Wound Healing and Antimicrobial Activities of Extracts and a Major Compound Isolated from the Tuber of *Arisaema schimperianum* Schott. (Araceae)

By: Betelhem Awoke (B.pharm.)

Advisors: Professor Kaleab Asres (PhD)

Dr. Daniel Bisrat (PhD)

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This is to certify that the thesis prepared by Betelhem Awoke, entitled: "**Wound Healing and Antimicrobial Activities of Extracts and a Major Compound Isolated from the Tuber of *Arisaema schimperianum* Schott**" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacognosy complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Examiner (External) Mr. Abyot Endale Signature_____ Date _____

Examiner (Internal) Dr. Wondemagegn Tamiru Signature ———————— Date _____

Advisors: Professor Kaleab Asres Signature _____Date _____

Dr. Daniel Bisrat Signature _____Date _____

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Abstract

Wound Healing and Antimicrobial Activities of Extracts and a Major Compound Isolated from the Tuber of *Arisaema schimperianum* Schott.

Betelhem Awoke
Addis Ababa University, 2024

Globally, wounds are a growing health concern due to rising vascular diseases. Traditionally, Ethiopians have used *A. schimperianum* to treat wounds and infections. This study aimed at evaluating the wound healing and antimicrobial activities of extract and a major compound isolated from the tubers of *A. schimperianum* Schott. Wound healing was determined in mice using excision and incision wound models, whilst *in vitro* antimicrobial activities were evaluated by broth dilution method. The acute oral toxicity test result of the hydroalcoholic extract of *A. schimperianum* tuber was safe at a dose of 2000 mg/kg. Furthermore, topical application of a maximum concentration (10% (w/w)) ointment of the extract was safe showing mild irritation. The study also revealed that the 80% methanol extract and solvent fractions of *A. schimperianum* tuber formulated into a simple ointment at concentrations of 5% (w/w) and 10% (w/w) are endowed with variable but significantly greater wound contraction compared to the negative control group ($p < 0.001$), with a faster epithelialization period and improved tensile strength. Notably, the water fraction exhibited a stronger wound healing effect and higher tensile strength than the other fractions. Similarly, the hydroalcoholic extract and all the solvent fractions inhibited growth of all the tested bacteria, with a more pronounced effect on Gram-positive than Gram-negative bacteria. In particular, the water fraction displayed the lowest minimum inhibitory concentration of 0.5 mg/ml against MRSA. Further phytochemical analysis of the water fraction using reverse thin-layer chromatography led to the isolation of a glyceryl

glycoside tentatively identified as 3-O-[α-D-galactopyranosyl-(1"→6')-O-β-O-galactopyranosyl] glycerol (GGG) based on spectral analysis. The 10% (w/w) formulation of the isolated compound GGG significantly increased wound contraction, decreased epithelializtion time and increased hydroxyproline content and tensile strength ($p < 0.001$) as compared to controls. GGG displayed stronger activity against Gram-positive bacteria than Gram-negative bacteria but failed to show any effect against the fungal strains tested. On the whole, results of the present study demonstrated that in all parameters, the effects of GGG were superior to those of the positive control (0.2% (w/v) nitrofurazone ointment). In conclusion, the experimental determination of the extract was safe on topical application would justify the use of the plant as a natural therapeutic agent for wound management supporting the traditional claims of the plant for the treatment of wounds.

Key words: *Arisaema schimperianum* tuber, Araceae, Wound healing, Antibacterial, Antifungal, 3-O-[α-D-galactopyranosyl-(1"→6')-O-β-O-galactopyranosyl] glycerol

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List of abbreviations and acronyms

^{13}C NMR	Carbon thirteen nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance
2D	Two dimensions
AMR	Antimicrobial resistance
ANOVA	One-way analysis of variance
ATCC	American type culture collection
BP	British pharmacopoeia
CDC	Centers for disease control and prevention
CLSI	Clinical and laboratory standards institute
DMSO	Dimethyl sulfoxide
ECM	Extracellular matrix
EPHI	Ethiopian public health institute
FT-NMR	Fourier transforms nuclear magnetic resonance
HSQC	Heteronuclear single quantum coherence
HIV/AIDS	Human immune virus acquired immune deficiency disease
IP	Intrapertonial
HE	Hematoxylin and eosin
LD50	Lethal dose kills to 50% of exposed mice
MEBO	Moist exposed burn ointment
MHA	Muller hinton agar
MHB	Mueller hinton broth
MIC	Minimum inhibitory concentration

MS	Mass spectrometry
NB	Nutrient broth
OECD	Organization for economic cooperation and development
RP-TLC	Reverse phase thin layer chromatography
SDA	Sabouraud dextrose agar
SDB	Sabouraud dextrose broth
SEM	Standard error of mean
UV	Ultraviolet
WHO	World health organization

1. Introduction

1.1 Overview about skin and wound

The skin, which is the largest organ of the body, makes up about 15% of total body weight (Kolarsick *et al.*, 2006). It serves as the body's primary barrier against external threats, rendering it highly vulnerable to foreign substances and infections (Leong *et al*., 2016). Wound is a break in the integrity of the skin disrupting its normal function (Velnar *et al*., 2009). Wound is classified as open or closed wound based on its cause, and as acute or chronic wound based on the time it takes to heal. Acute wounds heal normally after external injury while chronic wounds often are linked to underlying conditions and fail to heal in a timely and orderly manner (Chhabra *et al.*, 2017). Chronic wound has been a long-standing problem in clinical practices with early and late complications (Mekonnen *et al.*, 2013).

The prevalence and associated healthcare costs of chronic wounds have risen globally, primarily due to escalating rates of vascular diseases. Currently, it is estimated that approximately 6 million individuals develop chronic wounds annually. This surge places a significant economic impact, estimated nearly 2-4% of health budgets (Järbrink *et al.*, 2016). In developing regions, chronic wounds affect around 1% to 2% of the population during their lifetime (Nussbaum *et al.*, 2018). Wounds can result from diverse causes such as mechanical injury, chemical contact, thermal exposure and microbial infections (Thakur *et al.*, 2011).

Various treatment options are available for wound management but majority of these therapies produce numerous unwanted side effects. In recent years, herbal remedies have emerged as a promising alternative due to their potential safety and effectiveness (Dan *et al.*, 2018). In Ethiopia numerous medicinal plants are known for their wound healing properties such as *Acacia*

abysinica, Achyranthes aspera Brucea antidysentrica, Croton macrostachyus, Datura stramonium, Eucalyptus globules and *Rumex abyssinicus* (Alemu *et al.*, 2024).

1.1.1 Physiology of wound healing process

Wound healing is a biological process to maintain normal anatomical structure and function of the skin (Guo and DiPietro, 2010). The various processes of acute tissue repair, may be united into a sequence of time dependent phases (Table 1) namely, coagulation and hemostasis, inflammation, proliferation and remodeling (Velnar *et al.*, 2009). Hemostasis stops bleeding by constricting blood vessels, activating the coagulation cascade and forming a fibrin plug, protecting the vascular system and providing a matrix for healing cells (Kolarsick *et al*., 2006). Inflammatory (debridement) phase is critical for the maintenance of normal structure (Leong *et al*., 2016). It can roughly be divided into an early phase, characterized by increasing of neutrophils that starts with the critical task of phagocytosis in order to remove bacteria and foreign particles. The late phase is characterized by the appearance of macrophages that have multiple actions, such as debridement, antimicrobial and wound regulation (Guo and DiPietro, 2010). The proliferation (granulation) phase is characterized by extracellular matrix (ECM) formation, angiogenesis, epithelialization and wound contraction. Granulation tissue which fills up the wound gap provide a scaffold for cell migration, growth and differentiation during wound repair and serve as antigen presenting cells as well as enhance angiogenesis (Pastar *et al.*, 2014). Remodeling (maturation) phase is the final healing process which is characterized by wound contraction and collagen collection to produce greater tensile strength and maintaining balance between synthesis and degradation (Childs and Murthy, 2017).

Table 1: The four phases of wound healing process (Chhabra *et al*., 2017)

Phase of healing	Time post injury	Cells involved	function phase
Homeostasis	Immediately	Platelets	Clotting
Inflammation	Day 0-5	Neutrophils or macrophages	Phagocytosis
Proliferation (granulation or contraction)	Day 3-14	Macrophages Lymphocytes Neurocytes Fibroblasts Keratinocytes	Fill defect Re-establish Skin function closures
Remodeling (maturation)	Day 7-2years	Fibrocytes	Develop tensile strength

1.1.2 Factors that can interfere with wound healing

Naturally the wound healing system back up the structure and functional state of injured tissue in a predictable and timely manner. However, several factors can interfere with this process, leading to delayed, incomplete, or fail entirely healing which can result in abnormal scarring (Thakur *et al.*, 2011). Contributing factors encompass microbial infection, insufficient oxygenation and the existence of external substances, collectively called local factors. Systemic factors involve age, hormonal fluctuations, stress, diabetes, obesity, alcohol use, smoking, genetic conditions, nutritional deficiencies, and medications such as steroids and chemotherapy agents (Guo and DiPietro, 2010; Childs and Murthy, 2017). In developing countries, infectious wounds are common due to inadequate hygiene practices and sanitation (Mekonnen *et al.*, 2013).

1.1.3 Infectious wound

Wound infection worsens and delays the healing process of chronic wound. The majority of wounds are invaded with polymicrobial involving both Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes* and Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*. In some cases, fungi and viruses may also be involved (Dowd *et al.*, 2011). More than 85% of spinal cord injured patients develop an infected pressure ulcer at least once in their life (Espejo *et al.*, 2018). Patients with diabetes have at least a 10-fold greater risk of being hospitalized for foot infection than individuals without diabetes (Reddy *et al.*, 2012). Additionally, the incidence of surgical site infections in Africa is high and varies significantly, ranging from 2.5% to 30.9% (Legesse *et al*., 2017). According to the World Health Organization (WHO) and the Centers for Disease Control and Prevention**,** antibiotic-resistant infections currently account for at least 1.27 million deaths worldwide each year. In the context of wound infections, antibiotic resistance has become particularly concerning in cases involving methicillin-resistant *S. aureus* (MRSA) and resistant strains of *E. coli* and *P. aeruginosa* (WHO, 2023; CDC, 2024). Hospitalized patients with diabetes, compromised immune systems or HIV/AIDS are among the most vulnerable groups when it comes to antibiotic resistance (Sisay *et al*., 2019).

1.2 The genus *Arisaema*

The genus *Arisaema* (commonly known as "Cobra Lilies" or "Jack in the Pulpit") is one of the largest genera in the family Araceae, subfamily of Aroideae. It consists more than 250 species (Tran *et al*., 2022). Members of the family are monocotyledonous flowering plants widely distributed in the world. Members of the genus mainly grow in the evergreen and deciduous forests mainly populated in China and Japan and others in Southern Asia, East Africa, Central

Africa, Eastern North America, Mexico and Malaysia (Kant *et al*., 2020). The genus is unique due to its resemblance with the pitcher plant and lives 100 years and characterized by being seasonal, sequential hermaphroditism, having beautiful leaves, an unusual flower and tuberous stem. Many species are used for different medicinal purposes and some of them are used as vegetable or food (Ali and Yaqoob, 2021; Tran *et al.*, 2022).

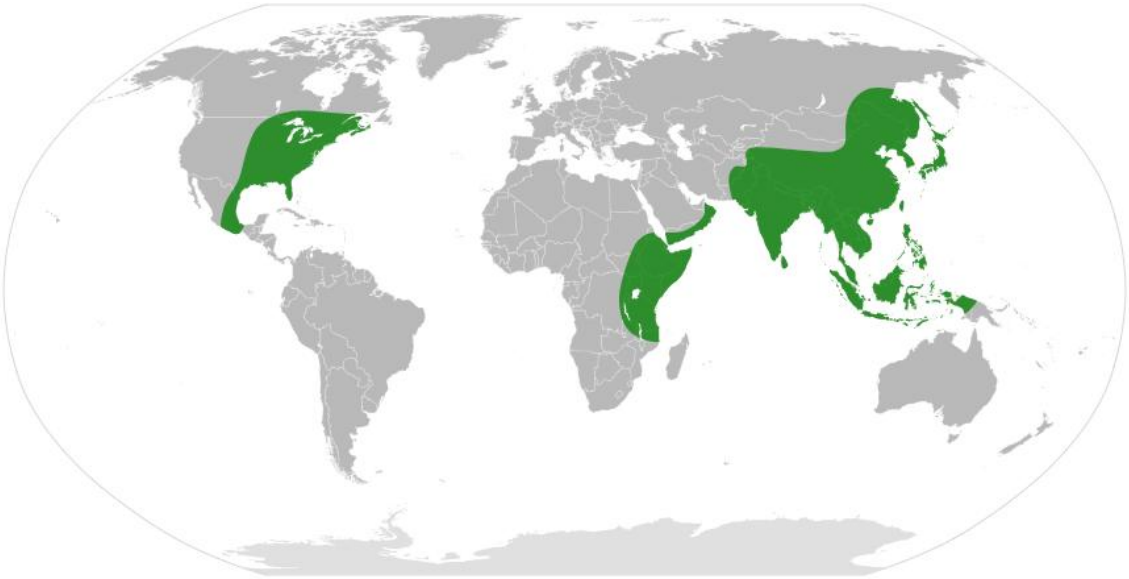


Figure 1: The distribution of plants belonging to the genus *Arisaema* (Ninjatacoshell, 2024)

1.2.1 Ethnobotanical uses

Arisaema species is widely used in the Asian traditional medicine, particularly in countries such as China, India, Nepal, Pakistan and Maynamar. Most of them are majorly used for the treatment of skin infections, nematode infections, inflammation, stroke, cancer, epilepsy, stomach problems, tetanus and sterility (Kant *et al.*, 2020). Uses of the most widely known members of the genus are depicted in Table 2.

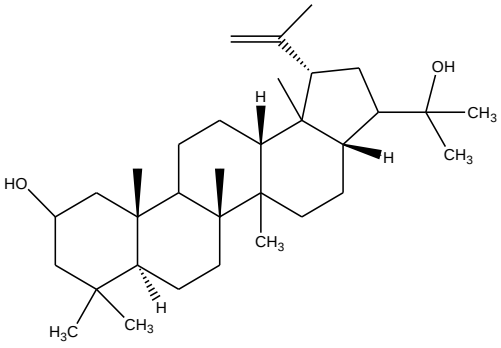
Table 2: Ethnobotanical uses of common species in genus *Arisaema*

No	Species	Part used	Disease treated	Reference
1.	*A. amurense*	Rhizome	Pain, tumor and snake poison	(Zhu *et al.*, 2013)
2.	*A. erubescens*	Stem	Lung, pituitary and brain cancers	(Du *et al.*, 2011)
		Root	Gastric upset, convulsions	(Wang *et al.*, 2023)
3	*A. franchetianum*	Tuber	Inflammation and swelling	(Su *et al*., 2013)
4.	*A. heterophyllum*	Tuber	Stroke, epilepsy and paralysis	(Sun *et al*., 2023)
5.	*A. jacquemontii*	Whole plant	Wound, infection, obstruction, Infertility and stomach problems	(Roshan *et al*., 2017) (Bala *et al*., 2019)
6.	*A. propinquum*	Rhizomes	Diabetic neuropathy, rheumatoid arthritis,	(Mir *et al.*, 2019)
		Fruit	Chronic boils and skin eruptions	
7.	*A. triphyllum*	Root	Sore eyes, sterility, headaches, boils, abscesses and ringworm	(Kant *et al.*, 2020)
8.	*A. tortuosum*	Whole plant	Constipation, indigestion, snake poison	(Kant *et al*., 2018)
			Dysentery, parasites, hepatoxic and cancer	(Verma *et al.*, 2012)
10.	*A. utile*	Rhizome	Infection in blood, liver and bile, parasitic and cancer	(Bhat *et al.*, 2019)

1.2.2 Phytochemistry

Arisaema species are rich in bioactive components that fall under various classes of secondary metabolites. The literature reveals that terpenoids, flavonoids, and glycosphingolipids are the primary chemical components of the genus (Kant *et al.*, 2020). Tanveer *et al*. (2013) showed that *A. amurense* and *A. flavum* are adept in the biosynthetic elaboration of pentacyclic triterpenoids like 2-hydroxydiplopterol **(1)**. The authors reported that it was for the first time that this compound was isolated from plants. Similarly, the leaves of *A. tortuosum* were shown to contain several flavonoids including the C-glycoside schaftoside **(2)** (Rittà *et al*., 2020). Diacylglyceryl-

galactosides have also been reported as constituents of *Arisaema* spp. Jung *et al*. (1996) isolated several 1,2-O-diacyl-3-O-β-D-galactopyranosylglycerols and 1,2-O-diacyl-3-O-[α-D-galacto-pyranosyl-(1→6')-O-beta-D-galactopyranosyl] glycerols as single components from *Arisaema amurense*. Of these, (2S)-1-O-octadecanoyl-2-O-(9Z, 12*Z*-octadecadienoyl)-3-O-[α-D-galacto-pyranosyl-(1"→6')-O-β-O-galactopyranosyl] glycerol **(3)** was the prominent compound. Furthermore, Rozema *et al*. (2012) reported the presence of glycosphingolipids which are important components of animal muscle and nerve cell membranes in the rhizomes of *A. amurense*. A bioactive cerebroside, 1-O-β-D-glucopyranosyl-(2S,3R,4E,8Z)-2-[(2(R)-hydroxyicosanoyl)-amido]-4,8-octadecadiene-1,3-diol **(4)** (Jung *et al.*, 1996) and a sphingosine, 4-*trans*-8-*trans*-sphingadiene **(5)** were isolated from *A. amurense* (Rozema *et al.*, 2012)**.**



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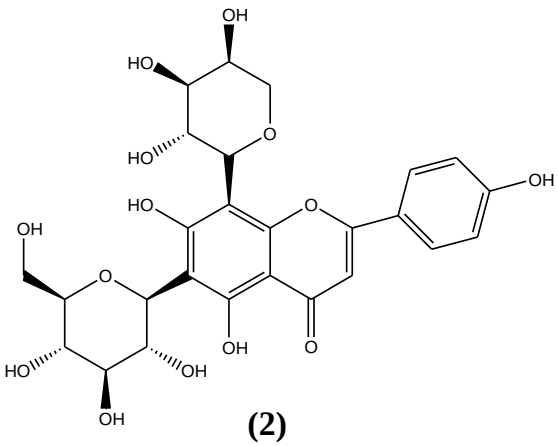

