HAWASSA UNIVERSITY

COLLEGE OF MEDICINE AND HEALTH SCIENCE

SCHOOL OF PHARMACY

WOUND HEALING ACTIVITY OF 80% METHANOL EXTRACT AND SOLVENT FRACTIONS OF *PLECTRANTHUS CYLINDRACEUS* HOECHST. EX. BENTH LEAVES IN MICE

EMEBET NIGATU BIRHANU (B. Pharm)

NOVEMBER, 2023

HAWASSA, ETHIOPIA

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A THESIS SUBMITTED TO DEPARTMENT OF PHARMACOLOGY, SCHOOL OF PHARMACY, COLLEGE OF MEDICINE AND HEALTH SCIENCES, SCHOOL OF GRADUATE STUDIES, HAWASSA UNIVERSITY, HAWASSA, ETHIOPIA

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ADVISORS' APPROVAL SHEET

This is to certify that the research thesis entitled **"Wound healing activity of 80% methanol extract and solvent fractions of *Plectranthus cylindraceus* hoechst. Ex. Benth leaves in mice"** was submitted in partial fulfillment of the requirements for the degree of Master's with specialization in Pharmacology, the Graduate Program of the School of Pharmacy, and has been carried out by **Emebet Nigatu**, ID. No. **GPPhaR/0010/14**, under our supervision. Therefore, we recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to the department.

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EXAMINERS' APPROVAL SHEET

We, the undersigned, members of the Board of Examiners of the final open defense by **Emebet Nigatu**, have read and evaluated her thesis entitled **"Wound healing activity of 80% methanol extract and solvent fractions of *Plectranthus cylindraceus* hoechst. Ex. Benth leaves in mice"** and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree of Master of Science in Pharmacology.

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Declaration Statement

I, **Emebet Nigatu**, hereby declare that this MSc thesis, with the title "**Wound healing activity of 80% methanol extract and solvent fractions of *Plectranthus cylindraceus* Hoechst. Ex. Benth leaves in mice,**" is my original work. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis, and compilation of the thesis. It has not been presented for the MSc degree at any other university, and all scholarly sources of material included in this thesis have been duly acknowledged.

Emebet Nigatu (B. Pharm)	_____	_____
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LIST OF ABBREVIATIONS AND ACRRONYMS

AQF	Aqueous Fraction
ANOVA	Analysis of variance
BP	British Pharmacopoeia
CEO	Crude extract ointment
EAF	Ethyl acetate fraction
ECM	Extracellular Matrix
EPHI	Ethiopian Public Health Institute
FGF	Fibroblast Growth Factor
HU	Hawassa university
IBM	International Business Machines
IP	Intraperitoneally
IV	Intravenous
LD50	Median Lethal Dose
NF	Nitrofurazone
HF	Hexane fraction
OECD	Organization of Economic Corporation and Development
SEM	Standard error of mean
SO	Simple ointment
SPSS	Statistical package for social science
TM	Traditional medicine
TS	Tensile of strengths
US	United state
V/V	Volume by volume
WHO	World Health Organization
W/W	Weight by weight

ABSTRACT

Background: The leaves of *Plectranthus cylindraceus* are traditionally used to treat wounds. Even though, there have been claims supporting wound healing effect, there are no scientific data on wound healing activities of the leaves of *Plectranthus cylindraceus*.

Objective: This study aimed to evaluate wound healing activity of 80% methanol crude extract and solvent fractions of *Plectranthus cylindraceus* in mice.

Method: The leaves of *Plectranthus cylindraceus* were dried, ground and macerated with 80% methanol three times successively. The crude extract was fractioned by water, ethyl acetate, and n-hexane, separately. Acute dermal toxicity tests were done by applying 2000 mg/kg of 10% (w/w) crude extract ointment. The wound healing activity of the crude extract was evaluated on excision and incision wound models. While, the fractions were evaluated on the excision wound model. The evaluation consisted of assessing the wound contraction rate, epithelialization period and histopathological analysis compared to simple ointment negative control. One-way ANOVA followed by the post-hoc Tukey test was conducted using IBM SPSS software version 27.0 for data analysis, and a p-value of <0.05 was considered statistically significant.

Results: Acute dermal toxicity test result showed that topical application of 2000 mg/kg of 10%(w/w) crude extract ointment (CEO) of *P.cylindraceus* did show any sign of toxicity and hence considered safe. Both the 5% (w/w) and 10% (w/w) crude extract ointment exerted significant($p < 0.001$) wound contraction from day 4 onwards compared to the simple ointment (SO) and resulted in reduced periods of epithelialization. The crude extract ointment treated mice exhibited a significantly increased tensile strength ($p< 0.001$) compared to untreated and negative control groups. The n-hexane, ethyl acetate, and aqueous fractions of the crude extract also demonstrated significant improvement in wound contraction and epithelialization time reduction on the excision wound model compared to the negative control group. The aqueous fraction demonstrated better activity compared to others.

Conclusion: The positive results of the study, which include the advancement of wound contraction, reduction in the period of epithelialization, and improvement of tensile strength, validate the traditional use of *Plectranthus cylindraceus* for wound healing.

Keywords: Crude extract, fraction, methanol, mice, *Plectranthus cylindraceus*, wound.

INTRODUCTION

1.1. Background

Wound is a disruption in the normal continuity of anatomic structure and functionality of living tissues (Gebremeskel *et al.*, 2018). It is break in the skin and formed due to physical, chemical, thermal, microbial, or immunological factors (Tessema and Molla, 2021). Symptoms of wound or injury include swelling, stiffness, tenderness, discoloration, skin tightness, scabbing, and itching (Dubey *et al.*, 2017).

Based on etiology, type of injury or presenting symptoms, its depth, and tissue loss or clinical appearance;wounds could be injuries, cuts or bites, diabetic, gastric, and duodenal ulcers (Tottoli *et al.*, 2020). Wound may be classified as open or closed depending on the underlying cause of wound creation and acute or chronic based on the physiology of its healing (Agyare *et al.*, 2015).

In open wounds, the skin is cracked open, leaving the underlying tissue exposed to the outside environment, which makes it more vulnerable to bleeding and infection (Zhao *et al.*, 2016). It is further classified as incised wound, laceration or tear wound, abrasions or superficial wounds, puncture wounds and penetration wounds (Chhabra *et al.*, 2017). However, in closed wounds, the skin is intact and the underlying tissue is not directly exposed to the outside world. Even with the skin intact, the damage can reach down to the underlying tissues, muscles, internal organs and bones (Sen, 2021). The blood escapes the circulatory system in some cases but remains in the body. It includes contusion or bruises, haematomas or blood tumor, crush injury (Sinno and Prakash, 2013; Agyare *et al.*, 2015).

Based on physiology or the time period required to be recovered, wound can be classified as acute and chronic (Wilkinson and Hardman, 2023). Accordingly, in acute wound the tissue injury caused by cuts or surgical incisions is going on through orderly and timely reparative process, results in sustained restoration of anatomic and functional integrity and the healing process has been completed within the predictable time frame (Tessema and Molla, 2021). If this orderly and timely reparative process of healing is failed and goes to a state of pathologic inflammation; the wound is considered as chronic (Trostrup *et al.*, 2013).

In this type wound healing process is delayed, incomplete, and does not proceed in a coordinated manner, resulting in poor anatomic and functional integrity over 3 months (Vitale *et al.*, 2022). This situation happens when the normal wound healing process is compromised due to numerous phenomenon such as microbial infection, metabolic disturbances, systemic problems such as diabetes mellitus, malnutrition and immuno deficiency (Jarbrink *et al.*, 2017).

Wound contaminating organisms prolong the duration of wound healing process by producing toxins that may further destroy the wounded tissues and/or degrade the biochemical substances that enhance wound healing (Dubey *et al.*,2017). Therefore, drugs that may be applied topically, orally or systemically are often used to shorten the duration of wound healing by overcoming microbial wound contamination and to achieve optimum healing (Karki and Maharjan, 2021).

Research on drugs that increase wound healing is a developing area in modern biomedical sciences and several drugs obtained from plant sources are known to increase the healing of different types of wounds (Farahpour and Habibi, 2012). The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Tiwari *et al.*, 2023). Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds and many more. These natural compounds are the foundations of modern drugs (Dhawale, 2013; Vitale *et al.*, 2022).

1.1.2. Overview of the Experimental Plant

The genus *Plectranthus*, is a large genus containing about 300 species worldwide (Pa and Buchbauer, 2009). The genus is known for its aromatic nature and essential oil with interesting biological activities (Khalik, 2016). *Plectranthus spp* are well known for their various pharmacological activities such as antibacterial, antifungal, anti-inflammatory, antioxidants, antiseptics and anti-plasmodial activities (Marwah *et al.*, 2007; Musila *et al.*, 2017; Mothana *et al.*, 2018). The main phytochemical constituents of the genus *Plectranthus* are triterpenoids, flavonoids, steroids, abietane diterpenoids, essential oils and phenols (Pa and Buchbauer, 2009; Botanical and Lamiaceae, 2021).

Plectranthus cylindraceus Hoechst. Ex. Benth is a shrub that belongs to a family of Lamiaceae. It is strongly aromatic, succulent, and highly branched shrub that attains 50cm-1m in height

(Ryding, 2015). The plant is widely distributed in tropical and sub-tropical regions of African countries in east and south Africa, Saudi Arabia, Yemen and India (Khalik, 2016). In Oman and Saudi Arabia, the leaves of this plant are used as disinfectant, deodorant and to treat; sore throats, skin, digestive, respiratory, and inflammatory disease (Kassim, 2013). The leaves of *P. cylindraceus* grown in Oman exhibited high antimicrobial activity against various bacterial and fungal strains (Marwah *et al.*, 2007).

In Ethiopia, *P.cylindraceus* is known by various vernacular names including; Dachet,(A) Amharic (Asfaw, 2021), Qintele sat (G) in gurage (Asfaw and Demissew, 2020), Dumio (Maale) (Kidane *et al.*, 2014). In Ethiopia, the plant is used traditionally for the treatment of inflammatory conditions and for its flavour (Asres *et al.*,2013), for swelling, evil spirit (Likift, Buda), rheumatism (Teka and Demissew, 2020), lower extremity weakness (Kidane *et al.*,2014), diarrhoea (Woldeab *et al.*, 2018) and wound healing (Asfaw, 2021).

In previously done study, sesquiterpenes and flavonoids have been reported from this plant (Khalik, 2016). And its oil possessed antibacterial and antifungal activities (Marwah *et al.*, 2007; Asres *et al.*, 2013). Despite this study and claims, no scientific data reported about the wound healing activity of the study plant on laboratory animals, therefore, it is considered crucial to prove scientifically whether extracts of the plant have wound healing activity or not.

Figure 1: Image of *Plectranths cylindraceus* taken from site of collection (Bui, Ethiopia).

1.2. Statement of the Problem

It has been estimated that 14 million people suffer from wounds worldwide with over 80% of these living in low and middle-income countries (Namunana *et al.*, 2018). For every million individuals affected by wounds, approximately 10,000 die due to complications arising from microbial infections and other factors (Mengie et, 2021).

Over the past few years, there has been a growing trend in the incidence of chronic wounds worldwide, which has been referred to as a "silent epidemic" (Jarbrink *et al.*, 2016). This increase is primarily attributed to the concurrent rise in comorbidity and lifestyle diseases such as diabetes, obesity, venous hypertension, and peripheral vascular diseases (Tottoli *et al.*, 2020). The treatment of these wounds is not only costly in terms of monetary expenditure, but also in terms of the time and resources required the impact on the productivity of afflicted individuals and their families, and the negative effect on their quality of life, which places a significant burden on society (Monika *et al.*, 2022).

It has been reported that the prevalence of chronic wounds ranges from 1% to 2% of the general population in developed countries (Nussbaum *et al.*, 2018). Developing countries, such as Sub-Saharan Africa and South Asian nations, are disproportionately affected by wound infections due to inadequate hygienic conditions (Getahun *et al.*, 2021). In Africa, wounds account for a significant portion of hospital attendance and deaths, with an estimated 30-42% of hospital visits and 9% of annual deaths attributable to wounds (Builders, 2016).

