Analgesic and Antinflammatory Activities of the Root extract of *Grewia schweinfurthii* and its Constituent and Determination of Nutritional and Antinutritional Compositions of its Fruit

By: Abdi Leta (B. Pharm)

Advisors: - Prof. Ariaya Hymete (PhD)

Dr. Solomon Tadesse (PhD)

Prof. Mirutse Giday (PhD)

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This is to certify that the thesis prepared by Abdi Leta entitled "**Analgesic and Antinflammatory activities of Root extract of *Grewia schweinfurthii* and its Constituent and Determination of Nutritional and Antinutritional compositions of its Fruit**" 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.

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External examiner: Dr. Solomon Mequanente	Signature_____	Date_____
Internal examiner: Dr. Daniel Bisrat	Signature_____	Date_____
Prof. Ariaya Hymete (Advisor)	Signature_____	Date_____
Dr. Solomon Tadesse (Advisor)	Signature_____	Date_____
Prof. Mirutse Giday (Advisor)	Signature_____	Date_____

Chair of Department or Graduate Program Coordinator

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List of abbreviations and acronyms

^{1}H NMR	Proton nuclear magnetic resonance
^{13}C NMR	Carbon thirteen nuclear magnetic resonance
AOAC	Association of Official Agricultural Chemists
CCl_4	Carbon tetrachloride
COX-2	Cyclooxygenase 2
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric (Fe^{3+}) reducing antioxidant power
GSH	Glutathione
IC_{50}	Half maximal inhibitory concentration
MTT	4,5-dimethylthiazol-2-yl
MDA	Malondialdehyde
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
NASADS	Non-steroidal anti-inflammatory drugs
NSCLC	Non-small cell lung cancer
OCED	The Organization for Economic Cooperation and Development
PGE2	Prostaglandin E2
STZ	Streptozotocin
SOD	Superoxide dismutase
TNF-α	Tumor necrosis factor alpha
WEP	Wild edible plant

Abstract

Analgesic and Antinflammatory activities of Root extract of *Grewia schweinfurthii* and its Constituent and Determination of Nutritional and Antinutritional compositions of its fruit

Abdi Leta

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The utilization of current treatments can often result in harmful side effects, and cause patients to experience pain and inflammation. In Ethiopian, traditional healers have relied on medicinal herbs, such as *Grewia schweinfurthii*, which possess analgesic and anti-inflammatory properties. Despite their widespread use, studies regarding the nutritional content of wild edible plants (WEPs) in low-income nations like Ethiopia are limited. This study aimed to evaluate the analgesic and anti-inflammatory effects of 80% methanolic root extract of *G. schweinfurthii* and its chemical constituents, as well as to examining the nutritional and antinutritional content of its fruit. The root extract of *G. schweinfurthii* and its constituents were evaluated for their analgesic activity using acetic acid-induced writhing and hot plate tests. The root extract was subjected to column chromatography (silica gel) to isolate a compound coded as AL-03 and tentatively identified as 4-(2"-(4'-isopropylphenyl) propan-2"-yl)-2,3-dihydrofuran based on spectroscopic (^{1}H, ^{13}C NMR & HSQC) data. The root extract *G. schweinfurthii* demonstrated significant analgesic activity ($p < 0.001$) in the acetic acid-induced writhing test at doses of 200 and 400 mg/kg. Additionally, in the hot plate method, a dose of 400 mg/kg of root extract showed noteworthy analgesic activity ($p < 0.001$). In the carrageenan-induced acute inflammation model, all doses (100, 200, 400 mg/kg) of the root extract resulted in a significant decrease in paw edema compared to the negative control. Compound AL-03 also exhibited antinflammatory activities in a dose-dependent manner against carrageenan-induced paw edema.

Proximate analysis was used to assess various nutritional parameters of *G. schweinfurthii* fruit, including crude protein, fat, total ash, crude fiber, and carbohydrates. In this regard, *G. schweinfurthii* fruit revealed the following composition: moisture (4.09 ± 0.10%); crude protein (11.24 ± 0.68%); fat (1.99 ± 0.01%); total ash (5.36 ± 0.20%); crude fiber (32.50 ± 0.50%); carbohydrate (44.82 ± 0.82%); and total energy (242.12 ± 1.72 kcal). Furthermore, phytate (62.97 ± 0.83%), tannin (3.97 ± 0.12%), and oxalate (0.28 ± 0.03%) were identified as antinutritional components. Overall, *G. schweinfurthii* fruit was found to be a reliable source of nutrients and bioactive compounds. The findings support the traditional medicinal use of the plant for analgesic and antinflammatory activities. Further research is recommended to explore additional bioactive compounds from the root extract of *G. schweinfurthii* and to analyze the mineral composition, such as amino acids, and vitamin C content of *G. schweinfurthii* fruit, as well as to conduct sub-acute and chronic toxicology testing.

