HARAMAYA UNIVERSITY

POSTGRADUATE PROGRAM DIRECTORATE

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF ALOE PUBESCENS (ASPHODELACEAE) LEAF GEL IN MICE MODELS

MSC Thesis

BY: SAMUEL SILESHI (MSc candidate)

JANUARY, 2026

HARAR, ETHIOPIA

HARAMAYA UNIVERSITY

POST GRADUATE PROGRAM DIRECTORATE

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF ALOE PUBESCENS (ASPHODELACEAE) LEAF GEL IN MICE MODELS

MSC THESIS

BY: SAMUEL SILESHI (MSc candidate)

College: Health and Medical Sciences

School: Pharmacy

Program: Pharmacology

Major advisor: Abraham Nigussie (PhD, Assistant Professor of pharmacology)

Co-advisor: Abraham Degaga (PhD, Assistant Professor of pharmacology)

A thesis submitted to the School of Pharmacy, College of Health and Medical Sciences, Haramaya University in partial fulfilment of the requirement for the Master of Science degree in Pharmacology.

JANUARY, 2026

HARAR, ETHIOPIA

APPROVAL SHEET

HARAMAYA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

I hereby certify that I have read and evaluate this thesis entitled "Anti-inflammatory and analgesic activities of *Aloe pubescens* (Asphodelaceae) leaf gel in mice models" prepared under my guidance by Samuel Sileshi. I recommended that it be submitted as fulfilling the thesis requirements.

Dr. Abraham Nigussie	_____	_____
Name of Major Advisor	Signature	Date
Dr. Abraham Degaga	_____	_____
Name of Co-Advisor	Signature	Date

As a member of the Board of Examiners for the MSc thesis-open defense examination, I certify that I have read and evaluated the thesis prepared by Samuel Sileshi and have examined the candidate. I recommend that the thesis be accepted as fulfilling the thesis requirements for the degree of master in Pharmacology.

_____	_____	_____
Chair Person	Signature	Date
_____	_____	_____
Internal Examiner	Signature	Date
_____	_____	_____
External Examiner	Signature	Date

Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the thesis to the School of Graduate Studies (SGS) through the Department/School Graduate Committee (DGC/SGC) of the candidate's department

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this thesis is my own work. I have followed all ethical principles of scholarship in the preparation, data collection, data analysis and completion of this thesis. All scholarly matter that is included in the thesis has been given recognition through citation.

This thesis is submitted in partial fulfillment of the requirement for a MSc degree at Haramaya University. The thesis is deposited in the Haramaya University Library and is made available to borrowers under the rules of the library. I solemnly declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

Brief quotations from this thesis may be used without special permission provided that accurate and complete acknowledgement of the source is made. Requests for permission for extended quotations from or reproduction of this thesis in whole or in part may be granted by the Head of the School when, in his or her judgment, the proposed use of the material is in the interest of scholarship. In all other instances, however, permission must be obtained from the author of the thesis.

Name: Samuel Sileshi

Signature: _____

Date: _____

School: Pharmacy

Department: Pharmacology

BIOGRAPHICAL SKETCH

I am Samuel Sileshi Fufa. I was born in Metekel, Benishangul gumiz region, Ethiopia in 1997 according to GC. I completed primary school at Senkora primary school in 2011. I have attended my secondary school (9-10) in Senkora secondary and Preparatory school and 11-12 in Wembera Secondary and preparatory school. After completion of secondary school, I joined the Mizan-Tepi University in 2015 and got Bachelor of Science degree in Pharmacy on February 05, 2021. After graduation, I got a chance to join Bisidimo Hospital Health Sciences Collage as an assistant lecturer from October 19, 2021 to date. On October 29, 2022 I started my post graduated study in Pharmacology.

ACKNOWLEDGEMENTS

First and foremost, I sincerely thank Almighty God for His unwavering grace, love, mercy, and provision in my life. I am deeply grateful to my mentor, Dr. Abraham Nigussie, for his insightful guidance, encouragement, and constructive feedback on this research. My heartfelt appreciation also goes to my co-advisor, Dr. Abraham Degaga, for his invaluable support, patience, and understanding. Additionally, I extend my gratitude to Haramaya University, College of Health and Medical Sciences, and the department of Pharmacology at the School of Pharmacy for providing me with the opportunity to develop this research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LISTS OF ABBREVIATIONS/ ACRONYMS

ANOVA……..Analysis Of Variance

ARRIVE…….Animal Research Reporting of *In vivo* Experiments

COX…….Cyclooxygenase

DW……...Distilled Water

GACP……...Good Agricultural and Collection Practices

IBS……Irritable Bowel Syndrome

I.P……..Intraperitoneal

NSAIDs…….Non-Steroidal Anti-Inflammatory Drugs

PGE_2…….. Prostaglandin E_2

PGF_2……. Prostaglandin F_2

P.O…….....Per Oral

SPSS……..Statistical Package for the Social Sciences

TMs……...Traditional Medicines

UV…….Ultraviolet

WHO…….World Health Organization

ABSTRACT

Pain and inflammation are associated with number of diseases or conditions as symptoms, and considered as a major clinical, social, and economic problem around the world. In Ethiopia, *Aloe pubescens* has been traditionally used to alleviate wounds, pain, and inflammation, but its medicinal benefits have not been scientifically validated. The present study aimed at the evaluation of the anti-inflammatory and analgesic activities of *Aloe pubescens* leaf gel in mice models.

Objective: To evaluate the anti-inflammatory and analgesic activities of *Aloe pubescens* leaf gel in mice.

Methods: Experimental study design was conducted. The study was conducted in Haramaya University, College of Health and Medical Sciences, School of Pharmacy, Harar, Eastern Ethiopia. The leaf gel powder of the *Aloe pubescens* was prepared, by using vacuum oven at 40 ˚c. The mice randomly divided into five groups (negative control, positive control and three test groups). Anti-inflammatory effect of *Aloe pubescens* leaf gel was evaluated using carrageenan-induced acute paw edema and formalin-induced sub-acute paw edema models. While, analgesic activity of *Aloe pubescens* leaf gel was tested via the acetic acid-induced writhing test for peripheral pain and the hot plate test for central pain. Additionally, phytochemical screening was conducted to identify the active compounds. The data were analyzed using SPSS version 25. A one-way ANOVA test was used to determine significance and results were expressed as mean ± SEM, with significance determined at $p < 0.05$.

Results: The leaf gel of *Aloe pubescens* produced significant analgesic and anti-inflammatory effects in experimental mice models. In the acetic acid–induced writhing test, the gel significantly reduced the number of writhes in a dose-dependent manner, indicating peripheral analgesic activity. In the hot plate test, the extract significantly prolonged reaction latency, demonstrating centrally mediated analgesic effects. Furthermore, the gel markedly suppressed carrageenan-induced acute paw edema and formalin-induced sub-acute paw edema across all tested doses (100, 200, and 400 mg/kg), with maximal inhibition observed at higher doses and later time points. Overall, the extract exhibited significant and dose-dependent analgesic and anti-inflammatory activities compared with the negative control.

Conclusion: The findings of this study demonstrate that *Aloe pubescens* leaf gel possesses significant analgesic and anti-inflammatory activities in experimental mouse models. The observed effects were dose-dependent and evident in both peripheral and central pain models, as well as in acute and sub-acute inflammation models. These results scientifically support the traditional use of the plant for the management of pain and inflammatory conditions and suggest that *Aloe pubescens* may serve as a potential source of bioactive compounds for the development of novel analgesic and anti-inflammatory agents.

Keywords: *Acetic Acid-Induced Writhing Test, Aloe pubescens, analgesic activity, Anti-inflammatory activity, Carrageenan-Induced Paw Edema, Formalin-Induced paw Edema, Hot plate*

1. INTRODUCTION

1.1. Background

Pain and inflammation are associated with number of diseases or conditions as symptoms, and considered as a major clinical, social, and economic problem around the world (Theanphong and Somwong, 2022). Globally, 30% of adults suffer from pain and inflammatory diseases and 20% get diagnosed with chronic ailments each year, increasing the incidence and prevalence of pain and associated disorders day by day (Javed *et al.*, 2020). Chronic pain was found to affect 18% of the general population in developing countries (Ashagrie, 2023a). Although no research has specifically investigated the prevalence of pain in Ethiopia, an urban population-based study revealed that 21.6% of individuals experienced primary headache disorders, while 10% were affected by migraines (Solanaceae, 2019).

Anti-inflammatory drugs help reduce swelling and pain, whereas analgesics are specifically designed to alleviate pain. A standard analgesic regimen is primarily composed of aspirin, codeine, and morphine. Common anti-inflammatory drugs include aspirin, ibuprofen, naproxen, and indomethacin (Kumar *et al.*, 2023). The most commonly available medicine in modern practice are cyclooxygenase (COX) inhibitors i.e. Non-steroidal anti-inflammatory drugs (NSAIDs) and opioids (Tatiya *et al.*, 2017). NSAIDs are among the most commonly prescribed drugs due to their consistent effectiveness in the treatment of pain, fever, inflammation and rheumatic disorders (Bhowmick *et al.*, 2014).

Despite the advance in modern pharmacotherapy, in many parts of the world, medicinal plants have an important role as traditional therapies for a wide range of human ailments, including inflammation and pain (Ashagrie, 2023b). One of the popular plants used for treatment is Aloe spp. (Kazeem *et al.*, 2022). Aloe is the largest genus in the Asphodelaceae family and comprised of more than 400 species, ranging from diminutive shrubs to large trees distributed across Africa, with the major diversity in South Africa. Aloe is represented in East Africa by 83 species, of which 38 grow naturally in Ethiopia, including 15 endemic species (Abdissa, Gohlke, *et al.*, 2017). In Ethiopia, the local names of Aloe vary by region and language. The general name for Aloe at the genus level is: "Ere" in Tigrigna (spoken in Tigray), "Eret" in Amharic (spoken in Amhara) (Fentaw *et al.*, 2022) and "Argiisa" in Oromic (spoken in Oromia) (Abera, 2014; Feyisa *et al.*,

2021). However, "Haamaaresaa" (in Oromia) specifically refers to *Aloe pubescens* (Wondimu *et al.*, 2007).

In Ethiopia, Aloes are primarily used in soap production, jute sacks production, anti-microbial activities in cotton fabric, as thickening agent, degraded land rehabilitation and source of food for animals (Oda and Erena, 2017). *A. pubescens* is used in traditional medicine of Ethiopia to treat wounds, stomach aches, muscle cramps, and anthrax (Wondimu *et al.*, 2007). However, the therapeutic potentials of the plant has not been scientifically evaluated and validated so far (Yimer et al., 2020). Therefore, the aim of the present study was to assess the anti-inflammatory and analgesic activities of *A. pubescens* (Asphodelaceae) leaf gel in mice models.

