journal of Advanced Research in Chemical Science*, 2(4), 25-32.

Barreto, R. S., Albuquerque-Junior, R. L. & Quintans-Junior, L. J. (2014). A systematic Review of the Wound-Healing Effects of Monoterpenes and Iridoid Derivatives. *Molecules*, 19(1), 846-862.

Basak, S., Samanta, K. K., Chattopadhyay, S. K., & Pandit, P. (2018). Sustainable coloration and value addition to textiles. *Handbook of Renewable Materials for Coloration and Finishing*, 523-543.

Bhanja, A., Sutar, P. P., & Mishra, M. (2022). Inulin-A polysaccharide: Review on its functional and prebiotic efficacy. *Journal of Food Biochemistry*, 46(12), 143-150.

Bhattacharjee, B., Lakshminarasimhan, P., Bhattacharjee, A., Agrawala, D. K., & Pathak, M. K. (2013). *Vernonia amygdalina* Delile (Asteraceae)–An African medicinal plant introduced in India. *Zoo's Print*. Retrieved from https://d1wqtxts1xzle7.cloudfront.net/31297939/Vernonia_amygdalina_paper

Biosecurity Act (2020). Restricted invasive plant, Siam weed. Retrieved from https://www.daf.qld.gov.au/__data/assets/pdf_file/0015/50028/siam-weed.pdf#:~:text=All%20other%20Siam%20species%20are%20prohibited%20invasive%20plants,plan%20that%20covers%20invasive%20plants%20in%20its%20area.

Britannica. (2023). Tumeric. Retrieved from Encyclopedia Britannica: https://www.britannica.com/plant/tumeric

Chanda, S. & Ramachandra, T. V. (2019). Phytochemical and Pharmacological Importance of Turmeric (*Curcuma longa*): A Review. Research & Reviews: *A Journal of Pharmacology*, 9(1), 16-23.

Chandel, S., Das, S., Mazumder, A., & Chauhan, V. S. (2023). An overview on phytoconstituents and multiple biological activities of *Euphorbia hirta*. *Plant Science Today, 10 (3),* 281-299.

Che, C. T., Wang, Z. J., Chow, M. S. S., & Lam, C. W. K. (2013). Herb-herb combination for therapeutic enhancement and advancement: theory, practice and future perspectives. *Molecules,* 18(5), 5125-5141.

Chokotho, L. & Hasselt, E. (2005). The use of tannins in the local treatment of burn wounds - a pilot study. Malawi Medical Journal: *The Journal of Medical Association of Malawi*, *17 (1),* 19 - 20.

Cooper, R. & Nicola, G. (2014). Natural products chemistry: sources, separations and structures. *CRC press.*

CSIR (2023). Indigenous Knowledge on Forest Foods & Medicinal Plants in Ghana. Retrieved from The Council of Scientific and Industrial Research: https://csir-forig.org.gh/tikfom/*sida-acuta*.

Da-Silva, L. M., Allemand, A., Mendes, D. A. G., Dos Santos, A. C., André, E., De Souza, L. M., . & Werner, M. F. (2013). Ethanolic extract of roots from *Arctium lappa* L. accelerates the healing of acetic acid-induced gastric ulcer in rats: Involvement of the antioxidant system. *Food and Chemical Toxicology*, 51, 179-187.

Dapson, R. W. & Bain, C. L. (2015). Brazilwood, sappanwood, brazilin and the red dye brazilein: from textile dyeing and folk medicine to biological staining and musical instruments. *Biotechnic and Histochemistry*, 90(6), 401-423.

Darko, I. N. (2009). *Ghanaian Indigenous Health Practices: The Use of Herbs*. A thesis submitted in for the degree of Master of Arts, 1-4. Retrieved from https://central.bac-lac.gc.ca/.item?id=MR59644&op=pdf&app=Library&oclc_number=784285012

De-Souza, A. R. C., De-Oliveira, T. L., Fontana, P. D., Carneiro, M. C., Corazza, M. L., de Messias Reason, I. J., & Bavia, L. (2022). Phytochemicals and biological activities of burdock (*Arctium lappa* L.) extracts: A review. *Chemistry and Biodiversity*, 19(11), 615-640.

Dhawan, D. & Gupta, J. (2017). Comparison of Different Solvents for Phytochemical Extraction Potential from Datura metel Plant Leaves. *International Journal of Biological Chemistry*, *11 (1),* 17 - 22. Retrieved from https://scialert.net/abstract/?doi=ijbc.

Editorial staff (2023). Important Health Benefits of Bitter Leaf (*Vernonia amygdalina*). Retrieved from Pharmchoices: https://pharmchoices.com/health-benefits-bitter-leaf-vernonia-amygdalina/?expand_article=1

Eisendrath, E. R. (1961). Portraits of Plants. A Limited Study of the" Icones". *Annals of the Missouri Botanical Garden,5,* 291-327. doi:https://doi.org/10.2307/2395120

Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, *4*, 177-180.

Eming, S. A., Krieg, T., & Davidson, J. M. (2007). Inflammation in wound repair: molecular and cellular mechanisms. *Journal of Investigative Dermatology*, 127(3), 514-525.

Ezeja, M. I., Anaga, A. O., & Asuzu, I. U. (2012). Effect of *Chromolaena odorata* leaf extract on wound healing in rats. *African Health Sciences*, *151*(3), 506-513.

Ezike, A. C., Akah, P. A., Okoli, C. O., Udegbunam, S., Okwume, N., Okeke, C. & Iloani, O. (2010). Medicinal plants used in wound care: A study of *Prosopis africana* stem bark. In *Indian Journal of Phamaceutical Sciences*. *72*, 334-340. Nigeria: Wolters Klower-Medknow Publications.

Flanagan, G. J., Hills, L. A. & Wilson, C. G. (1999). The successful biological control of spinyhead sida, *Sida acuta* [Malvaceae], by *Calligrapha pantherina* (Col: Chrysomelidae) in Australia's Northern Territory. *Proceedings of the X International Symposium on Biological Control of Weeds*, *4,* 4-14.

Froldi, G. (2023). The Use of Medicinal Plants in Blood Vessel The Use of Medicinal Plants in Blood Vessel Diseases: The Influence of Gender. *Life*, 13, 866-870.

Frost, J. (2023). F - table. Retrieved from Statistics by Jim: https://statisticsbyjim.com/hypothesis-testing/f-table/

Ganapathy, A. A., Priya, V. H., & Kumaran, A. (2021). Medicinal plants as a potential source of Phosphodiesterase-5 inhibitors: A review. *Journal of Ethnopharmacology*, 267, 113536. doi:https://doi.org/10.1016/j.jep.2020.113-120.

Gao, Q., Yang, M., & Zuo, Z. (2018). Overview of the anti-inflammatory effects, pharmacokinetic properties and clinical efficacies of arctigenin and arctiin from *Arctium lappa* L. Acta Pharmacologica Sinica, 39(5), 787-801.

GBIF secretariat (2022). *Euphorbia hirta.* Retrieved from GBIF Backbone Taxonomy: https://doi.org/10.15468/39omei

Gbotolorun, S. C., Osinubi, A. A., Noronha, C. C., & Okanlawon, A. O. (2006). Effect of *Sida acuta* leaf extract on wound healing in rats. *African Journal of Biomedical Research,5,* 163-167.

Ghanaweb (2020, July 30th). 10 indigenous plants that have saved Ghanaian lives for centuries. Retrieved from Ghana web: https://mobile.ghanaweb.com/GhanaHome.

Ghosh, A., Chowdhury, N., & Chandra, G. (2008). Laboratory evaluation of a phytosteroid compound of mature leaves of Day Jasmine (Solanaceae: Solanales) against larvae of Culex quinquefasciatus (Diptera: Culicidae) and nontarget organisms. *Parasitology Research,* 103, 271-277.