Key words: *Grewia schweinfurthii*, analgesic, antinflammatory, hot plate, carrageenan-induced paw edema, nutritional, antinutritional

1. Introduction

1.1. Background

Pain is an unpleasant sensory and emotional experience related to or resembling an actual or potential tissue injury (Tamrat *et al*., 2017). Despite being an unpleasant sensation, it offers protective advantages by serving as a warning indicator of a problem or hazard (Ashagrie, 2023). In the US, there are almost 100 million people who have chronic pain. An estimated $560 to $635 billion in medical expenses and lost productivity are a result of this illness every year (Hoeksema & Hobbs, 2013). Globally, pain is becoming a bigger issue. According to estimates, 10% of adults receive a new diagnosis of chronic pain every year, while 20% of adult's worldwide report experiencing some form of pain (Ayanaw *et al*., 2023).

Nociceptive pain and neuropathic pain are the two main classifications used to categorize pain. Nociceptive pain is caused by regular neural activity in response to real or probable tissue injury, as in the case of surgical pain, osteoarthritis-related pain, or mechanical low back pain. Neuropathic pain is pain that develops as a result of nerve damage, as is the case with unpleasant diabetic peripheral neuropathy, central post-stroke pain, and post-herpetic neuralgia (Beal & Wallace, 2015; Rosenblum *et al*., 2008;).

Inflammation is a process of the body's immune defenses in response to an attack, the goal of which is to eliminate the pathogen and repair tissue damage. At the tissue level, the inflammatory response is characterized by increased vascular permeability, increased protein denaturation, and alteration of cell membranes (Obiang *et al.,* 2021). Excessive inflammation contributes to many acute and chronic human diseases, including rheumatoid arthritis, atherosclerosis, psoriasis, inflammatory bowel disease, retinitis, and multiple sclerosis (Sagnia *et al.,* 2014).

Acute inflammation is a quick reaction to a harmful substance. It is distinguished by fluid and plasma protein exudation as well as a buildup of mostly neutrophilic leukocytes. It has a minimally helpful effect, especially when faced with pathogenic difficulties; instead, it makes infections worse (Hoeksema & Hobbs, 2013). Chronic inflammation is defined as a sustained inflammatory process (weeks or months) in which active inflammation, tissue damage, and healing efforts are occurring at the same time (Ayanaw *et al.*, 2023).

Pain and inflammation are symptoms of numerous diseases. Opiates and nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used in these situations. However, after taking these drugs, users have reported side effects such as dependence, kidney damage, respiratory depression and gastrointestinal disturbances (Karthik *et al*., 2022). Opioids, non-opioid analgesics (mainly non-steroidal anti-inflammatory drugs), antidepressants, anticonvulsants, cannabinoids, and topical agents are medications used to treat both chronic and acute pain (Beal & Wallace, 2015).

There are numerous anti-inflammatory drugs on the market such as NSAIDs, COX -2 selective inhibitors (e.g., celecoxib, rofecoxib, valdecoxib, etoricoxib, and lumiracoxib), glucocorticoids, and antihistamines, that are used to treat different types of inflammatory diseases. However, the side effects of currently available anti-inflammatory drugs pose a major problem for their clinical use. For example, due to their non-selective inhibition of both isoforms of the enzyme COX, NSAIDs cause several serious adverse consequences such as gastric injury and ulceration, renal damage, and bronchospasm (Masresha *et al*., 2012; Yonathan *et al.*, 2006).

A variety of plants are increasingly being used to treat pain and inflammation (Paviaya *et al.*, 2013). There are several plants species reported to have promising antinflammatory activity Some examples are *Alstonia scholaris* (Oguntibeju, 2018); *Carica papaya, Eremomastax*

spectiosa, Eleusine indica, *Cassia alata* (Sagnia *et al.,* 2014); *Genista quadriflora* (Boubekri *et al.,* 2014); *Ipomoea palmata* (Pongprayoon *et al.,* 1991); *Maytenus senegalensis, Mondia whitei* (Matu & Van Staden, 2003).

There are around 800 million individuals worldwide who suffer from chronic undernourishment. Undernutrition (including deficiencies in micronutrients as well as macronutrients) affects an estimated two billion individuals who are deficient in micronutrients, which increases their risk of illness and can be a major barrier to economic growth (Gedle, 2015 ; Lvers, *et al*., 2009).