In Ethiopia, injury is a leading cause of emergency department visits, with 55.6% of visits attributed to injury, according to a study conducted in 2015 in Amhara Regional State Referral Hospitals (Abayneh *et al.*, 2022). Wound infections carry substantial financial and human costs, which are estimated to range from $28.1 billion to $96.8 billion annually in the United States. Furthermore, the market for wound care products is anticipated to grow by 2024 (Sen, 2019). These costs can be further exacerbated by prolonged hospital stays and costly antimicrobial treatments (Degu and Ashenafi, 2022).

Studies indicated that many of the bacteria isolated from wounds showed high levels of resistance to antimicrobials and antimicrobial resistance can complicate treatment of wound infections and cause prolonged debility and significantly increase healthcare costs (Abayneh *et*

al., 2022). As resistance rates increase, the efficacy of antimicrobial agents in treating bacterial infections decreases (Orrett and Land, 2006). Emergence of resistant strains along with lack, high cost and retarded rate of newly generated antibiotics together increased wound related mortality and morbidity (Wilkinson and Hardman, 2023).

Only 1-3% of the medications listed in western pharmacopoeias are intended for use on wounds, despite the enormous advancements made in the pharmaceutical drug business. This is due to the restricted availability of medications that can stimulate the healing process (G/giorgis *et al.*, 2022). In addition many of the conventional wound healing drugs currently used for treating wounds are not only expensive but also pose numerous side effects such as toxic effects, allergy and drug resistance (Yiblet *et al.*, 2022).

Management of wound healing is a complicated and expensive program and thus research on drugs that increase wound healing is a developing area in modern biomedical science (Thakur *et al.*, 2011). Therefore, there is a need for medicinal plants research as alternative treatment for wound care and the World Health Organization (WHO) urged researchers to examine traditional medicines and/ or promoting traditional medicine as a source of less expensive, comprehensive medical care, especially in developing countries (Beyi, 2018; Sisay and Bussa, 2019).

1.3. Significance of the study

Plectranthus cylindraceus Hoechst. Ex. Benth, is one of the conventionally used medicinal herbs for wound healing (Asfaw, 2021).

The aim of this research was to validate the traditional use of *Plectranthus cylindraceus* Hochst. Ex. Benth as a wound healing agent and provide further evidence of the efficacy of the crude extract and solvent fractions.

The study also aimed to identify the qualitative phytochemical constituents of the plant in order to gain insight into the nature of the phytochemical responsible for its action. The results of this research could contribute to the development of new agents for wound healing and provide valuable information for the search for new natural products.

The findings of this study could serve as a reference for researchers, students, health professionals, and traditional medicine practitioners and support the traditional healthcare system with scientific evidence to optimize the traditional use of medicinal plants.

1.4. OBJECTIVES OF THE STUDY

1.4.1. General objective

- To evaluate wound healing activity of 80% methanol crude extract and solvent fraction of the leaves *Plectranthus cylindraceus Hochst. Ex. Benth* in mice.

1.4.2. Specific objectives

- To assess acute dermal toxicity of the 80% methanol crude extract of the leaves of *Plectranthus cylindraceus Hochst. Ex. Benth* in mice
- To evaluate the wound healing activity of 80% methanol crude extract of the leaves of *Plectranthus cylindraceus Hochst. Ex. Benth* on excision wound model
- To evaluate the wound healing activity of 80% methanol crude extract of the leaves of *Plectranthus cylindraceus Hochst. Ex. Benth* on incision wound model
- To evaluate wound healing activity of solvent fractions on excision wound model
- To identify phytochemical classes of compounds of *Plectranthus cylindraceus* leaf extracts

2: LITERATURE REVIEW

2.1. Epidemiology of Wounds and cost of wound healing

According to a 2019 retrospective examination of Medicare members, it was reported that there were 10.5 million individuals with wounds globally, expected to grow at a compound annual growth rate of 3.9% from 2019 to 2026 (Carter *et al.*, 2023). Despite the belief that injury-related morbidity is largely preventable, it is increasing worldwide (Kloth, 2009). Road traffic injuries, violence, and self-inflicted harm are projected to be among the top 20 global causes of disease burden in 2020, and it is anticipated that these causes will rise by 28% by 2030 (Prevention and Yorkshire, 2021).The estimated cost to Medicare for both acute and chronic wound care is projected to rise annually (Sen, 2021).

In the USA, chronic wounds are reported to affect 6.5 million patients with more than $25 billion each year spent by the healthcare system on treating wound-related complication (Jarbrink *et al.*, 2016).This figure is projected to grow due to the escalating healthcare expenses, aging populationand the rising prevalence of diabetes and obesity (Ongarora, 2022).

The United Kingdom'sNational Health Service managed 2.2 million patients with wounds at a cost of £5.3 billion according to the study of 2012/2013 (Shivani Gupta, 2021). It is estimated that 420,000 cases in Australia each year had a chronic wound at any given time and costs $2.85billion and in general in Europe, it has been estimated that 2%–4% of healthcare budgets are spent on wound management (Jarbrink *et al.*, 2016).

Wounds are a significant contributor to hospital visits in Africa, accounting for approximately 30-42% of attendance and 9% of annual deaths (Diamond *et al.*, 2018). Furthermore, wounds are often under reported as a health challenge in many parts of the continent due to limited access to hospitals and other factors (Builders, 2017).

In Ethiopia, there is a lack of comprehensive research and data on the epidemiology, social, psychological, and financial burden of chronic wounds make it difficult to monitor the healthcare resources consumed by wound care.Only a few studies have reported the incidence and prevalence of chronic wounds and most of the studies were conducted on Surgical site infections

(Shiferaw *et al.*, 2020). According to a study conducted in 2015 in Amhara Regional State Referral Hospitals, Ethiopia, 55.6% of emergency visits were due to injury (Abeje *et al.*, 2022).

2.2. Wound Healing Process

The wound healing process is a complex series of interrelated events that are mediated in its different phases by a wide range of chemically coordinated cellular processes, as well as hormonal influences (Taddese *et al.*, 2021).The process can be broadly categorized into four stages: homeostasis (coagulation) phase, inflammatory phase, proliferative phase (formation of granulation tissue and collagen synthesis), and finally the remodeling phase, which ultimately determines the strength and appearance of the healed tissue (Alemzadeh and Moshiri, 2018).

2.2.1. Haemostasis Phase

Immediately after injury, coagulation and hemostasis take place in the wound. The principal aim of these mechanisms is to prevent exsanguinations (Alemzadeh and Moshiri, 2018). It is a way to protect the vascular system, keeping it intact, so that the function of the vital organs remains unharmed despite the injury. A second aim is a long-term one, which is to provide a matrix for invading cells that are needed in the later phases of healing (Chen and Kirsner, 2007).

A dynamic balance between endothelial cells, thrombocytes, coagulation, and fibrinolysis regulates hemostasis and determines the amount of fibrin deposited at the wound site, (Tottoli *et al.*, 2020) thereby influencing the progress of this processes .These events are central to initiating the entire wound-healing cascade by providing the substances and communication that transition the wound to the next phase of healing (Bailey and Smrkolj, 2009).

2.2.2. Inflammatory Phase

The inflammatory phase is the immune system’s reaction to injury, inflammation begins shortly after hemostasis and aims to remove invading pathogens, as well as necrotic and damaged tissue, to provide a clean wound base in preparation for the proliferative phase of healing (Wolde *et al.*, 2022). The inflammatory response which begins 6 - 8 h after injury causes the blood vessels to become leaky, releasing plasma and neutrophils into the surrounding tissue (Gupta and Kumar, 2015). The neutrophils phagocytose debris and microorganisms provide the first line of defense against infection. As they digest bacteria and debris, neutrophils die and release intracellular

enzymes into the surrounding matrix, which further digest tissue. The neutrophils are replaced by macrophages which further digest and remove cell debris and other foreign bodies (Chen and Kirsner, 2007).

2.2.3. Proliferative Phase

After hemostasis and inflammatory phases are completed, the process of rebuilding the damaged tissue is intensified (Nagar *et al.*, 2016). The specific mechanisms of this stage are to ensure the wound surface coverage with new skin(re-epithelization), restoring vascular integrity to the region (neovascularization), and repairing the structural integrity of the tissue defect by filling it with new connective tissue (granulation) (Mulisa,et.al., 2015). Cells that regulate the inflammatory stage are also involved in the initiation of the proliferative phase once most of the necrotic and damaged tissue is removed. The goal is to fill the wound defect with new tissue and to restore the integrity of the skin (Janis and Attinger, 2006).

2.2.3. Neovascularization

The process of restoring the vascular network is called neovascularization or angiogenesis. This process restores blood circulation in the place of damage and prevents the development of ischemic necrosis simultaneously stimulating the tissue repair process (Strodtbeck, 2001). Fibroblasts which are the primary synthetic element in the repair process and are responsible for production of the majority of structural proteins (Lulseged *et al.*, 2022). They first appear in significant numbers in the wound on the third day post-injury and achieve peak numbers on the seventh day. This rapid expansion in the fibroblast population at the wound site occurs via a combination of proliferation andmigration of fibroblasts to the wound site is assisted by contraction of extra cellular matrix (ECM) and the formation of granular tissue (Houreld and Abrahamse, 2012).

2.2.4. Re-Epithelialization

It is the resurfacing of the wound. The primary cell is the keratinocyte, derived predominantly from epidermal stem cells located in the bulge area of the hair follicle and the epidermis at the edges of the wound (Thiruvoth *et al.*, 2015). Keratinocytes respond to signals from the macrophages, neutrophils, and other factors within hours after injury (Mutsaers *et al.*, 1997). The

cell then contracts, pulling itself forward across the wound surface. This process is repeated again and again until the migrating cells from opposite sides of injured area touch each other (Gurtner *et al.*, 2008). Once migration is completed, the keratinocytes stabilize themselves by forming firm attachments to each other and the new basement membrane close the wound (Pastar *et al.*, 2014).

2.2.5. Granulation

Granulation is the term used to describe the new wound matrix made up of collagen and an extracellular material called ground substance (Alemu,et.al., 2020).The predominant cell type found in this process is the fibroblast that produces collagen and numerous other substances that comprise the ECM (Palpandi *et al.*, 2010). Extracellular matrix is composed of substances that have different roles in wound healing cascade such as fibronectin which promote adhesion and migration (Edwin *et al.*, 2008). As the wound closes, the immature fibrin matrix and granulation tissue are replaced by collagen and scar. However, wound healing as a process does not end at wound closure (Guo and DiPietro, 2010).

2.2.6. Remodeling

The final stage of wound healing marked by the activities of growth factors, matrix, metalloproteinases, fibroblasts, macrophages, and epidermal cells to rebuild scar tissue under the reformed epidermis (Bailey and Smrkolj, 2009). The result is increased tensile strength, but decreased vascularity (Gebrehiwot *et al.*, 2015). Mechanisms used in remodeling are cell maturation, programed cell death or apoptosis and ECM reshaping by cross-linking collagens (Jimi *et al.*,2017). Remodeling is thus a balance between synthesis of new collagen and degradation of the old one. Eventually they will regain a structure similar to that seen in unwounded tissue (Sinno and Prakash, 2013).