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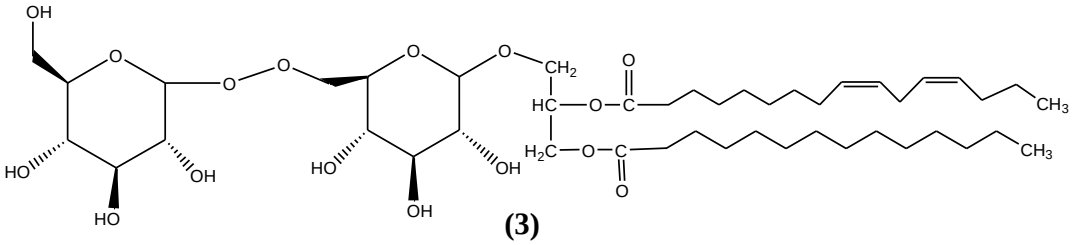

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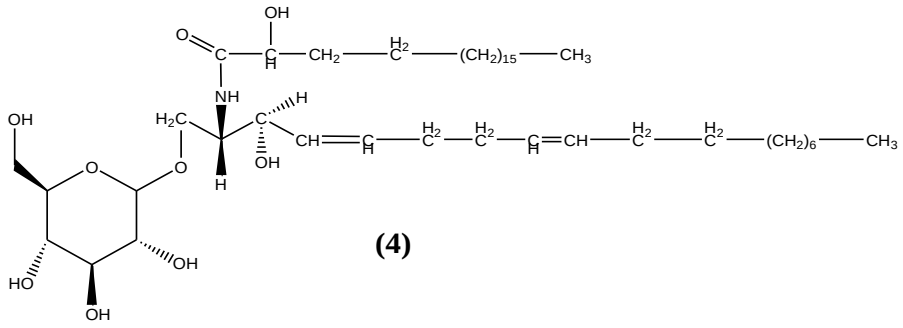

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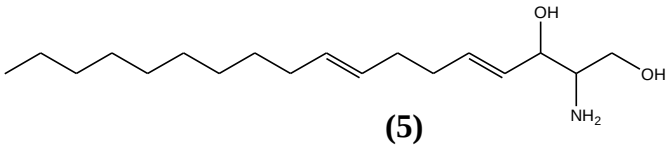


(5)

1.2.3 Pharmacological activities

There are several reports in the literature on pharmacological activities of the genus *Arisaema*. The crude extract of *A. flavum* showed antimicrobial activity against several bacterial strains within the range of (10-13.9 mm) zone of inhibition (Bibi *et al*., 2021). The water soluble disaccharide sucrose isolated from the rhizomes of *A. utile* demonstrated an excellent inhibition against *Candida paropsilosis* (MIC= 128 µg/mL), a fungal species known to cause sepsis, wound and tissue infections in immunocompromised people (Bhat *et al.*, 2019). Extracts and flavonoids such as schaftoside and isoschaftoside isolated from the rhizomes of *A. erubescens* displayed nematicidal activity against *M. incognita* (Du *et al.*, 2011). At a concentration of 250 µg/mL, the solvent fractions of *A. jacquemontii* tuber caused 100% mortality on *Leishmania tropica in vitro* (Tabassum *et al.*, 2019). Banyal (2014) reported that the tuber, leaf and fruit ethanol extracts of *A. jacquemontii* possess antimalarial activity with significant inhibition of *Plasmodium berghei.* Similarly, the leaf extract of *A. tortuosum* and its constituents displayed strong activity against herpes simplex virus type 2 (Rittà *et al.*, 2020). A lectin derived from *A. flavum* and *A. jacquemontii* demonstrated antiproliferative effects on murine cancer cell lines (Kaur *et al*,

2006). Anticonvulsant, phytotoxic, hemagglutination, analgesic, anti-inflammatory, sedative and antioxidant effects of extracts of plants belonging to the genus *Ariseama* were reported (Bhat *et al.*, 2019; Tabassum *et al.*, 2019). Kant *et al.* (2020) reported that many *Arisaema* species are promising candidates for the treatment of cancer, parasitic diseases and microbial infections.

1.3 *Arisaema schimperianum* Schott

1.3.1 Botanical description and distribution

Arisaema schimperianum Schott known by several vernacular names including *Amoch* in Amharic, *Qolxo* in Sidama, *Cherana* in Oromifa, *Hidha* in Burji, is a herbaceous tuber plant that belongs to the family Araceae. It is distributed mainly in tropical or subtropical region. The plant is widespread in Ethiopia, Imatong Hills on the Uganda-Sudan border, Democratic Republic of Congo, Kenya and Southern Arabia (Oman, Yemen and Saudi Arabi) (Mayo and Gilbert, 1986). It is an erect herb that grows up to 2 m in height. It has 2-3 large overlapping leaves and 5-15 leaflets which are narrowly elliptic to elliptic or oblanceolate to obovate. The spadix is usually unisexual rarely with flower of both sexes. Its unique flower stalk holds a single, colorful spathe (8-13 cm) and a bisexual spadix (3-5 cm) with separate male and female sections. The fruit is a bright scarlet berry (Mayo and Gilbert, 1986). It is reproduced mostly by its seed. It is seasonal plant which grows in rainy season but remain dormant in the soil during other seasons. The main flowering time is June and July (Hazo and Yirgalem, 2021).

(A) (B)

Figure 2: Photograph of *Arisaema schimperianum* Schott (A) aerial part, (B) tuber (Photographed by Betelhem Awoke, Sedimuja, South Gondar Zone, Ethiopia- July, 2023)

1.3.2 Ethnobotanical uses

Several ethnobotanical investigations have documented the medicinal uses of *A. schimperianum*. Some studies report that the dried tuber of *A. schimperianum* mixed with Vaseline is used for the treatment of wounds associated with glandular tuberculosis, in addition to its use for ring worm, scabies and skin lesion (Yineger *et al.*, 2008; Getachew *et al.*, 2022). In west Gojjam zone of the Amhara region, fresh rhizomes are crushed and mixed with water for oral administration to human and cattle, serving as a remedy for abdominal pain (Enyew *et al.*, 2014). In Fiche District, Central Ethiopia, the stem of *A. schimperianum* is used for gastrointestinal disorders (Alemneh, 2021). Furthermore, in Gondar town, northern Ethiopia, this plant is used for chronic sinusitis by making a powder from the dried root, transforming it into a paste with butter and applying it on the nasal area (Birhanu *et al*., 2015). Apart from its medicinal use, the boiled tubers are widely used as food by indigenous people especially in southern Ethiopia during drought (Dhakal *et al.*, 2020).

1.4. Statement of the problem

Chronic wound and microbial infections present substantial challenges with clinical, social, and economic implications for both the recovery process and overall patient well-being. Currently, the prevalence and cost of acute and chronic wounds are escalating globally. The expected cost for both types of wounds ranges from $28.1 billion to $96.8 billion, including infection management by 2026 (Nussbaum *et al*., 2018). Bacterial infections are one of the major causes for delayed wound healing. Most wound infections can be treated with antibiotics but currently serious side effects and antimicrobial resistance account for a significant risk of developing chronic wound and mortality. The spread of multi drug resistant bacteria in hospital and community settings remains a widely unresolved problems and a heavy burden to health services (Alemayehu, 2021). Studies show that for every million wound patients, at least 10,000 die from microbial infections (Wong *et al.*, 2015).

The drugs currently available for wound treatment face challenges regarding their effectiveness in promoting rapid wound healing (Wong *et al*., 2015). Moreover, several emerging drugs are not only costly but also are associated with allergies and drug resistance (Sisay *et al*., 2019). Addressing this gap is crucial for developing alternative therapies that complement existing treatments and contribute to improved patient outcomes.

The tubers of *A. schimperianum* are traditionally used for the treatment of wound and infection in various parts of Ethiopia. Therefore, scientific investigation of the wound healing effect of the plant may help to identify indigenous sources of drugs that could be produced at low cost.

2. Objectives

2.1 General objective

- The aim of this study is to investigate the wound healing and antibacterial activities of the tuber extracts of *Arisaema schimperianum* and its major constituent.

2.2 Specific objectives

- To investigate the wound healing and antibacterial activities of the 80% methanol extract of the tubers of *A. schimperianum*;
- To carry out acute oral and dermal toxicity tests of the 80% methanol extract of *A. schimperianum* tuber;
- To evaluate the wound healing effect and *in vitro* antibacterial and antifungal activities of solvent fractions of *A. schimperianum* tuber;
- To isolate the major compound from the most active fraction;
- To elucidate structure of the isolated compound; and
- To determine the wound healing and *in vitro* antibacterial and antifungal activities of the isolated compound.

3. Materials and Methods

3.1 Materials

3.1.1 Plant material

The fresh tubers of *A. schimperianum* were gathered from Sedie Muja district (11°30′ 0″ North Latitude and 38°30′ 0″ East Longitude) in South Gondar Zone, Amhara Region, about 774 km north of Addis Ababa in the month of July in 2023. It was authenticated by Ato Melaku Wondafrash, the National Herbarium, Department of Plant Biology and Biodiversity Management, College of Natural and Computational Sciences, Addis Ababa University (AAU), where a botanical specimen was deposited (Voucher number BA-001) for future reference.

3.1.2 Chemicals, media and drugs

Methanol, chloroform and *n*-hexane (LOBA Chemie Pvt. Ltd., India), TLC silica gel 60 RP-18 $F_{254}S$ (Merck KGeA, Darmstadt, Germany), wool fat, hard paraffin, cetostearyl alcohol (BDH Chemicals Ltd, England), white soft paraffin (Anonchem Ltd, China), 2,3,5-triphenyltetrazolium chloride, Mueller Hinton Broth, Sabouraud Dextrose Broth, Sabouraud Dextrose Agar (Sigma-Aldrich, Germany), Mueller Hinton Agar (Oxoid Ltd, UK), Nutrient Broth (Merck, Germany), nitrofurazone ointment USP 0.2% (Galentic Pharma, Pvt. Ltd, India), ketamine hydrochloride injection USP and diazepam (Neon Laboratories Ltd, India), ciprofloxacin 5 µg discs (Twin Brook Pkwy, Germany) and ketoconazole (Thermo Scientific Chemicals, U.S.A.) were used as received.

3.1.3 Instruments and apparatuses

Rota vapor (Heidolph Instruments GmbH and Co., Germany), UV spectrophotometer (Thermo Scientific Evolution 60S, USA), 1D and 2D nuclear magnetic resonance spectrophotometer

(Bruker, Billerica, MA, USA), high-resolution electrospray ionization mass spectrometry (HRESIMS) (Waters Corp., Milford, USA), digital weighing balance (Mettler Toledo, Switzerland), mini orbital shaker (Bibby Scientific Limited, UK), light microscope (Olympus CX41, Japan), oven, incubator (Memmert, Germany) and autoclave (JSR JSAT-105, Korea) were used.

3.1.4 Experimental animals

Healthy adult Swiss albino mice of both sexes weighing 25 - 30 g and aged 8-10 weeks were used. For dermal toxicity, female rats (150 - 250 g) were procured from the Animal House of the Department of pharmacology and clinical pharmacy, Addis Ababa University. The animals were housed at room temperature (22 °C) and a 12 h light/12 h dark cycle and were provided with water and food pellets *ad libtum* before and till the end of the experimental period. All the experiments were conducted in accordance with the internationally accepted laboratory animal use and care guideline (NRC, 2011). The animals were acclimated for one week prior to the study, and during the experiment, they were kept individually in separate cages to avoid fighting (Mulisa *et al*., 2015).

3.1.5 Test organisms

In vitro antibacterial and antifungal activity tests were conducted on the following clinical strains or drug sensitive standard strains of American Type Culture Collection (ATCC) that were obtained from Armauere Hanssen Research Institute (AHRI) Microbiology laboratory. Gram-positive bacteria strains used were *Staphylococcus aureus* (ATCC 25923), Methicillin-resistant *Staphylococcus aureus* (clinical isolate) and *Streptococcus epidermides* (ATCC 12228). *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 35659) and *Pseudomonas aeruginosa* (ATCC 27853) were the Gram-negative bacterial strains employed. *Candida albicans* (clinical

isolate) and *Aspergillus niger* (ATCC 10535) were the fungal stains used for the study. The microbial strains were stored at -70 to -80°C in Triptosoya + 20% glycerol broth until they are used.

3.2 Methods

3.2.1 Preparation of plant material

The plant material was collected, placed in a plastic bag free of chemicals , transported to the department of medicinal chemistry and pharmacognosy laboratory, rinsed with tap water, and then shaded at room temperature to dry for two weeks. Once dried, it was ground into a coarse powder using an electric mill and stored in a refrigerator until ready for extraction.

3.2.2 Extraction

Cold maceration using 80% (v/v) methanol was employed to extract the powdered *A. schimperianum* tubers, as recommended by a previous study for a higher yield (Azam *et al.*, 2016). Powdered tuber (800 g) was soaked in 4 L of 80% methanol for 72 h. The mixture was initially filtered using a nylon cloth, and the marc was re-macerated three times (each time for 24 h) to maximize the yield. The combined filtrate was then allowed to pass through Whatman No. 1 filter paper using a pressurized suction system, concentrated at a low-pressure rotary evaporator set to 121 mbar, 45 rpm, and a temperature of 40 °C. The extract was dried further in an oven at a temperature not exceeding 40 °C to remove the residual solvent. The resulting dried extract was weighed, transferred into an amber-coloured vial and stored in a refrigerator.

3.2.3 Solvent fraction

Further fractionation of the hydroalcoholic extract was performed using solvent-solvent partitioning. The process involved successive solvent-solvent fractionation using *n*-hexane,

chloroform and water in a separatory funnel. Initially, the hydroalcoholic extract (30 g) was suspended in 100 ml of distilled water. An equal volume of *n*-hexane was added, and the mixture was vigorously shaken in a funnel. After allowing the mixture to stand until clear separation occurred, the process was repeated three times and the *n*-hexane layer was collected. The aqueous layer was further partitioned with equal volume of chloroform. The lower chloroform layer was collected in a beaker and the process was repeated two more times and the organic layer combined. The *n*-hexane and chloroform fractions were separately concentrated in a rotavapor (Heidolph Instruments GmbH and Co., Schwabach, Germany) at a temperature not exceeding 40 °C. The remaining aqueous layer was dried in an oven at 40 °C. All the fractions were then packed in airtight amber-coloured bottles, labeled and stored in a refrigerator until use.

3.2.4 Isolated compound

Reverse-phase C-18 flash column chromatography was used to isolate the major compound. The water fraction (2 g) was dissolved in distilled water and subjected to C-18 reverse-phase flash column chromatography, using a gradient solvent system in the following sequence: H_2O (100), H_2O-MeOH (100:5), H_2O-MeOH (90:10), H_2O-MeOH (85:15), H_2O-MeOH (80:20), H_2O-MeOH (75:25), H_2O-MeOH (70:30), H_2O-MeOH (65:35), H_2O-MeOH (60:40), H_2O-MeOH (55:45), H_2O-MeOH (50:50), and MeOH (100). The purity of each fraction was monitored using analytical RP-TLC and viewed under UV light of 254 nm and 366 nm. Fractions eluted with the H_2O-MeOH (55:45) solvent gradient, which displayed a single spot on analytical TLC using a solvent system H_2O: MeOH in a ratio of 1:1, were combined to yield a sticky substance, coded BA. This substance was measured, placed into an amber-colored vial, and stored in a refrigerator at 4°C for future use.

3.2.5 Spectroscopic analysis

NMR spectral data were generated at room temperature using a Bruker FT-NMR spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. The scanning range was 0 - 15 ppm for ^{1}H NMR and 0 - 205 ppm for ^{13}C NMR. Signals were referenced to the internal standard tetramethylsilane (TMS). Chemical shifts are reported in δ units (ppm), and coupling constants (*J*) in Hz. The multiplicities of ^{1}H NMR signals are denoted as *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), *dd* (doublet of doublets), and *m* (multiplet). The mass of the isolated compound was analyzed using a positive-ion mode time-of-flight mass spectrometer (+ mode TOF-MS).

3.2.6 Ointment formulation

A simple ointment was prepared using the hydroalcoholic extract and each of the solvent fractions, following the formula outlined in the British Pharmacopoeia (Table 3) (BP, 1948). The simplified formula from the master recipe was employed to create three different ointment formulations: two containing the extract at concentrations of 5% and 10% (w/w), while the simple ointment served as the control.

Table 3: Master and reduced formulae used to for preparation of simple and medicated ointment

Ingredient	Master formula (g)	Reduced formula (g)
Wool fat	50	1
Hard paraffin	50	1
Cetostearyl alcohol	50	1
White soft paraffin	850	17
Total	1000	20

To prepare a simple ointment, the measured amounts of hard paraffin and cetostearyl alcohol were blended, followed by the addition of soft paraffin and wool fat based on their respective

melting points. The mixture was stirred continuously to ensure even blending. Once removed from the water bath, the mixture was stirred until it cooled, forming a medicated ointment. The ointment was then levigated on an ointment slab to achieve a smooth texture and uniform consistency.

For the preparation of a 5% (w/w) ointment, 1 g each of the hydroalcoholic extract, *n*-hexane fraction, chloroform fraction, water fraction and isolated compound GGG was incorporated into 19 g of the ointment base. Similarly, for 10% (w/w) ointment, 2 g of each of the test substances were mixed with 18 g of the ointment base. Then the ointments were used for the experiment.

3.2.7 Grouping and dosing of experimental animals

Twelve groups of mice, each group containing six animals, were employed for the excision model. Group I animals were treated with simple ointment base (negative control), Group II with 5% (w/w) hydroalcoholic extract, Group III with 10% (w/w) hydroalcoholic extract, Group IV with 5% (w/w) *n*-hexane fraction, Group V with 10% (w/w) *n*-hexane fraction, Group VI with 5% (w/w) chloroform fraction, Group VII with 10% (w/w) chloroform fraction, Group VIII with 5% (w/w) water fraction, Group IX with 10% (w/w) water fraction, Group X with 5% (w/w) the isolated compound, Group XI with 10% (w/w) isolated compound, and Group XII with 0.2 w/v nitrofurazone (positive control). For the incision model, twelve groups of animals were used in the same way as described for excision model except that Group XIII animals were added to serve as left untreated negative control group. All these groups were repeated for histological analysis and hydroxyproline determination.

3.2.8 Toxicity studies

Acute oral toxicity study: Acute oral toxicity study was carried out following OECD/OCDE 425 guideline (OECD/OCDE, 2022). Accordingly, five female mice (25 - 35 g) were used for the study. Each mouse received 2000 mg/kg of the 80% methanolic extract of *A. schimperianum* tuber solubilized in 5% Tween 80 by oral gavage. There are two phases of the study. In the first phase, one female mouse was weighed and received a single dose as described above, after fasting for 8 h. Food was then withheld for further 2 h. Following the administration of the required dose, the animals were monitored at least once during the initial 30 minutes and periodically over the next 24 hours, with particular focus during the first 4 hours. Since no death was observed, for phase two study the same dose was given for four female mice on the next day. Generally, they were observed for gross behavioral changes such as change in skin and fur, eye, loss of appetite, hair erection, lacrimation, tremors, convulsions and mortality for a period of 14 days.