Food insecurity is described as a persistent lack of access to food in the amount and quality needed. The security of food and nutrition is currently a big issue for our planet. There is a serious problem with food security, particularly in sub-Saharan African nations that depend heavily on imports (Duguma, 2020).

Wild edible plants are those growing naturally on farmland and fallow or uncultivated land and have edible components. Throughout human history, various wild edible plants have had a profound impact on various geographic areas of the world (Duguma, 2020 ; Teketay, *et al*., 2010).

In many rural communities around the world, wild vegetables help improve people's health and access to food. According to research, a large number of wild edible plants are abundant suppliers of one or more nutrients, including proteins, carbohydrates, vitamins, fiber, fatty acids, and minerals. Some of them additionally contain sizeable amounts of a range of health-promoting chemicals, like phenolic compounds, in addition to the nutritional components (Sir Elkhatim *et al*., 2018; Zhang *et al*., 2018; Seifu *et al*., 2017; Lulekal *et al*., 2011; Dansi *et al*., 2008; Tesfaye, 2007; Pieroni *et al*., 2007 ; Cavender, 2006).

In Africa, little is known about the underutilized and untapped plant resources that are employed in the food chain or in traditional medicine to treat various diseases (Adebiyi *et al*., 2015).

It is generally known that people experiencing extreme food shortages during natural and man-made calamities may end up becoming incredibly dependent on wild food plants to survive. While traditional agriculture is working hard to increase food production, a lot of attention is now being paid to the potential of utilizing the enormous quantities of less recognizable plant materials that are present in the wild (Elhassan & Yagi, 2010).

1.2. The genus *Grewia*

The genus *Grewia* is one of the genera found in the family of Malvaceae (Sub Famy. Tiliaceae). It was established by Linnaeus in 1737 in honor of Nehemiah Grew, an English physician and plant anatomist (Narayanaswami and Rao, 1950).

The genus *Grewia* (Malvaceae) has about 321 species of small trees and shrubs. It is distributed in the tropical and subtropical regions of the old world, namely, Tropical Africa, Madagascar, Arabia, India, China, Thailand, the Pacific islands, and North Australia (Zeb, 2019; Narayanaswami and Rao, 1950).

Grewia is the only genus in the family that produce edible fruits (Githinji *et al.,* 2020). The genera are one of the best examples of adaptable multipurpose plant species, important sources of food, fodder, fiber, fuel wood, timber, and a wide variety of traditional medicines (Dev *et al.,* 2018).

The edible fruits of certain *Grewia* species, including *Grewia tenax,* are prized for their local commercial value. *Grewia* drupes are well-liked in temperate regions of the world because they have astringent and cooling effects (Bari *et al*., 2019).

As provided in the Flora of Ethiopian and Eritrea, 22 species are found in Ethiopia under the genus *Grewia*. These include:- *G. arborea, G. asiatical, G. bicolor, G. erythraea, G. ferruginea, G. f1avescens, G. forbesii, G. gillettii, G. kakothamnos, G. lilacin, , G. mollisjus, G. ogadenensis , G. occidentalis , G. pennicillata, G. similis, , G. schweinfurthii, G. tristis, G. trichocarpa, G. tembensis, G. tenax, G. velutina, G. villosa* (Haile *et al.*, 2020*;* Mohammed *et al.,* 2018; Hedberg, 1996).

1.2.1. Botanical description of the genus *Grewia*

Members the genus *Grewia* are trees or shrubs. The leaves are simple, alternating, elliptic, oblong, ovate, orbicular, with a border that is whole to crenate or serrate and 3-5 nerves at the base. The stipules might be permanent or quickly detach. Flowers are (4-)5-merous and can be found singly or in axillary, extra-axillary, leaf-opposed, or terminal cymes. Sepals are hooded at the tip, have stellate hairs on the outside, and are glabrous on the inside. Petals are shorter than sepals and can be yellow, white, lilac, purple, or pink. The base frequently has a nectar-producing claw. There are several stamens that emerge from a high receptacle. Each locule in the ovary contains two or more ovules. The ovary has a simple, capitate, lobed, or laciniate stigma. A single fruit or a drupe with four or more lobes has one to four stones (pyrenes), each of which has numerous seeds. Brownish is the color of the seeds (Berhan *et al,*2012; Germishuizenand and Meyer, 2003).

Table 1: Systematic and common names of *Grewia* species found in Ethiopia.