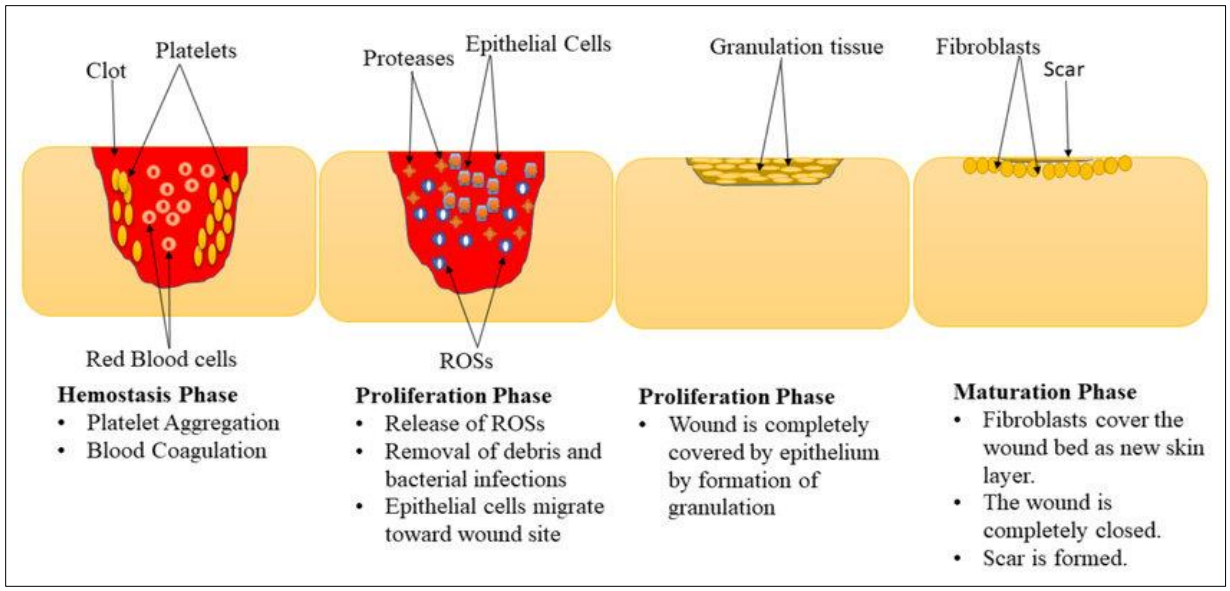


Figure2: Phases of the wound healing process *https://www.researchgate.net/profile/Alven-Sibusiso/publication/358593171/figure/fig1/AS:1123656923127812@1644912095953/*

2.3. Factors that can interfere with wound healing.

There are multiple factors that can lead to impaired wound healing. These can be categorized into local (extrinsic) and systemic (intrinsic) (Beshir *et al.*, 2017). Local factors are those that directly influence the characteristics of the wound itself, while systemic factors are the overall health or disease state of the individual that affect his or her ability to heal (Guo and DiPietro, 2010). Local factors affect the features of the wound and they are mainly oxygenation, infection, presence of a foreign body and venous insufficiencywhile, the systemic factors are provoked bythephysiological state of an individual which may impair wound healing (Giri, 2019).

Some other factors which affect wound healing include age, gender, temperature, chemicals, sex, hormones, stress, moisture, nutritional status, diabetes, cancer, autoimmune diseases and obesity. Alcoholism, smoking, and certain medications such as steroids and chemotherapy also affect the wound healing processes (Ayu and Abrahamse,2012). An infection mostly from *S. aureus*,*E.coli*, *P. aeruginosa*and Bacillus species delay natural healing process by protracting the inflammatory phase or by disrupting the normal clotting mechanism. Hence, it interferes and delays epithelialization, contraction, collagen deposition and angiogenesis (Arun *et al.*, 2016).

2.4. Management of Wound

The primary target of wound treatment is to promote wound healing in the short time as soon as possible, with minimum pain, discomfort, and scarring to the patient (Jarbrink *et al.*, 2017).Assessment of the patient starting with a diagnosis of the wound‟s aetiology and continues with optimizing the patients medical condition, particularly blood flow to the wound area is considered to be the first stage in wound management (Velnarand Smrkolj, 2009).The other aim of wound care is to control microbial colonization that may hinder subsequent proliferation and thus promoting the healing of the wounds (Sasidharan *et al.,* 2010).There are plenty of topically applied antibiotics in the form of lotions, creams, ointments, powders, gels and sprays (Gurtner *et al.*, 2008).

Management of infection and inflammation are the keys to a successful wound healing (Jahandideh *et al.*, 2017). Medical treatment of wound includes administration of drugs either locally (topical) or systemically (oral or parenteral) in an attempt to aid wound repair. The topical agents used include antibiotics and antiseptics (Kloth, 2009). Modern wound care medications all play an active role in some phase of wound healing. Each product has its own individual indications, including the type, condition, and age of the wound (Tatarusanu *et al.*, 2023). Using topical antimicrobials and antiseptics,in the contaminated or infected wound reduce the number of microorganisms present in the wound and they are also used to decrease the bacterial burden in chronic wounds (Patel and Hebert, 2023).

Some examples of topical antimicrobials are bacitracin, clindamycin (cream, lotion, and foam), gentamicin (ointment or cream), and nitrofurazone (0.2% ointment) (Sasidharan *et al.*, 2010). Antiseptics include, (chemical debridement, e.g. hydrogen peroxide, eusol and collagenase ointment) (Raina *et al.*, 2008). Antiseptics are used to cleanse or lavage animal wounds.Thus,use of antiseptics is for wound cleansing and to flush nonviable tissue and bacteria from the wound surface mechanically without damaging viable tissue or driving bacteria into underlying tissue (Doughty, 2005).

Systemic antimicrobial therapy should be used in all chronic wounds in which there is active infection beyond that manageable with local wound therapy (Krahwinkel and Boothe, 2006).Principles of antimicrobial selection and use should be based on culture and susceptibility

testing results, knowledge of expected flora, ability of the antimicrobial to reach the wound at appropriate concentrations, bacterial resistancepatterns, and antimicrobial pharmacokinetics (Douglass, 2003).Whereas, topical debridement applied for wound bed preparation, enhances wound assessment, decreases infection potential, activates cellular activity, and removes physical barriers to healing (Beitz, 2005; LA, A and JS, 2017).

2.5. Perspectives of Techniques and Drug Development for Wound Healing

Due to the complexity of wound assessment and management, which involves a number of aspects that are crucial to the healing process, wound healing is an unmet therapeutic challenge in the medical community (Okur *et al.*, 2020).The U.S. Food and Drug Administration now regulates a wide range of wound healing products for use in the country, medicated, or interactive dressings are included in these items (Sollott, 2017).

The vast amount of recent research on this topic demonstrates the importance of drug delivery and the challenge for translational medicine in developing efficient for wound healing applications (Atiyeh and Hayek, 2005).To overcome the challenges posed by the systemic circulation and the challenging wound environment, various medication delivery methods call for strategies (Sollott, 2017).

2.6. Herbal medicines used in the management of wound

The World Health Organization (WHO) has disclosed that approximately 80% of the global population relies on plant-based remedies for basic health care and skin-related concerns, with wounds being the most common reason for medical consultations in developing countries (Pr *et al.*, 2017). There has been several reports of medicinal plants having wound-healing properties. A great deal of research has been done on the topic of using medicinal herbs to manage wound healing (Nigussie *et al.*, 2020). According to WHO, around 21,000 plant species have the potential for being used as medicinal plants. Treatment with medicinal plants is considered very safe as there is no or minimal side effects (Hassan *et al.*, 2021).

In Ethiopia, it has been estimated that traditional remedies are the most important and sometime the only source of therapeutics for nearly 80% of the population of which 95% of traditional

medicinal preparations are of plant origin (Seyoum and Zerihun, 2014). Many Ethiopian medicinal plants are claimed to have wound healing activities (Gebremeskel *et al.*, 2018).

There is evidence for use of medicinal plants for wound healing which suggests that they may be cost-effective promoters of wound healing (Mulisa and Engidawork, 2015). Numerous plants and their preparations have been utilized historically to treat wounds in Ethiopia; few of them listed in the table below:

Table 1: Some herbal medicines used for the management of wound in Ethiopia

plant name	plant part studied	Wound models used	Reference
Vernonia auriculifera Hiern	leaves	Excision, incision, and burn wound models	(Lambebo *et al.*, 2021)
Justicia schimperiana	leaves	Excision, incision, and burn wound models	(G/giorgis *et al.*, 2022),
Croton macrostachyus	leaves	Excision and incision wound models	(Abraham *et al.*, 2016)
Acokanthera schimperi	leaves	Excision, incision and infected models	(Alemu,et.,al., 2020)
Brucea anti dysenterica	leaves	Excision, incision wound models	(Wolde *et al.*, 2022)
Bersama abyssinica	leaves	Excision, incision, and burn wound models	(Taddese *et al.*, 2021)
Kalanchoe petitiana	leaves	Excision, incision and dead space model	(Mekonnen *et al.*,2013)
Brassica carinata seeds	seeds	Excision and incision wound models.	(Alemu,et.,al., 2020)
Rumex abyssinicus	rhizomes	Excision and incision wound models	(Mulisa,et,.al., 2015)

3: MATERIALS AND METHODS

3.1. Chemicals and Reagents

Methanol (AppliChem, Germany), ketamine hydrochloride50 mg/ml injection (Neon laboratories limited, India), diazepam 5mg/mL injection (Gland pharm limited, India),Nitrofurazone USP 0.2% ointment (shanghai general pharmaceutical CO., LTD, China), hard paraffin (Lab tech chemicals, Ethiopia),whitesoft paraffin (Ethiopian pharmaceuticals manufacturing, SH.CO, Ethiopia),Ceto stearyl alcohol (Blulux Laboratories (P) Ltd., India), wool fat (Uni-Chem chemical reagents, Serbia), ethanol alcohol (Blulux Laboratories (P) Ltd., India), normal saline (IV infusion BP Medsol pharmaceuticals), sodium hydroxide (Norbright, China), lead acetate (Guanghua chemicals factories, China), hydrochloric acid (Blulux Laboratories (P) Ltd., India), Wagner's reagent (Research-Lab Fine Chem Industries, India), sulphuric acid (HiMedia Laboratories Pvt. Ltd., India), ferric chloride (Blulux Laboratories (P) Ltd., India),n-hexane (pentokey organy (india).LTD), chloroform (loba chemie PVT.LTD), ethylacetate (loba chemie PVT.LTD), hematoxylin (Alpha Chemika, India), eosin (Alpha Chemika, India),formalin 10% (Yilmana Chem- icals, Ethiopia) and distilled water were used.

3.2.Instruments and Apparatus

Sensitive digital weighing balance (Abron Exports, India),rotary evaporator (Yamato, Japan), dry oven (Abron Exports, India),Whatman filter paper No 1, water bath, ointment slab, sharp sterilized scissors, surgical threads with curved needles, forceps, surgical scalpel blade, conical flask, beaker, Buchner funnel, adhesive plaster, gauze, elastic bandage, mortar and pestle,refrigerator,permanent marker, transparent polythene sheet, syringe with needle, glove, cotton, graph paper and thermometer were used.

3.3. Plant Collection and Identification

Plectranthus cylindraceus Hoechst. Ex. Benth leaves were collected from Bui town, Gurage Zone, Ethiopia in early March, 2023. The plant was identified and authenticated by Mr. Melaku Wondafrash at the National Herbarium,College of Natural and Computational Sciences, Addis Ababa University and a voucher specimen (number EN-001) was deposited for future reference.

3.4. Experimental Animals

Healthy adult Swiss albino mice of weight 25–30 g and age 6–8 weeks of either sex was used for the actual experiment. Healthy female adult Swiss albino mice were used for determining acute dermal toxicity test.The animals were obtained from the Animal house of the Department of Pharmacology, School of Pharmacy, College of Medicine, and Health Sciences, Wolaita Sodo University, Wolaita Sodo, Ethiopia.

The animals were housed under standard conditions with 12-hours light and 12 hours dark cycles in polypropylene cages with free access to standard food and water ad libitum. The cages were kept in a well-ventilated room (22 ± 3)°C, 50–60% humidity (OECD, 2017). The experimental animals were acclimatized with the laboratory environment for a week before the initiation of the experiment. All the way through the experiment, the animals were handled according to the international and East African Laboratory animal use and care guidelines (Mohr *et al.*, 2017; Ogden *et al.*, 2017).