Guest, G. (2014). Sampling and selecting participants in field research. *Handbook of methods in cultural anthropology*, 2, 215-250.

Gupta, M., Mishra, V., Gulati, M., Kapoor, B., Kaur, A., Gupta, R., & Tambuwala, M. M. (2022). Natural compounds as safe therapeutic options for ulcerative colitis. *Inflammopharmacology*, 30, 397-434.

Gutierrez-Reyes, J. E., Caldera-Villalobos, M., Claudio-Rizo, J. A., Cabrera-Munguía, D. A., Becerra-Rodriguez, J. J., Soriano-Corral, F., & Herrera-Guerrero, A. (2023). Smart collagen/xanthan gum-based hydrogels with antibacterial effect, drug release capacity and excellent performance in vitro bioactivity for wound healing application. *Biomedical Materials*, 18(3), 11-20.

Harborne, J. B. (1998). A guide to modern techniques of plant analysis (3rd ed.). London: Chapman & Hall. Retrieved from https://books.google.com.gh/books?hl=en&lr=&id=2yvqeRtE8CwC&oi=fnd&pg=PR7&dq=Harborne,+J.+B.+(1998).+A+guide+to+modern+techniques+of+plant+analysis+(3rd+ed.).+London:+Chapman+%26+Hall.&ots=xAjjP1TlT7&sig=JNYNtnrJqCFzYOAVsdWNHoAiDps&redir_esc=y#v=onepage&q

Hartzell, V., & Wilkes-Barre. (2001). Medical Attributes of *Arctium sp.* - Burdock. Retrieved from https://klemow.wilkes.edu/Arctium.html

Hassanshahi, N., Masoumi, S. J., Mehrabani, D., Hashemi, S. S., & Zare, M. (2020). The healing effect of *Aloe vera* gel on acetic acid-induced ulcerative colitis in rat. *Middle East Journal of Digestive Diseases*, 12(3), 154-160.

Helmy, S. A., Mohamed, R. H., & Omar, A. G. (2020). Complementary and alternative medicine use among patients with metabolic syndrome in Egypt: A cross-sectional survey. *BMC Complementary Medicine and Therapies*, *5*, 1-11.

Hill, F. A. (1952). Economic Botany, A textbook of useful plants and plant products. New York: McGraw-Hill Book Company Inc.*12,* 560-580.

Igwe, R. O., Udo, H., Ogunnupedi, J. T., Olorunleke, S. O., Aikpitanyi, K. U. & Nwose, R. N. (2020). Efficacy of *Chromolaena odorata* (Siam weed) on performance, haematological and serum biochemical indices of broiler chicken. *Journal of Animal Science,* 22 (2), 106-113.

iNaturalist. (n. d.). *Prosopis africana*. Retrieved from iNaturalist: https://www.inaturalist.org/guide_taxa/491127

Isely, D. (2002). One hundred and one botanists. Retrieved from https://books.google.com.gh/books?hl=en&lr=&id=an6r8m0JfV8C&oi=fnd&pg=PR9&dq=The+tree+is+classified+under+the+genus+Caesalpinia,+which+is+named+after+the+Italian+botanist+Andrea+Cesalpino&ots=5pZarwtcpK&sig=SwtdVF_hCEMhh07elQw3VYgSFLw&redir_esc=y#v=onepage

Iwu, M. M., Duncan, A. R., Okunji, C. O., & Janick, J. (1990). New antimicrobials of plant origin. *Perspectives on New Crops and New Uses*, *13 (4)*, 457-462.

Jangale, V. J. (2022). Single Case Report on Effect of Ayurvedic Treatment Regimen in the Management of Psoriasis. *International Journal of Ayurvedic and Herbal Medicin, 12 (4)*, 279-283 . Retrieved from https://doi.org/10.47191/ijahm/v12i405.

Jeong, I. Y., Jin, C. H., Park, Y. D., Lee, H. J., Choi, D. S., Byun, M. W., & Kim, Y. J. (2008). Anti-inflammatory Activity of an Ethanol Extract of Caesalpinia sappan L. in LPS-induced RAW 264.7 Cells. Preventive Nutrition and Food Science, 13(4), 253-258.

Joao -De- Masi, E., Campos, A., Joao- De- Masi, F. D. & Joao- De -Masi, R. D. (2016). The influence of growth factors on skin wound healing in rats. *Brazilian Journal of Otorhinolaryngology*, *82,* 512 - 521.

Joseph, B., & Raj, S. J. (2010). Pharmacognostic and phytochemical properties of *Aloe vera* linn an overview. *International Journal of Pharmaceutical Sciences*

Review and Research, *4*(2), 106-110. Retrieved from https://www.erboristeriarcobaleno.it/pdf/aloe/article_017.pdf

Kandhare, A. D., Dalvi, G., Ghosh, P., Venkata, S., Raygude, K. S., & Bodhankar, S. L. (2016). Ameliorative effect of berberine against gentamicin-induced nephrotoxicity in rats via attenuation of oxidative stress, inflammation, apoptosis and mitochondrial dysfunction. *Renal Failure*, *38*(6), 996-1006

Kanmegne, J., Duguma, B., Henrot, J. & Isirimah, N. (1999). *Soil fertility enhancement by planted tree-fallow species in the humid lowlands of Cameroon*. Cameroon: *Agroforestry Systems*, *46*, 239-249.

Kaur, D., Kaur, N., & Chopra, A. (2019). A comprehensive review on phytochemistry and pharmacological activities of *Vernonia amygdalina*. *Journal of Pharmacognosy and Phytochemistry*, 8(3), 2629-2636. Retrieved from https://www.phytojournal.com/archives/2019.v8.i3.8407/a-comprehensive-review-on-phytochemistry-and-pharmacological-activities-of-ltemgtvernonia-amygdalinaltemgt

Khan, S., Malik, F., Suri, K. A., Singh, J., & Singh, N. (2011). A standardized herbal composition (OA-F2) improves type II diabetes-induced metabolic endotoxemia. *Human and Experimental Toxicology*, *8,* 399-408.

Kim, Y. S., Cho, I. & Bae, C. S. (2011). Therapeutic Effect of Total Gingseng Saponin on Skin Wound Healing. *Journal of Gingseng Research*, *35*(3), 360-370.

Kshirsagar, A. D., Panchal, P. V., Harle, U. N., Nanda, R. K., & Shaikh, H. M. (2014). Anti-inflammatory and antiarthritic activity of anthraquinone derivatives in rodents. *International Journal of Inflammation*, *14,* 1-12.

Kujath, P. & Michelsen, A. (2008). Wounds -from physiology to wound dressing. *Deutsches Ärzteblatt International*, *105*(13), 239-250.

Kumar, D., Arya, V., Kaur, R., Bhat, Z. A., & Gupta, V. K. (2010). Hypoglycemic activity of *Coccinia indica* and *Cassia auriculata* in diabetic rats. *Pharmaceutical Biology*, *4*, 44-63.

Kumar, M., Sharma, S., & Gupta, Y. K. (2014). Efficacy of a novel herbal formulation (RAFO) in patients with coronary artery disease: A randomized, double-blind, placebo-controlled study. *Evidence-Based Complementary and Alternative Medicine* *27*(23), 8316.

Kumar, S., Yadav, M., Yadav, A., & Yadav, J. P. (2017). Impact of spatial and climatic conditions on phytochemical diversity and in vitro antioxidant activity of Indian *Aloe vera* (L.) Burm. f. *South African Journal of Botany*, *8,* 50 - 59.

Lindholm, C. & Searle, R. (2016). Wound management for the 21st century: combining effectiveness and efficiency. *International Wound Journal, 13*, 5-15.