Systematic name	Common (local) name
Grewia bicolor Juss.	Daiyta, Dahita, Dawaita (Konso), Adibi'ato (Afar), Harroreesaa (Oromifa)
Grewia erythraea Schweinfurth.	Chaqlessa (Konso)
Grewia ferrginea Hochst. exA. Rich.	Lenquata (Amharic), Daieta-Damale, Kocheta (Konso), Adibi'ato/fo (Afar); Harroreesaa (Oromifa)
Grewia f1avescens Juss.	Daiyta-arba (Konso), Caamurjji (Oromifa)
Grewia lilacina K Schum.	Kocheta (Konso), Irga harree (Oromifa)
Grewia mollis Juss.	Daiyta (Konso)
Grewia schweinfurthii Burret	Adibi'ato (Afar), Mudhe gure (Oromifa)
Grewia trichocarpa Hochst. exA. Rich	Dawaita, Daiyta, Ahawteta-Daiyta (Konso) Roboy (Tigrigna)
Grewia tembensis Fresen.	Dheekkaa (Oromifa)
Grewia tenax (Forssk.) Fiori	Chaqlessa, Daiyta, Horma-Daiyta, Daieta (Konso), Saarkama (Oromifa)
Grewia villosa Willd.	Qoffisa, Offisa, Hoppissa, Ogomteta (Konso), Agobdie (Tigrigna), Ogomdii (Oromifa)

http://www.the plantlist.org/browse/A/Malvaceae/Grewia/ , (Addis *et al.*, 2013, Bahru *et al.*, 2012, Wondimu *et al*., 2007, Addis *et al.*, 2005, Gemedo-Dalle *et al.*, 2005, Hedberg, 1996).

1.2.2. Ethnopharmacological uses

According to reports, *Grewia* species are used as a traditional medicine to cure a variety of ailments, including malaria, dysentery, diarrhea, typhoid fever, smallpox, cough, and irritable bowel and bladder conditions (Githinji *et al.,* 2020).

In traditional medicine, the leaf of *G. ferruginea* is used to treat intestinal parasite infestation, renal infection, dandruff, constipation, and retention of fetal membrane. Moreover, *G. ferruginea* bark is utilized for washing hair (Tessema & Wubneh, 2020; Tura *et al*., 2017).

The dried fruits from *G. bicolor* are eaten as sweets, the roots are utilized as a poultice to treat infected skin sores, and a decoction is given to humans and occasionally cattle to help them remove their placentas. Moreover, the root is a sedative (Mohamed, 1990; Jasper *et al.,* 1986).

G. mollis is used to treat chronic illnesses and the discomfort they cause in Nigerian traditional medicine. For tiny skin cuts, body sores and snake bites, extracts of the bark and leaves are administered. The Yoruba people of Nigeria traditionally utilize the bark's mucilage to ease childbirth, while the leaf, bark, and root are eaten to treat cough and fever (Adamu & Adebayo, 2020).

G. tiliaefolia has long been used in India to treat chronic wounds, gastric ulcers, burning, itching, and other allergic conditions (Khadeer Ahamed *et al.,* 2010). In Bangladesh, *G. paniculata* has long been used as a remedy for wound healing, heat stroke, dyspepsia, colds, diarrhea, and fever, and as an insecticide (Nasrin *et al.,* 2015). *G. asitica* in India is used to alleviate blood disorders, inflammation, cardiac and respiratory diseases (Khattab *et al.*, 2015).

In Afar, Yalo Werda Ethiopia, *G. erythraea* is traditionally used for head wounds, warts, flue, typhoid, broken bone, dyspepsia, and arthritis (Teklehaymanot, 2017). *G. asiatica* (phalsa) has

been used in the treatment of a diverse array of medical conditions such as diarrhea, jaundice, stomach upsets, intestinal infection, cough, fever, wound healing, skin diseases, and osteoporosis (Mehmood *et al.,* 2020).

1.2.3. Pharmacological activities

1.2.3.1. Anticancer activities

The cytotoxic effect of the benzene extract leaf of G. *tiliaefolia* was evaluated by MTT assay and it significantly inhibited the proliferation of human (NSCLC) A549 cells in a concentration and time-dependent manner. Cytotoxicity against A549 cells was only at a maximum concentration (500 µg/ml) with the IC_{50} of 192.57 ± 5.22 and 121.12 ± 3.44 µg/ml at 24 and 48 h respectively (Rajavel *et al.,* 2017).