Acute dermal toxicity study: Skin irritation test of the 80% methanolic extract of *A. schimperianum* tubers was carried out on 3 adult male and 3 adult female Wistar rats weighing 150 - 250 g for each group. Following an acclimatization period of 7 days, a skin area of about 500 mm^2 of each animal was shaved on the dorsal side. Simple ointment was applied to the shaved site of the 1^{st} rat, whilst 5% and 10% ointments were applied thinly and uniformly to the shaved sites of the 2^{nd} and 3^{rd} rats, respectively. The sites were covered by gauze and wrapped with a non-occlusive bandage. The animals were returned to their individual cages and provided with food and water. After 24 hours, the bandage and gauze were taken off, and the ointments applied were washed off with distilled water (OECD/OCDE, 2017). The sites were examined at 24, 48 and 72 h according to the scoring system for skin reactions (Table 4)

Table 4: System for classifying skin reactions

Reaction Erythema	Score	Reaction Oedema	Score
No erythema	0	No oedema	0
Very slight erythema	1	Very slight oedema	1
Well defined erythema	2	Well defined oedema (edge by define raising)	2
Moderate to severe erythema	3	Moderate oedema (raising ~ 1 mm)	3
Severe erythema (beet redness) to scare formation	4	Severe oedema (raising more than 1 mm and extended beyond the area of exposure)	4

The score of primary irritation (SPI) was calculated for each rat as follows. Score for erythema and oedema at 24, 48, and 72 h was summed and divided by the number of observations for the treated sites (Annex 1). The SPI for the control sites was calculated in the same way as the test substances.

$$\text{SPI for each rat} = \frac{\sum \text{Erythema and oedema grade at 24, 48 and 72 h}}{\text{Number of observation}}$$

The difference between the total SPI scores of six animals from the treated and control sites was calculated and used to determine the primary irritation index (PII). The PII was then calculated as the arithmetic mean of the SPI values from the six rats. The irritation degree was categorized as negligible, slight, moderate or severe irritation based on the PII as shown in Table 5.

$$PII = \frac{\sum \text{SPI (test)} - \sum \text{SPI (control)}}{\text{Number of animals}}$$

Table 5: Response categories of irritation in rat

Category	Primary irritation index
Negligible	0 - 0.4
Slight irritation	0.5 - 1.9
Moderate irritation	2 - 4.9
Severe irritation	5 – 8

3.2.9 Wound healing studies

Wound healing activities were carried out in mice by using excision and incision models. In the excision model, activity was evaluated by the rate wound contraction, period of epithelialization which is the number of days required for the complete falling of scab without any residual raw wound (Kumar *et al.*, 2013), histological analysis and hydroxyproline content, whilst tensile strength was used to assess wound healing effect in the incision model (Thakur *et al.*, 2011).

3.2.9.1 Excision wound model

On the day of wounding, the animals were anesthetized with ketamine (50 mg/kg) plus diazepam (5 mg/kg) through intraperitoneal (IP) route of administration (Thakur *et al.*, 2011). After the mice were anesthetized, the fur from their dorsothoracic region was shaved using an electric hair clipper. A circular area, approximately 314 mm^2 in size, was marked with a permanent marker and carefully excised using sterilized scissors. The animals were then left to recover from anesthesia. Once recovered, they were returned to their individual cages, and the procedure day was considered day 0. Starting from day 1, the wound was treated daily with the topical application of the ointment preparations (Velnar *et al.*, 2009;Yesuf and Asres, 2013).

Measurement of wound contraction: The animals were monitored for wound closure, and the wound area was assessed every other day by tracing it onto transparent paper, which was then

transferred to graph paper. The changes in the wound area were calculated. The percentage of wound contraction was calculated taking the initial size of wound, i.e., 314 mm^2 as 100% as follows;

$$\text{Percent wound contraction} = \frac{\text{Wound area on day 0} - \text{Wound area on day n}}{\text{Wound area on day 0}} \times 100$$

Where n = number of days i.e., 2^{nd}, 4^{th}, 6^{th}, etc. up to complete wound healing

Histopathological analysis: After the animals were killed by an IP injection of ketamine and diazepam with high dose, the skin samples from each group were taken on the 12^{th} day after the wounding (Belachew *et al.*, 2020). Samples were fixed in 10% buffered formalin, treated, blocked with paraffin, sectioned into 5 μm sections and stained with hematoxylin and eosin (HE) stains. A light microscope attached to a Camera Digital Image Analyze System was used to look at the stages of wound healing (inflammation, proliferation, angiogenesis, and remodeling). Re-epithelization, fibroblast proliferation, collagen depositions, polymorphonuclear cells, mononuclear cells and neovascularization were examined and rated as mild (+), moderate (++) and high concentration (+++) to assess the epidermal or dermal remodeling (Süntar *et al*. 2010).

Estimation of hydroxyproline: Collagen content was estimated by measuring the levels of hydroxyproline, a modified amino acid found almost exclusively in collagen (Murthy *et al*., 2004). Standard hydroxyproline was used to produce a calibration curve and determine hydroxyproline content of the granulation tissue (Mekonnen *et al*., 2013). After applying the extract ointment to the round wounds in the excision model for 10 days, the animals were killed on the 11^{th} day with a high dose of anesthesia. Granulation tissues from the wounds were collected, cleaned, weighed for consistency and dried at 60 °C for 12 h. The dried tissue samples

were then hydrolyzed with 6N HCl for 24 h at 110 °C in sealed glass tubes (Nagar *et al*., 2016). Supernatant solution (1 ml) was taken from each tissue hydrolysate, which was treated in the same way as the standard hydroxyproline. The absorbance was read on a UV spectrophotometer (Jenway 6500, England), at a wavelength of 572 nm in 1 cm cell. The hydroxyproline content in each of sample solution was calculated using the equation obtained from the calibration curve (Figure 3) (Mulisa *et al*., 2015).

Preparation of calibration curve: Standard L-hydroxyproline (0.05 g) was dissolved in water and diluted to approximately 400 ml. Concentrated HCl (20 ml) was added, and the solution was then made up to 500 ml with water to obtain a 100 µg/ml solution. An aliquot portion of this solution was further diluted to prepare concentrations of 5, 10, 15, 25, and 50 µg/ml of hydroxyproline, with three test tubes for each concentration and one test tube for blank. Each test tube was gently mixed with 1 ml of 0.05 M $CuSO_4$ and 1 ml of 2.5 N NaOH. The tubes were placed in a water bath at 40°C for 3-5 minutes. Then, 1 ml of 6% hydrogen peroxide was added to each test tube, and the contents were thoroughly mixed by swirling before moving to the next tube. The tubes were left in the water bath for 10 minutes, with occasional swirling, and then cooled with tap water. Next, 4 ml of 3N H_2SO_4 and 2 ml of 5% p-dimethylaminobenzaldehyde solution were added to each tube, with thorough mixing and swirling after each addition. The caps were placed on the tubes, which were then kept in the water bath at 70°C for 16 minutes. Afterward, the tubes were cooled, mixed, and their absorbance was measured against the blank solution at a wavelength of 572 nm using a 1 cm cell. The average absorbance for each set of tubes was used to construct a standard absorbance versus concentration curve. The hydroxyproline concentration in the unknown samples was determined based on this standard curve (Yesuf and and Asres, 2013).

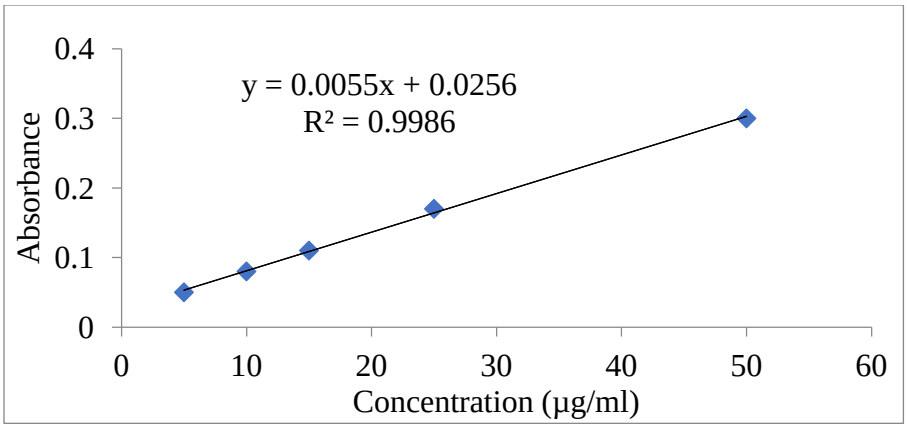


Figure 3: Standard curve for determination of hydroxyproline concentration

3.2.9.2 Incision wound model

On the day of wounding, the animals were anesthetized as described for the excision wound model. The dorsal fur was shaved, and a 3 cm longitudinal paravertebral incision was made through the skin and subcutaneous tissue. The incision was sutured using silk surgical thread (no. 000) and a curved needle (no. 9), with stitches placed 1 cm apart. Starting 24 hours post-wounding (day 1), the animals were treated with the respective ointments as per the grouping and dosing protocol, except for the last group (Thakur *et al.*, 2011). On day 8 post-wounding, the sutures were removed, and treatment continued. Tensile strength was measured on day 10 using the continuous water flow technique. The percent strength was then calculated based on the tensile strength values using the following formulas.

$$\text{Tensile strength (TS) of test sample (\%)} = \frac{\text{TS of test sample} - \text{TS of SO}}{\text{TS of SO}} \times 100$$

$$\text{Tensile strength (TS) of reference (\%)} = \frac{\text{TS of referance} - \text{TS of SO}}{\text{TS of SO}} \times 100$$

$$\text{Tensile strength (TS) of simpe ointment(SO)(\%)} = \frac{\text{TS of of SO} - \text{TS of left untreated}}{\text{TS of left untreated}} \times 100$$

Figure 4: Water flow technique for measurement of tensile strength

3.2.10 Antibacterial and antifungal activity test

Media and inoculums preparation: Culture media were prepared to subculture the test organisms and evaluate their susceptibility to test substances. Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB) for bacteria, Sabouraud Dextrose Broth (SDB) and Sabouraud Dextrose Agar (SDA) for fungi and Nutrient Broth (NB) for general bacterial growth comparison (as a control) were used. To prepare these media, 38 g of MHA, 65 g of SDA, 30 g SDB and 8 g of NB were weighed and each dissolved in 1 L of distilled water then boiled by shaking frequently in a separate media bottle according to manufacturer's instructions and autoclaved for 15 min at 121°C. After media preparation, the selected microorganisms were refreshed using the respective culture media. Three to five well isolated colonies from the refreshed agar plate culture were selected and transferred to the broth media using inoculating loop for standardized inoculum preparation to make the inocula needed for susceptibility testing. For this; UV spectrophotometer was used to adjust the bacterial or fungal suspension by measuring its absorbance with 1 cm path length at the wavelength of 625 nm. The absorbance reading in the range from 0.08 to 0.10 optical densities is proportional to 1×10^{8} CFU/ml bacteria and 1×10^{7}

CFU/ml fungi concentration. These suspensions were diluted with an appropriate broth to achieve a concentration of 5×10^5 CFU/ml for bacteria and 5×10^4 CFU/ml for fungi to determine minimum inhibitory concentration (MIC) (Alemu *et al*., 2024; Tabassum *et al.*, 2019).

Determination of minimum inhibitory concentration (MIC): MIC was determined by micro broth dilution method according to CLSI guideline using 96-micro-well plates (Wayne, 2020). A determined concentration of the test substance was prepared by dissolving it in 5% Tween 80. For every microbial strain, three 96-well micro-plates were prepared and 100 µl of MHB or SDB were added to each well. Then, 100 µl of the prepared solution, positive control (Ciprofloxacin 200 mg for bacteria) and (Ketoconazole 200 mg for fungi) and negative control (vehicle) were added on the first row on the wells. After 10 to 15 times thorough mixing two-fold serial dilutions with multichannel micropipettes were made down from row A to row H. Different concentrations of the test substance and positive control starting from a highest concentration of 10 mg/ml to 0.0625 mg/ml (for bacteria) and 50 µg/ml to 0.078125 µg/ml (for fungi), were prepared. Additionally, growth control (broth and tested microorganism) and sterility control (only broth) were run parallel to the experimental tests on the remaining columns. Following that, the bacterial and fungal suspensions containing approximately 5×10^5 and 5×10^4 CFU/ml, respectively, were prepared from a refreshed culture. From this suspension, except the sterility control, 100 µl aliquot was inoculated into each well within 10 - 15 min of standardization followed by incubation of the plates at 37 °C for 24 h using incubator for bacterial pathogens and at 25 °C up to 7 days for fungal pathogens. After incubation, 40 µl of a 0.4 mg/ml solution of 2,3,5-triphenyltetrazolium chloride were added to each well as an indicator of microbial growth and re-incubated at 37 °C for 30 min. Then, the MIC values were visually determined by observing the presence or absence of pink colour. The lowest concentration of each test

substance displaying no visible pink colour was recorded as the MIC (Alemu *et al*., 2024). Generally, during the antimicrobial assay positive controls, negative controls, sterility controls and growth controls were run parallel to the experimental tests. Tween 80 (5%) was used as negative control. All assays were performed in triplicate and conducted in biological safety cabinet (TELSTAR T Nr 16325).

3.2.11 Statistical analysis

The experimental data were presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test, with significance set at $p < 0.05$. All statistical calculations were conducted using SPSS version 23.

4. Results

4.1 Extract and fractions yields

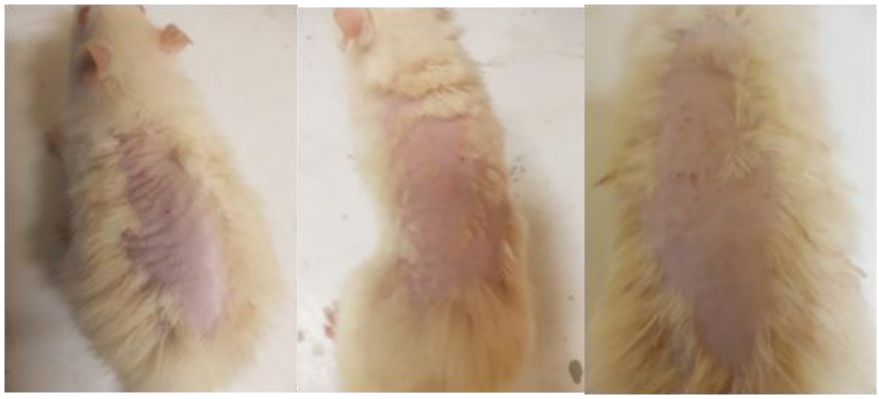
Extraction of *A. schimperianum* powder with 80% methanol resulted in a dark brown viscous substance with a yield of 4.75% (w/w). Subsequent fractionation of the hydroalcoholic extract yielded 33.33 %, 16.66 %, 50 % of *n*-hexane, chloroform$_3$ and water fractions, respectively.

4.2 Acute oral toxicity test

A single oral administration of the 80% methanol extract of *A. schimperianum* tuber at a dose of 2000 mg/kg appeared to be safe. No deaths were observed, and there were no signs of gross or behavioral toxicity during the 14-day observation period. Therefore, the LD_{50} of the extract is estimated to be greater than 2000 mg/kg.

4.3 Acute dermal toxicity test

In skin irritation test, topical application of 5% (w/w) ointment of the 80% methanol extract of *A. schimperianum* tuber did not show any irritation or noticeable inflammation. However, the 10% (w/w) ointment preparation showed slight redness after 24 h of application (Figure 5), which disappeared after a short period of time. Furthermore, 72 h after application of the 10% ointment, no inflammation or oedema was observed. There was no sign of toxicity or mortality during the 14 days of observation. Based on the dermal irritation scoring system, the PII values of the ointment preparations were found to be in the range of (0 - 0.4) which explains the non-irritant nature of the test samples. This gives an indication that *A. schimperianum* tuber extract is safe when used for the treatment of wound.



(A) **(B)** **(C)**

Figure 5: Photograph of skin 24 h after topical application of (A) 5% (w/w) 80% methanol extract of *Arisaema schimperianum* tuber, (B) 10% (w/w) 80% methanol extract of *Arisaema schimperianum* tuber, (C) simple ointment (negative control) for acute dermal toxicity study.

4.4 Wound healing effects

4.4.1 Effect on excision wound

4.4.1.1 Hydroalcoholic extract and solvent fractions

Effect on wound contraction: As shown in Table 6 the 10% ointment of 80% methanol extract of *A. schimperianum* tuber and standard drug exhibited significant wound contraction ($p < 0.001$ and $p < 0.05$, respectively) starting from the 4th day post wounding, compared to the negative control. Among the solvent fractions, the 10% (w/w) water fraction displayed significant ($p < 0.001$) wound contraction from the 4th post-wounding day onwards compared to the negative control and *n*-hexane fractions. In addition, except 5% (w/w) hexane fraction, all fractions showed significant effect from the 6th post wounding day ($p < 0.01$ to 0.001) compared to the negative control (Figure 6). Moreover, the 10% formulation increased wound contraction as compared with the 5% ointment. The 10% (w/w) water fraction, 10% ointment of 80% methanol extract of *A. schimperianum* tuber and the standard drug caused complete wound closure on 14th, 16th and 18th day, respectively, whilst simple ointments fully closed the wound on the 20th day.