Lonsdale, W. M., Farrell, G., & Wilson, C. G. (1995). Biological control of a tropical weed: a population model and experiment for *Sida acuta*. Journal of Applied Ecology, *5,* 391-399.

Majewska, I., & Gendaszewska-Darmach, E. (2011). Proangiogenic activity of plant extracts in accelerating wound healing-a new face of old phytomedicines. *Acta Biochimica Polonica,* 58(4), 300-321.

Matthew, S. & Brand, K. (2004). Africa invaded: the growing danger of invaded aliwn species. In *The Global Invasive Species Programme Secretariat*. South Africa: www.gisp.org/downloadpubs/gipsAfrica.pdf.

Maver, T., Kurecic, M., Smrke, D. M., Kleinschek, K. S. & Maver, U. (2017). Plant - Derived Medicines with Potential Use in Wound Treatment. *Herbal medicine. 529*(2), 576-588.

Merriam-Webster dictionary (2022). Merriam-Webster dictionary. *Retrieved from https://www.merriam-webster.com/dictionary/wound*

Miraldi, E., Ferri, S., & Mostaghimi, V. (2001). Botanical drugs and preparations in the traditional medicine of West Azerbaijan (Iran). *Journal of Ethnopharmacology*, *75*(2-3), 77-87.

Mohan, V., Khanna, P., & Pant, B. (2011). A herbal combination of *Gymnema sylvestre*, *Momordica charantia*, and *Trigonella foenum-graecum* for type 2 diabetes: a randomized controlled trial. *Journal of Clinical Biochemistry and Nutrition*, *12,* 72-77.

Mohd, J., Shah, Y., Omar, E., Pai, D. R., & Sood, S. (2012). Cellular events and biomarkers of wound healing. *National Library of Medicine*, *45*(2), 220 - 228.

Mukherjee, P. (2002). Quality control of herbal drugs. In *An Approach to Evaluation of Botanicals*. New Delhi, India: Business Horizons Publisher.

Nafiu, A. B., Akinwale, O. C., Akinfe, O. A., Owoyele, B. V., Abioye, A. I. R., Abdulazeez, F. I., & Rahman, M. T. (2016). Histomorphological evaluation of wound healing-comparison between use of honey and *Vernonia amygdalina* leaf juice. *Nigeria. Trop. J. Health Sci*, *23*(3), 10-11.

Nagori, B. P., & Solanki, R. (2011). Role of medical plants in wound healing. In *Research Journal of Medicinal Plant*. 5(4), 392-405.

Nandi, K., Ghosh, R., Mondal, S., Sen, D. J., & Saha, D. (2021). Source, isolation & impact of glycone and aglycone in human body. *World Journal of Pharmaceutical Sciences, 5,* 103-113. Retrieved from https://doi. org/10.54037/WJPS. 2021.91107

Nava-Tapia, D. A., Cayetano-Salazar, L., Herrera-Zúñiga, L. D., Bello-Martínez, J., Mendoza-Catalán, M. A., & Navarro-Tito, N. (2022). Brazilin: Biological activities and therapeutic potential in chronic degenerative diseases and cancer. *Pharmacological Research*, doi:https://doi.org/10.1016/j.phrs.2021.106023, *175*, 106023.

Nirmal, N. P., Rajput, M. S., Prasad, R. G., & Ahmad, M. (2015). Brazilin from Caesalpinia sappan heartwood and its pharmacological activities: A review. Asian Pacific Journal of Tropical Medicine, 8(6), 421-430.

Nwokocha, L. M. (2021). Galactomannans. In *Handbook of Hydrocolloids, 23,* 273-293). Woodhead Publishing.

Obode, O. C., Adebayo, A. H., & Chunyang, L. (2020). Phytochemical and toxicological evaluations of *Prosopis africana* (GUILL. and PERR.) extract on albino wistar rats. *National Library of Medicine, 37,* 183-195. doi:10.1007/s43188-020-00052-3

Oduwegwu, E., Mgbenka, B. O., Onyishi, G. C., Okafor, F. C., Ejere, V. C. & Eyo, J. E. (2017). Wound healing potentials of wire weed (*Sida acuta* Burman, 1768) in guinea pig (*Cavia porcellus* linnaeus, 1758). *Pharmacology online*, *2,* 130-140.

Okaiyeto K. & Oguntibeju. O. O., (2021). African herbal medicines: Adverse effects and cytotoxic potentials with different therapeutic applications. *International Journal of Environmental Research And Public Health.*

Omeroglu, P. Y., Acoglu B., Ozdal T., Tamer, C.E. & Çopur, O.U. (2019). Extraction techniques for plant based bioactive compounds (Vol. 2). Singapore: Springer Nature. doi:https://doi.org/10.1007/978-981-13-7205-6

Omokhua, A. G., McGaw, L. J., Finnie, J. F., & Van-Staden, J. (2016). *Chromolaena odorata* (L.) RM King & H. Rob.(Asteraceae) in sub-Saharan Africa: A synthesis and review of its medicinal potential. *Journal of Ethnopharmacology, 183*, 112-122.

Pace, D. S. (2021). Probability and non-probability sampling-an entry point for undergraduate researchers. *International Journal of Quantitative and Qualitative Research Methods*, 9(2), 1-15.

Panyaphu, K., Van-On, T., Sirisa-Ard, P., Srisa-Nga, P., ChansaKaow, S., & Nathakarnkitkul, S. (2011). Medicinal plants of the Mien (Yao) in Northern Thailand and their potential value in the primary healthcare of postpartum women. *Journal of ethnopharmacology, 135(2)*, 226-237.

Pawar, C. R., Landge, A. D., & Surana, S. J. (2008). Phytochemical and pharmacological aspects of *Caesalpinia sappan*. *Journal of Pharmacy Research*, *1*(2), 131-138.

Payghan, S. P., Shrikhande, M. N., & Rushikesh, B. A. (2023). Comprehensive Review of 20 Herbal Drugs for Wound Healing. *International Journal of Pharmaceutical Research and Applications*, 8, 2090-2099. Retrieved from https://ijprajournal.com/issue_dcp/A%20Comprehensive%20Review%20of%20 20%20Herbal%20Drugs%20for%20Wound%20Healing.

PDF4PRO (2017). Table of Critical Values for Pearson's r. Retrieved from PDF4PRO: https://pdf4pro.com/amp/view/table-of-critical-values-for-pearson-s-r-59198f.html

Percival, N. J. (2002). Classification of Wounds and their Management. *Surgery (Oxford)*, *20* (5), 114-117. Retrieved from ScienceDirect: https://www.sciencedirect.com/science/article/abs/pii/S0263931906702277

Prabhavathi, R. M., Prasad, M. P. & Jayaramu, M. (2016). Studies on qualitative and quantitative phytochemical analysis of *Cissus quadrangularis*. *Advances in Applied Science Research,* 7(4), 11-17.

Rafiei, V., Vélëz, H., & Tzelepis, G. (2021). The role of glycoside hydrolases in phytopathogenic fungi and oomycetes virulence. *International Journal of Molecular Sciences, 22*(17), 935-940.

Raziyeva, K., Kim, Y., Zharkinbekov, Z., Kassymbek, K., Jimi, S., & Saparov, A. (2021). Immunology of acute and chronic wound healing. *Biomolecules*, 11(5), 700-733.

Riaz, S., Hussain, S., Syed, S. K., & Anwar, R. (2021). Chemical Characteristics and Therapeutic Potentials of *Aloe vera*. *RADS Journal of Biological Research and Applied Sciences*, *12*(2), 160-166.

Roghini, R. & Vijayalakshmi,K. (2018). Phytochemical screening, quantitative analysis of flavonoids and minerals in ethanolic extract of *Citrus paradisi*. *International Journal of Pharmaceutical Science and Research*, *9*(11), 4859-4864.