1.2.3.2. Free radical scavenging activity

The free radical scavenging activity of compounds isolated from the methanol root extract of *G. optiva* was determined through DPPH and ABTS assays. Compounds 3,5 dihydroxy phenyl acrylic acid (28) and 2,5 dihydroxyl phenyl (3',6',8' trihydroxy-4H chromen-4'-one (29) showed the highest scavenging capabilities (85.21±1.10 and 84.13±2.1 at 1000 µg/mL) of DPPH radical with IC_{50} values of 65 and 64 µg/mL, respectively, followed by compound glutaric acid (27) (IC_{50}=72 µg/mL, percent inhibition = 82.13±1.21). The compounds that most potently scavenged ABTS-free radicals were 3,5 dihydroxy phenyl acrylic acid (28) and 2,5 dihydroxyl phenyl (3',6',8' trihydroxy-4H chromen-4'-one (29) again showing excellent percent inhibition of 84.13±2.11 and 86.13±2.31 at 1000 µg/mL, respectively, with an IC_{50} of 67 and 65 µg/ml. Ascorbic acid was used as a positive control, and its percent inhibition was 94.88±1.63 at 1000 µg/mL with IC_{50} value of about 35 µg/ml (Bari *et al.,* 2019).

1.2.3.3. Analgesic and antinflammatory activity

A study by Paviaya *et al*., (2013) showed the methanolic and aqueous extracts of *G. asiatica* have a significant inhibition of writhing response compared to standard (at 400 mg/kg extract, 41.14% and 46.24%, respectively, and for indomethacin, 36.04%), an increase in hot plate reaction time compared to standard (at 400 mg/kg extract, reaction time increased from 4.00 to 12.37 s and 3.40 to 12.00 s, respectively, and for pentazocine reaction time from 4.80 s to 13.00 s), and also caused a decrease in paw edema compared to standard (at 400 mg/kg extract, the percent inhibition of 59.14 and 53.04%, respectively, and for indomethacin, 64.02%).

1.2.3.4. Wound healing

The wound healing activities of the methanolic extract of *G. tiliaefolia* stem bark and the isolated constituents of gluonic acid γ-lactone were tested using three different cutaneous models: excision, incision, and dead space wounds in Wistar rats. The extract, gluonic acid γ-lactone, and standard reference drug nitrofurazone-treated animals showed significant reductions in the wound area (95.71, 97.06, and 94.02% of wound contraction, respectively) with an 18.40 ± 0.16, 18.62 ± 0.21, and 18.14 ± 0.25 faster rate of epithelialization, respectively, compared with control 79.53 ± 0.97 wound contraction and 22.59 ± 0.15 epithelization. The methanol extract and gluonic acid γ-lactone showed significant increases in breaking strength (565.10 ± 5.56 and 561.12 ± 5.18, respectively) compared with the reference drug Nitrofurazone (588.71 ± 2.96) (Ahamed *et al*., 2008).

1.2.4. Phytochemistry

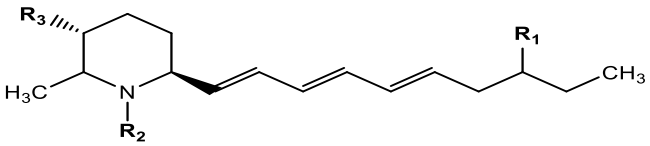
The crude methanol root extract of *G. nervosa* fractionated by n-butanol resulted in the isolation of four alkaloids namely N-Methyl microcosamine **(1)**, Microgrewiapine **(2)**, Homomicrogrewiapine **(3)** and Microcosamine **(4)** (Palareti *et al.,* 2016). The methanol root extract of *G. bicolor* resulted in the

isolation of 3 alkaloids namely harman (**5**), 6-methoxyharman (**6**) and 6-hydroxyharman (**7**) (Githinji *et al.,* 2020). The bioassay-directed fractionation of the $CHCl_3$-soluble fraction of the MeOH extract prepared from a sample of the combined leaves, twigs, and stems of *G. bilamellata* led to the isolation of Grewin **(8)**, Bilagrewin **(9)** and Nitidanin **(10)** (Ma *et al.,* 2006). Chemical investigation of n-butanol extract from the methanol extract of leaves of *G. damine* lead to the isolation of flavone C-glycosides vitexin **(22)** and isovitexin **(23)** (Jayasinghe *et al.,* 2004)**.**