3.5. Crude Extract Preparation

The leaves of *Plectranthus cylindraceus* herb were collected ,washed and dried under shade then the dried plant materials were ground to a coarse powder with a mortar and pestle. Then, 80% methanol was used to extract the grounded plant material. A total of 1000g powder was macerated in 6 L of 80% methanol (1:6 w/v) (Tripathi and Pandey, 2014). After 72 hours the extract was filtered using surgical gauze and then through whatman filter paper (No.1) consecutively.The marc was re-macerated twice in the same manner, and the filtrates were collected in one vesseland the methanol was removed by evaporationusing a rotary evaporator set at 40°C. The remaining concentrated aqueous solution was further evaporated using a dry oven at 40°C (G/giorgis *et al.*, 2022). The dried extract was weighed and packed in air tight container then the proportion yield of the extract was determined and stored in a refrigerator at 4°C until use for the preparation of crude topical formulation and solvent fractionation for the intended experiment (Poojar *et al.*, 2017).

$$Percent\ yield = \frac{Weight\ of\ the\ extrac}{Weight\ of\ the\ plant\ material} \times 100$$

3.6. Solvent Fractionation of the Crude Extract

Seventy grams of the 80% hydro-methanol crude extract was subjected to a successive fractionation using a separator funnel with n-hexane, ethyl acetate and water in the order of increasing polarity (Poojar *et al.*, 2017). 70-gram of crude extract was suspended in 420 ml of distilled water (1: 6 w/v) using a separatory funnel. Then, equal volume of n-hexane was added, after being gently shaken, the extract was allowed to settle and separated into two distinct layers according to their density. Once the distinct layers were formed ,the upper n- hexane layer was separated byeluting the bottom aqueous layer from the separatory funnel, and thus identical procedure was repeated two more times and the n-hexane fractions were collected in the same container.

To get ethyl acetate fractions, an equal amount of ethyl acetate to that of distilled water was added to the aqueous fraction followed by vigorous shaking. Then the ethyl acetate fraction was separated after a distinct layer was formed between ethyl acetate and aqueous fraction,by collecting the bottom aqueous layer and the procedure was repeated two more times and the ethyl acetate fractions were collected in the same container. Finally, the aqueous fraction was collected. The filtrates of n-hexane and ethyl acetate fractions were concentrated by rotary evaporator and dried by dry oven at 40°C and the aqueous fraction was concentrated using dry oven at 40°C. The proportion yield was calculated and stored at 4°Cin the refrigerator until ointment formulation (Poojar *et al.*, 2017; Taddese *et al.*, 2021)

3.7. Preliminary Phytochemical Screening

Qualitative screening for Preliminary phytochemical of the crude extract and its solvent fractions of the leaves of *Plectranthus cylindraceus* were carried out to ascertain the plant for the presence of alkaloids, saponins, flavonoids, terpenoids, phenols, glycosides, tannins and steroids using commonly employed standard screening procedures (Peiris *et al.*, 2023).

3.8. Sample Size Calculation

From a previous study (Abeje *et al.*, 2022) by finding means and standard deviation, the effect size was 1.67. Based on this, the sample size was calculated by using G Power Software. The t test is used to compare the differences between two dependent means (machine pairs). The

number of animals per group became 6 and, based on this, the total number of animals used for the experiment were 102 mice.

3.9. Study Designs

Complete randomized study design

3.9.1 Randomisation Technique

The animals were randomly assigned to treatment and control groups by the simple random sampling method. And also the order of treatment was also done by using the simple random sampling method.

3.10. Ointment Formulation

Simple ointment was first prepared from hard paraffin, cetostearyl alcohol, white soft paraffin, and wool fat by the formula and proportion described in table2 (‘British Pharmacopoeia 2020’). All the ingredients were weighed using an electronic weighing balance. Hard paraffin was placed first into an evaporating dish and melted over a water bath then the other ingredients were added in descending order of melting point until all are melted (the order being cetostearyl alcohol, then wool fat, and lastly white soft paraffin). The mixture was continuously stirred to ensure homogeneity (Amalia and Rahmawati, 2020).Then, the mixture was removed from heat source and stirred until being cool.

All ointments were prepared by levigation on the surface of the ointment slab to formulate ointment of uniform consistency and smooth texture. 300 gm of simple ointment base was prepared without active ingredient. This prepared simple ointment was used as vehicle for the preparation of ointments of the crude extract and solvent fractions and as a treatment for the negative control. The medicated ointment containing 5%w/w and 10% w/w strengths of the crude extract was prepared by using 95g and 90g of simple ointment and then adding 5g and 10g of the test substances,respectively (Taddese *et al.*, 2021).

Similarly, 5% (w/w) and 10% (w/w) of the three fraction ointments were prepared by mixing 1.5 g and 3g each of n-hexane, ethyl acetate, and aqueous fractions into 28.5 g and 27 g of simple ointment base, respectively, to get 30 g medicated ointments. Finally, the crude extract and

solvent fractions ointment and simple ointment base were transferred to a clean closed container for topical application during the experiment (Jayamohan and Jayachandra, 2013).

Table 2:Master and reduced formula used for simple ointment.('British Pharmacopoeia 2020').

Ingredients	Master formula	Reduced formula
Hard paraffin	50g	15g
Cetostearyl alcohol	50g	15g
White soft paraffin	850g	255g
Wool fat	50g	15g
Total	1000g	300g

3.11. Grouping and Dosing of Experimental Animals

102 mice with nine groups were used to evaluate the wound healing activities of both the crude extract and solvent fractions. The wound healing effect of the crude extract was evaluated using excision and incision wound models. In excision wound model 24 mice were grouped into four groups containing six mice per group, while 30 mice were grouped into the five groups each group contained six mice for the incision wound model. In both models, group I was treated with simple ointment (negative control), groups II and III were treated with 5% and 10% (w/w) extract ointment, respectively, and Group IV was treated with 0.2% w/v nitrofurazone ointment (positive control). GroupVof incision wound model was left untreated (served as an untreated negative control group (Taddese *et al.*, 2021).

The excision wound model was used to evaluate the solvent fractions, comprising eight groups, each consisting of six mice. Group I was treated with a simple ointment, whereas groups II and III were treated with 5% (w/w) and 10% (w/w) aqueous fraction (AQF) ointment formulations, respectively. Groups IV and V were treated with 5% (w/w) and 10% (w/w) ethyl acetate fraction (EAF) ointment formulations, respectively. Groups VI and VII were treated with 5% (w/w) and

10% (w/w) n-hexane fraction (NHF) ointment formulations, respectively, whereas group VIII was treated with 0.2% (w/v) nitrofurazone ointment. All the experiments were conducted in accordance with the internationally accepted guideline forlaboratory animal use and care Institute for Laboratory Animal Research (Rowan, 1979).

3.12. Acute Dermal Toxicity Test

Acute dermal toxicity test was conducted on the 80% methanol crude extract of the plant in compliance with the OECD 402 and 404 guidelines. Five female mice, aged 7 weeks and weighing between 25–30g, were acclimated to the laboratory for 5 days and caged individually before the test. The dorsal/fank area of the mice was shaved after they were anesthetized with 50 mg/kg ketamine and 5 mg/kg diazepam.The OECD guidelines were followed to ensure the proper conduct of the test (OECD, 2017).

On the following day, a 10% (w/w) ointment formulation of the extract, comprising 2000 mg/kg, was uniformly applied to the shaved area and covered with gauze and a non-occlusive bandage. Following a 24-hour period, the residual test substance was washed out, and the mice were observed daily for 14 days to assess the development of edema, erythema, and irritation. The skin reactions were evaluated using the scoring systems outlined in OECD 404 (OECD 404, 2015).

3.13. Wound Healing Activity Tests

3.13.1. Excision Wound Model

The mice were anesthetized with ketamine (50 mg/kg) and diazepam (5 mg/kg) through the intraperitoneal route, and the skin of the dorsolateral flank area was shaved with a shaving machine after being disinfected with 70% alcohol. The anticipated circular wound area of 300 mm2 was marked with thin permanent marker and 2 mm depth excised wound was created along the markings carefully by using forceps and small sharp sterilized scissors (figure 3) (Taddese *et al.,* 2021; Yiblet *et al.,* 2022). Hemostasis was achieved by blotting the wound with a cotton swab soaked in normal saline, and the wounding day was considered as day 0.

On the following day, day1, The mice were administered ointments once daily until the positive control group had fully healed (G/giorgis *et al.,* 2022). The mice were then observed for wound closure by measuring the wound area every two days using a transparent sheet and permanent

marker to mark the area, which was subsequently measured using one millimeter square graph paper (Wolde *et al.*, 2022). Wound healing progress was assessed by wound closure rate, period of epithelialization, and histopathological investigations. This procedure was repeated for the excision wound model of solvent fractions.

Figure 3: Images of circular excision wounds on day 0

3.13.1. Wound Healing Parameters

1. Wound Contraction

Wound contraction was quantified by determining the percentage of wound area reduction based on the initial wound size of 300 mm2, which was set as 100%. The percentage of wound contraction was calculated utilizing the following equation (Jimi *et al.*, 2017).

$$\%\ \text{wound contracation} = \frac{\text{Initial wound size} - \text{nth days of wound size}}{\text{Initial wound size}} \times 100$$

Where n = the days where measurement will be taken (2nd, 4th, and so forth)**.**

2.Epithelialization period: The number of days required for falling scar without any residue raw wound were observed (Pastar *et al.*, 2014).

3.Histopathological analysis

A histopathological examination was carried out on the healed wound at18th post wounding day of the experiment, after euthanasia of mice via injection of ketamine and diazepam (four times

the anesthetic dose) intraperitoneally (Leary*et al.*,2020). Full-thickness skin specimens were extracted from each group of mice, samples were fixed in 10% buffered formalin, processed, and blocked with paraffin and stained with hematoxylin and eosin (Gupta and Kumar, 2015).Then tissues were examined by microscope and analyzed for fibroblast proliferation, mononuclear, and/or polymorphonuclear cells and collagen depositions (Gupta and Kumar, 2015).

3.13.2. Incision wound model

In this model, 30 mice were anesthetized in the same way as described for excision wound model and the back fur was shaved after rubbed by 70% ethanol alcohol. Then, a 3 cm long and 2mm depth linear paravertebral incision was created with a sterile blade (Thakur *et al.,* 2011). The wound was closed with interrupted sutures 1 cm apart using surgical sutures (No. 00) and curved needle (no.11) (Taddese *et al.,* 2021). After stitching,wounds were left undressed. The wounding day is considered as day 0.

The animals in Group I–IV were given the respective treatments topically as described in grouping dosing section above. The treatments are given starting 24 hours after wound creation until 9thday. The mice in Group V were left untreated and are used as untreated negative control (G/giorgis *et al.,* 2022). On post-wounding day8, the sutures were removed and the healing progress was evaluated by means of the continuous water flow method on post-wounding day 10, in order to determine the tensile strength of the injured area (Aulia and Bangun, 2018).

Measurement of tensile strength

In order to carry out the experiment, mice were first anesthetized and positioned securely on a table. Two forceps were then applied to the mice, with one forceps being fixed to a stand and the other being tied to an empty bottle using a rope. A weight was placed on the bottle to ensure that the forceps remained in place. A continuous and slow flow of water was allowed to flow from an IV bag filled with 1000 ml of water into the container (Figure 4) (Taddese *et al.,* 2021).

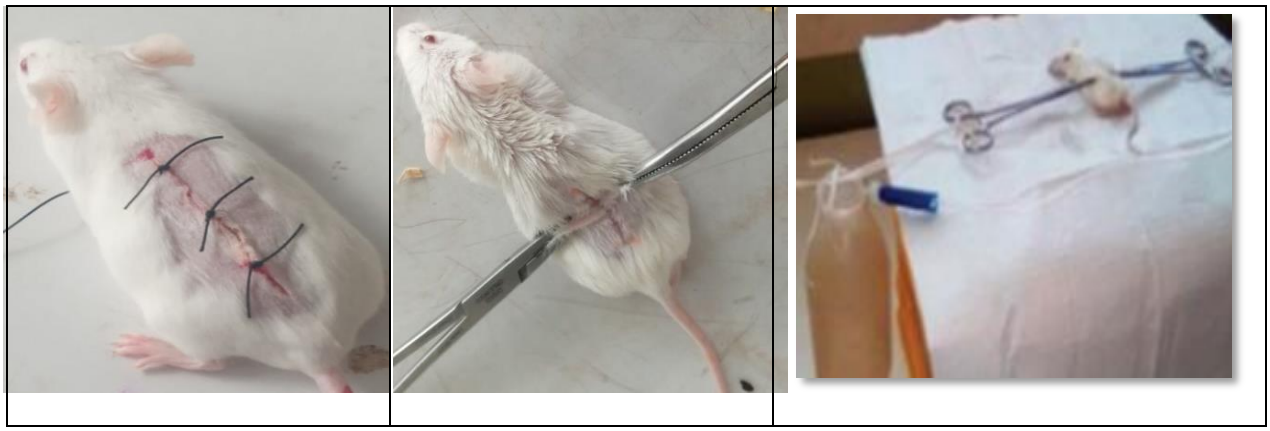
The water flow was then immediately stopped,just the woundunfolded and therefore, the volume of water collected in the container (approximately equal to its weight) was recorded as tensile strength (Nagar *et al.,* 2016). The tensile strengths of the test groups were compared with those of negative control, positive control and untreated negative control groups.The percent tensile strengths (% TS) of the groups were calculated using below formula (Belachew *et al.,* 2020).