Rojas-Sandoval, J., Acevedo-Rodriguez, P. & Clements, D. (2022). CABI. Retrieved from https://www.cabi.org/isc/datasheet/49985

Rolnik, A. & Olas, B. (2021). The Plants of the Asteraceae Family as Agents in the Protection of Human Health. *Molecular Plant Sciences, 22*(6), 3009-3349. Retrieved from www.mdpi.com/1422-0067/22/6/3009

Rwang, P. G., Fabiyi, J. P., Suleiman, M. & Mercy, K. P. (2016). Evaluation and phytochemical analysis of *Prosopis africana* and *Erythrina senegalensis* used against immature stages of *Schistosoma haematobium. European Journal of Medicinal Plants, 13*(1), 1-9. doi:10.9734/EJMP/2016/17675

Sadgrove, N. J., & Simmonds, M. S. (2021). Pharmacodynamics of *Aloe vera* and acemannan in therapeutic applications for skin, digestion, and immunomodulation. Phytotherapy Research, *35*(12), 657-668.

Salama, M., & Salama, S. G. (2016). *Arctium lappa* L. (Asteraceae); a new invasive highly specific medicinal plant growing in Egypt. *Pyrex Journal of Plant and Agricultural Research, 5*(1), 1-10. Retrieved from;https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=0317e80c7cf0a85fff3ece091fc342b3f1bee153

Saleem, A., Naureen, I., Naeem, M., Murad, H. S., Maqsood, S., & Tasleem, G. (2022). *Aloe vera* gel effect on skin and pharmacological properties. *Sch. Int. J. Anat. Physiol*, *5*(1), 1-8.

Santos-Buelga, C., Mateus, N., & De Freitas, V. (2014). plant pigments and beyond. *In Journal of Rural and Food Chemistry*, 62, 687-689.

Sayed, M. D. (1980). Traditional medicine in health care. *Journal of Ethnopharmacology*, *2*(1), 19-22.

Senthilkumar, R. P., Bhuvaneshwari, V., Malayaman, V., Ranjithkumar, R., & Sathiyavimal, S. (2018). Phytochemical screening of aqueous leaf extract of *Sida acuta* burm. F. And its antibacterial activity. *Journal of Emerging Technologies and Innovative Research*, *5*(8), 474-478.

Shang, X. F. N. (2018). Biologically active quinoline and quinazoline alkaloids part I. *Medicinal research reviews*, *38*(3), 775-828.

Sharma, A., Khanna, S., Kaur, G., & Singh, I. (2021). Medicinal plants and their components for wound healing applications. *Future Journal of Pharmaceutical Sciences.*

Sharma, P., Parmar, J., Verma, P., Sharma, P., Goyal, P. K., & Sharma, P. (2013). A Comprehensive Study on Anticancer Herbal Drugs. *Journal of Cancer Research and Therapeutics*, *39*(3), 265-273.

Sharrif- Moghaddasi, M., & Res, M. (2011). *Aloe vera* their chemicals composition and applications: A review. *Int. J. Biol. Med. Res,* *2*(1), 466-471

Shedoeva, A., Leavesley, D., Upton, Z. & Fan, C. (2019). Wound healing and the use of medicinal plants. *Evidence-based complementary and alternative medicine, 21*, 135-143. *https://doi/org/10.1155/2019/2684108*.

Sileyew, K. J. (2019). Research design and methodology. *Cyberspace*, 7, 1-12.

Sirinthipaporn, A. & Jiraungkoorskul, W. (2017). Wound healing property review of Siam weed, *Chromolaena odorata. Pharmacognosy review*. *11(21)*, 35-45

Smet, P. A., Keller, K., Hänsel, R., & Chandler, R. F. (1993). *Arctium* Species (1 ed.). Springer Berlin Heidelberg, *2*, 141-146

Steele, C. (2022). Collagen: A Review of Clinical Use and Efficacy. *Nutr. Med. J.*, *1*(2), 12-36.

Stephane, F. F., Jules, B. K., Batiha, G. E., Ali, I., & Bruno, L. N. (2021). Extraction of bioactive compounds from medicinal plants and herbs. *Nat. Med. Plants, 2, 27-50.*

Tcheghebe, O. T., Seukep, A. J., & Tatong, F. N. (2017). Ethnomedicinal uses, phytochemical and pharmacological profiles, and toxicity of *Sida acuta* Burm. F. a review article. *The Pharma Innovation*. 6,1-20.

The Plant List (2013). *Vernonia amygdalina* Delile. Retrieved from The plant list: http://www.theplantlist.org/

Tiamiyu, A. M. & Okunlade, O. A. (2020). Benefits and detriments of Siam weed (*Chromolaena odorata*): A review. *Biochemistry and Biotechnology Research*, 8(2), 21-28

Truong, D. H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H., & Nguyen, H. C. (2019). Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *Severinia buxifolia*. *Journal of Food Quality*, *8*, 25-28. doi:https://doi.org/10.1155/2019/8178294

Ukwueze, S. E., Duru, O., & Shorinwa, O. (2013). Evaluation of the cutaneous wound healing activity of solvent fractions of *Chromolaena odorata* Linn. *Indo American Journal of Pharm Research*, *3(4)*, 316-323.

Valizadeh, R., Hemmati, A. A., Houshmand, G., Bayat, S., & Bahadoram, M. (2015). Wound healing potential of *Althaea officinalis* flower mucilage in rabbit full thickness wounds. *Asian Pacific Journal of Tropical Biomedicine*, *5(11)*, 937-943.

Velnar, T., Bailey, T. & Smrkolj, V. (2009). The wound healing process: an overview of the cellular and molecular mechanisms. *Journal of International Medical Research.* Slovenia. *37(5)*, 528-542.

Vijayaraghavan, K., & Ashokkumar, T. (2017). Plant-mediated biosynthesis of metallic nanoparticles: A review of literature, factors affecting synthesis, characterization techniques and applications. *Journal Of Environmental Chemical Engineering*, *5(5),* 4866-4883.

Vithalkar, A., Kaiwartya, K., & Patel, Y. K. (2022). Aloe Vera: A Nature's Prodigy. *InternationalJournalof Recent Advances in Multidisciplinary Research*, *10*, 313-318. Retrieved from https://www.ijramr.com/sites/default/files/issues-pdf/4342.pdf

Wang, N., Du, N., Peng, Y., Yang, K., Shu, Z., Chang, K. & Zhou, X. (2021). Network patterns of herbal combinations in traditional Chinese clinical prescriptions. *Frontiers, 11*, 590-598.

Wikipedia (2023). Garlic. Retrieved from Wikipedia: https://en.wikipedia.org/wiki/Garlic

Wikipedia (2023). *Prosopis*. Retrieved from Wikipedia: https://en.wikipedia.org/wiki/Prosopis#cite_note-environment.gov.au-16.

Wikipedia (2023). Wound healing. Retrieved from Wikipedia: https://en.m.wikipedia.org/wiki/wound_healing.

Wilkins, R. & Unverdorben, M. (2013). Wound cleaning and wound healing, a concise review. *Adv Skin Wound Care, 26*(4), 160-163.

Wound Care Surgeons. (2022). Know About Different types of Wounds. Retrieved from Wound Care Surgeons: https://www.woundcaresurgeons.org/blogs/know-about-different-types-of-wounds

Yeap, S. K., Ho, W. Y., Beh, B. K., Liang, W. S., Ky, H., Yousr, A. H. N., & Alitheen, N. B. (2010). *Vernonia amygdalina*, an ethnoveterinary and ethnomedical used green vegetable with multiple bioactivities. *Journal of medicinal plants research*, *4(25)*, 2787-2812. Retrieved from https://academicjournals.org/article/article1380714162_Yeap%20et%20al.pdf

Yeoh, S. (2000). The influence of iron and free radicals on chronic leg ulceration. In *Primary Intention, 8*, 47-56

Zhang, N., Wang, Y., Kan, J., Wu, X., Zhang, X., Tang, S. & Jin, C. (2019). In vivo and in vitro anti-inflammatory effects of water-soluble polysaccharide from *Arctium lappa*. *International Journal of Biological Macromolecules, 135*, 717-724.