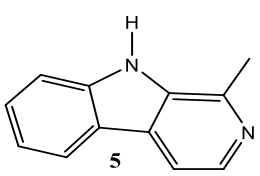
The crude methanol root extract of *G. optiva,* fractionated by ethyl acetate, resulted in the isolation of betulinic acid **(14)** (Bari *et al.,* 2019). The bioassay-directed fractionation of the $CHCl_3$-soluble fraction of the MeOH extract prepared from a sample of the combined leaves, twigs, and stems of *G. bilamellata* led to the isolation of 3R,20-lupandiol **(11)** and 2R,3â-dihydroxyolean-12-en-28-oic acid **(12)** (Ma *et al.,* 2006). The crude ethanolic extract of the stem bark of *G. optiva* fractionated by petroleum ether-ethyl acetate resulted in the isolation of four triterpenoids butelin (**13**), betulinic acid (**14**), oleanolic acid (**15**), ursolic acid (**16**) (Uddin, 2017). The CH_2Cl_2: MeOH (1:1) steam bark extract of *G. plagiophylla* eluting with hexane-ethyl acetate resulted in the isolation of three triterpenoids stigmasterol (**18**), butelin (**13**) and lup-20(29)-en-3-ol (**17**) (Githinji *et al.,* 2020).

The crude methanol root extract of *G. optiva* fractionated by ethyl acetate resulted in the isolation ofβ- sitosterol **(19)** (Bari *et al.,* 2019). The benzene leaf extract of *G. tiliaefolia* further fractionated by 10% ethyl acetate in petroleum ether and 30% acetone in chloroform respectively resulted in the isolation of two phytosterols β- sitosterol (**19**) and daucosterol (**20**) (Rajavel *et al.,* 2017).

The crude methanol root extract of *G. optiva,* fractionated by ethyl acetate resulted in the isolation of 7-O-methyl cathachin **(21)** (Bari *et al.,* 2019)**.** The bioassay-guided fractionation of methanolic extract of the *G. tiliaefolia* bark has resulted in the isolation of D-erythro-2-hexenoic acid γ-lactone (EHGL) **(24)** and gulonic acid γ-lactone (GAGL) **(25)** (Khadeer Ahamed *et al.,*2010)**.** The methanol extract of the leaves of *G. hirsuta* fractionated by chloroform resulted in the isolation of the (4Z, 12Z)-cyclopentadeca-4,12-dienone (**26**) (Natarajan *et al.,* 2015). The methanol root extract of *G. optiva* and chloroform and ethyl acetate fractions gave four compounds: glutaric acid (**27**), 3,5 dihydroxy phenyl acrylic acid (**28**), (2,5 dihydroxy phenyl) 3',6',8'-trihydroxyl-4H chromen-4'-one (**29**) and hexanedioic acid (**30**) (Bari *et al.,* 2019).



1. R_1 – OH R_2 – CH_3 R_3 - OH
2. R_1 – H R_2 – CH_3 R_3 - OH
3. R_1 – OH R_2 – CH_3 R_3 - CH_3OH
4. R_1 – OH R_2 – H R_3 - CH_3OH



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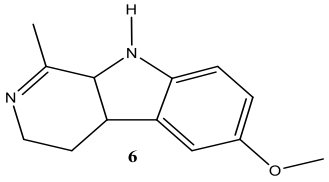

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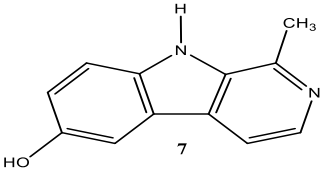

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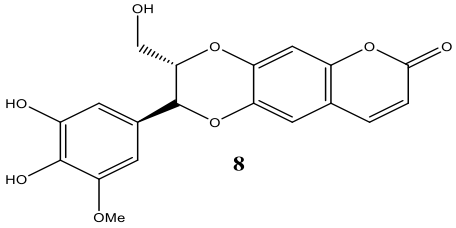

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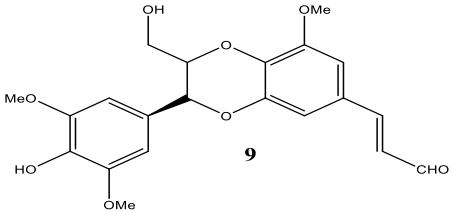