Table 6: Effect of topical application of ointment prepared from the 80% methanol tuber extract of *Arisaema schimperianum* and solvent fractions on wound area (mm^2) and percent of wound contraction in excision wound model

Group	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18
SO	3.33±2.96	283.50±2.62	260.66±2.77	224.33±4.08	164.50±7.10	102.50±4.34	71.83±4.49	30.16±2.70	15.16±2.02
	(3.39%)	(9.71%)	(16.98%)	(28.55%)	(47.61%)	(67.35%)	(77.12%)	(90.39%)	(95.17%)
5% HAE	301.00±4.56	275.50±2.27	236.66±2.21	192.50±6.61	120.00±2.22	90.50±8.06	33.50±1.76	12.66±1.22	5.00±1.06
	(4.14%)	(12.26%)	(24.63%) ^{d2}	(38.69%) ^{a2,d1}	(61.78%) ^{a1}	(71.17%) ^{d2}	(89.33) ^{a1}	(95.94) ^{a1}	(98.39) a^{1}
10% HAE	297.16±4.57	262.66±2.29	176.33±12.79	90.5±5.92	50.83±4.36	18.33±1.47	5.33±0.49	0	0
	(5.36%)	(16.35%) ^{a1,b3}	(43.83%)^{a1,d1,e1}	(70.99%) ^{a1,d1,e1}	(83.70%) ^{a1,d1,e1}	(94.12%) ^{a1,d1,e1}	(98.29%) ^{a1,e1}	(100%) ^{a1,e1}	(100) ^{a1}
5% HF	302.16±4.36	284.50±2.78	244.83±2.40	189.16±3.15	130.16±1.35	97.16±4.48	46.50±3.38	18.16±2.02	6.16±0.90
	(3.77%)	(9.39%)	(22.02%) ^{c1,d1}	(39.75%) ^{a1,c1,d1}	(58.54%)	(69.05%) ^{c1,d1}	(85.50%) ^{a1,c3,d1}	(94.21%) ^{a1,c3,d1}	(96.71%) ^{a1,c3,d1}
10% HF	298.33±3.79	272.66±2.47	194.83±2.18	156.66±2.92	108.66±2.89	60.00±2.43	23.16±11.00	5.50±0.42	0
	(4.99%)	(13.16%)	(37.95%) ^{a1,b1}	(50.10%) ^{a1,c1,d1}	(65.39%) ^{a1,b3,c1}	(80.89%) ^{a1,c1,d1}	(92.62%) ^{a1,b1}	(98.24%) ^{a1,b1}	(100%) ^{a1,b1}
5% CF	302.83±2.21	279.66±1.22	243.00±2.08	170.50±1.38	111.00±3.00	75.33±5.05	30.00±2.81	12.33±1.54	3.16±0.47
	(3.55%)	(10.93%)	(22.61%) ^{a2,c1,d1}	(47.70%) $^{a1, b1, c1}$	(64.64%) ^{a1,c1}	(76.00%) $^{a1, b1, c1}$	(90.00%) $^{a1, b1, c1}$	(96.04%) ^{a1,c1,d3}	(98.98%) ^{a1}
10% CF	295.83±3.52	275.33±1.83	226.50±2.66	110.00±0.68	62.33±6.69	16.83±0.79	5.50±1.20	0	0
	(5.78%)	(12.33%)	(27.86%) $^{a1,b2, c1}$	(64.96%) ^{a1,b1,d1}	(80.14%) ^{a1,b1,d1}	(94.60%) ^{a1,b1,d1}	(98.23%)^{a1,b1,dA1}	(100%) $^{a1, b}$	(100%) ^{a1,b1}
5% WF	301.00±4.56	273.16±2.97	218.00±2.22	175.50±3.90	97±3.47	24.67±2.78	6.67±0.84	0	0
	(4.14%)	(13.00%)	(30.57%) ^{a1,b1,c1,d1}	(44.10%) ^{a1,c1,d1}	(68.91%) ^{a1,a2,c1}	(92.09%) ^{a1,b1,d1}	(97.86%) ^{a1,b1,d1}	(100%) ^{a1,b1}	(100%) ^{a1,b1}
10% WF	285.16±4.57	216.50±8.92	103.83±6.60	36.33±1.52	13.16±1.35	3.20±0.50	0	0	0
	(8.60%)	(30.60%) ^{a1,b1,c1}	(66.72%)^{a1,b1,c1,d1}	(88.35%) ^{a1,b1,c1,d1,}	(95.78%) ^{a1,b1,c1,d1}	(98.97%)^{a1,b1,c1,d1}	(100%) ^{a1,b1,d1}	(100%) ^{a1,b1}	(100%) ^{a1,b1}
NF	300.50±3.30	270.66±1.76	183.83±3.32	116.50±2.39	65.33±6.60	25.00±1.91	13.16±1.42	2.16±0.47	0
	(4.29%)	(13.80%) ^{a3}	(41.45%) ^{a1,d1}	(62.66%) ^{a1,b1,d1,e1}	(79.06%) ^{a1,b1,d1,e1}	(91.98%) ^{a1,b1,d1,e1}	(95.78%) ^{a1,b1}	(98.80%) ^{a1,b1}	(100%) ^{a1,b1}

Data are presented as mean ± SEM (n = 6) and analyzed using a one-way ANOVA with a Tukey post hoc test; ^{a} compared with SO, ^{b} compared with 5% (w/w) HF ointment, ^{c} compared with 10% (w/w) HF ointment, ^{d} compared with NF, e: compared with 5% (w/w) HAE ointment;^{1} $p < 0.001$, ^{2} $p < 0.01$, $^{3}p < 0.05$, SO (negative control): simple ointment; HAE: 80% methanol extract of *Arisaema schimperianum* tuber, HF: *n*-hexane fraction, CF: chloroform fraction, WF: water fraction, NF (positive control): 0.2% (w/v) nitrofurazone ointment.

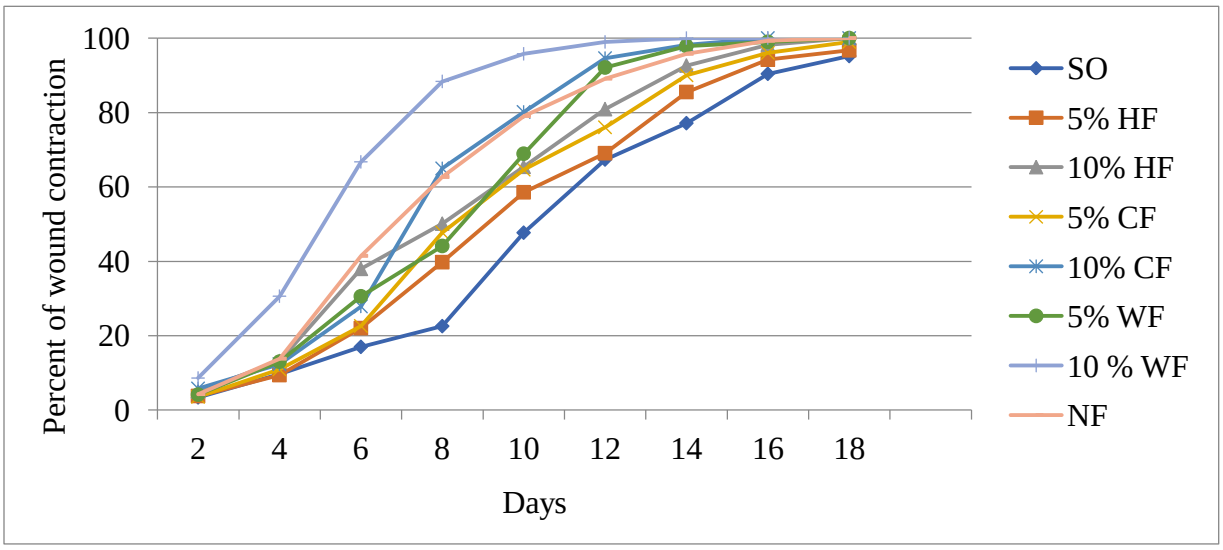


Figure 6: Effect of solvent fractions of *Arisaema schimperianum* tuber on the percentage wound contraction of excision wound model in mice; SO (negative control): simple ointment, HF: n-hexane fraction, CF: chloroform fraction, WF: water fraction, NF (positive control): 0.2% (w/v) nitrofurazone ointment.

Effect on epithelialization period: As shown in Table 7, the time for complete epithelialization was much shorter in extract ointments and standard drug-treated than the control group. Groups treated with 10% (w/w) water fraction ointment, 5% (w/w) water and 10% chloroform fraction treated groups showed a significant decrease ($p < 0.01$) in the epithelialization period compared with the negative control. Animals treated with 5% (w/w) chloroform and 10% (w/w) *n*-hexane fractions showed a significant decrease ($p < 0.05$) in epithelialization period as compared with the negative control. There was a difference between the epithelialization periods of the 5% and 10% (w/w) preparations of each fraction, but all lacked significance when compared with each other except the ointments prepared from the water fraction which showed significance ($p < 0.05$)

Table 7: Effect of topical application of the 80% methanol extract and solvent fractions from *Arisaema schimperianum* tuber on period of epithelialization

Group	Epithelialization period (days)
SO	18.67±0.51
5% HAE	16.00±0.44 [a2]
10% HAE	13.00±0.67 [a1]
5% HF	17.33±0.42
10% HF	16.00±0.73 [a1]
5% CF	16.00±0.51 [c1]
10% CF	15.83±0.54 [a1]
5% WF	15.67±0.61 [b1]
10% WF	13.67±0.61 [a1,b1,c2]
NF	14±0.73 [a1,b1,c2]

Data are presented as mean ± SEM (n = 6) and analyzed using a one-way ANOVA with a Tukey post hoc test; [a] compared with SO, [b] compared with 5% (w/w) HF ointment, [c] compared with 10% (w/w) HF ointment; [1] $p < 0.001$, [2] $p < 0.05$; SO (negative control): simple ointment, HAE: 80% methanol extract of *Arisaema schimperianum* tuber, HF; *n*-hexane fraction, CF: chloroform fraction, WF: water fraction, NF (positive control): 0.2% (w/v) nitrofurazone ointment.

Histological analysis: As shown in Table 8, histological studies determined the healing process of hydroalcoholic extracts and solvent fractions. the 10% (w/w) water, 10% (w/w) chloroform fractions and 10% hydroalcholic extract showed high fibroblasts cells, moderate collagen fiber and neovascularization also less inflammatory cells as compared to negative control (Figures 7A, 7B and 7C). Standard drug treated groups showed moderate fibroblast cell, high collagen fibers and blood vessels and reduced inflammatory cells compared with negative control (Figure 7D). Similarly, animals treated with 5% (w/w) hydroalcholic extract and water fraction ointment showed moderate fibroblast, collagen deposition, inflammatory cells and neovascularization as compared to negative control (Figure 7E and 7F), whilst simple ointment treated groups showed inflammatory cells, reduced collagen fibers, fibroblast cells and blood vessels (Figure 7J).

Table 8: Histological determination of wound healing process of the 80% methanol extract and solvent fractions of *Arisaema schimperianum* tuber

Group	RE	FB	CD	PMN	MNC	NV
SO	+	+	+	++	++	+
5% HAE	++	++	++	++	++	++
10% HAE	+++	+++	+++	++	+	++
5% HF	++	++	++	++	+++	+
10% HF	++	++	++	+++	++	+++
5% CF	++	++	++	+++	+++	+
10% CF	+++	+++	++	+++	+++	++
5% WF	++	++	++	++	++	++
10% WF	+++	+++	++	+++	+++	++
NF	++	++	+++	++	++	++

+ (mild), ++ (moderate), +++ (severe) for epidermal or dermal remodeling; RE: reepithelization, FB: Fibroblast, CD: collagen deposition, PMN: polymorphonuclear cells, MNC: mononuclear cells, NV: neovascularization; SO (negative control): simple ointment, HAE: 80% methanol extract of *Arisaema schimperianum* tuber, HF: *n*-hexane fraction, CF: chloroform fraction, WF: water fraction: NF (positive control): nitrofurazone 0.2% (w/v) ointment.

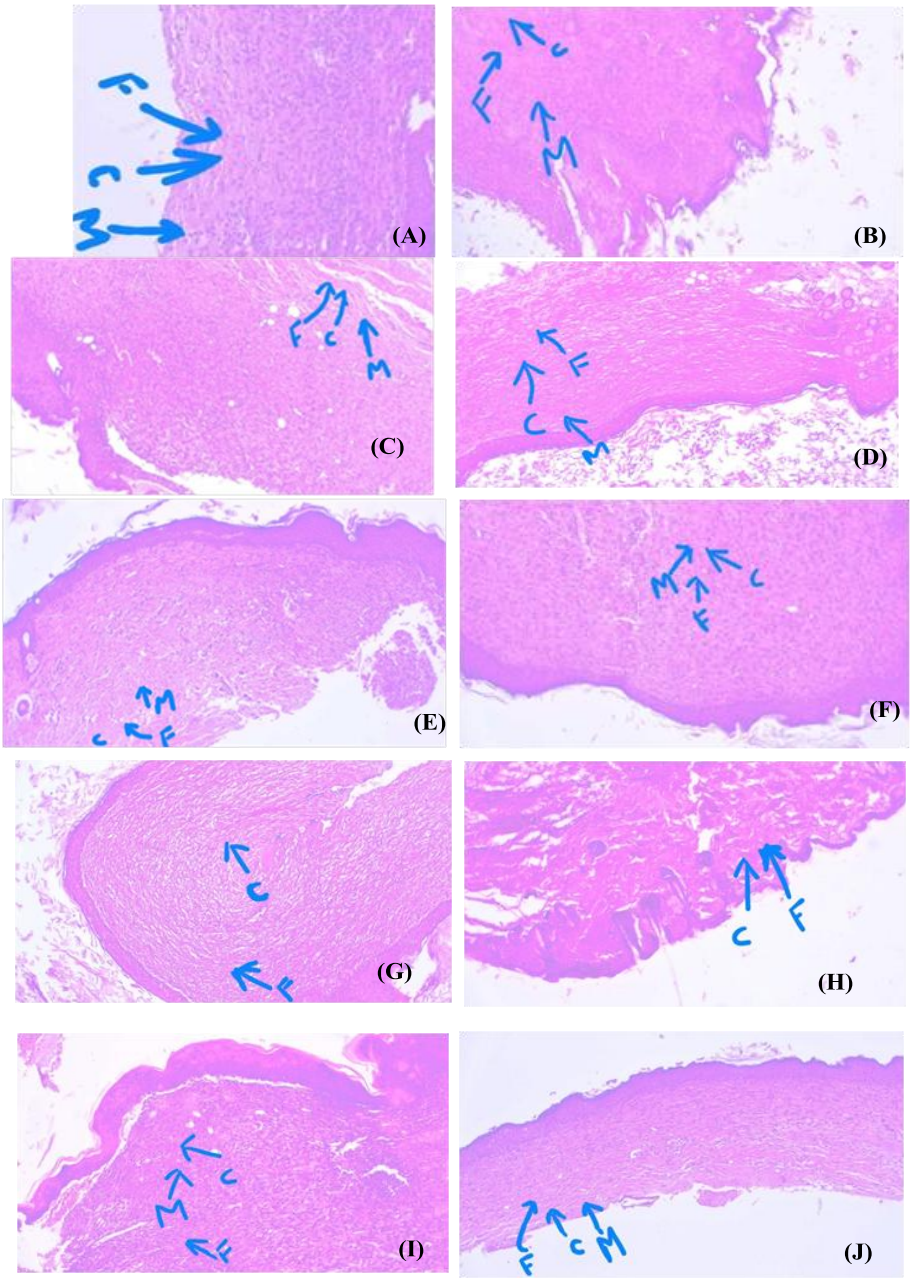


Figure 7**:** Photomicrograph of histopathological section of wound tissue (stained with H&E, 100× magnifications) obtained from mice treated with (A) 10% (w/w) water fraction of *Arisaema schimperianum* tuber, (B) 10% (w/w) chloroform fraction of *Arisaema schimperianum* tuber, (C) standard drug, (D) 10% (w/w) 80% methanol extract of *Arisaema schimperianum* tuber, (E) 5% (w/w) 80% methanol extract of *Arisaema schimperianum* tuber, (F) 5% (w/w) water fraction of *Arisaema schimperianum* tuber, (G) 10% (w/w) n-hexane fraction of *Arisaema schimperianum* tuber, (H) 5% (w/w) chloroform fraction of *Arisaema schimperianum* tuber, (I) 5% (w/w) n-hexane fraction of *Arisaema schimperianum* tuber, (J) simple ointment; C: collagen fibers F: fibroblasts, M: macrophages.

Effect on tissue hydroxyproline content: As shown in Table 9, hydroxyproline levels of the granulation tissue of animals treated with 10% ointment of hydroalcoholic extracts and all the solvent fractions were significantly greater ($p < 0.01$) than the levels in the negative control group. However, the levels displayed by the 10% water fraction and the standard drug nitrofurazone were much higher than those demonstrated by the other solvent fractions. The 10% water fraction increased the level of hydroxyproline to 61.00 mg/g tissue whilst the level attained by the standard drug was 58.02 mg/g tissue. The effects of 10% hydroalcoholic extracts and positive control were comparable.

Table 9: Effect of topical application 80% methanol extract and solvent fractions of *Arisaema schimperianum* tuber on tissue hydroxyproline content in excision wound model

Group	Hydroxyproline content (mg/g tissues)
SO	10.00±0.57
5% HAE	41.00±2.23 ^{a1,d1}
10% HAE	58.67±1.15^{a1,e1}
5% HF	12.33±1.20 ^{c1,d1}
10% HF	31.33±1.29 ^{a1,b1,d1}
5% CF	28.00±1.57 $^{a1, b1,d1}$
10% CF	34.33±0.881 ^{a1,b1,d1}
5% WF	43.67±1.20 ^{a1,b1,c1,d1}
10% WF	61.00±2.30 ^{a1,b1,c1,d1}
NF	58.02±1.11 $^{a1,b1,c1, e1}$

Data are presented as mean ± SEM (n = 6) and analyzed using a one-way ANOVA with a Tukey post hoc test; ^{a} compared with SO, ^{b} compared with 5% (w/w) HF ointment, ^{c} compared with 10% (w/w) HF ointment, ^{d} compared with NF, ^{e} compared with 5% (w/w) HAE ointment ;$^{1}p < 0.001$; SO (negative control): simple ointment, HAE: 80% methanol extract of *Arisaema schimperianum* tuber, HF: *n*-hexane fraction, CF: chloroform fraction, WF: water fraction, NF (positive control): 0.2% (w/v) nitrofurazone ointment.

4.4.2 Effect on incision wound

4.4.2.1 Tensile strength of hydroalcoholic extract and solvent fractions

As shown in Table 10, in the incision wound model, 5% and 10% (w/w) ointments of 80% methanol extract of *A. schimperianum* tuber and standard drug treated groups showed significant ($p < 0.001$) increase in tensile strength when compared to the negative control and untreated groups. All solvent fractions of *A. schimperianum* tuber except the 5% (w/w) *n*-hexane fraction showed significant ($p < 0.001$) increase in tensile strength when compared to simple ointment. Similarly, 5% (w/w) hexane fraction-treated groups recorded a significant ($p < 0.05$) increase in tensile strength as compared with a simple ointment base. However, there was a slight difference in tensile strength between 10% (w/w) hydroalcoholic extract and the standard nitrofurazone ointment, which failed to reach statistical significance.

Table 10: Effect of topical application of 80% methanol extract and solvent fractions of *Arisaema schimperianum* tuber on tensile strength

Groups	Tensile strength (g)	Percent tensile strength (%)
Untreated control	187.50±7.04	---
SO	230.83±19.07	18.86
5% HAE	304.50±15.01 ^{a1,d2,e1}	31.91
10% HAE	425.00±18.02 ^{a1,d1,e1}	84.11
5% HF	276.66±18.95 $^{c2,\ d1}$	19.85
10% HF	333.33±17.44 $^{a2,\ b1}$	44.40
5% CF	340.00±6.83 $^{a2,\ b1}$	47.29
10% CF	378.33±18.87 ^{a1,b1}	63.89
5% WF	359.16±9.69 ^{a1,b1}	55.59
10% WF	440.00±32.04 ^{a1,b1,c1}	90.61
NF	406.67±16.67 ^{a1,b1,c1,e1}	76.32

Data are presented as mean ± SEM (n = 6) and analyzed using a one-way ANOVA with a Tukey post hoc test; ^{a} compared with SO, ^{b} compared with untreated control, ^{c} compared with 5% (w/w) HF ointment, ^{d} compared with NF, ^{e} compared with 5% (w/w) HAE ointment; ^{1} $p < 0.001$, ^{2} $p < 0.05$; SO (negative control): simple ointment; HAE: 80% methanol extract of *Arisaema schimperianum* tuber, HF: *n*-hexane fraction, CF: chloroform fraction, WF: water fraction, NF (positive control): 0.2% (w/v) nitrofurazone ointment.