Zhao, Y., Wang, X., Yang, S., Song, X., Sun, N., Chen, C., Zhang, Y., Yao, D., Huang, J., Wang, J. & Zhang, Y., (2020). Kanglexin accelerates diabetic wound healing by promoting angiogenesis via FGFR1/ERK signaling. *Biomedicine and Pharmacotherapy, 132*, 933-950.

Zohary, D. & Hopf, M. (2000). Domestication of plants in the Old World. *The origin and spread of cultivated plants in West Asia, Europe and the Nile Valley*. 3, 47-56 Oxford university press.

APPENDICES

APPENDIX I

Plates of Herbal Plants

Plate 2: Image of *Vernonia amygdalina* Delile

Plate 3*: Euphorbia hirta* L.

Plate 4: *Caesalpinia sappan* L.

Plate 5*: Arctium lappa* L.

Plate 6: Image of *Aloe vera* (L.) Burm. F.

Plate 7: powdered form of *Curcuma longa* L.

Plate 8: Rhizome of *Curcuma longa* L.

Plate 9: Image of *Prosopis africana* (Guill., Perrott, & Rich.) Taub.

Plate 10: Image of the Garlic plant

Plate 11: Image of the bulb of Garlic

Plate 12*: Chromolaena odorata* L.

Plate 13: Image of *Sida acuta* Burm. F.

APPENDIX II

Molecular structures of the phytochemicals

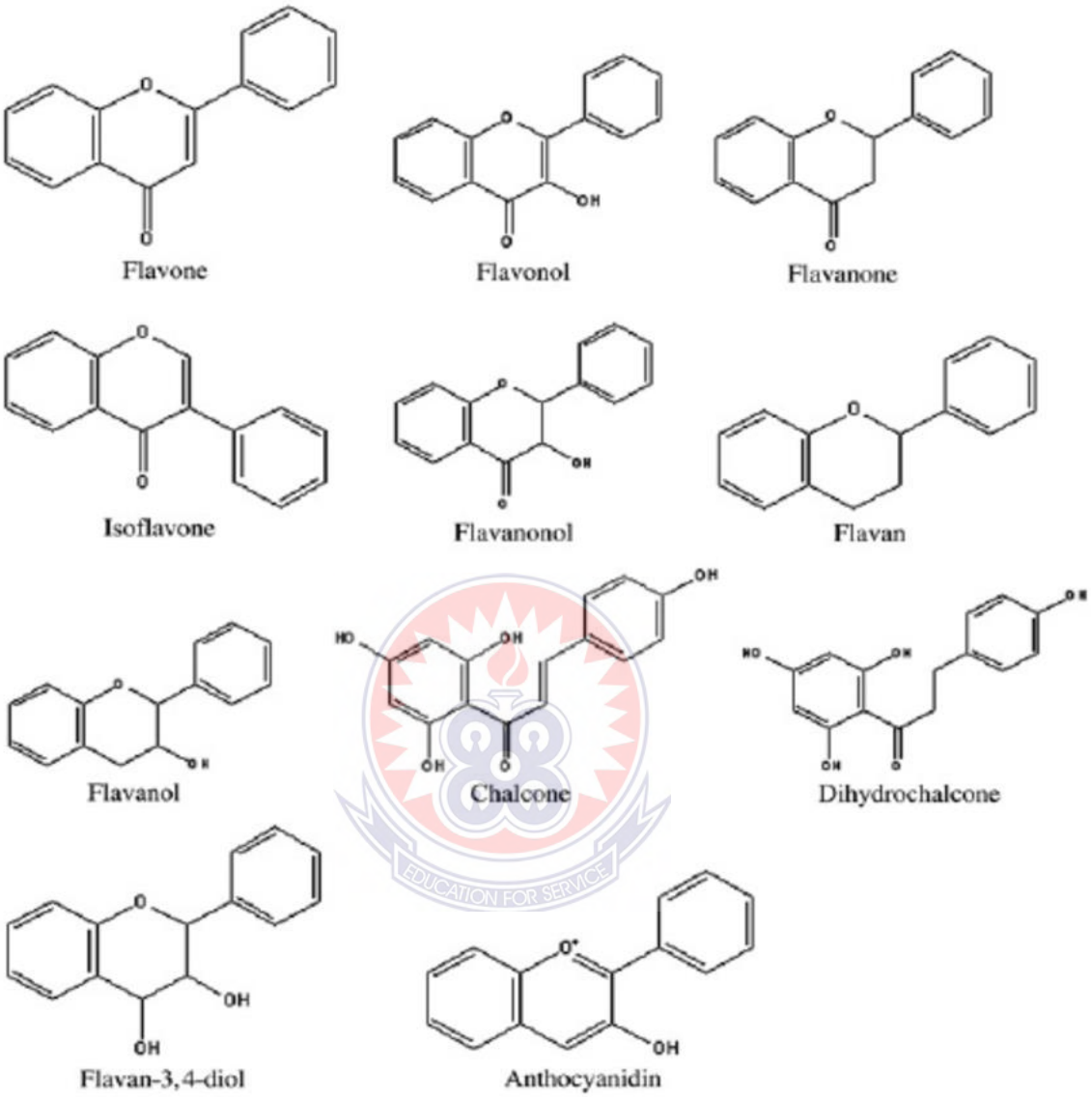
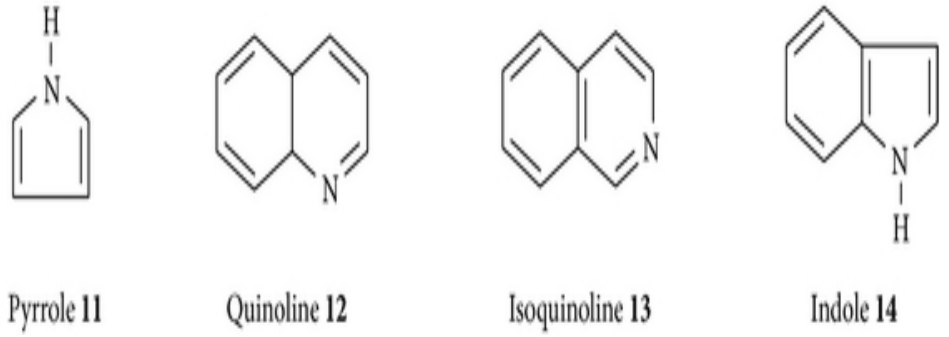
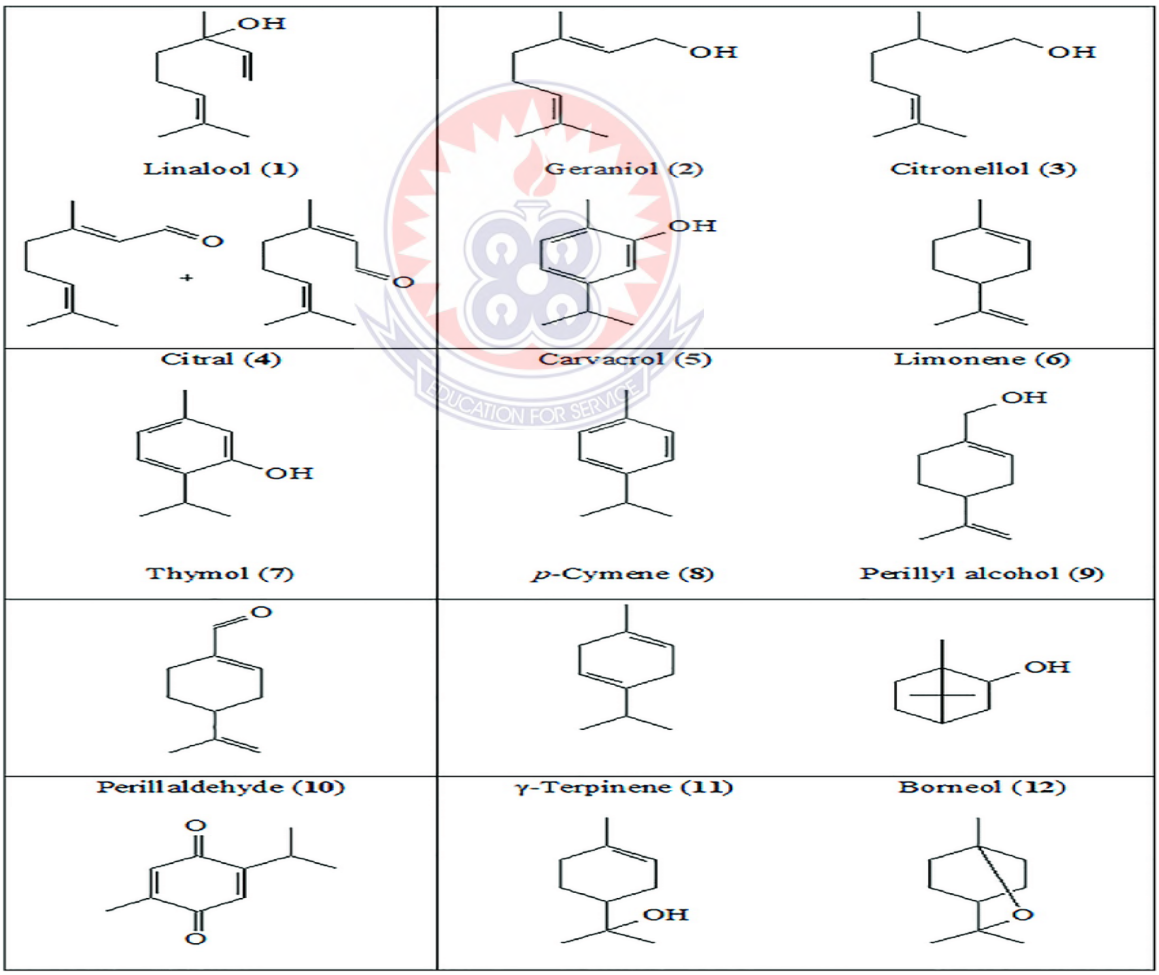