% Tensile strength of the extract = $\frac{\text{Tensile strength of extract - Tensile strength of SO}}{\text{Tensile strength of simple ointment}} * 100$,

% Tensile strength of the reference = $\frac{\text{Tensile strength of reference - Tensile strength of SO}}{\text{Tensile strength of simple ointment}} * 100$'

% Tensile strength of the SO = $\frac{\text{Tensile strength of SO - Tensile strength of left untreated}}{\text{Tensile strength of left untreated}} * 100$.

SO: simple ointment



A B C

Figure 4; Incision wound creation(a) and Tensile strength measurement (b) and (c)

3.14. Statistical analysis

The results obtained from the experiments were expressed as mean ± SEM (standard error of the mean) and statistically analyzed using one-way ANOVA followed by post hoc Tukey tests with SPSS version 27.0 computer softwareand $p < 0.05$, 95% confidence interval were considered as statistically significant.

3.15. Ethical consideration

All the experiments were conducted in accordance with the internationally accepted laboratory animal use care and guideline(Liu *et al.*, 2016).and ethical permission was acquired from College of Health,Medicine and Sciences,Hawassa University after review by institutional review board with reference No;IRB/374/15.

4. RESULTS

4.1. Yield of Crude Extract and Solvent Fractions

From 1000 g dried leaves of *Plectranthus cylindraceus* Hoechst. Ex.Benth,182g crude extract was obtained, the percentage yield of the crude extract being 18.2%. Also, from fractionation of 70 g crude extract, 7.5 g hexane, 12.5 g ethyl acetate and 41.0 g aqueous fractions were obtained.

4.2. Phytochemical Screening

The preliminary phytochemical screening of the crude extract and solvent fractions of the plant showed the presence of alkaloids, flavonoids, phenols, tannins, steroids, glycosides, terpenoids and saponins. All the phytochemicals found in crude extract were also present in the aqueous fraction except steroids. Flavonoids, saponins and glycosides were absent in the n-hexane fraction whereas, Alkaloids, phenols andsteroids were absent in ethyl acetate fraction (Table 3).

Table 3: Results of Phytochemical screening of 80 % methanol crude extract and its solvent fractions of the leaves of *Plectranthus cylindraceus* Hoechst. Ex. Benth

Phytochemical	Tests used	Crude extract	Hexane fraction	Ethyl acetate fraction	Aqueous fraction
Alkaloids	Wagner's test	+	+	-	+
Flavonoids	NaOH test	+	-	+	+
Saponins	Foam test	+	-	+	+
Phenols	Ferric chloride test	+	+	-	+
Tannins	Ferric chloride test	+	+	+	+
Terpenoids	Salowski's test	+	+	+	+
Glycosides	Keller–Kiliani test	+	-	+	+
Steroids	Salkowski's test	+	+	_	-

+: present; −: absent

4.3. Acute Dermal Toxicity Test

The topical application of a limit test dose of 2000 mg/kg of 10% crude extract ointment did not show any dermal toxicity (inflammation, irritation or redness) over 24 hours. After washing the applied ointment, there was also no death observed when the animals were monitored for 48 hr and for 14 consecutive days of cage side observation.Hence, the extracts were considered to be safe (Figure 5).

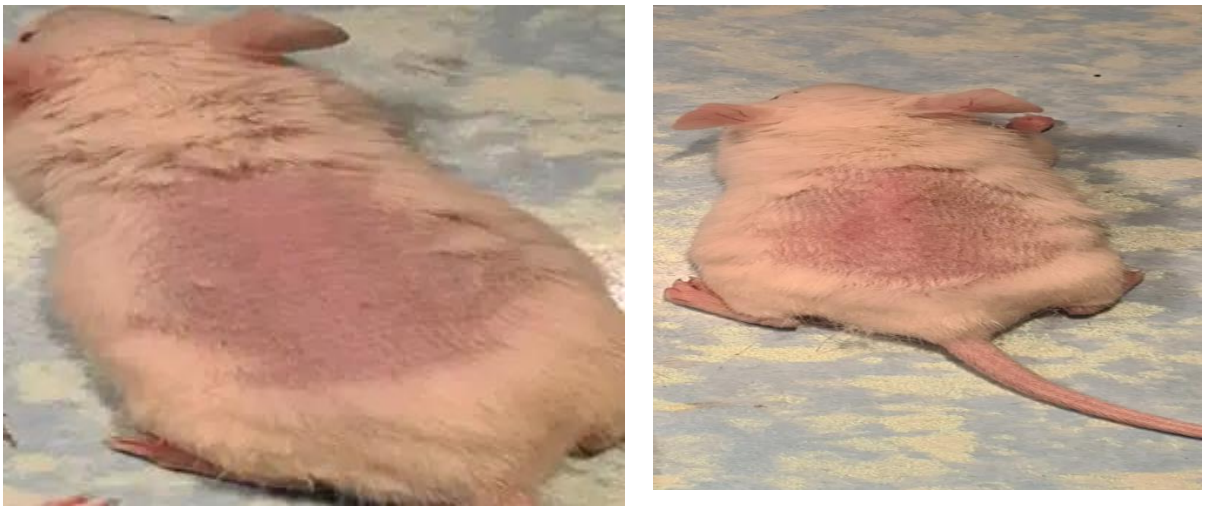


Figure 5: Images of acute dermal toxicity test results

4.4 Evaluation of Wound Healing Activity of the Crude Extract

4.4.1. Evaluation of the Crude Extract on Excision Wound Model

1. Wound Contraction

Treatment with a 10% (w/w) crude extract ointment and 0.2% w/v nitrofurazone ointment showed significant ($p < 0.01$) wound contraction on day 2 compared to a simple ointment. On days 4 and subsequently, the treatment showed a similar level of significance ($p < 0.001$) wound contraction compared to the simple ointment.The 5% (w/w) crude extract ointment also demonstrated significant wound contraction on day 2 ($p < 0.05$) and on day 4 and thereafter ($p < 0.001$) compared to the simple ointment. The 10% crude extract ointment and 0.2% w/v nitrofurazone ointment demonstrated significant wound contraction ($p < 0.001$) on days 4 and 6 compared to the 5% (w/w) crude extract ointment.During the treatment period, the 10% (w/w)

crude extract ointment demonstrated more notable wound contraction than the 0.2% nitrofurazone ointment, although the disparities failed to attain statistical significance (Table 4).

Table 4: Effects of the crude extract on the excision wound.

Wound area (mm2) ± SEM (% wound contraction)				
Days	Simple ointment	5% (w/w) crude extract ointment	10% (w/w) crude extract ointment	0.2% (w/v)nitrofurazone
Day 2	275.16 ± 0 .823 (8.28)	272.79 ± 0.413 (9.07) a*	271.90 ± 0.486 (9.37) a***	271.44 ± 0.589 (9.52) a**
Day 4	256.95 ± 0.746 (14.35)	232.12 ± 0.904 (22.63) a***	227.01 ± 0.728 (24.33) (ab)***	226.01 ± 0.624 (24.66)(ab)***
Day 6	238.48 ± 3.329 (20.51)	177.04 ± 0.988 (40.99) a***	163.44±0.383 (45.52) (ab)***	165.11± 0.992 (44.96) (ab)***
Day 8	211.03 ± 0.440 (29.66)	137.24 ± 0.612 (54.25) a***	135.79 ± 0.539 (54.74) a***	135.06 ± 0.694 (54.98) a***
Day 10	162.65 ± 1.50 (45.78)	128.44 ± 0.64 (57.19) a***	123.73 ± 1.270 (58.76) a***	125.69 ± 1.930 (58.10) a***
Day 12	124.86 ±1.060 (58.38)	84.44 ± 0.740 (71.85) a***	81.12± 0.857 (72.96) a***	82.26 ± 0.794 (72.58) a***
Day 14	81.80 ± 0.791 (72.73)	36.02 ± 0.507 (87.99) a***	34.64 ± 0.284 (88.45) a***	35.01 ± 0.581 (88.33) a***
Day 16	53.91 ± 0.626 (82.03)	7.16 ± 0.307 (97.61) a***	6.60 ± 0.171 (97.80) a***	6.64 ± 0.166 (97.79) a***
Day 18	23.52 ± 0.732 (92.16)	0.85 ± 0.173 (99.72) a***	0.00 ± 0.000 (100%) a***	0.00 ±0.000 (100%) a***

Notes: Wound areas are expressed as mean ± SEM (n = 6). a- Compared to Simple ointment, b- compared to 5% Crude extract ointment, *p<0.05, **p<0.01, ***p<0.001, # % wound contraction is from the initial wound area(300mm2)

2. Epithelialization period

The application of a 10% w/w crude extract ointment resulted in a statistically significant decrease in the duration of epithelialization when compared to both the negative control and the 5%w/w crude extract ointment ($p< 0.001$ and $p< 0.01$, respectively). Similarly, the 5% crude extract ointment found to have a statistically significant decrease in epithelialization period relative to the negative control ($p< 0.001$). Furthermore, the 0.2% nitrofurazone ointment demonstrated a significant reduction in epithelialization period in comparison to both the negative control and the 5%w/w crude extract ointment ($p< 0.001$ and $p< 0.05$, respectively (Table 5).

Table 5: Effects of the 80% methanol crude extract on epithelization period of excision wound

Treatment group	Period of Epithelialization (Days)	% Decrease the period of epithelialization
Simple ointment	20.50 ± 0.224	---
5% crude extract	16.67 ± 0.211 ***a	18.73
10% crude extract	15.50 ± 0.224**b***a	24.4
0.2% Nitrofurazone	15.67 ± 0.211 *b***a	23.6

Notes: Values are expressed as mean ± SEM (n = 6 in each group), analyzed by one-way ANOVA followed by Post hoc Tukey's test. a- Compared to SO, b- Compared to 5% CEO, *p < 0.05, **p< 0.01, ***p < 0.001, the % reduction in period of epithelialization is from the SO treated group.

4.4.2.Effect of the 80% methanol Crude Extract on Incision Wound Model

The tensile strength (wound breaking strength) of the incision wound was measured on the 10th day post-wounding, and mice treated with 10%w/w and 5%(w/w) extract ointment and 0.2% Nitrofurazone ointment showed statistically significant ($p< 0.001$) increases in tensile strength compared to simple ointments and left untreated groups. Treatment with 10% (w/w) crude extract ointment showed the highest percentage of tensile strength (67.2%), while the simple ointment group showed the least percentage of tensile strength (12.07%) compared to the left untreated group (Table 6).

Table 6: Effects of the crude extract on tensile strength of incision wound

Treatment group	Mean tensile strength ± SEM	Percent of tensile strength
Untreated	167.3 ± 0.667	--
Simple ointment	187.5 ± 0.847	12.07
5%w/w CEO	294.0 ± 0.577a, ***, b, ***	56.8
10%w/w CEO	313.50 ± 0.764a, ***, b, ***	67.2
0.2% NFO	310.83 ± 0.477a, ***, b, ***	65.8

Notes: Values are expressed as mean ± SEM (n = 6 mice in each group), analyzed by one-way ANOVA followed by the post hoc Tukey's test. a- Compared to left untreated, b- Compared to simple ointment, ***p<0.001.