4.5 Antibacterial and antifungal activities

4.5.1 Hydroalcoholic extract and solvent fractions

In vitro antibacterial and antifungal activities were evaluated by broth dilution method as described by (Alemu *et al*., 2024; Tabassum *et al.*, 2019). The lowest concentration of the extract that prevents the visible growth of each of the test microorganisms was determined as minimum inhibitory concentration (MIC). As shown in Table 11, the 80% methanol extract of *A. schimperianum* tuber inhibited growth of all the six bacterial strains tested. However, Gram-positive bacteria appeared to be more susceptible to the extract than the Gram-negative ones. The extract showed MIC value of 2 mg/ml against methicillin-resistant *S. aureus* (MRSA), whilst the highest value (MIC = 16 mg/ml) was showed against *P. mirabilis*. The Gram-positive bacteria were also found to be the most inhibited bacterial pathogens by the solvent fractions. The water fraction (MIC = 0.5 mg/ml) showed the strongest activity against MRSA, whilst *S. epidemidis* was most susceptible to the *n*-hexane fraction (0.5 mg/ml). All the bacterial strains tested, except MRSA, were sensitive to the positive control with MIC value of less than 0.6 µg/ml. Growth control and sterility control were not changed throughout the study. However, the hydroalcoholic tuber extract and solvent fractions of *A. schimperianum* failed to show any activity against the fungal strains tested at the tested doses.

Table 11: Antibacterial and antifungal activities of the 80% methanol extract and solvent fractions of *Arisaema schimperianum* tuber

Bacteria	Test substance	Growth in nutrient agar containing different concentrations (mg/ml) of the solvent fractions							
		32	16	8	4	2	1	0.5	0.25
Escherichia coli	Hydroalcoholic extract	-	-	-	+	+	+	+	+
	n-Hexane fraction	-	-	-	+	+	+	+	+
	Chloroform fraction	-	-	-	+	+	+	+	+
	Water fraction	-	-	-	+	+	+	+	+
Proteus mirabilis	Hydroalcoholic extract	-	-	+	+	+	+	+	+
	n-Hexane fraction	-	-	+	+	+	+	+	+
	Chloroform fraction	-	-	+	+	+	+	+	+
	Water fraction	-	-	+	+	+	+	+	+
Pseudomonas aeruginosa	Hydroalcoholic extract	-	-	-	+	+	+	+	+
	n-Hexane fraction	-	-	-	+	+	+	+	+
	Chloroform fraction	-	-	-	+	+	+	+	+
	Water fraction	-	-	-	-	+	+	+	+
Staphylococcus aureus	Hydroalcoholic extract	-	-	-	-	-	+	+	+
	n-Hexane fraction	-	-	-	+	+	+	+	+
	Chloroform fraction	-	-	-	+	+	+	+	+
	Water fraction	-	-	-	-	-	-	+	+
MRSA	Hydroalcoholic extract	-	-	-	-	-	+	+	+
	n-Hexane fraction	-	-	-	+	+	+	+	+
	Chloroform fraction	-	-	-	+	+	+	+	+
	Water fraction	-	-	-	-	-	-	-	+
Streptococcus epidermidis	Hydroalcoholic extract	-	-	-	-	-	+	+	+
	n-Hexane fraction	-	-	-	-	-	-	-	+
	Chloroform fraction	-	-	-	-	-	+	+	+
	Water fraction	-	-	-	-	-	-	+	+
Candida albicans	Hydroalcoholic extract	NA	NA	NA	NA	NA	NA	NA	NA
	n-Hexane fraction	NA	NA	NA	NA	NA	NA	NA	NA
	Chloroform fraction	NA	NA	NA	NA	NA	NA	NA	NA
	Water fraction	NA	NA	NA	NA	NA	NA	NA	NA
Aspergillus niger	Hydroalcoholic extract	NA	NA	NA	NA	NA	NA	NA	NA
	n-Hexane fraction	NA	NA	NA	NA	NA	NA	NA	NA
	Chloroform fraction	NA	NA	NA	NA	NA	NA	NA	NA
	Water fraction	NA	NA	NA	NA	NA	NA	NA	NA
All tested microorganism	Negative control	NA	NA	NA	NA	NA	NA	NA	NA

MRSA: methicillin-resistant *Staphylococcus aureus*, Negative control: 5% tween 80; + growth, -: no owth, NA: in active; MIC of bacteria positive control (Ciprofloxacin: 0.6 µg/ml), MIC of fungi positive control (Ketoconazole: 12.5 µg/ml).

4.6 Isolated compound

Normal-phase silica gel TLC was found to be not suitable for analyzing the tuber extracts of *A. schimperianum* due the high polarity of its constituents. Therefore, reversed-phase silica gel TLC was employed. Application of the water fraction on C-18 reversed-phase silica gel column chromatography led to the isolation of a major compound, coded as BA. BA quenched UV light of 254 nm, appearing as a dark brown spot, while the spot appeared blue under UV light at 366 nm. Figure 8 depicts the reversed-phase TLC chromatograms of BA under UV light of 254 and 366 nm.

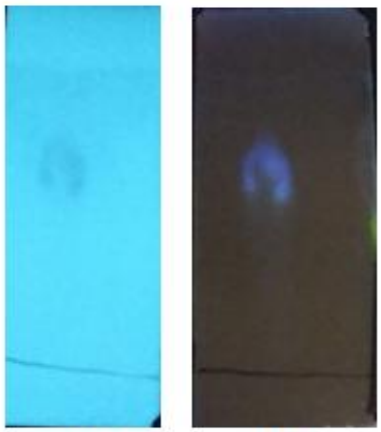


Figure 8: RP-TLC chromatograms of compound BA using H_2O: MeOH (1:1) as a solvent system, and viewed under UV 254 nm (A) and 366 nm (B)

4.7 Structural elucidation

BA was isolated as a brown substance with an *Rf*-value of 0.6 in on RP-TLC using MeOH/H_2O: (1:1) as solvent system. A molecular formula of $C_{15}H_{28}O_{14}$ was proposed for compound BA based on the pseudomolecular ion observed at *m/z* 433.0191 $[M+H]^+$ in the positive ion HR-ESI–mass spectrum (Figure 9) (exact calcd. = 433.1557 $[M+H]^+$). A tentative structure for BA was established on the basis of the HR-ESI-MS, ^1H and ^{13}C NMR spectral data.

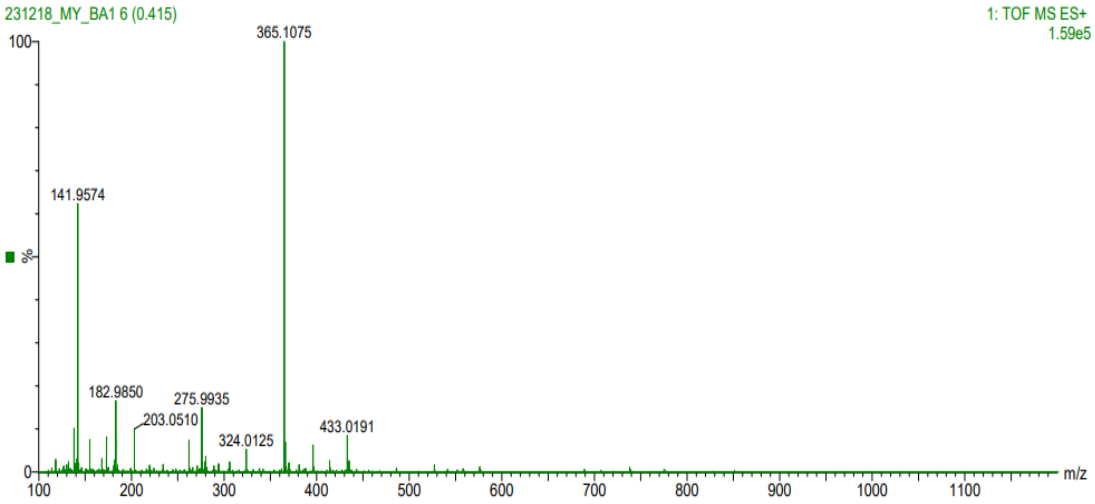


Figure 9: Positive mode HR-ESI-mass spectrum of BA

In the ^{1}H-NMR spectrum of BA (Figure 10), five key proton signals were observed at δ 3.89 and δ 3.62 (*m*, H-3a, H-3b); δ 3.59 and δ 3.53 (H-1a, H-1b) and δ 3.80 (*m*, H-2), indicating the presence of a glycerol moiety as a part of the structure of BA. Additionally, the presence of two sugar moieties was suggested by the observation of two anomeric proton signals in BA at δ 5.17 (*d*, *J* = 4.0 Hz, H-1″) and δ 3.92 (*m*, H-1′). Further examination of the ^{1}H-NMR spectrum revealed that BA does not contain any aromatic or olefinic protons.

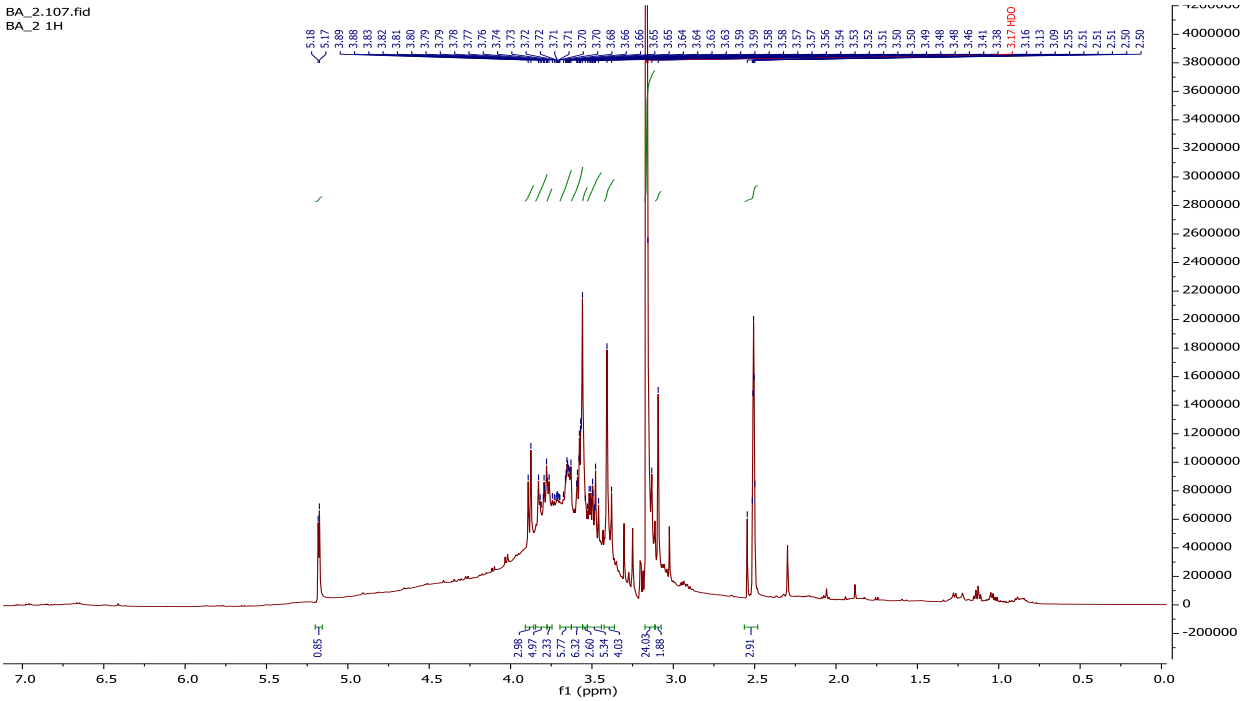


Figure 10: ^{1}H-NMR spectrum of BA

In the ^{13}C-NMR of BA (Figure 14), three carbon signals observed at δ 60.88 (C-1), δ 72.05 (C-2) and δ 63.36 (*m*, C-3) further supported the presence of a glycerol unit in BA. Additionally, two anomeric carbons signals were clearly visible in the ^{13}C-NMR spectrum at δ 92.19 (C-1″) and δ 104.42 (C-1′). The carbon signals are summarized in Table 11.

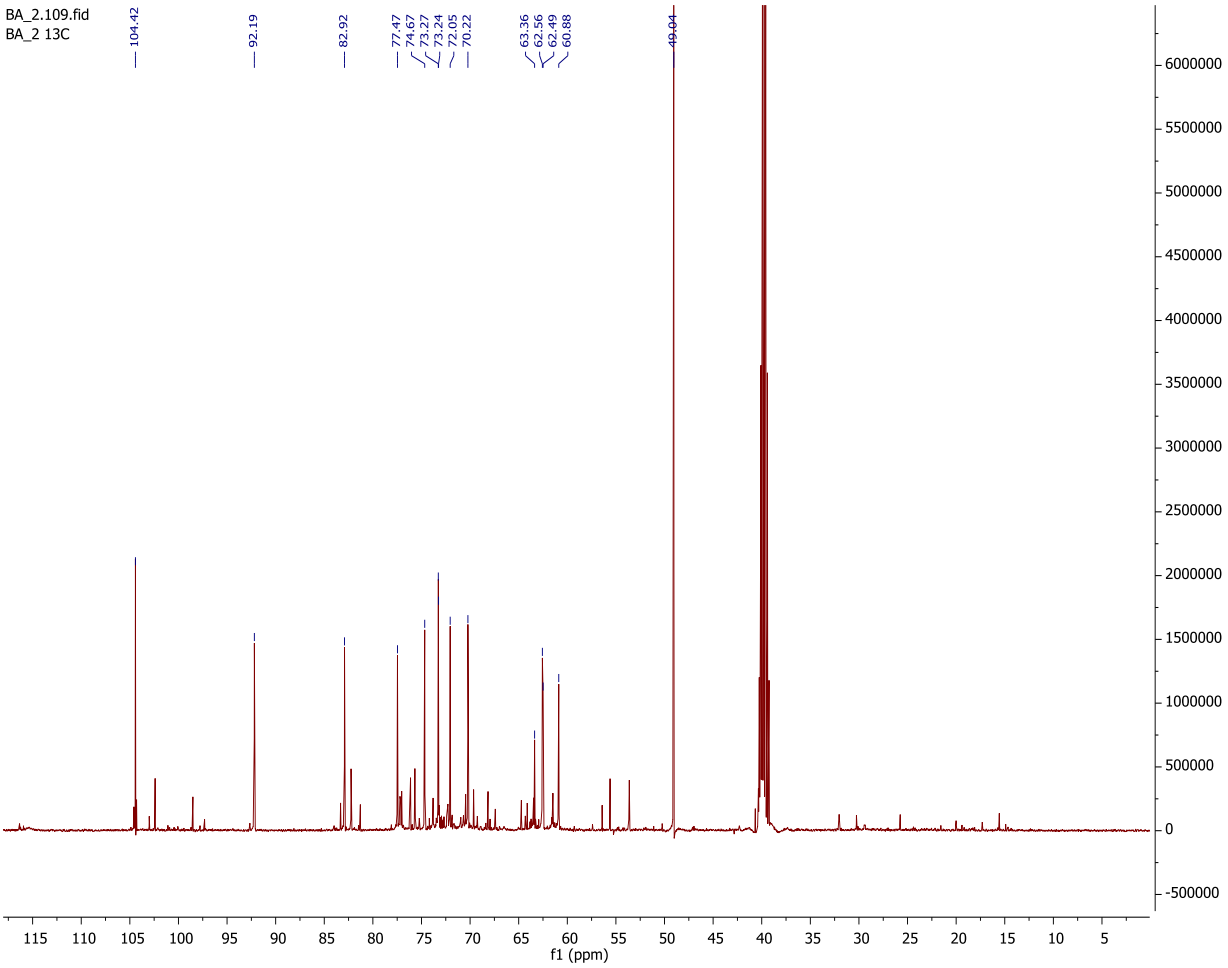


Figure 11: ^{13}C-NMR spectrum of BA

Table 12: ^{1}H and ^{13}C-NMR data of BA compared with diacylglycerylgalactosides (Jung *et al*., 1996)

Position	^{1}H; ppm*		^{13}C, ppm	
	BA	Literature report	BA	Literature report
1a	3.59 (*nr*)	3.59 (*dd*, 11.3, 5.4)	60.88	64.0
1b	3.53 (*nr*)	3.55 (*dd*, 11.3, 5.8)	60.88	64.0
2	3.80 (*m*)	3.78 (*m*)	72.05	72.4
3a	3.89 (*m*)	3.91 (*dd*, 10.1, 7.0)	63.36	68.7
3b	3.62 (*nr*)	3.73 (*dd*, 10.1, 3.2)	63.36	68.7
1′	3.92	4.24 (*d*, 7.5)	104.42	105.3
2′	3.62 (*m*)	3.53 (*dd*, 9.9, 7.5)	73.24	72.5
3′	3.71 (*m*)	3.49 (*dd*, 9.9, 3.5)	74.67	74.7
4′	3.59 (*m*)	3.85 (*dd*, 3.5, 1.0)	70.12	70.1
5′	3.72 (*m*)	3.75 (*dd*, 5.5, 4.2)	77.47	74.6
6a′	3.77 (*nr*)	3.72 (*dd*,11.6, 6.1)	62.49	62.8
6b′	3.52 (*nr*)	3.69 (*dd*, 11.6, 5.5)	62.49	62.8
1″	5.17 (*d*, 4.0)	4.84 (*d*, 3.5)	92.19	100.6
2″	3.64 (*m*)	3.46 (*dd*, 9.8, 3.2)	73.27	71.5
3″	3.70 (*m*)	3.77 (*dd*, 9.8, 3.5)	77.47	70.2
4″	3.60 (*m*)	3.88 (*d*, 3.2)	73.24	71.1
5″	3.68 (*m*)	3.70 (*dd*, 5.5, 4.1)	82.92	71.7
6a″	3.82 (*nr*)	3.83 (*dd*, 10.0, 5.5)	62.56	62.8
6b″	3.68 (*nr*)	3.68 (*dd*, 10.0, 4.1)	62.56	62.8

*: multiplicity and *J*-coupling constant in parenthesis (*J* in Hz); nr: not well resolved.

The 2D-HSQC spectrum of BA, shown in Figure 12, revealed the ^{1}J-coupling between carbon and proton. For instance, ^{1}J-coupling between carbon atom (δ 92.19; C-1”) and proton atom (δ 5.17, *d*, *J*=4.0Hz; H-1”).