1.2. Statement of the problem

Pain and inflammation remain among the most severe and widespread health challenges, affecting 80% of the global adult population (WHO, 2012). They are recognized as significant clinical, social, and economic burdens in communities worldwide. Chronic, untreated pain not only causes physical harm but also contributes to psychological disorders, while persistent inflammation impairs daily functioning by leading to missed work, school, and social activities. Furthermore, unresolved inflammation accelerates the progression of serious conditions such as asthma, autoimmune diseases, chronic inflammation, glomerulonephritis, inflammatory bowel disease, pelvic inflammatory disease, reperfusion injury, hypersensitivities, hay fever, atherosclerosis, and rheumatoid arthritis. These debilitating conditions are a leading cause of disability and, if left unmanaged, can result in severe health consequences, including death (Yimer et al., 2020).

NSAIDs, opioids, and glucocorticoids are frequently prescribed to manage inflammation and related conditions. However, long-term use of these drugs can lead to serious side effects, such as gastric irritation, ulcers, liver toxicity, and kidney damage (Gou *et al.*, 2017). Moreover, in Ethiopia, a large portion of the population lives in rural areas with limited healthcare access. The country's healthcare system is also grappling with challenges, including shortages of medical personnel, resources, and essential medications (Tafese Awulachew, 2021).

In developing countries, including Ethiopia, herbal remedies are widely used to treat pain and inflammation due to their affordability, availability, and environmental sustainability (Alemu *et al.*, 2018). Plant-based medicines are increasingly being sought as alternatives to conventional treatments. However, they can also pose risks of harmful effects (Bribi *et al.*, 2015). There are often no standardized guidelines for the dosage form, concentration, frequency of administration, dose, intensity, or duration of medicinal plant use (Nigussie *et al.*, 2021). In Ethiopia, many plant species are traditionally used to treat conditions like headaches, stomachaches, and wounds. However, only a few of these plants have been scientifically studied, while the majority remain unexplored (Alemu *et al.*, 2018).

A. *pubescens* is used in Ethiopian traditional medicine for gastric problems, wound healing, and anthrax treatment. Environmentally, it aids in soil conservation, serves as a boundary marker, and is planted in graveyards for cultural significance. Its gel is also consumed as food (Belayneh *et al.*, 2020). In Ethiopia, *A. pubescens* is traditionally used both orally and topically. For oral use, its leaf bud and flower are crushed, mixed with water, and consumed to treat stomach aches in humans and anthrax in livestock. For topical application, the crushed plant is directly applied to wounds and skin ailments to aid healing (Wondimu *et al.*, 2007). However, there is no scientific data on its anti-inflammatory and analgesic activities. Therefore, the present study was aimed to evaluate the anti-inflammatory and analgesic activities of *A. pubescens* (Asphodelaceae) leaf gel in mice models.

1.3. Significance of the study

- The findings of this study may serve as a source of information, open doors for future researchers to refine, expand further studies, that could potentially contribute to the development of innovative anti-inflammatory and analgesic treatments.
- The study could aid in the identification of novel bioactive compounds for pharmaceutical development.
- Scientific validation of its anti-inflammatory and analgesic activities would support its traditional uses and contribute to herbal medicine research

1.4. Objectives of the study

1.4.1. General objective

To evaluate the anti-inflammatory and analgesic activities of *A. pubescens* (Asphodelaceae) leaf gel in mice models.

1.4.2. Specific objectives

- To assess the acute oral toxicity of *A. pubescens* leaf gel in mice
- To evaluate the anti-inflammatory activity of *A. pubescens* leaf gel by using carrageenan induced paw edema test in mice
- To evaluate the anti-inflammatory activity of *A. pubescens* leaf gel by using Formalin-induced paw edema test in mice
- To investigate the analgesic effect of *A. pubescens* leaf gel by using acetic acid induced writhing test in mice
- To investigate the analgesic effect of *A. pubescens* leaf gel by using hot plate test in mice
- To conduct phytochemical screening tests of *A. pubescens* leaf gel

2. LITERATURE REVIEW

2.1. Medicinal plants in the management of inflammation and pain

According to the World Health Organization (WHO), 80% of the global population relies on traditional medicine, and there is a consistent demand for and widespread use of traditional and complementary medicine worldwide (Ayanaw and Yesuf, 2023; Jami *et al.*, 2014). The WHO also promotes traditional medicine as a source of less expensive, comprehensive medical care, especially in developing countries (Nagesh *et al.*, 2015). Furthermore, WHO estimated that majority of the population in sub-Saharan Africa depend solely on traditional medicinal plants for their primary healthcare needs because of their accessibility, cheapness and socio-cultural background (Abdissa, Geleta, *et al.*, 2017).

Africa has an immensely rich biodiversity and knowledge in the use of plants to treat various ailments (Abdissa, Geleta, *et al.*, 2017). For centuries, Africans have treated different disease conditions including inflammatory diseases using medicinal plants (Oguntibeju, 2018). In southern Africa, 555 medicinal plants identified from 118 families which were traditionally used to treat inflammation and pain. Based on the number of species represented, Fabaceae was the most prominent family with the highest number of species, followed by Asteraceae, Apocynaceae, Euphorbiaceae, Lamiaceae, and Asphodelaceae. The most frequently recorded genera for the treatment of inflammation and pain include Solanum, Aloe, Helichrysum , Ficus and Vachellia (Khumalo *et al.*, 2021).

Traditional Ethiopian healers employ a variety of plants for the treatment of inflammation and pain (Ayanaw and Yesuf, 2023). In Ethiopia, over 70% of the people depend on traditional medicines (TMs) for their healthcare, and more than 95% of the preparations are made from plant origin (Tuasha *et al.*, 2018). Many plants are used as analgesic and anti-inflammatory agents in traditional medicine practice of Ethiopia. Some of these plants include: *Allium sativum* (Amaryllidaceae), *Zingiber officinale* (Zingiberaceae), *Nigella sativum* (Ranunculaceae), *Albuca abyssinica* (Asparagaceae), *Ruta chalepensis* (Rutaceae), and *Moringa stenopetala* (Moringaceae) (Tamrat *et al.*, 2017), *Aloe megalacantha Baker* (Asphodelaceae) (Asmerom *et al.*, 2020; Belayneh *et al.*, 2020; Gebremeskel *et al.*, 2018), *Aloe trigonantha* (Asphodelaceae) (Leach *et al.*, 2021), *Acacia seyal* (Fabaceae) (Kedir and Ayele, 2024), *Acacia mellifera* (Fabaceae) (August *et al.*, 2017), *Echinops kebericho M.* (Asteraceae) (Yimer et al., 2020), *Uapaca togoensis*

(Euphorbiaceae) (Olorukooba *et al.*, 2020), *Ocimum suave* (Lamiaceae) (Masresha *et al.*, 2012), *Tectona grandis* Linn. (Lamiaceae) (Giri and Varma, 2015), *Aloe vera* (Asphodelaceae) (E and Galam, 2011), *Aloe ferox* Mill. (Asphodelaceae) (Mwale and Masika, 2010), *Impatiens rothii* (Balsaminaceae) (Ashagrie, 2023a), etc.

2.2. Plant extracts and phytochemicals with anti-inflammation and analgesic activities

Plant extracts can be an important source of natural and safer drugs for the treatment of painful and inflammatory conditions (Ishola *et al.*, 2014). For instance, the aqueous extracts of the whole leaf of *A. vera* at various concentrations (100, 200 and 400 mg/kg) significantly reduced formation of edema induced by carrageenan and formaldehyde in a dose dependent manner. Further, in the analgesic study of extract showed significantly ($P<0.5$) reduced the number of writhes induced by a 0.6% Acetic acid solution. The study revealed no mortality was observed during the acute toxicity test at the limited dose of 2000 mg/kg, indicating a good safety profile (Devaraj and Karpagam, 2011). Preliminary phytochemical screening test of the plant revealed that it contained tannins, flavonoids, anthraquinones, saponins, alkaloids and cardiac glycosides (Osagie and Omoregie, 2013).

On the other hand, the anti-inflammatory activity of *Aloe trigonantha* leaf gel was evaluated, the extract demonstrated a dose-dependent inhibition of inflammation at 100 mg/kg, 200 mg/kg, and 400 mg/kg doses. The preliminary phytochemical screening of the leaf gel of *A. trigonantha* showed the presence of flavonoid, glycoside, phenols, and tannins. Acute oral toxicity study results showed the plant leaf gel was safe at 2000 mg/kg dose(Leach *et al.*, 2021).

Furthermore, *Aloe ferox* leaf aqueous extract showed dose-dependent anti-inflammatory and analgesic effects, at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg (Mwale and Masika, 2010). An acute toxicity study on this plant extract revealed that it is safe, as no signs of overt toxicity were observed at the limit test dose of 2000 mg/kg in rat(Marizvikuru Mwale, 2012). *Aloe ferox* leaf contains key phytochemicals, including phenols, flavonoids, flavonols, proanthocyanidins, tannins, alkaloids, and saponins, which contribute to its medicinal properties (Wintola and Afolayan, 2011).

2.3. *In-vivo* preclinical models for anti-inflammation and analgesic studies

In vivo preclinical models are essential for evaluating the anti-inflammatory and analgesic properties of new therapeutic agents. These models help assess the efficacy, safety, and mechanisms of action before clinical trials in humans. Here are commonly used *in vivo* preclinical models for inflammation and analgesia: carrageenan-induced paw edema model, formalin test, acetic acid-induced writhing test and hot plate test (Sherif *et al.*, 2024).

2.3.1. Carrageenan-induced paw edema model

Carrageenan is a sulfated polysaccharide obtained from red seaweed (Rhodophyceae) and can trigger inflammatory activation in both humans and laboratory animals. Subcutaneous injection of carrageenan causes local inflammation characterized by inflammatory signs including swelling, heat, pain, redness, and loss of function (Widyarini *et al.*, 2023). Furthermore, Carrageenan injection induces inflammation through a biphasic phase. In the vascular phase, proinflammatory cytokines such as TNF-α, IL-6, and IL-β are involved from the first hour after injection, while prostaglandins play an important role in the cellular phase(second phase), which occurs in the 3-5th hour post injection. The mediators mentioned above are responsible for erythema, edema, and pain at the injection site (Reanmongkol *et al.*, 2009). Carrageenan-induced paw edema model is frequently used for studying acute inflammation and testing the efficacy of anti-inflammatory drugs due to its highly reproducible method with a well-characterized biphasic inflammatory response that makes it exceptionally sensitive for screening anti-inflammatory drugs compared to other models. However, as the limitations of this model, the investigator should be trained to record the stable and reproducible paw volumes using sophisticated equipment like plethysmometer, rise in paw edema is based on the concentration of injected carrageenan, and typically the maximum edema response produced by carrageenan is too difficult to inhibit. Therefore, the carrageenan type and preparation of its solution needs careful attention (Patil *et al.*, 2019).