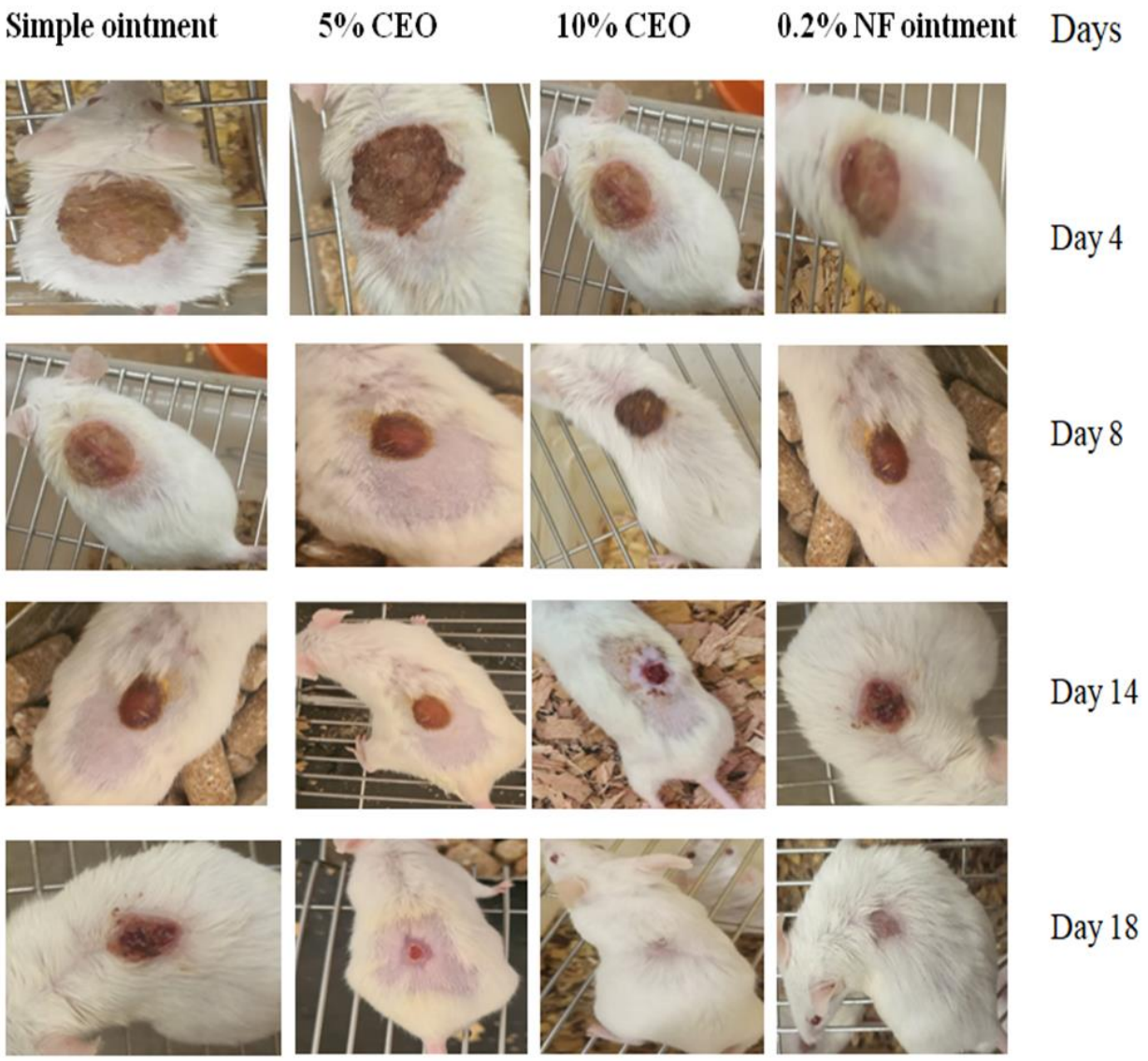


Figure 6: excision wound healing progress after treated with crude extract ointment

4.5. Wound Healing Activity of Solvent Fractions

1. Period of Epithelialization

Mice treated with 10% (w/w) aqueous fraction ointment reduced period of epithelialization significantly ($p < 0.001$) as compared to simple ointment, 5% (w/w) ethyl acetate fraction, 10% (w/w) ethyl acetate fraction, 5% (w/w) hexane fraction, and 10% (w/w) hexane fraction ointments. Treatment with 5%(w/w) aqueous fraction ointment also showed significant reduction in period of epithelialization ($p< 0.001$) as compared to simple ointment and 5% (w/w) n-hexane fraction ointment and ($p< 0.01$) as compared to 10% (w/w) n-hexane fraction ointment and 5% (w/w) ethyl acetate fraction.

Treatments with both 10% and 5% (w/w) ethyl acetate fraction ointments reduced the period of epithelialization significantly ($p< 0.001$ and $p< 0.01$ respectively) as compared to simple ointment. Groups treated with 10% (w/w) n-hexane fraction ointments reduced the period of epithelialization significantly ($p< 0.05$) as compared to simple ointment. Treatment with 10% (w/w) aqueous fraction ointment showed the highest percentage of reduction in the period of epithelialization, and treatment with 5% (w/w) n-hexane fraction ointment showed the lowest percentage reduction in the period of epithelialization as compared to simple ointment (Table 7).

Table 7:Effects of solvent fractions on the period of epithelization in excision wound

Treatment group	Mean period of epithelialization in days	% decrease in the period of epithelialization
Simple ointment	19.67 ± 0.21	---
5% (w/w) AQF ointment	16.67 ± 0.21(ab)***(cd)**	15.25
10% (w/w) AQF ointment	15.50 ± 0.22 (abcde)***	21.20
5% (w/w) n-HF ointment	18.50 ± 0.22	5.95
10% (w/w) HF ointment	18.33 ± 0.33 (a)*	6.81
5% (w/w) EAF ointment	18.17 ± 0.17 (a)**	7.63
10% (w/w) EAF ointment	17.83 ± 0.31 (a)***	9.35
0.2% (w/v) NF ointment	16.67 ± 0.33 (ab)***(cd)**	15.25

Values are expressed as mean ± SEM (n = 6), and one-way ANOVA followed by post hoc Tukey test was used for analysis. a, compared to the negative control, b compared to 5% (w/w) EAF ointment; c compared to 10% (w/w) EAF ointment; d compared to 5% (w/w) n-HF ointment; e compared to 10% (w/w) n-HF ointment. ∗p< 0.05; ∗∗p< 0.01; ∗∗∗p<0.001, AQF: aqueous fraction; EAF: ethyl acetate fraction; n-HF: n-hexane fraction, NF: nitrofurazone.

2. Wound contraction

Mice treated with 10% w/w aqueous fraction ointment showed significant (p< 0.001) wound contraction starting from day 2 onwards as compared to simple ointment,10 % w/w n-hexane fraction ointment ,5 % w/w n-hexane fraction ointment, and 5% w/w ethyl acetate fraction treated groups. The 10% w/w aqueous fraction ointment also showed statistically significant (p< 0.001) wound contraction starting from day 4 onwards as compared to 10% w/wethyl acetate fraction treated group.

Similarly, the 5%w/w aqueous fraction ointment showed statistically significant wound contraction (p < 0.001) as compared to simple ointment and (p < 0.01) as compared to 5% w/w ethyl acetate fraction and 5% w/w hexane fraction on day 2. From post-wounding day4 onwards, the 5% w/w aqueous fraction ointments showed more signifcant (p< 0.001) wound contraction as compared to simple ointment, 5% w/w n-hexane fraction, 10% w/w n-hexane fractionand 5% w/wethyl acetate fraction ointments.

Treatment with 10% (w/w) ethyl acetate fraction ointment showed significant wound contraction ($p< 0.05$) from day 2 to 4 as compared to simple ointment and from 6^{th} to 16^{th} ($p < 0.001$) post wounding days as compared to negative control and 5% w/w n-hexane fraction treated mice ,and ($p< 0.05$) from day 8onwards as compared to 10% w/w n-hexane fraction treated groups .while 5% (w/w)ethyl acetate fraction ointment showed significant wound contraction on day 8 and onwards and on day 12 onwards ($p< 0.001$) as compared to simple ointment and 5% w/w n-hexane fraction ointment respectively.

Treatment with 10% w/w n-hexane fraction ointment showed significant wound contraction from day 4 to 6 ($p< 0.05$), from day 8 to 10 ($p< 0.01$), and from day 12 onwards ($p< 0.001$) as compared to simple ointment Whereas, the 5% w/w n-hexane fractionointment showed significant wound contraction from day 8 to 10 ($p< 0.05$) and from day 12 to 16 ($p< 0.01$ as compared to simple ointment. Nitrofurazone 0.2%w/v ointment showed significant wound contraction ($p< 0.001$) on day2, and onward post- wounding days as compared to all extracts except to the 10% (w/w) aqueous fraction ointment. The effect of this extract was comparable to the effects of the standard treatment (Table 8&9).

Table 8: Effects of solvent fractions on excision wound contraction

Days	Wound Area (mm2)							
	SO	5% AQF	10% AQF	5% n-HF	10% n- HF	5% EAF	10% EAF	0.2% (w/v) NF
Day 2	289.01 ±0.75	276.77± 0.55 a***(bd)**	273.38 ± 0.45 (abcd)***	287.56 ± 0.63	286.65 ± 0.56	286.41± 0.94	286.02 ± 0.65 a*	271.95 ± 0.64 (abcde)***
Day 4	266.54 ±0.68	239.74 ± 0.89 (abcd)***	237.61 ± 0.47 (abcde)***	264.25 ± 1.34	262.39 ± 0.64 a*	263.23± 1.02	262.17± 0.58 a*	237.07± 0.70 (abcde)***
Day 6	228.82 ±1.58	196.30 ± 0.59 (abcd)***	192.64 ± 0.65 (abcde)***	225.97± 0.72	222.93 ± 0.70 a*	225.78 ± 0.87	219.13 ± 0 .85 (ab)***	191.24 ± 0.68 (abcde)***
Day 8	171.89 ±0.67	141.07± 0.73 (abcd)***	138.97 ± 0.41 (abcde)***	169.17± 0.41 a*	165.17 ± 0.83 a**	167.01 ± 0.77 a***	161.62 ± 0.90 (ab)***c*	141.10 ± 0.78 (abcde)***
Day 10	131.28 ±0.85	104.25±0.97 (abcd)***	100.28 ± 0 .63 (abcde)***	128.16 ± 0.94 a*	126.19 ± 0.66 a**	125.51 ± 0.72 a***	122.36 ± 0.55 (ab)***c*	102.33 ± 0.51 (abcde)***
Day 12	101.05 ±0.75	67.38 ± 0.53 (abcd)***	66.33 ± 0.61 (abcde)***	96.69 ± 0.74 a**	91.03 ± 0.40 a***	88.16 ± 0.71 (ab)***	87.74 ± 0.60 (ab)***c*	67.53 ± 0.54 (abcde)***
Day 14	60.73 ±.0.61	28.44 ± 0.63 (abcd)***	25.42 ± 0.56 (abcde)***	54.03 ± 0.59 a**	49.79 ± 0.24 a***	47.48 ± 0.51 (ab)***	47.13 ± 0.64 (ab)***c*	27.05 ± 0.65 (abcde)***
Day 16	26.06 ± 0.89	2.84 ± 0.36 (abcd)***	0.00 ± 0.00 (abcde)***	23.18 ± 0.38 a**	17.56 ± 0.64a ***	17.12 ± 0.78 (ab)***	12.45 ± 1.75 (ab)***c*	3.98 ± 0.40 (abcde)***
Day 18	11.22 ± 0.37	0.00 ± 0.00	0.00 ± 0.00	9.08 ± 0.40	1.84 ± 0.22 a***	3.80 ± 0.39 (ab)***	0.53 ± 0.35 (ab)***	0.00 ±0.00

Values are expressed as mean ± SEM (n = 6), and one-way ANOVA followed by post hoc Tukey test was used for analysis. a, compared to the negative control, b compared to 5% (w/w) EAF ointment; c compared to 10% (w/w) EAF ointment; d compared to 5% (w/w) n-HF ointment; e compared to 10% (w/w) n-HF ointment. ⁎p< 0.05; ⁎⁎p< 0.01; ⁎⁎⁎p< 0.001, SO: Simple ointment, AQF: aqueous fraction; EAF: ethyl acetate fraction; n-HF: n-hexane fraction