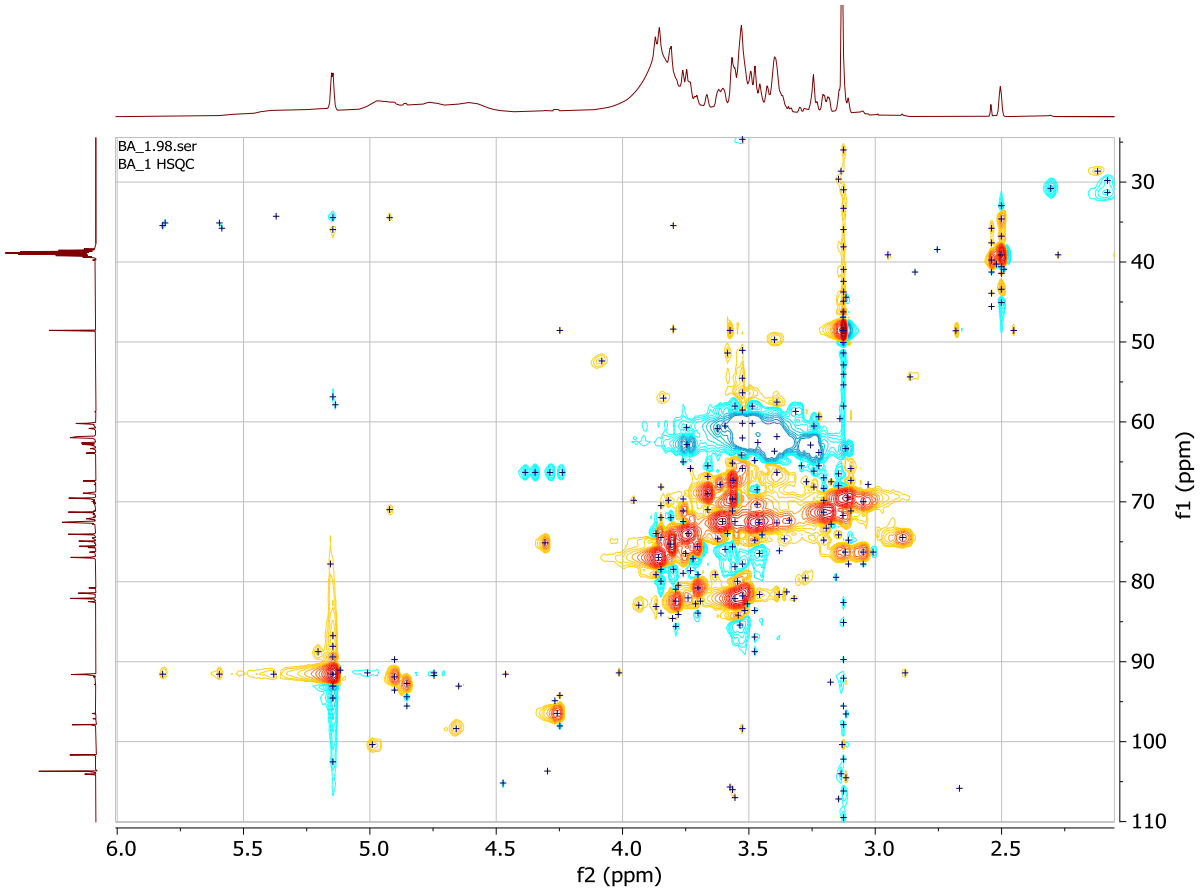


Figure 12**:** 2D-HSQC spectrum of BA

The above spectroscopic analysis (ESI-MS, ^{1}H; and ^{13}C-NMR) coupled with comparison of literature data led to tentative identification of BA as 3-*O*-[α-D-galactopyranosyl-(1”→6’)-*O*-β-*O*-galactopyranosyl] glycerol (GGG) (Figure 13).

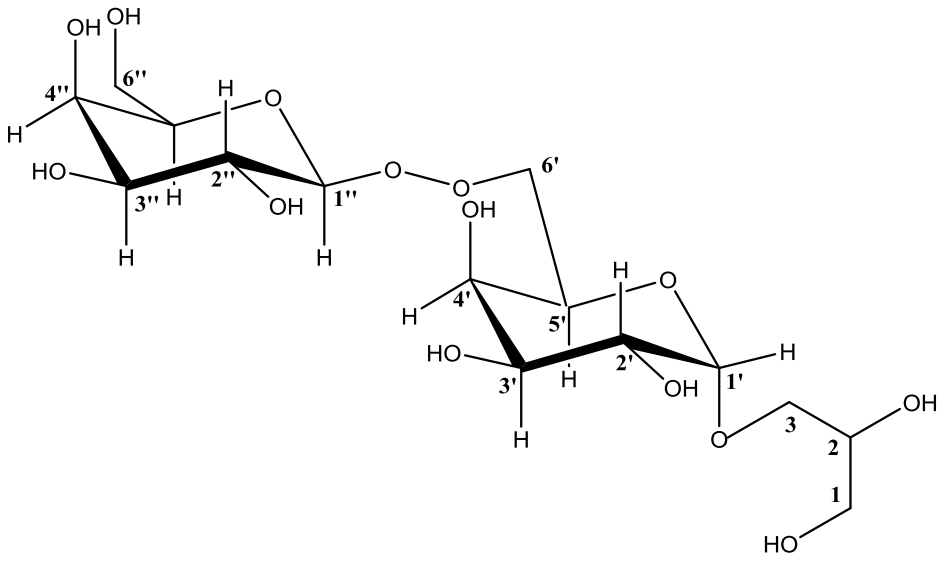


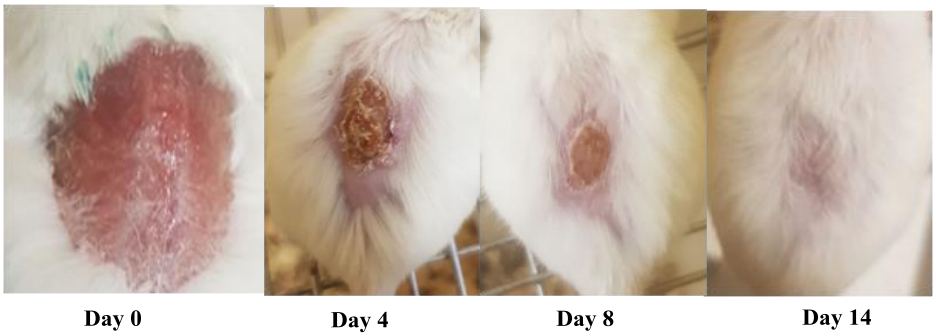
Figure 13: A tentative structure for the isolated compound 3-O-[α-D-galactopyranosyl-(1″→6′)-O-β-O-galactopyranosyl] glycerol (GGG)

4.8 Excision wound model

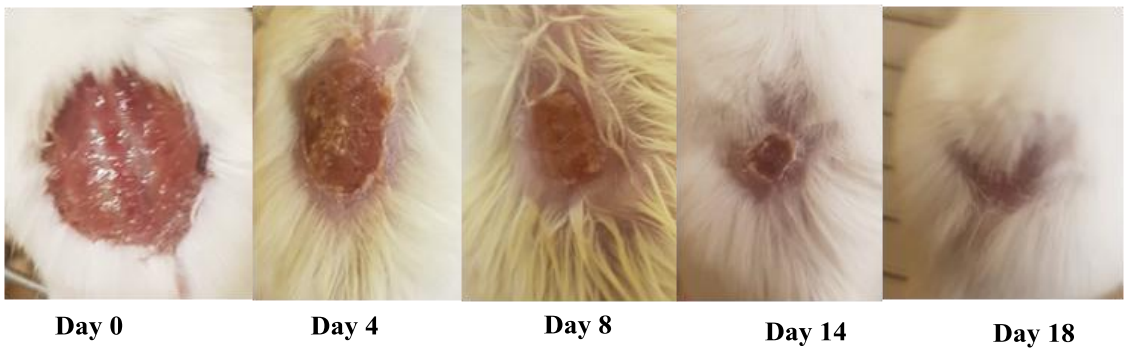
4.8.1 Effect of GGG on wound contraction

As shown in Figure 14, 5% and 10% w/w ointment preparations of GGG showed strong, dose-dependent wound healing effect comparable to nitrofurazone. From the 4th post-wounding day onward, 10% (w/w) GGG exhibited significant wound contraction ($p < 0.001$) compared to the negative control (Table 13). As shown in the Figure 15, 10% (w/w) GGG achieved complete wound closure on day 14, whilst the 5% (w/w) GGG and the standard drug closed the wound on day 18.

A. 5% (w/w) GGG



B. 10% (w/w) GGG



C. Nitrofurazone ointment 0.2% (w/v)

Figure 14**:** Photograph of skin after topical application of (A) 5% (w/w) 3-O-[α-D-galactopyranosyl-(1″→6′)-O-β-O-galactopyranosyl] glycerol, (B) 10% (w/w) 3-O-[α-D-galactopyranosyl-(1″→6′)-O-β-O-galactopyranosyl] glycerol, and (C) 0.2% (w/v) nitrofurazone ointment for wound contraction test.

Table 13: Effect of topical application of 3-O-[α-D-galactopyranosyl-(1″→6′)-O-β-O-galactopyranosyl] glycerol (GGG) on wound area (mm^2) and percent of wound contraction in excision wound model

Group	Day2	Day4	Day6	Day8	Day10	Day12	Day14	Day16	Day 18
SO	303.33±2.96	283.50±2.62	260.66±2.77	224.33±4.08	164.50±7.10	102.50±4.34	71.83±4.49	30.16±2.70	15.16±2.02
	(3.39%)	(9.71%)	(16.98%)	(28.55%)	(47.611%)	(67.35%)	(77.12%)	(90.39%)	(95.17%)
5% GGG	302.16±4.36	272.33±2.29	187.83±16.3	101.66±2.12	55.33±1.45	25.33±1.45	10.50±0.42	3.75±0.17	0.00
	(3.77%)	(13.27%)	(40.18%)[a1]	(67.62%)[a1,c1]	(82.37%)[a1,c1]	(91.93%)[a1]	(96.65%)[a1]	(98.80%)[a1]	(100%)[a1]
10% GGG	293.33±3.79	258.50±3.79	149.83±4.53	55.67±5.07	12.67±1.30	3.33±0.49	0.00	0.00	0.00
	(6.58%)	(17.14%)[a1,b1,c3]	(51.97%)[a1,b3,c3]	(82.15%) [a1,b1,c1]	(95.93%)[a1,b1,c1]	(98.93%)[a1,b1,c1]	(100%)[a1,b3,c1]	---	---
NF	300.50±3.30	270.66±1.76	183.83±3.32	116.50±2.39	65.33±6.60	25.00±1.91	13.16±1.42	2.16±0.47	0.00
	(4.29%)	(13.80%)[a3]	(41.45%)[a1]	(62.66%) [a1,b1]	(79.06%)[a1,b1]	(91.98%)[a1]	(95.78%)[a1]	(99.30%)[a1]	(100%)[a1]

Data are presented as mean ± SEM (n = 6) and analyzed using a one-way ANOVA with a Tukey post hoc test; [a] compared with SO, [b] compared with 5% (w/w) GGG, [c] compared with standard; [1] $p < 0.001$, [2] $p < 0.01$, [3] $p < 0.05$; SO: (negative control): simple ointment; NF: (positive control): 0.2% (w/v) nitrofurazone ointment.

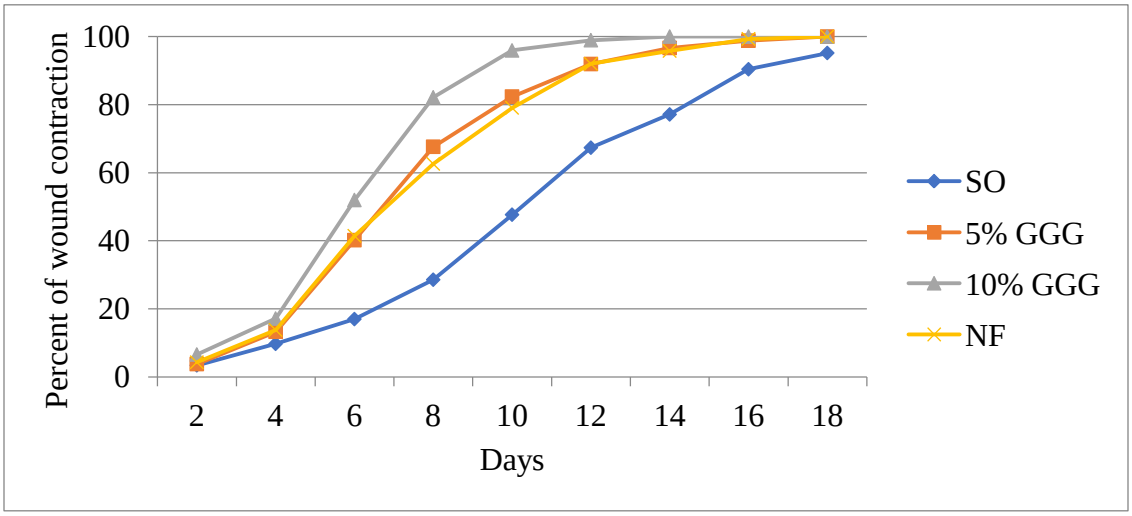


Figure 15: Effect of 3-O-[α-D-galactopyranosyl-(1″→6′)-O-β-O-galactopyranosyl] glycerol (GGG) on the percentage wound closure of excision wound model in mice; SO (negative control): simple ointment, NF (positive control): 0.2% (w/v) nitrofurazone ointment.

4.8.2 Effect of GGG on epithelization period

As shown Table 14, the 5% and 10% (w/w) GGG and standard drug treated groups showed a significant reduction ($p < 0.001$) when compared with the negative control. However, 10% (w/w) GGG showed faster rate of epithelialization compared to 5% (w/w) GGG and the positive control, but it was not statistically significant.

Table 14: Effect of topical application of 3-O-[α-D-galactopyranosyl-(1″→6′)-O-β-O-galactopyranosyl] glycerol (GGG)on period of epithelialization

Group	Epithelialization period (day)
SO	18.00±0.51
5% (w/w) GGG	14.00±0.73 [a1]
10% (w/w) GGG	12.66±0.84 [a1]
NF	14.00±0.73 [a1]

Data are expressed as mean ± SEM (n = 6); [a] compared with simple ointment; [1]$p < 0.001$; SO (negative control): simple ointment; NF (positive control): 0.2% (w/v) nitrofurazone ointment.

4.8.3 Histopathological analysis

As shown in Table 15 and Figure 16A, histological features indicated that the 10% (w/w) GGG showed complete healing tissue evidenced by high collagen fiber and fibroblast cells with moderate blood capillaries as compared with simple ointment treated group. Similarly, animals in the standard drug treated group (Figure 16B) also showed complete tissue repair, whilst the 5% (w/w) GGG treated group showed moderate fibroblast, collagen deposition, inflammatory cells and neovascularization (Figure 16C). On the contrary, mice treated with simple ointment base (Figure 16D) showed more inflammatory cells, reduced collagen fibers, fibroblast cells and blood vessels, thus showing delayed wound healing processes.

Table 15: Histological evaluation of wound healing process induced by 3-O-[α-D-galactopyranosyl-(1″→6′)-O-β-O-galactopyranosyl] glycerol (GGG)

Group	RE	FB	CD	PMN	MNC	NV
SO	+	+	+	++	++	+
5% (w/w) GGG	++	+++	++	++	++	++
10% (w/w) GGG	++	+++	+++	++	+	++
NF	++	++	+++	++	++	+

+: mild, ++: moderate, +++: severe for epidermal or dermal remodeling; RE: reepithelization, FB: Fibroblast, CD: collagen deposition, PMN: polymorphonuclear cells, MNC: mononuclear cells, NV: neovascularization; SO (negative control): simple ointment, NF (positive control): 0.2% (w/v) nitrofurazone ointment.

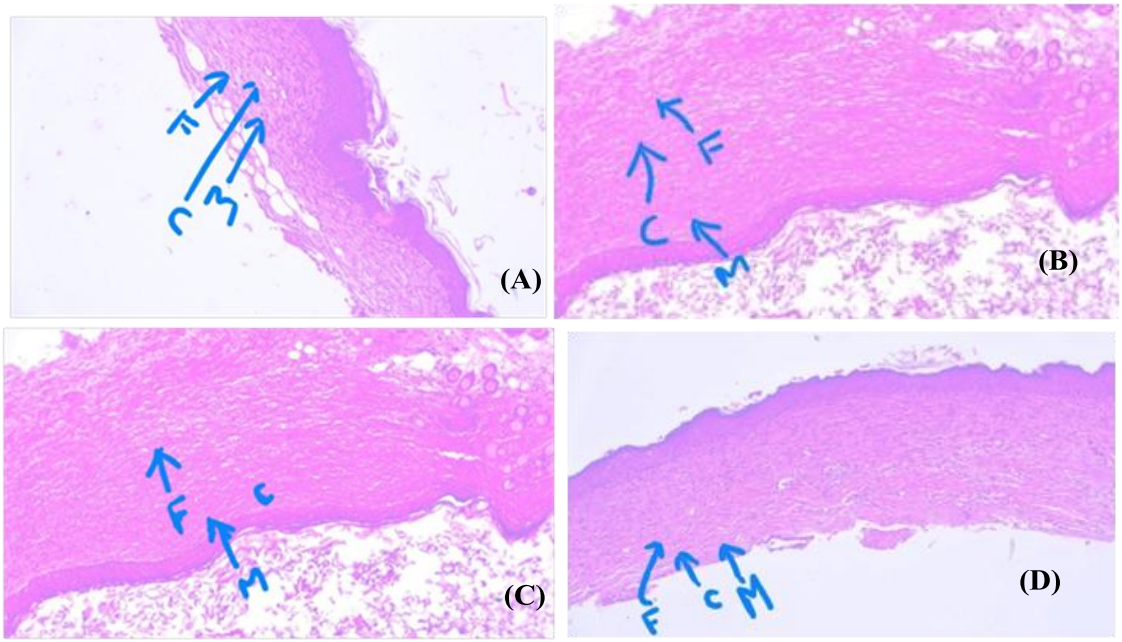


Figure 16: Photomicrograph of histopathological section of wound tissue (stained with H&E, 100× magnifications) obtained from mice treated with (A) 10% (w/w) 3-O-[α-D-galactopyranosyl-(1″→6′)-O-β-O-galactopyranosyl] glycerol, (B) positive control (0.2% (w/v) nitrofurazone ointment, (C) 5% (w/w) 3-O-[α-D-galactopyranosyl-(1″→6′)-O-β-O-galactopyranosyl] glycerol (D) simple ointment; C: collagen fibers F: fibroblasts, M: macrophages.

4.8.4 Effect of GGG on tissue hydroxyproline content

As shown in Table 16, the hydroxyproline content of mice treated with 10% GGG increased significantly ($p < 0.001$) in comparison with 5% GGG or simple ointment treated mice. The increase was also in a concentration dependent manner. The relative order of collagen deposition for the different groups was at 10% (w/w) GGG > standard nitrofurazone > 5% (w/w) GGG > simple ointment.