2.3.2. Formalin test

The formalin test is an experimental method used to study both pain and inflammation in animal model. The injection of formalin produces two distinct phases: the early phase (0-10 minutes) involves direct nociceptor activation causing immediate pain, while the late phase (15-60 minutes) is driven by inflammation, central sensitization, and edema formation due to the release of inflammatory mediators (Lee and Jeong, 2002). The formalin test simultaneously captures

immediate neurogenic pain and delayed inflammatory pain, enabling the evaluation of both analgesic and anti-inflammatory effects in a single model (Jang and Seong, 2014). Additionally, Formalin is a well-recognized and commonly used irritant to induce acute and sub-acute inflammation (Abebe, 2020).

2.3.3. Acetic acid-induced writhing test

The acetic acid induced writhing method is an effective method to evaluate peripherally active analgesics. The abdominal constriction response induced by acetic acid is a sensitive method to test peripherally acting analgesics. Hyperalgesia, induced by the injection of acetic acid, is characterized by contraction of the abdominal muscle accompanied by body elongation and an extension of forelimbs (Kouakou, 2016). Overall, its sensitivity and ability to detect antinociceptive effects of natural products and test compounds at dose levels which remains inactive for other methods, makes acetic acid-induced writhing test a well recommended model for screening the peripheral analgesic potentials of test compounds (Yimer et al., 2020).

2.3.4. Hot plate test

The Hot Plate Test is a straightforward, widely used to evaluate the central antinociceptive (pain-relieving) properties of drugs in rodents, typically mice or rats. In this test, an animal is placed on a heated surface usually maintained at a constant temperature between 50°C and 55°C and researchers measure the time it takes for the animal to display pain-related behaviors, such as licking its paws, jumping, or shaking (Gunn *et al.*, 2012). It has the advantage of being selective and sensitive only to analgesics of central action, but not peripheral; it is a selective test for opioid compounds (Silva-Correa *et al.*, 2021).

Morphine acting as a reference for central analgesia, demonstrating the strongest pain relief in the hot plate test while Aspirin was used as a peripheral analgesic standard, effectively reducing pain in the writhing test through prostaglandin inhibition (Ashagrie, 2023a). Indomethacin acting as a reference drug, in carrageenan and formalin models, it showed significant anti-inflammatory effects, reducing swelling likely by inhibiting cyclooxygenase (COX) enzymes and reducing prostaglandin-mediated inflammation (Sharma *et al.*, 2020).

2.4. Overview of the experimental plant

The word Aloe originated from the Arabic word 'Alloeh', which refers to a shining bitter substance (Boudreau and Beland, 2006; Guo and Mei, 2016). The bitterness results from the presence of aloin and Aloe-emodin. Additionally, Aloe is referred to as the 'Miracle Plant' and 'Healing Plant' (Al-wajih *et al.*, 2022). Asphodelaceae is a family of lily-related monocotyledonic flowering plants with 2 subfamilies, 16 genera and about 780 species distributed in arid and mesic regions of the temperate, subtropical and tropical zones of the world, with the main center of diversity in southern Africa. The Aloe genus, belonging to Asphodelaceae family and it comprises of more than 400 species that are widely distributed in Africa, India, and other arid areas, with the major diversity in South Africa (Diriba and Deresa, 2022). Aloes are traditionally used to treat several ailments such as skin infection, as laxatives, in wound healing, managing pregnancy, pain, and inflammation. Additionally, Aloes are also used in soap production, jute sacks production, antimicrobial activities in cotton fabric, as thickening agent, degraded land rehabilitation and source of food for animals (Oda and Erena, 2017).

Aloe plants can vary in size and appearance depending on the species, but most have a rosette shape with thick, fleshy leaves. The leaves can range in color from green to gray-green and are often covered in small white spots (Banjaw, 2024). Genus Aloe is native to the Arabian Peninsula and cultivated in several dry regions of the world, including the Americas, Asia, Europe and Africa (Kazeem *et al.*, 2022). Aloe plants have been widely known and used for centuries as topical and oral therapeutic agent due to their health, beauty, medicinal, and skin care properties (Guo and Mei, 2016). Several studies reported aloes' pharmacological potentials, including antioxidant, anti-inflammatory, antimicrobial, antimalarial, anticancer, and antidiabetic properties (Kazeem *et al.*, 2022).

Aloes are interesting sources of various classes of secondary metabolites. Regarding the different composition of these leaf portions, they are also likely to have distinct classes of bioactive compounds, which is believed to contribute to the different biological properties of leaves. Briefly, the outer green epidermis has been reported to contain anthraquinones, pre-anthraquinones, and their corresponding glycosides, while the outer pulp region below the epidermis contains latex that predominantly consists of phenolic compounds, including anthraquinones and pre-anthraquinones, anthrones, chromones, coumarins, flavonoids, and pyrones. The inner leaf pulp contains a high

acemannan polysaccharide content, as well as a wide variety of phytochemicals, among them alkaloids, anthraquinones, anthrones, chromones, coumarins, flavonoids, and pyrones. Pulp also contains vitamins, minerals, enzymes, and proteins (Salehi *et al.*, 2018).

Aloes has been used for the treatment of wounds and skin complaints, malaria, microbial infections, and complaints of the digestive system. Commercial preparations containing Aloe species include laxative drugs, health drinks and tonics, after shaving gel, mouthwash and toothpaste, hair tonic and shampoo, and skin moistening gel (Belayneh *et al.*, 2020). *Aloe* spp. has significant medicinal benefits, including digestive support as a laxative and treatment for ulcers, IBS, colitis, and hemorrhoids. It detoxifies the liver, supports kidney health, and promotes skin and wound healing with antimicrobial and anti-inflammatory effects. It aids respiratory health, alleviates asthma, and fights infections. With immune boosting and antiviral properties, it combats herpes and inflammation. Neurologically, it enhances memory, mood, and neuroprotection in conditions like Alzheimer's and Parkinson's. It treats genital ulcers, supports eye health by promoting corneal healing, and reduces inflammation. Furthermore, it relieves joint pain, gout, and fractures while promoting hair growth and UV protection (Akaberi *et al.*, 2016).

The Aloe species have been used for centuries in traditional Ethiopian medicine, and are known for their healing properties and nutritional value. Both humans and livestock benefited from the Aloe species through wild harvesting. Aloe species have high economic, social, health, and environmental values (Banjaw, 2024).

A. pubescens is found in the central highlands of Ethiopia (Shewa and Harerge regions). Occurs at altitudes between 1,800 and 2,550 m (Weber *et al.*, 2013). *A. pubescens* is widely used in Ethiopian ethnoveterinary medicine, particularly for treating yoke sores (wounds) in cattle. Its sap, extracted by squeezing the leaves, is directly applied to the affected area, promoting wound healing and reducing inflammation. This plant is valued for its antiseptic and soothing properties, making it a crucial remedy in traditional livestock care (Feyisa *et al.*, 2021).

Therefore, the aim of this study was to evaluate the anti-inflammatory and analgesic activities of the leaf of *A. pubescens* gel in mice models.

Figure 1: Picture of *A. pubescens*

3. MATERIALS AND METHODS

3.1. Materials and instruments

In this study, vacuum oven (Memmert, Germany), refrigerator, analytical balance (VEIP, Italy), hot plate (Stuart, UK), digital plethysmometer (Orchid Scientific, India), electrical blender, volumetric flask, measuring cylinder, funnels, glass rod, vacuum pump (Torontech, Canada), beakers, oral gavage, syringes with needles, spoon spatula, test tube, dropper, pipettes, animal cages, aluminum foil, gauze, surgical gloves, face mask, glass or porcelain type mortar and pestle, marker, plasticizer, and notepads were used.

3.2. Drugs and chemicals

Indomethacin (Cadila pharmaceuticals, Ethiopia), aspirin (Scott-Edil Pharmacia Ltd, India), morphine (Amino Ltd, Switzerland), distilled water (HU Pharmaceutical Lab, Ethiopia), normal saline (Sansheng Pharmaceutical P.L.C, Ethiopia), carrageenan (Sigma-Aldrich, Germany), formalin (Laba Chemicals, India), glacial acetic acid (Fluka PLC, Switzerland), Wagner's reagent (iodine solution in potassium iodide), ferric chloride (Neolan, India), acetic anhydride, sulphuric acid (Sigma Aldrich, USA), hydrochloric acid, diethyl ether (Loba Chemicals, India), ammonia (Wassie Pharma PLC, Ethiopia) and sodium hydroxide (FINCHEM, India) were used in the experiment.

3.3. Collection, identification and preparation of plant material

Fresh leaves of *A. pubescens* were collected from Karamile, East Hararghe, Oromia, Ethiopia. The plant was identified and authenticated by a botanist at College of Natural and Computational Sciences, Haramaya University and a sample specimen was deposited at the Herbarium of Haramaya University with a voucher number (AHU06) for future reference. The harvested plant materials were packed in plastic-coated sacs and transported to the laboratory at the School of Pharmacy, College of Health and Medical Sciences, Haramaya University. The fresh leaves were washed gently by rinsing with distilled water without squeezing to remove debris and dust particles then spikes and margins were avoided before slicing the leaf, and the cortex was carefully separated from the parenchyma using a knife. Fresh *A. pubescens* leaves gel were cut into smaller pieces and blended with electrical blender to homogenize. The homogenate was kept in a deep freezer to minimize enzymatic degradation, air oxidation, and loss of active ingredients. Then it was dried in vacuum oven at 40 ˚c. Once dried, the plant sample was milled into fine powder using

a mortar and pestle. Then the powdered product was stored in a clean plastic container in refrigerator at -4˚c until experimentation (Leach *et al.*, 2021).