Fig. 7: Flavonoids



Pyrrole **11** Quinoline **12** Isoquinoline **13** Indole **14**

Fig. 8: Alkaloids



Linalool (1) Geraniol (2) Citronellol (3)

Citral (4) Carvacrol (5) Limonene (6)

Thymol (7) *p*-Cymene (8) Perillyl alcohol (9)

Perillaldehyde (10) γ-Terpinene (11) Borneol (12)

Fig. 9: Monoterpenes

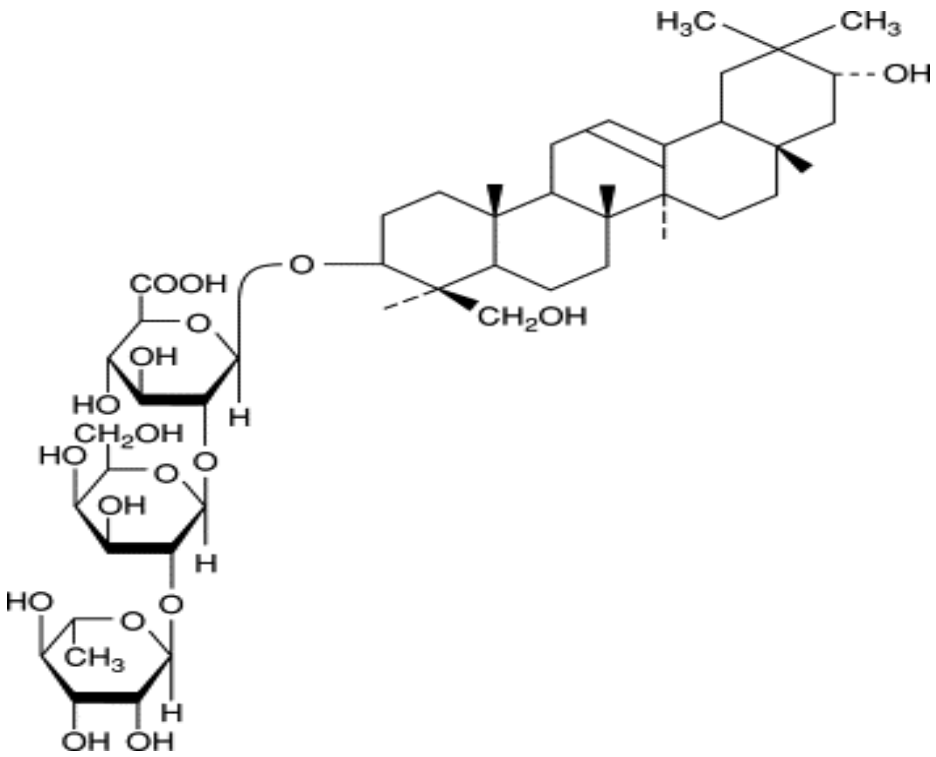
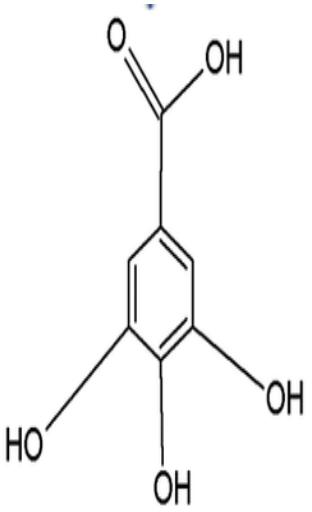
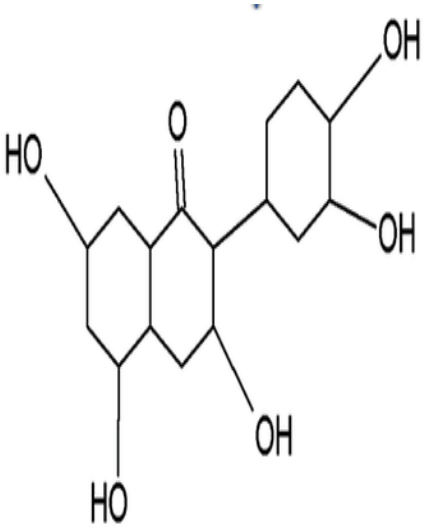