Table 16: Effect of 3-O-[α-D-galactopyranosyl-(1′′→6′)-O-β-O-galactopyranosyl] glycerol (GGG) on tissue hydroxyproline content in excision wound model

Groups	Hydroxyproline content (mg/g\ tissues)
SO	10.00±1.00
5% (w/w) GGG	52.67±1.15[a1]
10% (w/w) GGG	64.67±2.51[a1,b1,c1]
NF	58.89±[a1,b1]

Data are presented as mean ± SEM (n = 6) and analyzed using a one-way ANOVA with a Tukey post hoc test; [a] compared with negative control, [b] compared with 5% (w/w) GGG, [c] compared with nitrofurazone; [1] $p < 0.001$, [2] $p < 0.01$, [3] $p < 0.05$; SO (negative control): simple ointment, NF (positive control): 0.2% (w/v) nitrofurazone ointment.

4.10 Effect of GGG on incision wound

As shown in Table 17, the 5% and 10% (w/w) ointment preparations of GGG showed significant increase ($p < 0.001$) in tensile strength as compared to the negative control or untreated group. Mice treated with 5% (w/w) GGG and the standard drug showed comparable tensile strength, whilst the tensile strength of animals treated with 10% (w/w) GGG ointment showed the highest wound breaking strength.

Table 17: Effect of topical application of 3-O-[α-D-galactopyranosyl-(1′′→6′)-O-β-O-galacto-pyranosyl] glycerol (GGG) on tensile strength of incision wound model

Group	Tensile Strength (g)	Percentage of tensile strength
Untreated control	184.16±8.00	---
SO	230.83±19.07	22.86
5% (w/w) GGG	415.00±28.75 [a1]	79.78
10% (w/w) GGG	439.33±26.63 [a1]	90.32
NF	406.67±23.58 [a1]	76.32

Data are presented as mean ± SEM (n = 6) and analyzed using a one-way ANOVA with a Tukey post hoc test; [a] compared with simple ointment, [b] compared with untreated control; [1] $p < 0.001$; SO (negative control): simple ointment, NF (positive control): 0.2% (w/v) nitrofurazone ointment.

4.11 Antibacterial and antifungal activities of GGG

GGG displayed varying activity against the test organisms used in this study. In particular, it exhibited relatively good activity against *S. aureus* and MRSA (MIC = 1 mg/ml). As shown in Table 18, GGG inhibited the Gram-positive bacteria more than the Gram-negative ones.

Table 18: Determination of minimum inhibitory concentrations of 3-O-[α-D-galactopyranosyl-(1″→6′)-O-β-O-galactopyranosyl] glycerol

Bacteria	Growth in nutrient agar containing different concentrations (mg/ml)						
	32	16	8	4	2	1	0.5
Escherichia coli	-	-	-	+	+	+	+
Proteus mirabilis	-	-	+	+	+	+	+
Pseudomonas aeruginosa	-	-	-	-	+	+	+
Staphylococcus aureus	-	-	-	-	-	-	+
MRSA	-	-	-	-	-	-	+
Streptococcus epidermidis	-	-	-	+	+	+	+

MRSA: methicillin-resistant *Staphylococcus aureus*, Negative control: 5% tween 80; +: growth; -: no growth, NA: in active; MIC of bacteria positive control (Ciprofloxacin: 0.6 µg/ml).

5. Discussion

Wound infection remains a significant challenge in healthcare, particularly in developing countries like Ethiopia, where the rise in comorbidities further complicates the healing process. Effective wound healing treatments aim to speed up the progress of natural processes of wound healing and avoiding complications (Belachew *et al.*, 2020). Generally, herbal based ointments are considered to be safe and effective in addition to their low cost for wound treatment (Pastar *et al*., 2009). In Ethiopia, numerous medicinal plants include the tuber of *A. schimperianum* are used in wound management (Bitew *et al.*, 2019). Ethnobotanical studies give an account of the use of the dried tuber of *A. schimperianum* mixed with Vaseline to treat wound associated with

glandular tuberculosis as well as for ring worm, scabies and skin lesion (Yineger *et al.*, 2008; Getachew *et al.*, 2022). However, similar to other species of the Araceae family, the toxicity of *A. schimperianum* due to the presence of calcium oxalate needle crystals has been recorded (Hazo and Yirgalem, 2021).

The present study evaluated the wound healing activity of the 80% methanolic extract of *A. schimperianum* and its successive solvent fractions to determine the most active fraction with the aim of identifying the compound(s) responsible for bioactivity. Both acute oral toxicity and dermal toxicity studies were also carried out to determine safety of the extract(s). The results demonstrated that the hydroalcoholic extract of the plant is nontoxic to mice at an oral dose of 2000 mg/kg.

Furthermore, topical application of both 5% (w/w) and 10% (w/w) ointment preparations of the hydroalcoholic extract were safe for wound application, though mild irritation was noted with the 10% (w/w) ointment. This is likely due to the increased concentration of active compounds, leading to a stronger dose-dependent effect on the skin (Ali and Yaqoob, 2021). Previous studies have shown that topical application of *Arisaema leschenaultii* tuber extract causes skin irritation (Suruse *et al.*, 2011).

For a plant-based treatment to be scientifically supported as a wound healing agent, it should influence at least two different wound healing processes (Gebrehiwot *et al.*, 2015). These processes are commonly studied by excision and incision wound models (Mekonnen *et al.*, 2013). In this study wound contraction which pulls the edges of a wound inward from all sides and decreasing the amount of extracellular matrix required for repair is measured by excision model (Childs and Murthy, 2017). Ointments formulated with 5% and 10% (w/w) of 80% methanolic extract of *A. schimperianum* tuber enhanced wound contraction starting from the 10th

and 4th post-wounding day, respectively. In addition, the 10% formulation promotes faster formation of new epithelial tissue by encouraging keratinocyte migration from the wound edges across the wound surface (Chhabra *et al*., 2017). This increased efficacy may be attributed to the higher concentration of bioactive compounds in the extract, which likely accelerates cell proliferation and tissue repair (Ali and Yaqoob, 2021). The delayed effect in the 5% formulation suggests that lower concentrations may require more time to achieve similar healing outcomes.

In the incision wound model, the force required to break the healed wound are measured by water flow technique (Thakur *et al.*, 2011). Topical application of 5% (w/w) and 10% (w/w) ointments of the hydroalcoholic extract increased wound breaking strength indicating a better wound healing. However, stronger force was required to open the wound of animals treated with the 10% (w/w) ointment than those treated with 5% (w/w) ointment probably due to greater concentration of bioactive compounds, leading to enhanced biological activity and possible increased synergistic effects (Deshmukh *et al*., 2009). Furthermore, histological analysis of wound of animals treated with the medicated ointments revealed high amounts of collagen and fibroblast indicating a rebuild in strength and structure of the tissue (Kolarsick *et al*., 2006).

Owing to accelerated healing effect of *A. Schimperianum* tuber hydroalcoholic extract on both excision and incision wounds, the extract was further fractionated with solvents of different polarity. The results of the study demonstrated that the water fraction was more efficacious in promoting wound contraction rate, shortening period of epithelization and increasing tensile strength than the organic solvent fractions. This was supported by histological studies which revealed that the water fraction enhanced wound healing by reducing inflammation, boosting collagen and fibroblast activity and promoting tissue maturation. A previous study by Belachew *et al*. (2020) showed that such effects accelerate wound closure and support orderly tissue repair.

Enhanced rate of wound contraction and reduction in healing time caused by the water fraction of *A. schimperianum* is consistent with previous studies which reported that polar compounds that belong to different classes of secondary metabolites are endowed with anti-inflammatory, antioxidant and antimicrobial properties that are crucial for wound healing. Moreover, it was shown that water soluble compounds are absorbed by tissue with ease enhancing the wound healing process (Suntar *et al*., 2010).

Hydroxyproline is a unique amino acid predominantly found in collagen. Therefore, it is a common biochemical approach to estimate collagen content by determining hydroxyproline concentration (Murthy *et al*., 2004). The current study showed that the ointment prepared from 10% (w/w) water fraction increased hydroxyproline content more than that of the positive control (0.2% (w/v) nitrofurazone ointment), suggesting a marked enhancement in collagen synthesis and turnover which are crucial for wound healing. This likely occurs due to the active phytochemicals present in the aqueous fraction that accelerate collagen deposition (Childs and Murthy, 2017). Collagen, being a major structural protein, strengthens and supports the extracellular matrix around the wound, facilitating quicker tissue repair, speedy wound contraction and faster healing (Mulisa *et al*., 2015).

It is well known that wound infection is detrimental to wound healing, and is the most common cause of impairing the wound healing process. In this study the hydroalcoholic extract and solvent fractions of *A. schimperianum* displayed antibacterial activity. However, they inhibited growth of the Gram-positive bacteria tested better than the Gram-negative ones. This is probably due the difference in cell wall structure between Gram-positive and Gram-negative bacteria. In addition to the thick layer of peptidoglycan present in Gram-negative bacteria, their outer membrane is composed of lipopolysaccharides, making it harder for antimicrobial compounds to

penetrate (Breijyeh *et al*., 2020). It is interesting to note that the water fraction of *A. schimperianum* displayed the strongest effect against *S. aureus* (MIC = 1 mg/ml) and MRSA (0.5 mg/ml), which are among the most prevalent causative organisms associated with infected wound (Bala *et al*., 2019). However, the weaker effect of this polar fraction against the Gram-negative bacteria implied that the hydrophilic compound(s) present in it struggled to cross the outer lipophilic membrane. Generally, as plant extracts with MIC values of ≤ 8 mg/mL are regarded as effective antibacterial agents (Zouine *et al.,* 2024), the antibacterial effects displayed by the extracts of *A. schimperianum* cannot be overemphasized. Antibacterial studies previously done on *Arisaema jacquemontii* showed that the methanol extracts of the leaves and tubers possess good activity (MIC = 0.37 mg/ml) against *S. aureus* (Bala *et al*., 2019). Bhat *et al*. (2019) also demonstrated that the methanol extract of *Arisaema utile* tuber inhibits growth of both bacteria and fungi. However, in the present study neither the hydroalcoholic extract nor the solvent fractions of *A. schimperianum* tuber exhibited antifungal activity.

Phytochemical investigation of the aqueous fraction of *A. schimperianum* tuber, utilizing reverse-phase flash column chromatography, led to the isolation of an active compound tentatively identified as 3-*O*-[α-D-galactopyranosyl-(1”→6’)-*O*-β-D-galactopyranosyl] glycerol (GGG). Interestingly, this compound was isolated for the first time from *A. schimperianum*, marking a significant addition to the phytochemical profile of the species. However, structurally related diacylglycerylgalactosides have been previously reported from the *Arisaema* genus. For instance, Jung *et al*. (1995) identified similar compounds such as (2*S*)-1-*O*-hexadecanoyl-2-*O*-(9*Z*,12*Z*-octadecadienoyl)-3-*O*-[α-D-galactopyranosyl-(1"→6')-*O*-β-D-galactopyranosyl] glycerol and (2*S*)-1-*O*-octadecanoyl-2-*O*-(9*Z*,12*Z*-octadecadienoyl)-3-*O*-[α-D-galactopyranosyl-(1"→6')-O-β-D-galactopyranosyl] glycerol. These findings highlight the structural diversity of

glycerylgalactoside derivatives in the genus *Arisaema*, underscoring the genus as a valuable source of unique glycolipids based on glycerol structure. Moreover, diacylglycerylgalactosides have been isolated from various algae and higher plants, supporting their broad natural distribution. However, Jung *et al*. (1996) noted that these compounds are rarely reported as single entities, and the detailed sequence of their acyl chains is often left undetermined. This lack of specificity in characterization adds complexity to their study and limits the understanding of their precise biological roles.

In the present study, the isolated compound GGG was tested for its wound healing activity in both excision and incision wound models. In excision wound model, 10% (w/w) GGG levigated in simple ointment showed increase in percentage closure of wounds, decrease in epithelization time and enhanced rate of wound contraction which were better that of the positive control 0.2% (w/v) nitrofurazone ointment. For example, complete wound closure by the positive control was observed on the 18^{th} post wounding day, whereas 10% (w/w) GGG closed the wound on the 14^{th} post wounding day. Similarly, epithelization period was 12 days for 10% (w/w) GGG and 14 days for the positive control. It has been reported that shorter epithelization period is an indication of better wound healing activity (Süntar *et al*., 2010). In the incision wound model, deposition of newly synthesized collagens at the wound site increases collagen concentration per unit area and hence the tissue tensile strength (Deshmukh *et al*., 2009). Results of the present study demonstrated that wound treated with 10% (w/w) GGG had much higher tensile strength (439.33 g) than wound treated with the positive control (406.67 g). Higher tensile strength arises from high amount of collagen which facilitates wound healing by the formation of inter- and intra-molecular cross links (Reddy *et al*., 2008).

In all excision and incision wound model parameters, the effects of GGG were superior to those of the positive control but were comparable to the effects of the aqueous fraction. This implies that the wound healing activity of *A. schimperianum* tuber is attributed not to a single compound but to a combination of secondary metabolites present in the plant. It is possible that the minor components present in the aqueous fraction have stronger activity or may have acted synergistically with each other or with GGG making the water fraction to be strongly active.

As shown in Table 24, GGG displayed antibacterial activity against all the bacterial strains tested. However, the Gram-negative bacteria were shown to have limited susceptibility compared to the Gram-positive bacteria. It is of a particular significance that GGG is most active against *S. aureus* and its methicillin resistant type (MRSA), which is among the most common bacterial wound pathogens. Literature review reveals that *S. aureus* colonization impedes wound healing (Zielińska *et al*., 2023). Biswas *et al*. (2001) have also reported that bacterial infections can cause poor quality granulation tissue formation, reduced tensile strength of connective tissue, impaired epithelization and odour. Therefore, the wound healing activity of GGG could be attributed, at least in part, to its antibacterial effects. The wound healing and antibacterial activities of GGG suggest the compound's potential as a natural therapeutic agent. Furthermore, the discovery of this compound contributes to the growing body of evidence that glycerylgalactosides may possess significant bioactive properties. However, further investigations are necessary to elucidate the complete structure-activity relationships, mechanisms of action, and potential pharmaceutical applications of these compounds.

6. Conclusion

The present findings confirmed that the tubers of *A. schimperianum* possess effective wound healing properties, lending scientific support to folkloric or anecdotal use of the plant for the treatment of wound. The study further ascertained that the major compound 3-*O*-[α-*D*-galactopyranosyl-(1"→6')-*O*-β-*O*-galactopyranosyl] glycerol (GGG) isolated from the aqueous fraction of the tubers has the ability to improve wound contraction, enhance tensile strength and reduce epithelization time. However, results of the study also gave a clue that the wound healing effect of the plant was attributed not only to GGG but possibly to other strongly active minor secondary metabolites present in the plant. It can also be concluded that the ability of the tuber extract of *A. schimperianum* to enhance wound healing is, in part due to antibacterial effects. The extract was safe at a high dose and also caused minimal topical irritation which supports its use as a natural wound healing agent.

7. Recommendations

Based on the findings of the study, the following recommendations are proposed:

- Isolate the minor compound present in the tubers of A. *schimperianum*;
- Conduct sub-chronic, chronic and further dermal toxicity studies on the extracts and isolated compounds; and
- Test the wound healing activity of the minor compounds present in the active fractions.
- Formulate and standardize the plant extract ointment with a suitable base.

8. References

Alemayehu T (2021). Prevalence of multidrug-resistant bacteria in Ethiopia: a systematic review and meta-analysis. *J Glob Antimicrob Resist* **26**: 133-139.

Alemneh D (2021). Ethnobotanical study of medicinal plants used for the treatment of domestic animal diseases in Yilmana Densa and Quarit Districts, West Gojjam Zone, Amhara Region, Ethiopia. *Ethnobot Res Appl* **22**: 28.

Alemu M, Lulekal E, Asfaw Z, Warkineh B, Debella A, Abebe A, Degu S, Debebe E (2024). Antibacterial activity and phytochemical screening of traditional medicinal plants most preferred for treating infectious diseases in Habru District, North Wollo Zone, Amhara Region, Ethiopia. *PLoS One* **19**(**3**): e0300060.

Ali H, Yaqoob U (2021). Traditional uses, phytochemistry, pharmacology and toxicity of *Arisaema* (Araceae): a review. *Bull Natl Res Cent* **45**: 47.

Azam S, Saqib MS, Zar F, Ahmad B, Zeb Z, Khan I (2016). Antibacterial, antifungal, phytotoxic, antioxidant and hemagglutination activities of organic fractions of *Arisaema tortuosum*. *Pak J Pharm Sci* **29**(**3**): 991–997.

Bala K, Rana J, Sagar A (2019). Antibacterial and antioxidant potential of *Arisaema jacquemontii* Blume from Manali, Himachal Pradesh. *Bull Pure Appl Sci Bot* **38**(**1**): 23-33.

Belachew TF, Asrade S, Geta M, Fentahun E (2020). *In vivo* evaluation of wound healing and anti-inflammatory activity of 80% methanol crude flower extract of *Hagenia abyssinica* (Bruce) J.F. Gmel in Mice. *Evid Based Complement Altern Med* **2020**: 9645792.

Banyal HS, Tandon A, Nainta M (2014). Antimalarial effect of extract of *Arisaema Jacquemontii* Bl. on *Plasmodium berghei* Vincke and Lips (1948). *Asian J Biol Sci* **7**(**3**): 131–134.

Bhat AH, Alia A, Rather GM, Kumar M (2019). Isolation and characterisation of beta-sitosterol from the rhizomes of *Arisaema utile* and its evaluation for antioxidant activity. *Int J Sci Res Biol Sci* **6**(**2**): 111–118.

Bhat AH, Alia A, Rather GM, Kumar M (2019). Isolation and characterization of a water-soluble disaccharide from methanolic extract of *Arisaema utile* and its evaluation for antioxidant and antifungal activity. *Int J Pharm Biol Arch* **10**(**2**): 83–89.

Bibi Y, Nisa S, Chaudhary FM (2021). Antibacterial activity of some selected medicinal plants of Pakistan. *BMC Complement Altern Med* **11**: 52.

Birhanu Z, Endale A, Shewamene Z (2015). An ethnomedicinal investigation of plants used by traditional healers of Gondar town, North-Western Ethiopia. *J Med Plants Stud* **3**(**2**): 36–43.

Biswas TK, Auddy B, Bhattacharya NP, Bhattacharya S, Mukherjee B (2001). Wound healing activity of human placental extracts in rats. *Acta Pharmacol Sin* **22**: 1113–1116.

Bitew H, Gebregergs H, Tuem KB, Yeshak MY (2019). Ethiopian medicinal plants traditionally used for wound treatment: a systematic review. *Ethiop J Health Dev* **33**(**1**): 102-127.