3.4. Experimental animals

Healthy adult Swiss Albino mice of either sex (20–30g, and 6–8 weeks of age) were obtained from the animal house of School of pharmacy, Haramaya University. All mice were kept in cages at room temperature on a 12 h light/dark cycle with access to standard laboratory pellet food and water *ad libitum*. The mice were allowed to acclimatize to the laboratory condition for a week before beginning of the experiment to minimize stress. All experiments were carried out during the light phase. All mice used in this study were handled in accordance with the internationally accepted standard guidelines for use of laboratory animals (Worlein, 2011).

3.5. Acute oral toxicity study

Acute oral toxicity study for the leaf gel of *A. pubescens* was performed according to the internationally accepted protocol of Organization for Economic Cooperation and Development (OECD) Guideline 425 (OECD, 2008). Five nulliparous and non-pregnant female albino mice of 6-8 weeks were used. All mice were undergo fasting for 4 hours before and 2 hours after the extract administration with free access to water only. The mice were weighed immediately before administering the extract. A single female mouse was received 2000 mg/kg of the extract as a single dose in the initial screening test to determine the starting dose. Since no death was noticed within 24 hours, the remaining four mice received the same dose of extract. The animals were watched for the first four hours at intervals of 30 minutes, and then for the following 14 days at intervals of 24 hours to assess general toxic signs and symptoms like changes in skin and fur, somatomotor activities and behavioral patterns, eyes and mucous membranes, diarrhea and salivation, convulsions and tremor, food and water intake, weight loss, lethargy, paralysis and mortality.

Based on the results of the acute oral toxicity test, three dose levels were selected: a middle dose of the plant extract was one-tenth of the maximum dose determined in the acute oral toxicity study; a low dose of the plant extract was half of the middle dose; and a high dose of the plant extract was twice the middle dose (Ayanaw and Yesuf, 2023).

3.6. Animal grouping and dosing

To evaluate the leaf gel of the *A. pubescens*, Swiss albino mice of either sex (weighing 20–30 g and 6-8 weeks of age) were randomly divided into five groups, each consisting of five mice. Group I (negative control) was received solvent which used to dissolve the gel powder (distilled water at a dose of 10 ml/kg) and Group II (positive control) was received standard drugs: morphine (10 mg/kg) for the hot plate test, aspirin (150 mg/kg) for the writhing test, and indomethacin (10 mg/kg) for carrageenan-induced paw edema model and formalin-induced paw edema model. The remaining three groups (treatment groups) were received different doses (dose determined based on acute oral toxicity study) of the plant leaf gel. All agents were administered orally using an oral gavage (Ayalew *et al.*, 2022; Tamrat *et al.*, 2017). The powdered leaf gel was dissolved in distilled water and administered orally using an oral gavage at the required dose.

3.7. Induction of experimental inflammation and pain

3.7.1. Carrageenan-induced paw edema

Acute inflammation was induced by injecting 0.05mL of 1% w/v carrageenan in normal saline into sub-plantar tissue of the right hind paw of the mice that were fasted overnight with free access to water. Prior to inflammation induction, the paw was marked with ink at the lateral malleolus. To trigger inflammation, carrageenan were administered to each group of mice one hour after the oral administration of the crude leaf gel of *A. pubescens*, the vehicle and the standard drug. The extent of inflammation was measured in milliliters by assessing the displacement of water due to edema using a digital plethysmometer at 0, 1, 2, 3, 4, and 5 hours post-injection. The percentage of edema protection was calculated in comparison to the control mice using the specified formula (Ashagrie, 2023b).

$$\% \text{ Edema inhibition} = \frac{PEC - PET}{PEC} \times 100$$

Where PEC paw edema in control group, PET paw edema in test group.

3.7.2. Formalin-induced paw edema

In this model, sub-acute inflammation was induced using formalin. On day 1 and day 3, the overnight-fasted mice were injected in to the sub-plantar region of the right hind paw with 0.02 mL of 2% v/v formalin in normal saline. To ensure consistent measurement, the right hind paw of each mouse was marked at the lateral malleolus level before induction and immersed to the same depth in the plethysmometer chamber throughout the study. The leaf gel, standard drug, and vehicle were administered according to their respective groups, 1 hour prior to formalin injection, for seven consecutive days. Paw volume was measured daily using a plethysmometer, 1 hour after treatment administration, up to day seven (Yimer *et al.*, 2020). The percentage of edema inhibition was calculated using the above formula.

3.7.3. Acetic acid-induced writhing test

This test was performed to evaluate the peripheral analgesic effects of the crude leaf gel of *A. pubescens*. Swiss albino mice of either sex were fasted overnight with free access to water. The mice were randomly assigned to five groups, each consisting of five animals. The three treatment groups were received varying doses of the crude leaf gel of *A. pubescens*, which determined based on acute oral toxicity test, while one group was received a vehicle as a negative control, and another group was administered Aspirin (150 mg/kg), as a positive control, one hour before acetic acid injection. After 60 minutes, the analgesic effect of *A. pubescens* was assessed by counting the number of writhes induced by 0.6% acetic acid (10 mL/kg, i.p.). Five minutes after acetic acid administration, each animal was placed individually in an inverted flask, and abdominal muscle contractions along with hind limb stretching were recorded cumulatively over 20 minutes (Ayanaw and Yesuf, 2023).

The percentage of protection against writhing was used as an index

% inhibition of Paw edema = mean writhing count $\frac{(control\ group - treated\ group)}{mean\ writhing\ count\ of\ control} \times 100$

3.7.4. Hot plate method

The hot plate test was conducted to evaluate the central analgesic activities of *A. pubescens* leaf gel by placing a mouse in an open-ended cylindrical chamber with a metallic plate at the base, which was heated. The plate was kept at a steady temperature of 55°C ± 1°C, eliciting two behavioral responses; paw licking and jumping, both of which are supraspinally-integrated reactions. All animals were fasted overnight. Mice of either sex were administered varying doses of *A. pubescens* leaf gel (which determined based on acute oral toxicity study, P.O.), a vehicle (negative control, P.O.), or the standard drug morphine (10 mg/kg orally), depend on their assigned groups. Each mouse was then individually placed on the hot plate with a 15-second cutoff time to prevent paw injuries. The latency period before paw licking or jumping was recorded at 0, 30, 60, 90, and 120 minutes to assess reaction time. The percentage increase in reaction time or pain threshold inhibition was determined using the following formula (Ashagrie, 2023b).

$$\text{Elongation (\%)} = \frac{Latency(test) - Latency(control)}{Latency(test)} \times 100$$

3.8. Preliminary phytochemical screening

A standard phytochemical screening test was conducted to identify the presence or absence of secondary metabolites and examine their potential relationship with the anti-inflammatory and analgesic properties of the leaf of *A. pubescens*. As a result, standard testing procedures were employed to determine the presence or absence of alkaloids, saponins, phenols, steroids, anthraquinones, flavonoids, tannins, and glycosides (Yimer et al., 2020).

Test for alkaloids (Wagner's test): A few drops of Wagner's reagent (a solution of iodine in potassium iodide) were added to 0.25 g of plant dried gel sample that was taken in a test tube. The formation of a reddish-brown precipitate indicated the presence of alkaloids in the sample (Jacob, 2025).

Test for saponins: About 0.5 g of the gel was shaken with distilled water in a test tube. Frothing which persisted for 15 min indicated the presence of saponins (Musa *et al.*, 2009).

Test for phenols (Ferric chloride test): plant leaf gel was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols (Tyagi, 2017).

Test for steroids (Liebermann-Burchard's test): 2 mg of the gel powder was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green colour indicated the presence of steroids. (Pooja and Vidyasagar, 2016)**.**

Test for anthraquinones: Borntrager's test Approximately 50 mg of powdered gel was heated with a 10% ferric chloride solution and 1 ml of concentrated hydrochloric acid. After cooling and filtering the mixture, the resulting filtrate was shaken with diethyl ether. The ether extract was then further treated with strong ammonia. The appearance of a pink or deep red color in the aqueous layer indicated the presence of anthraquinone (Tyagi, 2017).

Test for flavonoids: 2 ml of the gel was added with few drops of 20% sodium hydroxide, formation of intense yellow color was observed. To this few drop of 70% dilute hydrochloric acid was added and yellow color was disappeared. Formation and disappearance of yellow color indicate the presence of flavonoids in the plant leaf gel (Reviewed and Page, 2021).

Test for tannins: About 0.25 g of plant gel powder was boiled in 10 ml of distill water in a test tube and then filtered with filter paper (Whatman No. 1). A few drops of 0.1% ferric chloride was added to the filtrate. A brownish green or a blue-black precipitate indicated the presence of tannins (Singh and Bag, 2013).

Test for cardiac glycosides: (Keller Killiani Test) – 0.5g of plant leaf gel was dissolved in 2ml of glacial acetic acid containing one drop of Ferric chloride solution and mixed. 1ml Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green indicate a positive test for glycosides (Maobe *et al.*, 2013).

3.9. Operational definition

A positive control group is a group in an experiment that receives a known effective treatment to validate the study and compare new treatments. It ensures the experiment is working correctly.

A negative control group is a group that does not receive the experimental treatment or any active substance, serving as a baseline to rule out external influences.

An experimental (treatment) group is a group in an experiment that receives the tested drug, intervention, or condition to measure its effects.

3.10. Data quality control

The experimental procedure data was regularly recorded in a laboratory logbook to minimize mistakes in the experimental process. All reagents and medications were analytical grade. The experimental implementation was routinely carried out and supervised by the principal investigator. At the laboratory, all process and safety protocols were regularly evaluated and maintained. To prevent the possibility of bias, animal groups were formed using a completely random selection process. Prior to entry, the data was tallied and double-checked.

3.11. Data processing and analysis

All statistical analyses were conducted using IBM SPSS (Statistical Packages for the Social Sciences), version 25 for Windows (SPSS Inc., Chicago, Illinois, USA). For the *in vivo* anti-inflammatory and analgesic activity, statistical differences between groups were assessed using one-way analysis of variance (ANOVA). Post-hoc Tukey's test was conducted to determine significance difference between control groups against each test groups separately. Dose-dependent effect was confirmed using multiple linear regression analysis. This test was performed to assess the effect of the plant material on edema volume, number of writhes and latency time. The results were presented as mean ± standard error of the mean (SEM), and $p\text{-}values<0.05$ were considered statistically significant.

3.12. Ethical consideration

The study protocol was approved by the Experimental Research Ethics Committee of the Haramaya University, College of Health and Medical Sciences, School of Pharmacy. The animals were handled and used in accordance with the international guidelines for the care and use of laboratory animals. The plant material was collected after obtaining consent from the person at the collection area, based on the WHO guidelines on Good Agricultural and Collection Practices (GACP) for medicinal plants. The study was conducted in compliance with the ARRIVE (Animal Research: Reporting of *In vivo* Experiments) guidelines.