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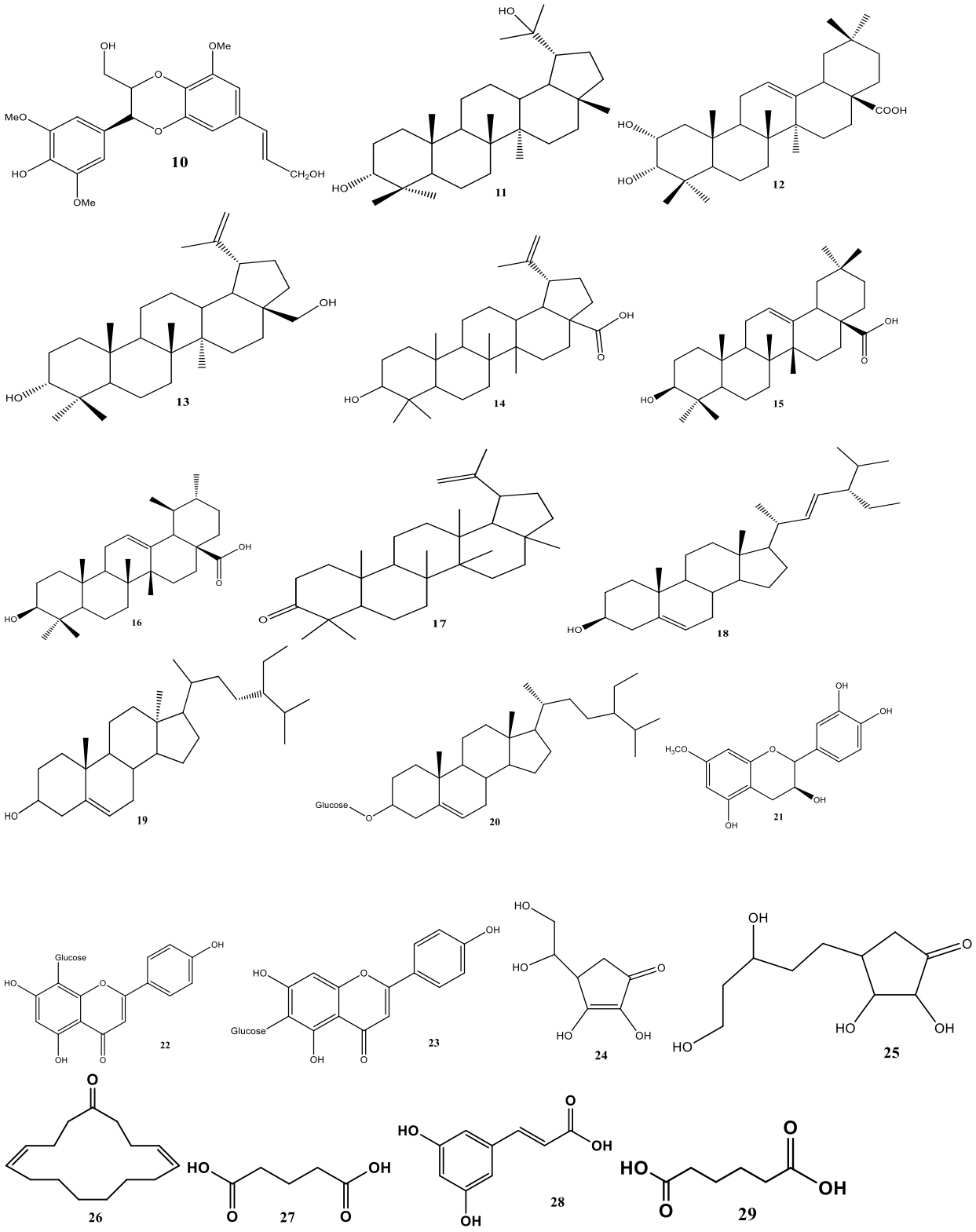

Figure 1:Chemical structure of compounds isolated from the genus *Grewia*

1.2.5. *Grewia schweinfurthii* Burret.

Morphological features: *G. schweinfurthii* Burret: is a shrub that can grow as tall as 4 meters. Round branches with dark red to greyish undertones, pubescence in the young, and pale lenticels. The leaf blade is oblong to trilobed, uniformly green, with stellate hairs on both sides, the apex is acute to obtuse; the base is rounded to truncate; and the border is crenate-serrulate. Inflorescences that are 1.5–2.5 cm long, 3-flowered, and 2-3 together terminal and/or solitary leaf-opposed. Yellow, elliptic-oblong to obovate, 7–11 mm long petals with claws that produce nectar. Yellow stamens. Two-locular ovary with eight ovules in each locule; subulate, four-branched stigma. Fruits are solitary, 4–8 mm long, and coated in a few long hairs; the stone is rugulose (Hedberg, 1996).

Habitat: Open grassland and Acacia-wooded terrain between basalt rocks as well as on rocky limestone slopes between 600 and 1600 meters. It can be found in Somalia, Ethiopia, and Yemen (Hedberg, 1996).