Breijyeh Z, Jubeh B, Karaman R (2020). Resistance of Gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules* **25**(**6**): 1340.

British Pharmacopoeia (BP) (1948). British Pharmacopoeia. Office of the British Pharmacopoeia Commission **2**: 1-713.

Centers for Disease Control and Prevention (CDC) (2024). Antimicrobial resistance facts and stats. Available at: https://www.cdc.gov/antimicrobial-resistance/data-research/facts-stats/index.html. Accessed on July 2024.

Chhabra S, Chhabra N, Kaur A, Gupta N (2017). Wound healing concepts in clinical practice of OMFS. *J Maxillofac Oral Surg* **16**(**4**): 403–423.

Childs DR, Murthy AS (2017). Overview of wound healing management. *Surg Clin N Am* **97**(**1**): 189–207.

Dan MM, Sarmah P, Vana DR, Dattatreya A (2018). Wound healing: concepts and updates in herbal medicine. *Int J Med Res Health Sci* **7**(**1**): 170–181.

Deshmukh PT, Fernandes J, Atul A, Toppo E (2009). Wound healing activity of *Calotropis gigantea* root bark in rats. *J Ethnopharmacol* **125**(**1**): 178–181.

Dhakal L, Aryal B, Prasad P, Rajpant D (2020). Nutritional potential of selected species of *Arisaema* Mart. from Nepal. *Biodiversitas* **21**(**12**): 5703–5709.

Dowd SE, Hanson DJ, Rees E, Wolcott RD, Zischau AM, Sun Y, White J, Smith DM, Kennedy J, Jones CE (2011). Survey of fungi and yeast in polymicrobial infections in chronic wounds. *J Wound Care* **20**(**1**): 40–47.

Du SS, Zhang HM, Bai CQ, Wang CF, Liu QZ, Wang YY, Deng ZW (2011). Nematocidal flavone-C-glycosides against the root-knot nematode (*Meloidogyne incognita*) from *Arisaema erubescens* tubers. *Molecules* **16**(**6**): 5079–5086.

Enyew A, Asfaw Z, Kelbessa E (2014). Ethnobotanical study of traditional medicinal plants in and around Fiche District, central Ethiopia. *Curr Res J Biol Sci* **6**(**4**): 154–167.

Espejo E, Andrés M, Borrallo RM, Padilla A, Padilla E, Garcia-Restroy E, Bella F; Complex wounds working group (2018). Bacteremia associated with pressure ulcers: a prospective cohort study. *Eur J Clin Microbiol Infect Dis* **37**(**5**): 969–975.

Gebrehiwot M, Asres K, Bisrat D, Mazumder A, Lindemann P, Bucar F (2015). Evaluation of the wound healing property of *Commiphora guidottii* Chiov. ex. Guid. *BMC Complement Altern Med* **15**: 282.

Getachew S, Medhin G, Asres A, Abebe G, Ameni G (2022). Traditional medicinal plants used in the treatment of tuberculosis in Ethiopia: a systematic review. *Heliyon* **8**(**5**): e09478.

Guo S, DiPietro LA (2010). Factors affecting wound healing. *J Dent Res* **89**(**3**): 219–229.

Hazo H, Yirgalem A (2021). Proximate composition of qolxo (*Arisaema schimperianum* Schott) and different processing effect on its composition. *J Food Process Technol* **12**(**8**): 903.

Järbrink K, Sönnergren H, Schmidtchen H, Pang C, Bajpai R, Car J (2016). Prevalence and incidence of chronic wounds and related complications: a protocol for a systematic review. *Syst Rev* **5**(**1**): 152.

Jung JH, Lee H, Kang SS (1996). Diacylglycerylgalactosides from *Arisaema amurense*. *Phytochemistry* **42**(**2**): 447–452.

Kant K, Lal UR, Ghosh M (2018). *In silico* prediction and wet lab validation of *Arisaema tortuosum* (Wall.) Schott extracts as antioxidant and anti-breast cancer source: a comparative study. *Pharmacogn Mag* **13**(**4**): 786–790.

Kant K, Lal UR, Rawat R, Kumar A, Ghosh M (2020). Genus *Arisaema*: a review of traditional importance, chemistry and biological activities. *Comb Chem High Throughput Screen* **23**(**7**):

624–648.

Kaur M, Singh K, Rup PJ, Kamboj SS, Saxena AK, Sharma M, Bhagat M, Sood SK, Singh J (2006). A tuber lectin from *Arisaema jacquemontii* Blume with anti-insect and antiproliferative properties. *J Biochem Mol Biol* **39**(**4**): 432-440.

Kolarsick P, Kolarsick MA, Goodwin C (2006). Anatomy and physiology of the skin. *J Dent Med Sci* **3**(**1**): 203-213.

Kumar V, Khan AA, Naharajan K (2013). Animal models for the evaluation of wound healing activity. *Int Bull Drug Res* **3**(**5**): 93-107.

Legesse LT, Gemeda HD, Abdella SH (2017). Incidence and predictors of surgical site infection in Ethiopia: prospective cohort. *BMC Infect Dis* **17**(**1**): 119.

Leong M, Murphy KD, Phillips LG (2016). Wound healing. In: *Current Diagnosis and Treatment: Surgery*, Doherty DM (ed), Chapter 6, McGraw Hill LLC, New York, USA, pp 130-162.

Mayo SJ, Gilbert MG (1986). A preliminary revision of *Arisaema* (Araceae) in tropical Africa and Arabia. *Kew Bulletin* **41**(**2**): 261-278.

Mekonnen A, Sidamo T, Asres K, Engidawork E (2013). *In vivo* wound healing activity and phytochemical screening of the crude extract and various fractions of *Kalanchoe petitiana* A. Rich (Crassulaceae) leaves in mice. *J Ethnopharmacol* **145**(**2**): 638–646.

Mir PA, Dar MA, Roohi M, Mohi-Ud-din R, Bader GN (2019). Evaluation of antioxidant and anti-inflammatory activity of methanolic and aqueous extract of *Arisaema propinquum* Schott rhizomes. *J Biomed Pharm Sci* **2**: 1.

Mulisa E, Asres K, Engidawork E (2015). Evaluation of wound healing and anti-inflammatory activity of the rhizomes of *Rumex abyssinicus* (Polygonaceae) in mice. *BMC Complement Altern Med* **15**: 341.

Murthy KN, Reddy VK, Veigas JM, Murthy UD (2004). Study on wound healing activity of *Punica granatum* peel. *J Med Food* **7**(**2**): 256-259.

Nagar HK, Kumar SA, Rajnish S, Lal M, Singh H, Singh M (2016). Pharmacological investigation of the wound healing activity of *Cestrum nocturnum* (L.) ointment in Wistar albino rats. *J Pharm* (Cairo): 2016: 9249040.

National Research Council (2011). Guide for the care and use of laboratory animals (8th ed.) Washington, DC: the National Academies Press. Retrived from https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals_prepub.pdf

Ninjatacoshell (2024). The distribution of the plant genus *Arisaema*. *BlankMap-World6.svg* https://commons.wikimedia.org/wiki/File:Arisaema_distribution.svg

Nussbaum SR, Carter MJ, Fife CE, DaVanzo J, Haught R, Nusgart M, Cartwright D (2018). An economic evaluation of the impact, cost and Medicare policy implications of chronic nonhealing wounds. *Value Health* **21**(**1**): 27-32.

Organization for Economic Cooperation and Development (OECD) (2017). OECD Test No. 402: Acute Dermal Toxicity, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

Organization for Economic Cooperation and Development (OECD) (2022). OECD Test No. 425: Acute Oral Toxicity- Up and Down Procedure, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, Patel SB, Khalid L, Isseroff RR, Tomic-Canic M (2014). Epithelialization in wound healing: a comprehensive review. *Adv Wound Care* **3**(**7**): 445-464.

Reddy BS, Reddy RKK, Naidu VGM, Madhusudhana K, Agwane SB, Ramakrishna S, Diwan PV (2008). Evaluation of antimicrobial, antioxidant and wound healing potentials of *Holoptelea integrifolia*. *J Ethnopharmacol* **115**: 249–256.

Reddy M, Gill SS, Wu W, Kalkar SR, Rochon PA (2012). Does this patient have an infection of a chronic wound? *J Am Med Assoc* **307**(**6**): 605-611.

Rittà M, Marengo A, Civra A, Lembo D, Cagliero C, Kant K, Lal UR, Rubiolo P, Ghosh M, Donalisio M (2020). Antiviral activity of a *Arisaema tortuosum* leaf extract and some of its constituents against herpes simplex virus type 2. *Planta Med* **86**(**4**): 267–275.

Roshan R, Ahmed S, Hasan MM (2017). *Arisaema jacquemontii* Blume (Araceae): a review of medicinal uses, phytochemistry and pharmacology. *J Pharmacogn Phytochem* **6**(**6**): 429–432.

Rozema E, Popescu R, Sonderegger H, Huck CW, Winkler J, Krupitza G, Urban E, Kopp B (2012). Characterization of glucocerebrosides and the active metabolite 4,8-sphingadienine from *Arisaema amurense* and *Pinellia ternata* by NMR and CD spectroscopy and ESI-MS/CID-MS. *J Agric Food Chem* **60**(**2**): 7204−7210.

Sisay M, Worku T, Edessa D (2019). Microbial epidemiology and antimicrobial resistance patterns of wound infection in Ethiopia: a meta-analysis of laboratory-based cross-sectional studies. *BMC Pharmacol Toxicol* **20**(**1**): 35.

Sun N, Yang H, Zhou M, Hou Y, Wu X, Li W, Zhang Y, Sun J, Xiong X, Huang PS (2023). Isolation and evaluation of antioxidants from *Arisaema heterophyllum* tubers. *Phytochem Lett*

53(**3**): 106–110.

Su Y, Xu J, Bi JL, Wang YH, Hu GW, Yang J, Yin G, Long C (2013). Chemical constituents of *Arisaema franchetianum* tubers. *J Asian Nat Prod Res* **15**(**1**): 71-77.

Süntar IP, Akkol EK, Yilmazer D, Baykal T, Kirmizibekmez H, Alper M, Yes-ilada E (2010). Investigations on the *in vivo* wound healing potential of *Hypericum perforatum* L. *J Ethnopharmacol* **127**: 468–477.

Suruse P, Kale M, Gunde M, Amnerkar N (2011). Evaluation of wound healing activity of *Arisaema leschenaultii* Blume in rats. *Scholars Res Libr* **3**(**4**): 200–206.

Tabassum S, Zia M, Esperanza J, Blanco EJ, Batool R, Aslam R, Hussain S, Wali Q, Gulzar MM (2019). Phytochemical, *in-vitro* biological and chemo-preventive profiling of *Arisaema jacquemontii* Blume tuber extracts. *BMC Complement Altern Med* **19**(**1**): 256.

Tanveer M, Rehman H-U, Sims J, Choudhary MI, Hamann MT (2013). First ever isolation of cytotoxic triterpenoid 2-hydroxydiplopterol from plant source. *J Med Plants Res* **7**(**27**): 2040–2042.

Thakur R, Jain N, Pathak R, Sandhu SS (2011). Practices in wound healing studies of plant: article review. *Evid Based Complement Altern Med* **2011**: 438056.

Tran VT, Hoang TPN,, Croat TB, Ma Z, Liu B, Nguyen VD, Le TC (2022) Reconstruction of the evolutionary biogeography reveals the origin and diversification of *Arisaema* (Araceae). *Acta Bot Bras* **36**: e2021abb0253.

Velnar T, Baliley T, Smrkolj V (2009). The wound healing process: an overview of the cellular and molecular mechanisms. *J Int Med Res* **37**(**5**): 1528–1542.

Verma H, Lal VK, Pant KK, Soni N (2012). A ethnomedicinal review on *Arisaema tortuosum* Hemlata. *Int J Adv Pharma Biol Chem* **1**(**2**): 176-179.

Wang J, Ding X, Guo, C-A, Zhang X, Feng H, Yang H, Wang Y (2023). An ethnobotanical study of wild edible plants used by the Tibetan in the Rongjia River Valley, Tibet, China. *J Ethnobiol Ethnomed* 1**9**: 49.

Wayne, PA (2020). Performance standards for antimicrobial susceptibility testing. 30th ed. Clinical and laboratory standards institute supplement M100. *CLSI* **40** : 1

WHO (2023). Antimicrobial resistance. Available at https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance. Accessed in July 2024.

Wong SY, Manikam R, Muniandy S (2015). Prevalence and antibiotic susceptibility of bacteria from acute and chronic wounds in Malaysian subjects. *J Infect Dev Ctries* **9**(**9**): 936-944.

Yesuf A, Asres K (2013). Wound healing activity guided isolation of active constituents from the leaves of *Allophylus abyssinicus*. *Phytopharmacol* **4**(**2**): 442-453.

Yineger H, Kelbessa E, Bekele T, Lulekal, E (2008). Plants used in traditional management of human ailments at Bale Mountains National Park, Southeastern Ethiopia. *J Med Plants Res* **2**(5): 132-153.

Zhu L, Dai J, Yang L (2013). Anthelmintic activity of *Arisaema franchetianum* and *Arisaema lobatum* essential oils against *Haemonchus contortus*. *J Ethnopharmacol* **148**(**1**): 311–316.

Zielińska M, Pawłowska A, Orzeł A, Sulej L, Muzyka-Placzyńska K, Arkadiusz-Baran A, Filipecka-Tyczka D, Pawłowska P, Nowińska A, Bogusławska J, Scholz A (2023). Wound microbiota and its impact on wound healing. *Int J Mol Sci* **24**(24): 17318.

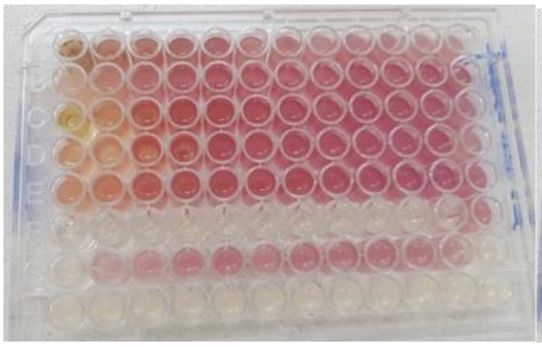
Zouine N, El Ghachtouli N, El Abed E, Koraichi SI (2024). A comprehensive review on medicinal plant extracts as antibacterial agents: Factors, mechanism insights and future prospects. *Sci Afr* **26**: e02395.

Appendices

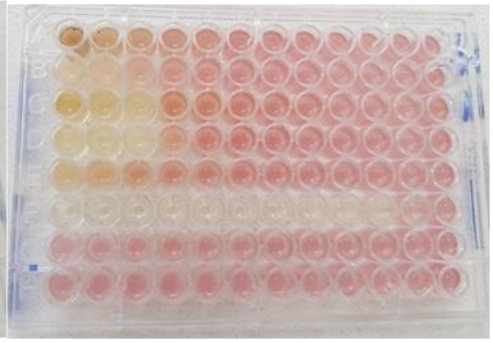
Appendix 1: Score of irritation and oedema after application of ointments of 80% methanol extract of the tuber of *Arisaema schimperianum* with their respective base.

Score of Skin irritation										
No. Rat		Ointment of tuber extract						Base ointment		
		5%			10%					
	Reaction	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
1	Erythema	0	0	0	1	1	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0	0
2	Erythema	1	0	0	0	1	0	1	0	0
	Oedema	0	0	0	0	0	0	0	0	0
3	Erythema	0	1	0	1	0	0	1	0	0
	Oedema	0	0	0	0	0	0	0	0	0
4	Erythema	0	0	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0	0
5	Erythema	0	1	0	1	0	0	1	1	0
	Oedema	0	0	0	0	0	0	0	0	0
6	Erythema	0	1	0	1	1	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0	0
	PII	**4/36=0.11**			**7/36=0.19**			**4/36=0.11**		

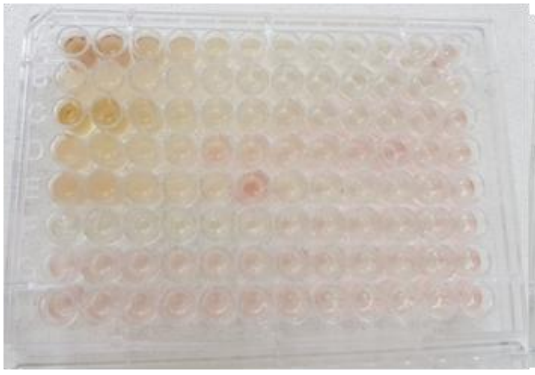
Appendix 2: 96 micro plate for minimum microbial inhibition



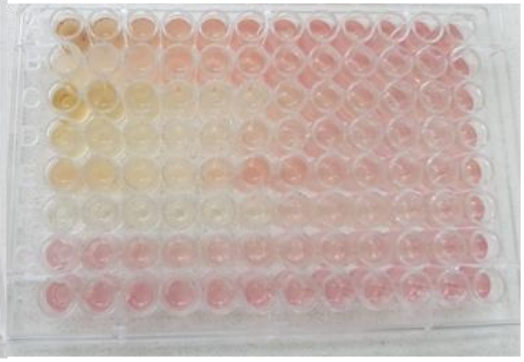
P. mirabilis



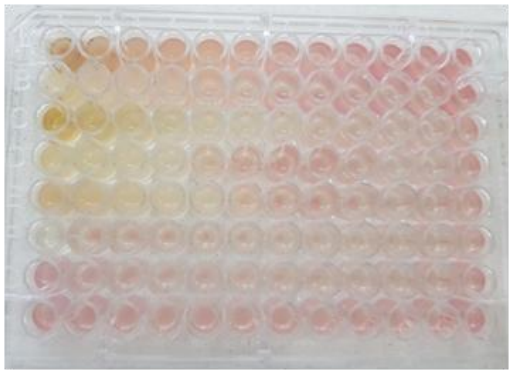
P. aeruginosa



E. coli



S. aureus



MRSA

Appendix 3: Ethical clearance approval letter

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Addis Ababa University

ቀን Date May 27, 2024

ቁ.ጥ.ር Ref. No. ERB/SOP/620/16/2024

To: Betelhem Awoke
School of Pharmacy

Re: Ethical Clearance

It is to be recalled that you submitted a research proposal entitled **"Wound healing and antimicrobial activities of the extract and major compounds from the tuber of *Arisaema schimperianum* Schott"**. The committee thoroughly reviewed the proposal based on its operational guideline and found that, it fulfills all the ethical requirements stipulated in the guideline. This is, therefore, to inform you that the proposal is ethically approved for implementation.

With best regards,

Shemsu Umer (PhD)
Chairperson, ERC
School of Pharmacy
College of Health Sciences

☎ 00251156 02 12 ✉ 1176 ቴሌክስ Telex: 21205 ፋክስ Fax: 00251(11)1558566 ቴ.ግ.ራ.ም Cable: AAUNIV

Appendix 4: Some photographs taken during laboratory work