4. RESULTS

4.1. Preliminary phytochemical screening test

The results of phytochemical screening showed, the presence of saponins, phenols, steroids, anthraquinones, flavonoids, tannins, and cardiac glycosides in the plant leaf gel. However, this qualitative screening could not detect the presence of alkaloids (Table 5).

Table 5: Preliminary phytochemical screening results of *A. pubescens* leaf gel

Secondary metabolites	**Absence (-) or presence (+) of compounds**
Alkaloids	-
Saponins	+
Phenols	+
Steroids	+
Anthraquinones	+
Flavonoids	+
Tannins	+
Cardiac glycosides	+

4.2. Acute oral toxicity study

The results of the acute oral toxicity study showed that the leaf gel of *A. pubescens* at dose of 2000 mg/kg did not cause any notable toxic manifestations, deaths within 24 hours, and during the next 14-days follow up period.

4.3. Anti-inflammatory activity test

4.3.1. Carrageenan-induced paw edema

As shown in Table 1, the leaves gel of the plant produced a significant reduction in paw edema at all tested doses from 2 to 5 hours after induction ($p<0.001$, $p<0.01$ or $p<0.05$) compared to the negative control. Intergroup comparison among lower and middle doses showed no significant difference. However, significant difference was observed between 100 mg/kg and 400 mg/kg ($p<0.05$ or $p<0.01$), 200 mg/kg and 400 mg/kg ($p<0.05$) of the plant leaf gel. Indomethacin (10 mg/kg) produced a statistically significantly anti-inflammatory activity than the lower and middle doses of the leaf gel of the plant ($p<0.01$ or $p<0.001$).

At all tested doses (100 mg/kg, 200 mg/kg and 400 mg/kg), the gel showed its strongest anti-inflammatory effect at 4 hours with percentage inhibition of 30.5%, 32.2% and 37.2% respectively. The anti-inflammatory activity at this time point was increased in dose dependent manner. The maximum dose of the plant leaf gel produced significantly greater inhibition than the middle dose ($p<0.05$) and lower dose ($p<0.05$ or $p<0.01$). However, it's less inhibition than the positive control (Table 1).

Table 1: Anti-inflammatory effect of the leaf gel of *A. pubescens* on carrageenan-induced paw edema in mice

Groups	**Mean paw volume(mL) (mean ± SEM) (% inhibition)**					
	0hr	**1hr**	**2hr**	**3hr**	**4hr**	**5hr**
DW	0.40 ± 0.011	0.42 ± 0.013	0.46 ± 0.011	0.56 ± 0.02	0.59 ± 0.02	0.47 ± 0.019
IND	0.36 ± 0.016	0.38 ± 0.022(9.5)	0.39 ± 0.019(15.2)^{c**d**e*}	0.41 ± 0.01(26.8)^{c**d**e*}	0.32 ± 0.024(45.8)^{c**d***e*}	0.29 ± 0,019(38.2)^{c***d**e**}
APG 100	0.38 ± 0.022	0.40 ± 0.013(4.7)	0.43 ± 0.021(6.5)^{a*e*}	0.47 ± 0.026(16)^{a**e**}	0.41 ± 0.016(30.5)^{a***e**}	0.40 ± 0.013(14.8)^{a***e*}
APG 200	0.38 ± 0.016	0.40 ± 0.024(4.7)	0.43 ± 0.014(6.5)^{a*c*}	0.46 ± 0.022(17.8)^{a**e*}	0.40 ± 0.013(32.2)^{a***c*}	0.39 ± 0.011(17)^{a**e*}
APG 400	0.37 ± 0.011	0.39 ± 0.019(7.1)	0.41 ± 0.015(10.9)^{a**}	0.44 ± 0.016(21.4)^{a**}	0.37 ± 0.019(37.2)^{a***}	0.36 ± 0.021(23.4)^{a**}

Notes: Results are expressed as mean ± SEM (n=5), analysis was performed with One-Way ANOVA followed by Tukey post Hoc test. [a] compared with negative control, [b] compared with positive control, [c] compared with APG 100mg/kg, [d] compared with APG 200mg/kg, [e] compared with APG 400mg/kg, ***$p<0.001$, **$p<0.01$, *$p<0.05$
Abbreviations: APG, *A. pubescens* gel; DW, Distilled water; IND, Indomethacin

4.3.2. Formalin-induced paw edema

The *A. pubescens* leaf gel at all test doses (100, 200 and 400 mg/ kg) significantly reduced formalin-induced paw edema in mice from day 1 to day 7 observation period as compared with the negative control group ($p<0.01$ or $p<0.001$). At all tested doses (100 mg/kg, 200 mg/kg and 400 mg/kg), the gel showed its strongest anti-inflammatory effect at day 7 with percentage inhibition of 42.9%, 49.1% and 55.1% respectively and the anti-inflammatory activity at this day was increased in dose dependent manner. The maximum dose of the plant leaf gel produced significantly greater inhibition than the middle dose ($p<0.05$ or $p<0.01$) and lower dose ($p<0.01$ or $p<0.001$) [Table 2].

Table 2: Anti-inflammatory effect of the leaves gel of *A. pubescens* on formalin-induced paw edema in mice

Groups	Mean Paw Edema(mL) (Mean ± SEM) (% inhibition)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DW	0.40 ±0.019	0.41 ± 0.011	0.50 ± 0.024	0.53 ± 0.020	0.52 ± 0.032	0.51 ± 0.036	0.49 ± 0.025
IND	0.27 ± 0.016 (32.5)^{c**d*}	0.25 ± 0.019 (39.2)^{c**d*}	0.28 ±0.015(44)^{c**d**}	0.26 ±0.011(50.9)^{c***d***}	0.25 ±0.008 (51.9)^{c***d*}	0.23 ±0.009 (54.9)^{c**d*}	0.21 ±0.008 (57.1)^{c**d*}
APG 100	0.34 ± 0.009 (15)^{a**d*e**}	0.33 ± 0.009 (19.5)^{a***d*e***}	0.35 ± 0.016 (30)^{a***d*e**}	0.34 ± 0.019 (35.8)^{a***d*e**}	0.33 ± 0.023 (36.5)^{a***d*e**}	0.30 ± 0.022 (41.2)^{a***d*e**}	0.28 ± 0.019 (42.9)^{a***d*e***}
APG 200	0.31 ± 0.011 (22.5)^{a**e*}	0.30 ± 0.013 (26.9)^{a***e**}	0.33 ± 0.008 (34)^{a***e**}	0.32 ± 0.008 (39.6)^{a***e*}	0.29 ± 0.020 (44.2)^{a***e**}	0.26 ± 0.016 (49)^{a***e*}	0.25 ± 0.024 (49.1)^{a***e*}
APG 400	0.28 ± 0.019 (30)^{a***}	0.27 ± 0.008 (34.1)^{a***}	0.29 ± 0.016 (42)^{a***}	0.27 ± 0.021 (49.1)^{a***}	0.26 ± 0.016 (50)^{a***}	0.24 ± 0.016 (52.9)^{a***}	0.22 ± 0.017 (55.1)^{a***}

Notes: Results are expressed as mean ± SEM (n=5), analysis was performed with One-Way ANOVA followed by Tukey post Hoc test. [a] compared with negative control, [b] compared with positive control, [c] compared with APG 100mg/kg, [d] compared with APG 200mg/kg, [e] compared with APG 400mg/kg, ***$p<0.001$, **$p<0.01$, *$p<0.05$
Abbreviations: APG, *A. pubescens* gel; DW, Distilled water; IND, Indomethacin

4.4. Analgesic activity test

4.4.1. Acetic acid-induced writhing test

The leaves gel of the plant at different doses (100 mg/kg, 200 mg/kg, 400 mg/kg) produced a significant peripheral analgesic effect in a dose dependent manner ($p<0.05$, $p<0.01$ and $p<0.001$) respectively as compared with the negative control group. However, the standard drug aspirin (150 mg/kg) produced a statistically significant peripheral analgesic activity compared to the three doses of the plant gel ($p<0.001$). Meanwhile, when the experimental groups compared, to each other, showed significant differences in 100 vs 200 mg/kg and 200 vs 400 mg/kg ($p < 0.01$), and 100 vs 400 mg/kg ($p < 0.001$). The maximum dose of the plant material (400 mg/kg) displayed statistically significant difference than the lower dose (100 mg/kg) and the middle dose (200 mg/kg) ($p < 0.001$ and $p<0.01$) respectively. Oral administration of the various doses (100 mg/kg, 200 mg/kg, 400 mg/kg) of the leaf gel of the plant and aspirin reduced the number of writhings in mice with percentage inhibition of 12.2%, 29.5%, 46.8%, and 73.7%, respectively (Table 3).

Table 3: Peripheral analgesic effect of the leaf gel of *A. pubescens* on acetic acid-induced writhing test in mice

Groups	Mean no. of writhing ± SEM	% Inhibition
DW 10 mL/kg	27.8 ± 1.33	
ASA 150mg/kg	7.2 ± 0.83^{c***d***e***}	73.7%
APG 100mg/kg	24.4 ± 1.34^{a*d**e***}	12.2%
APG 200mg/kg	19.6 ± 1.81^{a**e**}	29.5%
APG 400mg/kg	14.8 ± 0.83^{a***}	46.8%

Notes: Results are expressed as mean ± SEM (n=5), analysis was performed with One-Way ANOVA followed by Tukey post Hoc test. [a] compared to DW (10 ml/kg), [b] compared to ASA (150 mg/kg), [c] compared with APG 100mg/kg, [d] compared with APG 200mg/kg, [e] compared with APG 400mg/kg, ***$p<0.001$, **$p<0.01$, *$p<0.05$
Abbreviations: APG, *A. pubescens* gel; ASA, Aspirin 150 mg/kg; DW, Distilled water 10 ml/kg

4.4.2. Hot plate test

In this model, from three tasted doses, only the maximum dose of the plant leaf gel displayed significant central analgesic activity (p<0.05 or p<0.01 or 0.001) by increasing reaction time at all observation points compared to the negative control. Throughout the observation, the standard drug morphine produced significant analgesic effect (p<0.05 or p<0.01) by prolonged latency time from 30-120 minutes than the gel (Table 4). Meanwhile, the maximum dose of the *A. pubescens* leaf gel and the standard drug morphine produced strongest central analgesic activity at 120 minutes of observation with percentage inhibition results of 76.3 and 80.4, respectively.