Fig. 10: Saponins

Fig. 11: Steroids





Hydrolysable tannins **Condensed tannins**

Fig. 12: Structures of tannins

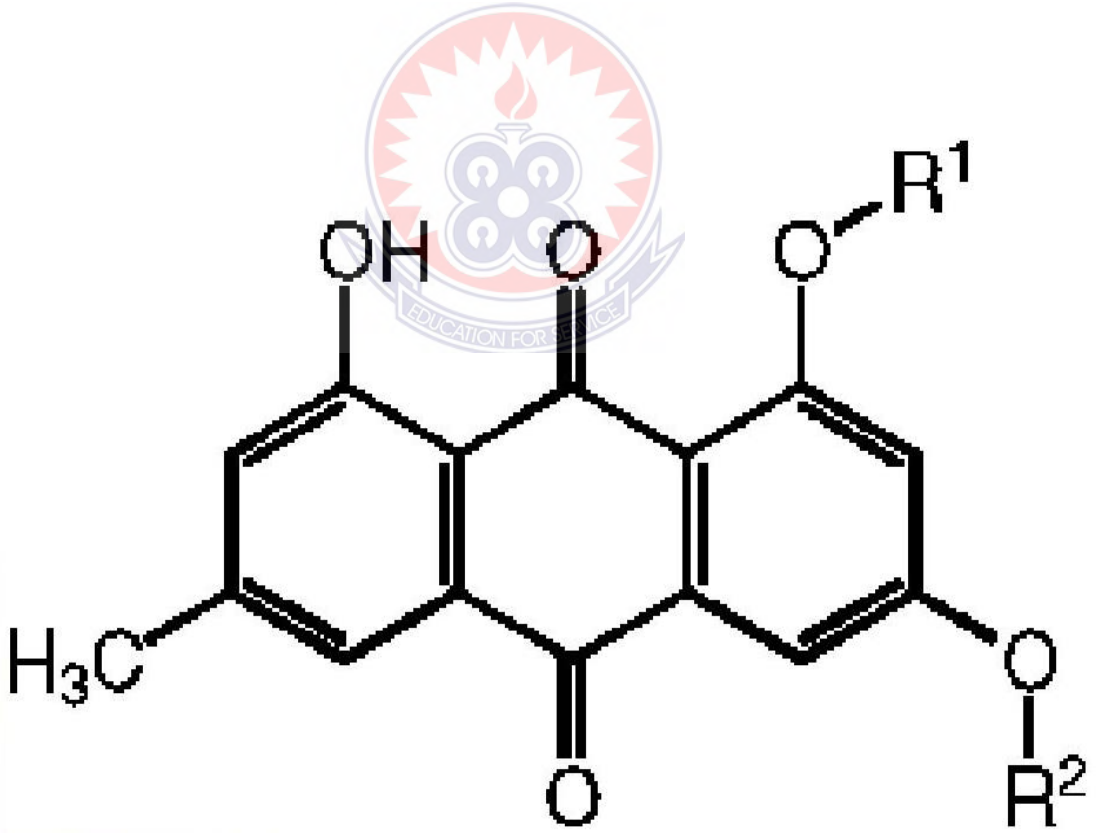


Fig. 13: Anthraquinone

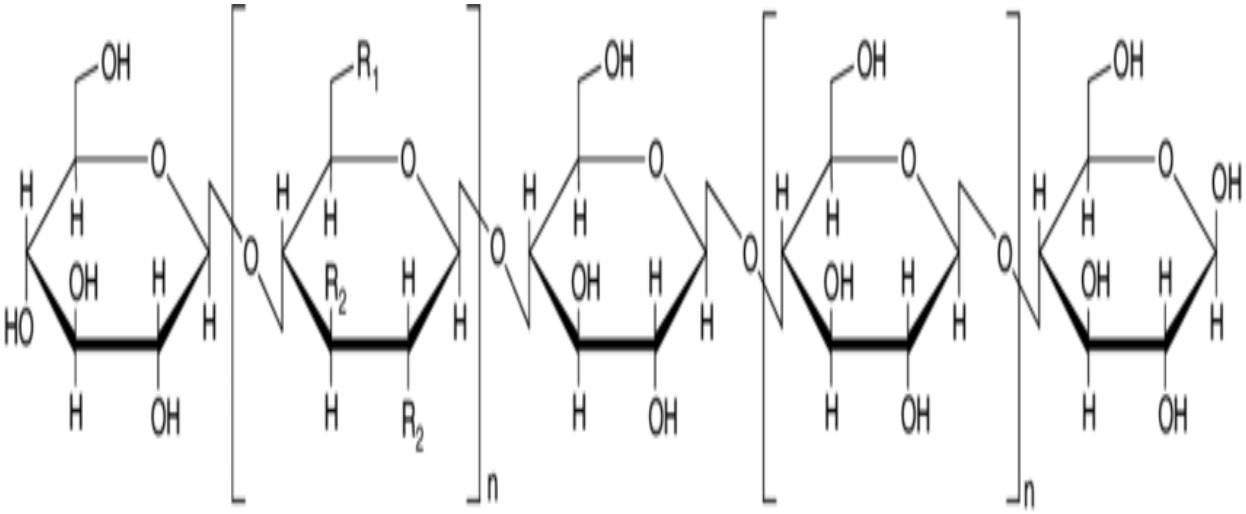


Fig. 14: molecular structure of mucilage in yellow mustard

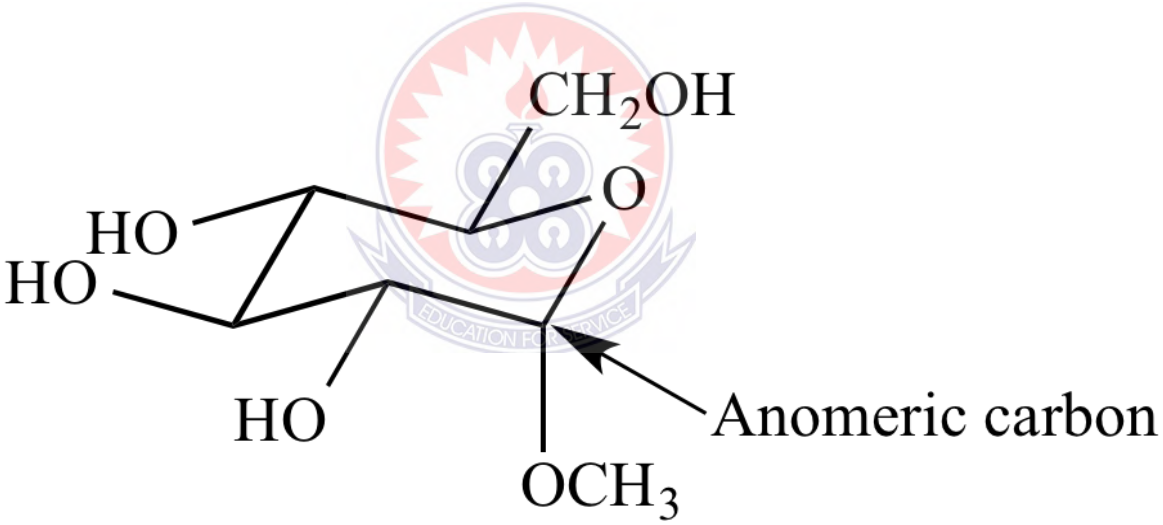


Figure 15: Glycosides

APPENDIX III

Table 9: Table of Critical Values for F - distribution

	F-table of Critical Values of α = 0.05 for F(df1, df2)																		
	DF1=1	2	3	4	5	6	7	8	9	10	12	15	20	24	30	40	60	120	∞
DF2=1	161.45	199.50	215.71	224.58	230.16	233.99	236.77	238.88	240.54	241.88	243.91	245.95	248.01	249.05	250.10	251.14	252.20	253.25	254.31
2	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38	19.40	19.41	19.43	19.45	19.45	19.46	19.47	19.48	19.49	19.50
3	10.13	9.55	9.28	9.12	9.01	8.94	8.89	8.85	8.81	8.79	8.74	8.70	8.66	8.64	8.62	8.59	8.57	8.55	8.53
4	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.91	5.86	5.80	5.77	5.75	5.72	5.69	5.66	5.63
5	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74	4.68	4.62	4.56	4.53	4.50	4.46	4.43	4.40	4.37
6	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	4.00	3.94	3.87	3.84	3.81	3.77	3.74	3.70	3.67
7	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.57	3.51	3.44	3.41	3.38	3.34	3.30	3.27	3.23
8	5.32	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35	3.28	3.22	3.15	3.12	3.08	3.04	3.01	2.97	2.93
9	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14	3.07	3.01	2.94	2.90	2.86	2.83	2.79	2.75	2.71
10	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98	2.91	2.85	2.77	2.74	2.70	2.66	2.62	2.58	2.54
11	4.84	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90	2.85	2.79	2.72	2.65	2.61	2.57	2.53	2.49	2.45	2.40
12	4.75	3.89	3.49	3.26	3.11	3.00	2.91	2.85	2.80	2.75	2.69	2.62	2.54	2.51	2.47	2.43	2.38	2.34	2.30
13	4.67	3.81	3.41	3.18	3.03	2.92	2.83	2.77	2.71	2.67	2.60	2.53	2.46	2.42	2.38	2.34	2.30	2.25	2.21
14	4.60	3.74	3.34	3.11	2.96	2.85	2.76	2.70	2.65	2.60	2.53	2.46	2.39	2.35	2.31	2.27	2.22	2.18	2.13
15	4.54	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54	2.48	2.40	2.33	2.29	2.25	2.20	2.16	2.11	2.07
16	4.49	3.63	3.24	3.01	2.85	2.74	2.66	2.59	2.54	2.49	2.42	2.35	2.28	2.24	2.19	2.15	2.11	2.06	2.01
17	4.45	3.59	3.20	2.96	2.81	2.70	2.61	2.55	2.49	2.45	2.38	2.31	2.23	2.19	2.15	2.10	2.06	2.01	1.96
18	4.41	3.55	3.16	2.93	2.77	2.66	2.58	2.51	2.46	2.41	2.34	2.27	2.19	2.15	2.11	2.06	2.02	1.97	1.92
19	4.38	3.52	3.13	2.90	2.74	2.63	2.54	2.48	2.42	2.38	2.31	2.23	2.16	2.11	2.07	2.03	1.98	1.93	1.88
20	4.35	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.39	2.35	2.28	2.20	2.12	2.08	2.04	1.99	1.95	1.90	1.84
21	4.32	3.47	3.07	2.84	2.68	2.57	2.49	2.42	2.37	2.32	2.25	2.18	2.10	2.05	2.01	1.96	1.92	1.87	1.81
22	4.30	3.44	3.05	2.82	2.66	2.55	2.46	2.40	2.34	2.30	2.23	2.15	2.07	2.03	1.98	1.94	1.89	1.84	1.78
23	4.28	3.42	3.03	2.80	2.64	2.53	2.44	2.37	2.32	2.27	2.20	2.13	2.05	2.01	1.96	1.91	1.86	1.81	1.76
24	4.26	3.40	3.01	2.78	2.62	2.51	2.42	2.36	2.30	2.25	2.18	2.11	2.03	1.98	1.94	1.89	1.84	1.79	1.73
25	4.24	3.39	2.99	2.76	2.60	2.49	2.40	2.34	2.28	2.24	2.16	2.09	2.01	1.96	1.92	1.87	1.82	1.77	1.71