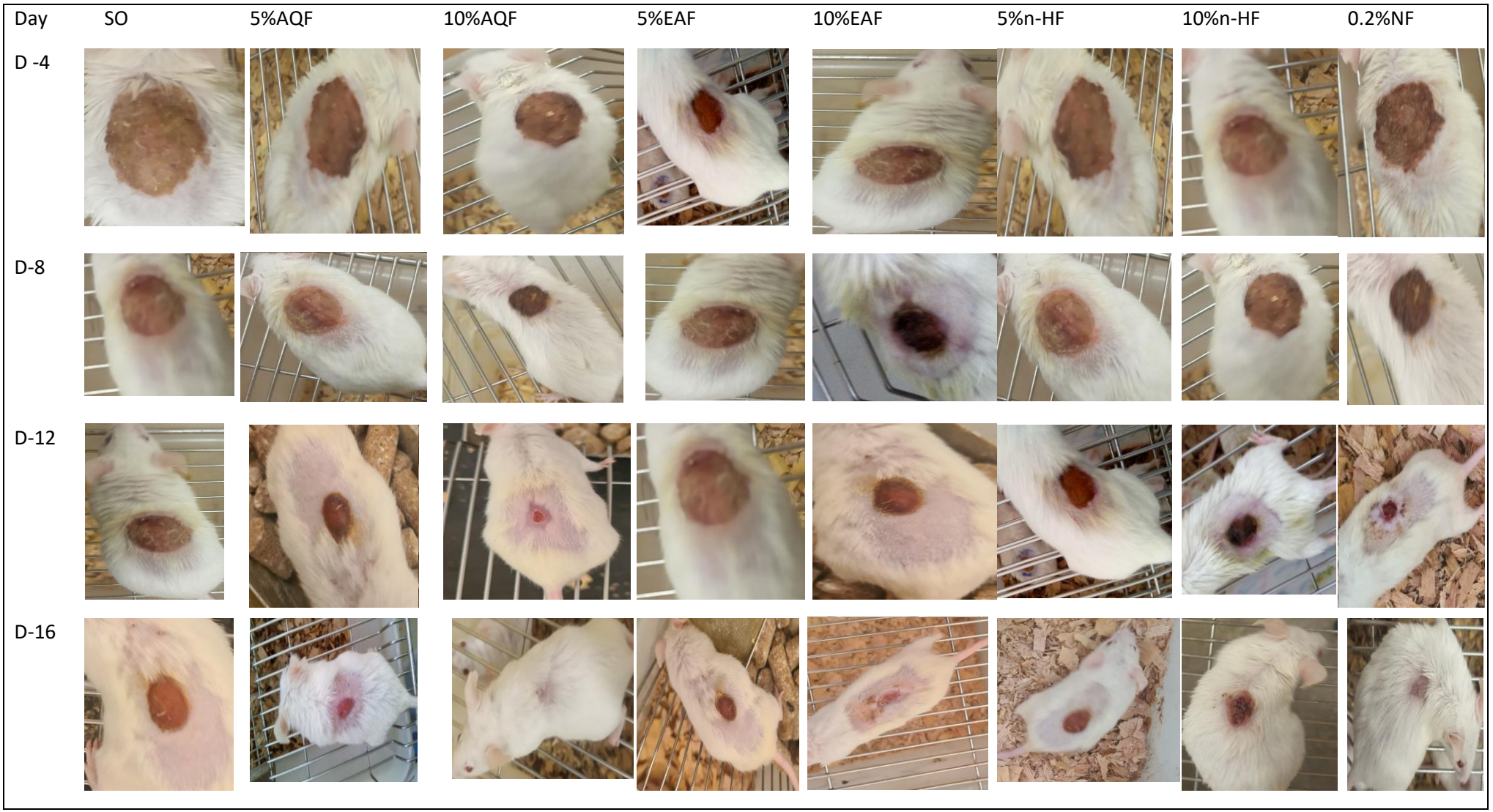


Figure 7; wound healing progress of excision wounds after treated with solvent fractions

Table 9: percent of wound contraction produced by solvent fractions

Groups	% Wound Contraction								
	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18
Simple ointment	3.66	11.15	23.73	42.70	56.24	66.32	79.76	91.31	96.26
5% (w/w) AQF ointment	7.74	20.09	34.57	52.98	65.25	77.54	90.52	99.05	100.00
10% (w/w) AQF ointment	8.87	20.80	35.79	53.68	66.57	77.89	91.53	100.00	100.00
5% (w/w) n-HF ointment	4.15	11.92	24.68	43.61	57.28	67.77	81.99	92.27	96.97
10% (w/w) n-HF ointment	4.45	12.54	25.69	44.94	57.94	69.66	83.40	94.15	99.39
5% (w/w) EAF ointment	4.53	12.26	24.74	44.33	58.16	70.61	84.17	94.29	98.73
10% (w/w) EAF ointment	4.66	12.61	26.96	46.13	59.21	70.75	84.29	95.85	99.82
0.2% w/v NF ointment	9.35	20.98	36.25	52.97	65.89	77.49	90.98	98.67	100.00

Note: #% wound contraction is from the initial wound area (300 mm2). Abbreviations: AQF: aqueous fraction; EAF: ethyl acetate fraction; n-HF: n-hexane fraction; NF, Nitrofurazone

4.6. Histopathological Studies

4.6.1. Histopathological evaluation of excised wound treated with crude extract

Wound treated with 10% w/w crude extract ointments showed high collagen deposition, fibroblast proliferation and neovascularization. Whereas wound treated with 5% w/w crude extract ointment showed moderate fibroblast proliferation, moderate collagen deposition, and neovascularization (Figure 7). Similarly, Nitrofurazone-treated wound showed high fibroblast proliferation, collagen deposition, and neovascularization while wound treated with simple ointment showed that moderate number of inflammatory cells.

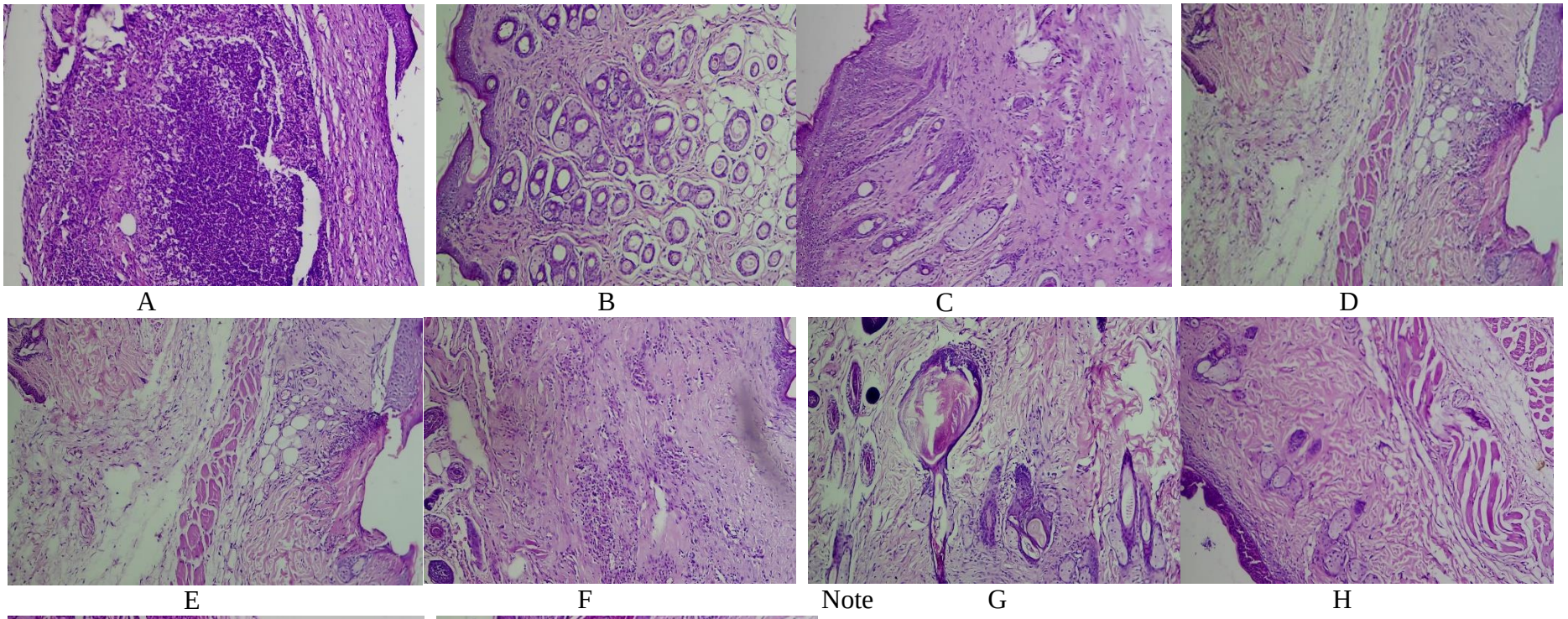
4.6.2. Histopathological evaluation of excised wound treated with solvent fractions.

Wound treated with 10% (w/w) aqueous fraction showed high fibroblast proliferation, collagen deposition, and neovascularization. Similarly, mice treated with 5% (w/w) aqueous fraction ointment showed high fibroblast proliferation, moderate collagen deposition, and high neovascularization whereas, Groups treated with 5% (w/ w)n-hexane fraction, 10% (w/w) n-hexane fraction,5%(w/w) ethyl acetate fraction, and 10% (w/w) ethyl acetate fraction ointments showed moderate fibroblast proliferation, moderate to high number of inflammatory cells, low collagen deposition, and low neovascularization.

Table 10: Histopathological results of the effects of the crude extract and solvent fractions on healed excision wound

Crude extract					
Group	FB	CD	MNC	PMNC	NV
Simple Ointment	+	-	++	++	++
5% w/w Crude Extract Ointment	++	++	+	+	++
10%w/w Crude Extract Ointment	+++	+++	+	+	+
0.2% nitrofurazone w/v ointment	+++	++	+	+	++
Solvent fractions					
Simple ointment	+	_	++	++	++
5% w/w AQF ointment	+++	++	+	+	+++
10% w/w AQF ointment	+++	+++	+	+	+++
5% w/w n-HF ointment	+	_	++	+++	+
10% w/w n-HF ointment	+	+	++	++	+
5% w/w EAF ointment	+	+	+++	+++	+
10% w/w EAF ointment	+	+	+	++	+
0.2% nitrofurazone w/v ointment	+++	++	+	+	++

Notes: absent (-) Low number (+), moderate number (++), and high number (+++), FP: fibroblast proliferation; CD: collagen deposition; MNC: mononuclear cells; PMNC: polymorphonuclear cells; NV: neovascularization, AQF: aqueous fraction; EAF: ethyl acetate fraction; n-HF: n-hexane fraction.



A: wound treated with simple ointment showing poor collagenization and fibroblasts. B: wound treated with 5% crude extract ointment, showing moderate collagen deposition, C:wound treated with 10% crude extract ointment showing good collagen deposition and neovascularization. D:wound treated with 0.2% nitrofurazone ointment, showing moderate level of collagen and fibroblasts. E: wounds treated with 5% aqoues fraction ointment , showing good collagen deposition, F:wound treated with 10% aqoues fraction showing higher collagen deposition, fibroblasts and neovascularization, G: wound treated with 5% ethyl acetate fraction showing moderate collagen,with mild inflammation. H: wound trated with 10% ethyl acetate fraction scant connective tissue deposition and moderate inflammation , I :wound treated with 5% n- hexane fraction showing intense inflammation, poor collagen deposition, J:wound treated with 10% n-hexane fraction showing moderate inflammation

Figure 8; Histophathological results of the effects of the crude extract and solvent fractions on healed excision wound

5. DISCUSSION

In this study, the wound healing properties of the methanolic crude extract of *Plectranthus cylindraceus* leaves and its solvent fractions were evaluated using two models, the excision and incision wound models, both models were used for the methanolic crude extract while the solvent fractions were assessed using only the excision wound model.