Table 4: Central analgesic effect of *A. pubescens* leaf gel on hot plate latency time in mice

Groups	Latency time in second (mean ± SEM) (percentage inhibition)				
	0 min	30 min	60 min	90 min	120 min
DW	3.4 ± 1.140	2.6 ± 0.548	2.4 ± 0.548	2.2 ± 0.447	1.8 ± 0.836
MRP	2.4 ± 0.548	7.2 ± 1.303 (63.9)^{c**d**e*}	8.8 ± 0.837 (72.7)^{c**d**e*}	9.6 ± 0.548 (77)^{c**d**e**}	9.2 ± 0.837 (80.4)^{c***d**e*}
APG 100mg/kg	2.6 ± 0.894	2.8 ± 0.836 (7.1)	3.4 ± 1.140 (29.4)	3.6 ± 1.140 (38.9)	3.8 ± 1.304 (52.6)
APG 200mg/kg	2.8 ± 0.837	3.2 ± 0.836 (18.8)	3.4 ± 1.140 (29.4)	3.8 ± 1.303 (42.1)	4.2 ± 1.303 (57.1)
APG 400mg/kg	3.2 ± 1.304	4.6 ± 1.140 (43.5)^{a*c*d*}	5.8 ± 1.483 (58.6)^{a*c**d*}	6.4 ± 1.140 (65.6)^{a**c**d*}	7.6 ± 1.140 (76.3)^{a***c**d*}

Notes: Results are expressed as mean ± SEM (n=5), analysis was performed with One-Way ANOVA followed by Tukey post Hoc test. [a] compared to DW (10 ml/kg), [b] compared to MRP (10 mg/kg), [c] compared with APG 100mg/kg, [d] compared with APG 200mg/kg, [e] compared with APG 400mg/kg, ***p<0.001, **p<0.01, *p<0.05
Abbreviations: APG, *A. pubescens* gel; DW, Distilled water 10 ml/kg; MRP, Morphine 10mg/kg

5. DISCUSSION

In light of the considerable clinical, social, and economic burdens imposed by pain and inflammation, coupled with the longstanding traditional use of herbal remedies, there is a clear imperative to develop safer and more effective agents derived from medicinal plants (Kedir and Ayele, 2024).

In this context, *A. pubescens* is a traditionally important medicinal plant widely used in local healthcare practices in Ethiopia. Traditionally, the plant is used to treat various human ailments such as stomachache, muscle cramps, wounds, and febrile conditions locally known as "Mich." In livestock, it is notably employed for the management of anthrax (Wondimu *et al.*, 2007). Thus, its application in both human and veterinary medicine indicates its broad therapeutic relevance and support its potential for further pharmacological investigation. However, to date, no scientific studies have reported the anti-inflammatory and analgesic activities of *A. pubescens* leaf gel in experimental animal models. Accordingly, this study aimed to scientifically assess the anti-inflammatory and analgesic effects of the leaf gel in mice in order to validate its traditional medicinal claims. The current study key findings include: the demonstration of significant anti-inflammatory and analgesic activities of *A. pubescens* leaf gel in mice models.

The preliminary phytochemical screening of the leaf gel of *A. pubescens* was carried out to detect the possible presence or absence of different phytoconstituents. The results showed, the presence of saponins, phenols, steroids, anthraquinones, flavonoids, tannins, and cardiac glycosides as well as the absence of alkaloids in the plant leaf gel were confirmed via qualitative color changes of test reagents. These findings will give hint on the possible mechanisms of analgesic and anti-inflammatory activities of the leaf gel.

To assess the anti-inflammatory activity, the carrageenan induced-paw edema model was used, as it shows excellent reliability for acute phase inflammation (Ashagrie, 2023b). In this model, acute inflammation was induced by sub-planar injection of carrageenan (1% w/v in normal saline) in to the right hind paws of the mice. Following the induction of carrageenan, an acute localized inflammation was induced through sequential release of various endogenous inflammatory mediators. The release of these endogenous mediators was biphasic (early phase and late phase) (Yimer *et al.*, 2020).

The early phase is mediated by histamine, serotonin, and bradykinin and is not significantly inhibited by NSAIDs, whereas, the late phase is driven by increased prostaglandin production and cyclooxygenase activation. The late phase is characterized by progressive swelling resulting from increased vascular permeability and prostaglandin-mediated edema, and this phase is effectively inhibited by NSAIDs (Sharma *et al.*, 2020). Likewise, the extract reduced paw edema during the late phase, suggesting that their anti-inflammatory effects may involve inhibition of cyclooxygenase (COX) in the carrageenan-induced inflammatory cascade. Natural compounds including phenols, flavonoids, alkaloids, terpenoids, and glycosides inhibit prostaglandin synthesis during the late phase of inflammation (Ashagrie, 2023b).

Consitent with this mechanism, the leaf gel exhibited significant anti-inflammatory activity from 2 to 5 hours following carrageenan injection compared to the negative group. The effect was most pronounced between this time period. This finding suggests that the gel is particularly effective at inhibiting the second phase of the inflammation, which is primarily driven by prostaglandins produced through COX-2 activity, and leukotrienes (Devaraj and Karpagam, 2011; Muhammad *et al.*, 2012). Similarly, previous studies have demonstrated the anti-inflammatory activity of different plant extracts using the carrageenan model. For instance, the extract of the leaves of *Moringa stenopetala* reduced paw edema during the late phase of inflammation (Tamrat *et al.*, 2017). In addition, *Echinops kebericho* extract showed activity starting from the first phase and continuing till the second phase (Yimer *et al.*, 2020). These findings suggest that bioactive constituents may suppress both phases of acute inflammation by interfering with the release and/or activity of the chemical mediators.

Moreover, at all the tested doses (100, 200, and 400 mg/kg), the gel exhibited its maximum anti-inflammatory effect at 4 hours, producing percentage inhibitions of 30.5%, 32.2%, and 37.2%, respectively. These effects confirmed that the anti-inflammatory activity increased in a dose-dependent manner. Additionally, the highest dose of the gel produced significantly greater inhibition than the middle dose ($p<0.05$) and lower dose ($p<0.05$ or $p<0.01$). Similarly, previous study showed that the maximum anti-inflammatory effect of the leaves of the *Leonotis ocymifolia* was observed at 6 hours after induction at all doses in dose dependent manner (Alemu *et al.*, 2018). Additionally, root extract of *Echinops kebericho* at the three doses, produced the maximum

percentage of edema inhibition at four hours of observation period in dose dependent manner (Yimer *et al.*, 2020).

In addition to acute inflammation, the formalin-induced paw edema was used to evaluate the effect of our extract during the sub-acute phase of inflammation. In this model, *A. pubescens* leaf gel significantly reduced paw edema in a dose-dependent manner over a 7-day observation period. The maximal anti-inflammatory effect was observed on day 7, with inhibition ranging from 42.9% to 55.1%. Furthermore, the highest dose produced significantly greater anti-inflammatory activity than the lower doses.

Taken together, the anti-inflammatory activity observed in this study is supported by previous reports indicating that plants containing alkaloids, flavonoids, saponins, tannins, phenolic compounds, glycosides, coumarins, and triterpenoids exhibit strong anti-inflammatory effects. Hence, the anti-inflammatory effect of *A. pubescens* leaf gel may be attributed to the presence of these secondary metabolites. These bioactive compounds may interfere with the synthesis, release, and activity of endogenous inflammatory mediators involved in both acute and sub-acute inflammation (Ashagrie, 2023a, 2023b; Kedir and Ayele, 2024; Tazeze *et al.*, 2021; Yimer *et al.*, 2020).With respect to analgesic activity, the acetic acid–induced writhing test was employed to assess peripheral analgesia (Abebe, 2020). In this model, *A. pubescens* leaf gel produced a significant, dose-dependent reduction in writhing responses compared with the negative control. The highest dose showed greater analgesic activity than the lower doses. However, its effect was lower than that of the positive control (46.8% vs. 73.7%). The increase in analgesic activity with increasing doses of the gel might be attributed with an increase in concentration of phytoconstituents that possess analgesic activity with the maximum dose (Ayanaw and Yesuf, 2023; Yimer *et al.*, 2020).

Furthermore, the hot plate test was used to evaluate central analgesic activity. In this test, only the highest dose of the leaf gel produced significant central analgesic effects. This was evidenced by increased reaction time at all observation points compared with the negative control. In contrast, the lower and middle doses did not show significant effects. This may be due to insufficient concentrations of active secondary metabolites at these doses. At 120 minutes of observation, the highest dose produced peak central analgesic activity closer to morphine, with percentage inhibitions of 76.3% and 80.4%, respectively.

Overall, the analgesic activity of *A. pubescens* leaf gel is probably due to its phytochemical constituents like phenols, flavonoids, tannins and steroids. These compounds are known to inhibit prostaglandin synthesis (Ashagrie, 2023b; Yimer *et al.*, 2020). Therefore, the combined anti-inflammatory and analgesic effects observed in this study support the traditional use of *A. pubescens* and highlight its potential as a source of bioactive therapeutic agents.

6. CONCLUSION

Findings from the present study indicate that the leaf gel of *A. pubescens* is safe in acute toxicity assessments in mice, supporting its potential therapeutic use. In addition, the leaf gel exhibited both anti-inflammatory and analgesic activities in mouse models. Overall, it can be suggested that the observed anti-inflammatory and analgesic activities of *A. pubescens* leaf gel may be attributed, at least in part, to the combined effects of multiple bioactive phytoconstituents, which could modulate the synthesis, release, or action of endogenous mediators involved in the initiation and progression of inflammation and pain.

7. RECOMMENDATIONS

Based on the results of the present study, the following recommendations are proposed for further studies:

- Further studies should focus on fractionation of the leaf gel of *A. pubescens* using different solvents to identify the most active anti-inflammatory and analgesic constituents.
- Isolation and structural characterization of bioactive compounds are recommended, along with mechanistic studies to elucidate their modes of action.
- In addition, sub-chronic and chronic toxicity studies, pharmacokinetic evaluations, and validation using additional experimental inflammation and pain models are necessary to further establish the safety, efficacy, and therapeutic potential of the plant.

8. REFERENCES

WHO (2012), "WHO guidelines on the pharmacological treatment of persisting pain in children with medical illnesses", *WHO Guidelines on the Pharmacological Treatment of Persisting Pain in Children with Medical Illnesses*, p. 166.

Abdissa, D., Geleta, G., Bacha, K. and Abdissa, N. (2017), "Phytochemical investigation of Aloe pulcherrima roots and evaluation for its antibacterial and antiplasmodial activities", *PLoS One*, Public Library of Science San Francisco, CA USA, Vol. 12 No. 3, p. e0173882.