26	4.23	3.37	2.98	2.74	2.59	2.47	2.39	2.32	2.27	2.22	2.15	2.07	1.99	1.95	1.90	1.85	1.80	1.75	1.69
27	4.21	3.35	2.96	2.73	2.57	2.46	2.37	2.31	2.25	2.20	2.13	2.06	1.97	1.93	1.88	1.84	1.79	1.73	1.67
28	4.20	3.34	2.95	2.71	2.56	2.45	2.36	2.29	2.24	2.19	2.12	2.04	1.96	1.91	1.87	1.82	1.77	1.71	1.65
29	4.18	3.33	2.93	2.70	2.55	2.43	2.35	2.28	2.22	2.18	2.10	2.03	1.94	1.90	1.85	1.81	1.75	1.70	1.64
30	4.17	3.32	2.92	2.69	2.53	2.42	2.33	2.27	2.21	2.16	2.09	2.01	1.93	1.89	1.84	1.79	1.74	1.68	1.62
40	4.08	3.23	2.84	2.61	2.45	2.34	2.25	2.18	2.12	2.08	2.00	1.92	1.84	1.79	1.74	1.69	1.64	1.58	1.51
60	4.00	3.15	2.76	2.53	2.37	2.25	2.17	2.10	2.04	1.99	1.92	1.84	1.75	1.70	1.65	1.59	1.53	1.47	1.39
120	3.92	3.07	2.68	2.45	2.29	2.18	2.09	2.02	1.96	1.91	1.83	1.75	1.66	1.61	1.55	1.50	1.43	1.35	1.25
∞	3.84	3.00	2.60	2.37	2.21	2.10	2.01	1.94	1.88	1.83	1.75	1.67	1.57	1.52	1.46	1.39	1.32	1.22	1.00

Source: (Frost, 2023)

APPENDIX IV

Table 10: Critical Values for Pearson's r

df \ α	0.2	0.1	0.05	0.02	0.01	0.001
1	0.951057	0.987688	0.996917	0.999507	0.999877	0.999999
2	0.800000	0.900000	0.950000	0.980000	0.990000	0.999000
3	0.687049	0.805384	0.878339	0.934333	0.958735	0.991139
4	0.608400	0.729299	0.811401	0.882194	0.917200	0.974068
5	0.550863	0.669439	0.754492	0.832874	0.874526	0.950883
6	0.506727	0.621489	0.706734	0.788720	0.834342	0.924904
7	0.471589	0.582206	0.666384	0.749776	0.797681	0.898260
8	0.442796	0.549357	0.631897	0.715459	0.764592	0.872115
9	0.418662	0.521404	0.602069	0.685095	0.734786	0.847047
10	0.398062	0.497265	0.575983	0.658070	0.707888	0.823305
11	0.380216	0.476156	0.552943	0.633863	0.683528	0.800962
12	0.364562	0.457500	0.532413	0.612047	0.661376	0.779998
13	0.350688	0.440861	0.513977	0.592270	0.641145	0.760351
14	0.338282	0.425902	0.497309	0.574245	0.622591	0.741934
15	0.327101	0.412360	0.482146	0.557737	0.605506	0.724657
16	0.316958	0.400027	0.468277	0.542548	0.589714	0.708429
17	0.307702	0.388733	0.455531	0.528517	0.575067	0.693163
18	0.299210	0.378341	0.443763	0.515505	0.561435	0.678781
19	0.291384	0.368737	0.432858	0.503397	0.548711	0.665208
20	0.284140	0.359827	0.422714	0.492094	0.536800	0.652378
21	0.277411	0.351531	0.413247	0.481512	0.525620	0.640230
22	0.271137	0.343783	0.404386	0.471579	0.515101	0.628710
23	0.265270	0.336524	0.396070	0.462231	0.505182	0.617768
24	0.259768	0.329705	0.388244	0.453413	0.495808	0.607360
25	0.254594	0.323283	0.380863	0.445078	0.486932	0.597446
26	0.249717	0.317223	0.373886	0.437184	0.478511	0.587988
27	0.245110	0.311490	0.367278	0.429693	0.470509	0.578956
28	0.240749	0.306057	0.361007	0.422572	0.462892	0.570317

29	0.236612	0.300898	0.355046	0.415792	0.455631	0.562047
30	0.232681	0.295991	0.349370	0.409327	0.448699	0.554119
35	0.215598	0.274611	0.324573	0.380976	0.418211	0.518898
40	0.201796	0.257278	0.304396	0.357787	0.393174	0.489570
45	0.190345	0.242859	0.287563	0.338367	0.372142	0.464673
50	0.180644	0.230620	0.273243	0.321796	0.354153	0.443201
60	0.164997	0.210832	0.250035	0.294846	0.324818	0.407865
70	0.152818	0.195394	0.231883	0.273695	0.301734	0.379799
80	0.142990	0.182916	0.217185	0.256525	0.282958	0.356816

Source: (PDF4PRO, 2017)