We employed excisional wound healing models in our study, as they more accurately reflect the healing process of human wounds compared to incisional models. This model enables the assessment of the various events that occur during skin wound healing. It closely simulates the process of acute wound healing in humans, thereby providing a well-defined and easily quantifiable wound bed for histological analysis (Moura Estevão *et al.*, 2019; Sun *et al.*, 2020).

With 80% methanol, the maceration technique was used for extraction of the study plant. Maceration is a very popular, economical, practical, and more appropriate method of extracting medicinal herbs than other extraction processes. To achieve the highest level of flavonoid and phenolic content, the most popular solvent for maceration was methanol (Butsat and Siriamornpun, 2016; Peiris *et al.*, 2023)

Both the crude extract and the fractions were made into ointments in order to guarantee the continuous and extended release of active components in the area of the wound. Topical treatment is the preferred method of treating wounds because it is simple to remove, has enough concentration in the area where it is applied, and has minimal systemic side effects. This helps the healing of wounds. Wool fat, cecostearyl alcohol, and other ointment ingredients give the product thickness and stability, and soft and hard paraffin keeps the skin from drying out (Yiblet *et al.*, 2022).

The study showed that the methanolic crude extract and the aqueous solvent fraction exhibited fast wound healing activities in the excision wound model, as characterized by an accelerated rate of wound contraction and a reduced period of epithelization. The rapid wound contraction exhibited by the plant extract is likely due to the enhanced proliferation of fibroblasts, collagen deposition, and neovascularization, as revealed by histopathological analysis in Figure 5 (A-J).

The proliferation of fibroblasts results in the differentiation into myofibroblasts, which increases the pulling forces between cells on the opposite side of the wound.whereas collagen contributes to the physical strength of the healing tissues by binding them together. The newly formed blood capillaries in the healing excision wounds provide the granular cells with oxygen and nutrient supplies, ultimately leading to the complete healing of the wound (Thiruvoth *et al.*, 2015).

Furthermore, the wound healing effect of the 10% w/w crude extract and aqoues fraction were shown to be of comparable percentage of wound contraction to other studies conducted on the same genus *(Plectranthus tenuiflorus*), which reported significant wound healing activity (Khorshid *et al.*, 201). This suggests that the genus (*Plectranthus*) is endowed with wound healing activity.

Additionally , in the excision wound models of both the crude extract and the aqueous fraction, the period of epithelialization was observed to be significantly reduced in comparison to that of the simple ointment.This reduction in the period of epithelialization demonstrated by the plant extract is likely attributed to the accumulation of secondary metabolites that play a crucial role in determining its medicinal value.

Plants may contain bioactive compounds with antioxidant, anti-inflammatory, and antibacterial properties (Casadiego *et al.*, 2023). In previous studies it was reported that the leaves and the Aerial part of *Plectranthus cylindraceus,* possesses antioxidant activity (Ali *et al.*, 2014; Mothana *et al.*, 2019). Hence, the antioxidant property of *Plectranthus cylindraceus* might have contributed to its wound healing activity.

Wound healing activity of the crude extract was also evaluated using the incision wound model, and the results indicated that the crude extract ointment displayed a statistically significant increase in tensile strength compared to both the untreated and simple ointment-treated groups. This might be due to increased collagen synthesis per cell (as evidenced by histopathological analysis) and facilitated cross-linking of the proteins since the tensile strength of a wound is primarily dependent on collagen concentration and fiber cell stability (Aulia and Bangun, 2018).

The present study revealed that the aqueous fraction showed a higher wound healing activity compared to the crude extract. Specifically, the 10% w/w aqueous fraction showed complete wound closure on the 16th day of treatment, whereas the 10% w/w crude extract ointment demonstrated 97.8% wound closure on the same day, with a statistically significant difference ($p<0.001$). In terms of the epithelialization period, there was no significant difference between the crude extract and aqueous fractions. Furthermore, the crude extract ointment demonstrated more rapid wound contraction and an enhanced period of epithelization compared to the ethyl acetate and n-hexane fraction ointments, with statistical significance ($p<0.001$).

The higher therapeutic efficacy of the aqueous fraction in our study may be attributed to the facilitated liberation of polar constituents from the simple ointment base. In particular, the most polar components in the aqueous fraction are more effectively released than non-polar matrices compared to less polar compounds (Mekonnen *et al.*,2012 ; Botanical and Lamiaceae, 2021). Polar solvents such as water are often employed in the extraction of polar compounds including flavonoids, glycosides, tannins, and certain alkaloids.The application of these solvents facilitates the effective isolation and identification of these compounds, which are of considerable interest in a variety of fields of study (Sticher, 2008). This suggests that more phytochemicals with wound healing properties are present in polar solvents.

While the n-hexane fraction exhibited the least wound healing activity compared to the other fractions, crude extract, and aqueous fraction, it is important to note that the delayed and reduced effect may be attributed to the varying concentration of active components responsible for wound healing, which is influenced by solubility differences (A. Hussein and A.El-Anssary, 2019). Additionally, the preliminary phytochemical study conducted on the n-hexane fraction revealed lower levels of phenolic compounds, which are known to possess wound healing properties, compared to the other fractions.

In the current study, the phytochemical analysis of the crude extract and the solvent fractions of *Plectranthus cylindraceus* leaves were found to contain alkaloids, flavonoids, phenols, tannins, steroids, glycosides, and terpenoids in both crude extract and aqueous fraction. Furthermore, it was reported that leaves of *Plectranthus cylindraceus* contain terpenoids such as carvacol, thymol, diterpenoids, and triterpenoids in previes studies (Ali *et al.*, 2014; Abdissa, et al., 2017) which are known to promote wound healing due to their antioxidant and antimicrobial properties.

Additionally, triterpenoids have been known to enhance collagen content, which is a vital factor in the wound healing process (Albahri *et al.*, 2023).

The observed wound healing activity of the crude extract and solvent fractions may be primarily attributed to the individual or additive effects of phytoconstituents. Plants which are rich in a wide variety of secondary metabolites belonging to chemical classes such as tannin,terpenoid, alkaloid and polyphenol are generally superior in their wound healing activities (Justin *et al.*, 2014).

Tannins have been demonstrated to expedite wound healing through their ability to chelate with free radicals and promote the contraction of the wound. This is achieved by the astringent effect of tannins on proteins, which causes them to shrink and leads to the formation of capillary vessels and fibroblasts (Jiang *et al.*, 2010).Phenols possess a range of biological activities, including strong antioxidant and antibacterial properties. Many studies have shown that phenols are highly effective in scavenging free radicals due to their redox properties (Albahri *et al.*,2023). Additionally, steroids have been found to possess both antibacterial and anti-inflammatory activities (A. Hussein and A. El-Anssary, 2019).

Flavonoids are known to promote the wound-healing process mainly due to their anti-microbial and free radical scavenging activities, which may be responsible for wound contraction and increased rate of epithelialization (Grayer *et al.*, 2010). Additionally, flavonoids have been reported to lower the synthesis of inflammatory mediators, increase collagen synthesis, and promote the cross-linking of collagen (Maheswari *et al.*, 2016).

Terpenoids have been noted to exhibit a wound-healing effect by disrupting bacterial membranes, preventing DNA damage through antioxidant activity, and neutralizing free-radical scavenging enzymes that result from free radicals (Chen and Hou, 2019). Alkaloids possess wound-healing activity due to their antibacterial and anti-inflammatory effects sincethey inhibit the synthesis of inflammatory mediators such as prostaglandins from arachidonic acid (Hamzah *et al.*, 2022).

Previous studies have established that the leaves of *Plectranthus cylindraceus* possess antimicrobial properties against a variety of pathogens, including *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Candida albicans*, which are commonly associated with

wound infections (Marwah *et al.*, 2007; Ali *et al.*, 2014; Frese and Sewald, 2017). Those *in-vitro* studies conducted on the extract of the plant have indicated that the antimicrobial activity may exert a protective effect on the wounded area by preventing microbial growththereby inhibiting infection and creating a clean wound bed for the initiation and progression of the natural tissue repair process. This also supports the wound healing activity of the plant extract.

As previously mentioned,a study conducted in Oman found that *Plectranthus cylindraceus* contains significant amounts of carvacrol following phytochemical screening (Marwah *et al.*, 2007). Carvacrol has previously been established to possess numerous medicinal properties, exhibits a broad spectrum of activity against bacteria and fungi.The antibacterial effect of carvacrol is attributed to its positive impact on wound healing (Costa *et al.*, 2019; Marinelli *et al.*, 2022). Therefore, the presence of phytochemicals in the extract, such as carvacrol, and their antibacterial activities may contribute to the wound healing processeither independently or in synergistic fashion.

It has been observed that, in all the crude extract and solvent fractions used in the wound healing models,ointment with a higher concentration (10% w/w) showed a better effectiveness than ointment with a lower concentration (5% w/w) of the plant. This may imply their wound healing effect is proportional to the dose of the chemical ingredients in the plant with pharmaceutical value (Hamzah *et al.*, 2022). All the results discussed in this study provide evidence that supports the claim regarding the wound-healing properties of the leaves of *Plectranthus cylindraceus.*

Limitations of the study

This study has revealed an important ethnopharmacological finding on the traditional use of *Plectranthus cylindraceus* in the mangemnet of wound lending support to the traditional use. The study applied current state of art science for the study, however, due to limitations in in resources, some important studies have not been conducted.These includes: determining the active components of the plant, deterimination of the safety of the plant extract on long term use,determine the mechanism of action of the plant extract, and estimation of the hydroxyproline content.

6.CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

The current study showed that the 80% methanol extract and solvent fractions of the leaves of *Plectranthus cylindraceus Hochst. Ex. Benth*, prepared as ointment formulations with strengths of 5% w/w and 10% w/w, displayed noteworthy wound healing activity compared to the negative control group (simple ointment). Among the solvent fractions, the aqueous and ethyl acetate extracts showed significant wound healing activities, as evidenced by rapid wound contraction, decreased period of ephethelazation, and histophathological assessment.These findings suggest that the presence of bioactive metabolites within the extract may be responsible for its wound healing properties. Therefore, the finding lends support for the traditional use of the plant's leaves for wound treatment.

6.2. RECOMMENDATIONS

Basedo n the findingsof this study, we recommend the following for further study:

- Conducting quantitative phytochemical study to clearly quantify potential active components against wound from the plant
- Chronic toxicity studies should be performed
- Establishing the mechanism for wound healing activity of the plant
- It is imperative that the results of the current study should be confirmed through hydroxyproline assay

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APPENDICES

Phytochemical Screening procedure

1. Saponins test: - To 0.5 gm of crude and fractions 2 ml of distilled H_2O was added. After shaking vigorously, a stable foam was observed for 10 minutes. A stable persistent foam was observed that indicated the presence of saponins.
2. Terpenoids test: - To 0.25 gm of crude and fractions chloroform was added. Then, a layer was created after 3ml concentrated H_2SO_4 added. At the interface the appearance of reddish-brown color indicated the presence of terpenoids.
3. Test for flavonoids: - To 0.2gm of the crude and fractions, 5ml 95% ethanol, few drops of concentrated HCL and 5gm of magnesium turnings were added. The pink color appearance indicated presence of flavonoids.
4. Test for glycosides: - To 2 ml of the extract, 2ml of glacial acetic acid, $FeCl_3$, and concentrated H2SO4were added. At the junction of two liquids reddish brown color and the bluish color at the upper layer were formed indicated the presence of glycosides.
5. Test for alkaloids: - After a mixture of 2-3 drops of the crude extract and fraction and 1ml of 1% HCl heated few drops of Wagner´s reagent was added to a mixture. A precipitate was observed taken as a positive for presence of alkaloids.
6. Test for tannins: -The 1ml the crude extract and fraction diluted with water then 2 drops of ferric chloride added the transient greenish to black color indicates the presence of tannins
7. Test for steroids: - To 2ml of the extract 1ml of chloroform, 1ml of H_2SO_4 were added. Then after shaking, the chloroform layer become red and the acid to yellow with green florescence. This color appearance was an indication of the presence of steroids.
8. Test for phenols: - The 2ml of 2%$FeCl_3$ solution was mixed with 2ml of the extract. Appearance of bluish black color was as positive indication for the presence of phenols.