Abdissa, N., Gohlke, S., Frese, M. and Sewald, N. (2017), "Cytotoxic Compounds from Aloe megalacantha", *Molecules (Basel, Switzerland)*, Vol. 22 No. 7, pp. 1–9, doi: 10.3390/molecules22071136.

Abebe, B. (2020), "Evaluation of Analgesic and Anti-inflammatory Potential of 80 % Methanol Leaf Extract of Otostegia integrifolia Benth (Lamiaceae)", pp. 1175–1183.

Abera, B. (2014), "Medicinal plants used in traditional medicine by Oromo people, Ghimbi District, Southwest Ethiopia", *Journal of Ethnobiology and Ethnomedicine*, Vol. 10 No. 1, pp. 1–15, doi: 10.1186/1746-4269-10-40.

Akaberi, M., Sobhani, Z., Javadi, B., Sahebkar, A. and Emami, S.A. (2016), "Therapeutic effects of Aloe spp. in traditional and modern medicine: A review", *Biomedicine and Pharmacotherapy*, Elsevier Masson SAS, Vol. 84, pp. 759–772, doi: 10.1016/j.biopha.2016.09.096.

Al-wajih, A.M., El-shaibany, A.M., Aasser, M. El and Raweh, S.M. (2022), "Preliminary phytochemical screening for various secondary metabolites , quantitative and qualitative analysis of Yemeni Aloe vera and Aloe vacillans flower extracts".

Alemu, A., Tamiru, W., Nedi, T. and Shibeshi, W. (2018), "Analgesic and Anti-Inflammatory Effects of 80 % Methanol Extract of Leonotis ocymifolia (Burm . f .) Iwarsson Leaves in Rodent Models", Hindawi, Vol. 2018, doi: 10.1155/2018/1614793.

Ashagrie, G. (2023a), "Evaluation of Analgesics and Anti-Inflammatory Activity of the Root Extract of Impatiens rothii Evaluation of Analgesics and Anti-Inflammatory Activity of the Root Extract of Impatiens rothii (Balsaminaceae) in Rodents", doi: 10.2147/JEP.S410024.

Ashagrie, G. (2023b), “Analgesic and Anti-Inflammatory Activities of 80 % Methanol Extract and Solvent Fractions of Ehretia cymosa Thonn (Boraginaceae) Leaves in Rodents”, No. February, pp. 63–79.

Asmerom, D., Kalay, T.H. and Tafere, G.G. (2020), “Antibacterial and Antifungal Activities of the Leaf Exudate of Aloe megalacantha Baker”, *International Journal of Microbiology*, Vol. 2020, doi: 10.1155/2020/8840857.

August, J. *et al*. (2017), “Antiinflammatory , analgesic and antipyretic effects of dichloromethane stem bark extract of Acacia mellifera”, Vol. 6 No. 4, pp. 239–246.

Ayalew, M., Bekele, A., Mengistie, M.G. and Atnafie, S.A. (2022), “Evaluation of the antidiarrheal activity of 80% methanol extract and solvent fractions of the leaf of Bersama abyssinica fresen (Melianthaceae) in mice”, *BMC Complementary Medicine and Therapies*, BioMed Central, Vol. 22 No. 1, pp. 1–9, doi: 10.1186/s12906-021-03498-6.

Ayanaw, M.A. and Yesuf, J.S. (2023), “Evaluation of Analgesic and Anti-inflammatory Activities of Methanolic Leaf and Root Extracts of Gomphocarpus purpurascens A . Rich (Asclepiadaceae) in Mice”, No. December 2022, pp. 1–11.

Banjaw, D.T. (2024), “Underutilized Medicinal Plant in Ethiopia : The Case of Aloe Species”, Vol. 10 No. 1, pp. 1–5.

Belayneh, A., Demissew, S., Bussa, N.F. and Bisrat, D. (2020), “Ethno-medicinal and bio-cultural importance of aloes from south and east of the Great Rift Valley floristic regions of Ethiopia”, *Heliyon*, Elsevier Ltd, Vol. 6 No. 6, p. e04344, doi: 10.1016/j.heliyon.2020.e04344.

Bhowmick, R.. *et al*. (2014), “In vivo analgesic , antipyretic , and anti-inflammatory potential in Swiss albino mice and in vitro thrombolytic activity of hydroalcoholic extract from Litsea glutinosa leaves”, pp. 1–8.

Boudreau, M.D. and Beland, F.A. (2006), “An evaluation of the biological and toxicological properties of Aloe barbadensis (Miller), Aloe vera”, *Journal of Environmental Science and Health - Part C Environmental Carcinogenesis and Ecotoxicology Reviews*, Vol. 24 No. 1, pp. 103–154, doi: 10.1080/10590500600614303.

Bribi, N. *et al.* (2015), "Antinociceptive and anti-inflammatory effects of total alkaloid extract from fumaria capreolata", *Evidence-Based Complementary and Alternative Medicine*, Vol. 2015, doi: 10.1155/2015/736895.

Devaraj, a and Karpagam, T. (2011), "Evaluation of anti-inflammatory activity and analgesic effect of aloe vera leaf extract in rats", *International Research Journal of Pharmacy*, Vol. 2 No. 3, pp. 103–110.

Diriba, T.F. and Deresa, E.M. (2022), "Botanical description , ethnomedicinal uses , phytochemistry , and pharmacological activities of genus Kniphofia and Aloe : A review", *Arabian Journal of Chemistry*, The Author(s), Vol. 15 No. 9, p. 104111, doi: 10.1016/j.arabjc.2022.104111.

E, E.U.G.C.K. and Galam, N.Z. (2011), "Anti-inflammatory and Analgesic Effects of Aqueous Extract of Aloe Vera (Aloe barbadensis) in Rats", Vol. 14 No. September.

Fentaw, E. *et al*. (2022), "Uses and perceived sustainability of Aloe L. (Asphodelaceae) in the central and northern Highlands of Ethiopia", *South African Journal of Botany*, Vol. 147, pp. 1042–1050, doi: 10.1016/j.sajb.2020.11.001.

Feyisa, M., Kassahun, A. and Giday, M. (2021), "Medicinal Plants Used in Ethnoveterinary Practices in Adea Berga District, Oromia Region of Ethiopia", *Evidence-Based Complementary and Alternative Medicine*, Vol. 2021, doi: 10.1155/2021/5641479.

Gebremeskel, L., Bhoumik, D., Sibhat, G.G. and Tuem, K.B. (2018), "In vivo wound healing and anti-inflammatory activities of leaf latex of aloe megalacantha baker (Xanthorrhoeaceae)", *Evidence-Based Complementary and Alternative Medicine*, Vol. 2018, doi: 10.1155/2018/5037912.

Giri, S.P. and Varma, S.B. (2015), "Analgesic and anti-inflammatory activity of Tectona grandis Linn . stem extract", doi: 10.1515/jbcpp-2014-0043.

Gou, K., Zeng, R., Dong, Y., Hu, Q. and Hu, H. (2017), "Anti-inflammatory and Analgesic Effects of Polygonum orientale L . Extracts", Vol. 8 No. August, doi: 10.3389/fphar.2017.00562.

Gunn, A., Bobeck, E.N., Weber, C. and Morgan, M.M. (2012), "in Rats", Vol. 12 No. 2, pp.

222–227, doi: 10.1016/j.jpain.2010.06.011.The.

Guo, X. and Mei, N. (2016), “Aloe vera: A review of toxicity and adverse clinical effects”, *Journal of Environmental Science and Health - Part C Environmental Carcinogenesis and Ecotoxicology Reviews*, Vol. 34 No. 2, pp. 77–96, doi: 10.1080/10590501.2016.1166826.

Ishola, I.O., *et al*. (2014), “Analgesic and anti-inflammatory effects of the methanol root extracts of some selected Nigerian medicinal plants Analgesic and anti-inflammatory effects of the methanol root extracts of some selected Nigerian medicinal plants”, Vol. 0209, doi: 10.3109/13880209.2014.880487.

Jacob, C.V.K.C. (2025), “Phytochemical Profiling And Spectroscopy Characterization Of”, Vol. 11 No. 7, pp. 521–536.

Jami, S.I., Sultana, Z., Ali, E., Begum, M.M. and Haque, M. (2014), “Evaluation of Analgesic and Anti-Inflammatory Activities on Ethanolic Extract of Terminalia chebula Fruits in Experimental Animal Models”, Vol. 2014 No. January, pp. 63–69.

Jang, J.Y. and Seong, Y.H. (2014), “Anti-nociceptive and anti-inflammatory effect of an ethanol extract of the leaf and stem of Aralia cordata”, *Natural Product Sciences*, Vol. 20 No. 4, pp. 301–305.

Javed, F., Jabeen, Q., Aslam, N. and Mehmood, A. (2020), “Pharmacological evaluation of analgesic , anti-in fl ammatory and antipyretic activities of ethanolic extract of Indigofera argentea Burm . f .”, *Journal of Ethnopharmacology*, Elsevier Ireland Ltd, Vol. 259 No. January, p. 112966, doi: 10.1016/j.jep.2020.112966.

Kazeem, M.I., Bankole, H.A., Fatai, A.A., Saibu, G.M. and Wusu, A.D. (2022), “Genus Aloe as sources of antidiabetic, antihyperglycemic and hypoglycemic agents: A review”, *South African Journal of Botany*, Elsevier B.V., Vol. 147, pp. 1070–1077, doi: 10.1016/j.sajb.2022.05.041.

Kedir, G. and Ayele, A.G. (2024), “In-vivo evaluation of analgesic and anti- inflammatory activities of the 80 % methanol extract of Acacia seyal stem bark in rodent models”, Vol. 1, pp. 1–8.

Khumalo, G.P., Erik, B., Wyk, V., Feng, Y. and Cock, I.E. (2021), “A review of the traditional

use of southern African medicinal plants for the treatment of inflammation and inflammatory pain", *Journal of Ethnopharmacology*, Elsevier B.V., No. July, p. 114436, doi: 10.1016/j.jep.2021.114436.

Kouakou, Y.K.F. (2016), "Evaluation of antalgic activity and trace elements analysis of Trema guineensis extracts in acetic acid induced in rats", *Open Access Library Journal*, Scientific Research Publishing, Vol. 3 No. 05, p. 1.

Kumar, S., Alam, M., Chandra, M. and Roy, E. (2023), "An Assessment of Analgesic and Anti-inflammatory Activity of Manilkara zapota on Rat Model", Vol. 6 No. 3, pp. 177–184.

Leach, L.C., Tazeze, H., Mequanente, S. and Mengie, T. (2021), "Investigation of Wound Healing and Anti-Inflammatory Activities of Leaf Gel of Aloe", pp. 5567–5580.

Lee, I.O. and Jeong, Y.S. (2002), "Effects of different concentrations of formalin on paw edema and pain behaviors in rats.", *Journal of Korean Medical Science*, Vol. 17 No. 1, pp. 81–85, doi: 10.3346/jkms.2002.17.1.81.

Maobe, M.A.G., Gatebe, E., Gitu, L. and Rotich, H. (2013), "Preliminary phytochemical screening of eight selected medicinal herbs used for the treatment of diabetes, malaria and pneumonia in Kisii region, southwest Kenya", *European Journal of Applied Sciences*, Vol. 5 No. 10, pp. 1–6.

Marizvikuru Mwale. (2012), "Toxicological studies on the leaf extract of Aloe ferox Mill. (Aloaceae)", *Scientific Research and Essays*, Vol. 7 No. 15, pp. 1605–1613, doi: 10.5897/sre09.334.

Masresha, B., Makonnen, E. and Debella, A. (2012), "In vivo anti-inflammatory activities of Ocimum suave in mice", *Journal of Ethnopharmacology*, Elsevier, Vol. 142 No. 1, pp. 201–205, doi: 10.1016/j.jep.2012.04.041.

Muhammad, N., Saeed, M. and Khan, H. (2012), "Antipyretic , analgesic and anti-inflammatory activity of Viola betonicifolia whole plant".

Musa, A.M. *et al*. (2009), "Preliminary phytochemical, analgesic and anti-inflammatory studies of the methanol extract of Anisopus mannii (NE Br)(Asclepiadaceae) in rodents", *African Journal of Pharmacy and Pharmacology*, Vol. 3 No. 8, pp. 374–378.

Mwale, M. and Masika, P.J. (2010), "Analgesic and anti-inflammatory activities of Aloe ferox Mill . aqueous extract", Vol. 4 No. June, pp. 291–297.

Nagesh, H., Basavanna, P. and Kishore, M. (2015), "Evaluation of wound healing activity of ethanolic extract of Azadirachta indica leaves on incision and excision wound models in Wister albino rats", *International Journal of Basic and Clinical Pharmacology*, Vol. 4 No. 6, pp. 1178–1182, doi: 10.18203/2319-2003.ijbcp20151354.

Nigussie, D. *et al*. (2021), "Systematic review of Ethiopian medicinal plants used for their anti-inflammatory and wound healing activities", *Journal of Ethnopharmacology*, Elsevier B.V., Vol. 276, p. 114179, doi: 10.1016/j.jep.2021.114179.

Oda, B.K. and Erena, B.A. (2017), "Aloes of Ethiopia: A Review on Uses and Importance of Aloes in Ethiopia", *Int J Plant Biol Res*, Vol. 5 No. 1, p. 1059.

Oguntibeju, O.O. (2018), "Medicinal plants with anti-inflammatory activities from selected countries and regions of africa", *Journal of Inflammation Research*, Vol. 11, pp. 307–317, doi: 10.2147/JIR.S167789.

Olorukooba, A.B., Khan, F., Sani, Y.M. and Hamza, A.N. (2020), "Analgesic , anti-inflammatory and antipyretic studies on the residual aqueous fraction of Uapaca togoensis (Pax) stem bark in rodents", Vol. 17 No. 2, pp. 189–199.

Organisation for Economic Cooperation and Development. (2008), "Test guideline 425: acute oral toxicity - Up-and-Down Procedure", *Guideline for Testing of Chemicals*, No. December, p. 26.

Osagie, A. and Omoregie, E. (2013), "Proximate composition, phytochemical screening and antianaemic effect of *Aloe barbadensis (Aloe)* leaves in nutritionally stressed rats", *Journal of Pharmacy & Bioresources*, Vol. 9 No. 2, doi: 10.4314/jpb.v9i2.7.

Patil, K.R.. *et al.* (2019), "Animal models of inflammation for screening of anti-inflammatory drugs: implications for the discovery and development of phytopharmaceuticals", *International Journal of Molecular Sciences*, MDPI, Vol. 20 No. 18, p. 4367.

Pooja, S. and Vidyasagar, G.M. (2016), "Phytochemical screening for secondary metabolites of Opuntia dillenii Haw", *Journal of Medicinal Plants Studies*, Vol. 4 No. 5, pp. 39–43.

Reanmongkol, W., Noppapan, T. and Subhadhirasakul, S. (2009), "Antinociceptive, antipyretic, and anti-inflammatory activities of Putranjiva roxburghii Wall. leaf extract in experimental animals", *Journal of Natural Medicines*, Springer, Vol. 63, pp. 290–296.

Reviewed, P. and Page, C. (2021), "Qualitative and quantitative phytochemical screening of three plants stem bark and", Vol. 816 No. 6, pp. 6–11.

Salehi, B., *et al.* (2018), "Aloe genus plants: from farm to food applications and phytopharmacotherapy", *International Journal of Molecular Sciences*, MDPI, Vol. 19 No. 9, p. 2843.

Sharma, V.C., Kaushik, A., Dey, Y.N., Srivastava, B. and Wanjari, M. (2020), "Analgesic , anti-inflammatory and antipyretic activities of ethanolic extract of stem bark of Anogeissus latifolia Roxb", Clinical Phytoscience.

Sherif, A.E., Sajid-ur-rehman, M., Asif, M., Qadeer, I. and Khan, R. (2024), "antipyretic potential of Oxystelma esculentum (L . f .) Sm . using in vitro , in vivo , and in silico studies", No. January, pp. 1–17, doi: 10.3389/fphar.2023.1326968.

Silva-Correa., *et al.* (2021), "Potential activity of medicinal plants as pain modulators: A Review", *Pharmacognosy Journal*, Vol. 13 No. 1, pp. 248–263, doi: 10.5530/pj.2021.13.35.

Singh, K.L. and Bag, G.C. (2013), "Phytochemical analysis and determination of total phenolics content in water extracts of three species of Hedychium."

Solanaceae, L. (2019), "Evaluation of Analgesic Activities of 80 % Methanol Leaf Extract of Solanum Journal of Drug Delivery and Therapeutics Evaluation of Analgesic Activities of 80 % Methanol Leaf Extract of Solanum incanum L . (Solanaceae) in Mice", No. September, doi: 10.22270/jddt.v9i5.3235.

Tafese Awulachew, M. (2021), "Hand Book of Common Ethiopian Traditional Medicinal Plants: Their Parts and Uses for Human and Animal Treatments", *Journal of Diseases and Medicinal Plants*, Vol. 7 No. 3, p. 48, doi: 10.11648/j.jdmp.20210703.11.

Tamrat, Y., Nedi, T., Assefa, S., Teklehaymanot, T. and Shibeshi, W. (2017), "Anti-inflammatory and analgesic activities of solvent fractions of the leaves of Moringa stenopetala Bak . (Moringaceae) in mice models", BMC Complementary and Alternative

Medicine, pp. 1–10, doi: 10.1186/s12906-017-1982-y.

Tatiya, A.U., Saluja, A.K., Kalaskar, M.G., Surana, S.J. and Patil, P.H. (2017), "Journal of Traditional and Complementary Medicine Evaluation of analgesic and anti-in fl ammatory activity of Bridelia retusa (Spreng) bark", *Journal of Traditional Chinese Medical Sciences*, Elsevier Ltd, Vol. 7 No. 4, pp. 441–451, doi: 10.1016/j.jtcme.2016.12.009.

Tazeze, H., Mequanente, S., Nigussie, D., Legesse, B., Makonnen, E. and Mengie, T. (2021), "Investigation of wound healing and anti-inflammatory activities of leaf gel of aloe trigonantha l.C. leach in rats", *Journal of Inflammation Research*, Vol. 14, pp. 5567–5580, doi: 10.2147/JIR.S339289.

Theanphong, O. and Somwong, P. (2022), "Combination of selected Thai traditional pain relief medicinal plants with anti-inflammatory abilities in a protein denaturation assay", *Pharmacia*, Vol. 69 No. 3, pp. 745–753, doi: 10.3897/pharmacia.69.e86904.

Tuasha, N., Petros, B. and Asfaw, Z. (2018), "Medicinal plants used by traditional healers to treat malignancies and other human ailments in Dalle District , Sidama Zone ", Journal of Ethnobiology and Ethnomedicine, pp. 1–21.

Tyagi, T. (2017), "Phytochemical screening of active metabolites present in Eichhornia crassipes (Mart.) Solms and Pistia stratiotes (L.): Role in ethanomedicine", *Asian Journal of Pharmaceutical Education and Research*, Vol. 6 No. 4, pp. 40–56.

Weber, O., Kelbessa, E. and Kalema, J. (2013), "Aloe pubescens. The IUCN Red List of Threatened Species 2013: e.T201330A2700027.", No. February 2021, doi: 10.2305/IUCN.UK.2013-2.RLTS.T201330A2700027.en.

Widyarini, S., Akrom, A.M. and Paryuni, A.D. (2023), "Carrageenan-Induced Acute Inflammation on Back-Skin of Mice: Histopathological Features, Number of Inflammatory Cells, and Expression of COX-2, COX-1, and IL-6", *World's Veterinary Journal*, Scienceline Publication Ltd., No. 4, pp. 520–530.

Wintola, O.A. and Afolayan, A.J. (2011), "Phytochemical constituents and antioxidant activities of the whole leaf extract of Aloe ferox Mill.", *Pharmacognosy Magazine*, Vol. 7 No. 28, pp. 325–333, doi: 10.4103/0973-1296.90414.

Wondimu, T., Asfaw, Z. and Kelbessa, E. (2007), "Ethnobotanical study of medicinal plants around 'Dheeraa' town, Arsi Zone, Ethiopia", *Journal of Ethnopharmacology*, Vol. 112 No. 1, pp. 152–161, doi: 10.1016/j.jep.2007.02.014.

Worlein, J.M. (2011), "The eighth edition of the guide for the care and use of laboratory animals (2011); implications for behavioral management", *American Journal of Primatology*, Vol. 73, p. 98.

Yimer, T., Birru, E.M., Adugna, M., Geta, M. and Emiru, Y.K. (2020), "Evaluation of analgesic and anti-inflammatory activities of 80% methanol root extract of echinops kebericho m. (asteraceae)", *Journal of Inflammation Research*, Vol. 13, pp. 647–658, doi: 10.2147/JIR.S267154.