Figure 2: Image of *G. schweinfurthii* in its natural habitat

1.2.6. Ethnopharmacological use of *G. schweinfurthii*

G. schweinfurthii Burret: The leaf/stem and root of *G. schweinfurthii* are used as antimalarials in the Afar region of Ethiopia in the Awash-Fentale district. The root is used in various parts of Ethiopia to treat colds and severe coughs. The plant is also known for its edible fruits in various regions of Ethiopia, including Tigray, SNNPR, and Oromia (Teklehaymanot, 2017, Seta *et al*., 2014, Bahru *et al*., 2014, Addis *et al.,* 2013).

1.3. Statement of the problem

Pain and inflammation remain a major public health concern globally, affecting 80% of adults, despite the availability of medications (Yimer *et al*., 2020). This issue is viewed as the primary clinical, social, and economic problem in most communities around the world (Calati *et al*., 2015). Medicinal plants claimed to have analgesic and anti-inflammatory uses are being researched because the standard drugs have severe side effects and toxicity, such as gastric irritation, gastric ulcers and many others. For instance, NSAIDs result in an increased risk of cardiovascular adverse events, especially in patients taking COX-2 inhibitors (Yimer *et al*., 2020, Geremew *et al*., 2015). Opioid analgesics are also linked to harmful side effects, such as drowsiness, nausea and vomiting, pruritus, constipation, disruption of hormonal equilibrium, hearing loss, tolerance, physical dependency, addiction, and respiratory issues (Tamrat *et al*., 2017). Therefore, researchers are pursuing the development new analgesics and anti-inflammatory agents, particularly from medicinal plants.

Wild edible plants (WEPs) are also an alternative and nutritious source of food, particularly significant in sub-Saharan Africa (Duguma, 2020). *G. schweinfurthii* is a medicinal plant used to treat cough, cold and malaria in Ethiopia; it also has edible fruits. This study is aimed to evaluate

analgesic, antinflammatory, nutritional and antinutritional properties of *G. schweinfurthii* and its major constituents.

1.4. Significance of the study

The current investigation on the analgesic, anti-inflammatory properties, proximate composition, and antinutritional content of the root and fruit extracts and key components of G. schweinfurthii can offer valuable insights into the chemistry of the plant as well as its analgesic and anti-inflammatory properties. Consequently, the purpose of the research endeavors was to support the traditional medical claim that the herb has analgesic and anti-inflammatory properties. Also, the discovery will guide and provide background knowledge for people interested in the chemistry of natural products as well as analgesic and anti-inflammatory agents. These findings would also fill the knowledge gap regarding the proximate composition and antinutritional content present in *G. schweinfurthii* and thus be helpful in quantifying consumption and improving food security.

2. Objectives

2.1. General objectives

- To evaluate the analgesic, and antinflammatory activities of 80% methanolic extracts and isolated compound of *G. schweinfurthii* and determination of nutritional and antinutritional composition of its fruit.

2.2. Specific objectives

- To evaluate anti-inflammatory activity of the 80% methanolic root extract *G. schweinfurthii*
- To evaluate analgesic activity of the 80% methanolic root extract *G. schweinfurthii*
- To isolate compound/s from *G. schweinfurthii*
- To determine proximate composition of fruit of *G. schweinfurthii*
- To determine the antinutritional content of fruit of *G. schweinfurthii*

3. Materials and Methods

3.1. Materials

3.1.1. Plant material

The root and fruits of *G. schweinfurthii* were collected from Awash National Park located about 231.3 km east of Addis Ababa, Ethiopia in June 2022. Taxonomic identification was done by Mr. Melaku Wondafrash at National Herbarium, College of Natural and Computational Sciences, Addis Ababa University.

3.1.2. Chemical, reagent and drug

Methanol, n-hexane, ethyl acetate, chloroform (all from Sigma-Aldrich Co., MO, USA) were used for extraction and chromatography. Pre-coated silica gel 60 F_{254} plates (aluminum backed, 200 μg, Merck KGaA, Darmstadt, Germany) were used for analytical TLC, and silica gel F_{254} was used for preparative TLC, carrageenan (Sigma Chemicals Co., St Louis, USA), normal saline(H. R. Leuven, Belgium), distilled water, glacial acetic acid (Sigma-Aldrich laborchemikalien, Germany), indomethacin (Cadila pharmaceuticals Ethiopia), aspirin, morphine (Ethiopian Pharmaceutical Manufacturing Factory, Ethiopia), sulfuric acid (Fisher Scientific, UK), K_2SO_4, $CuSO_4$, NaOH, HCl, H_2O_2, H_3BO_3, Diethyl ether, porcelain dish, muffle furnace, , celite, $FeCl_3$. $6H_2O$, Sulfosalcilic acid, HCl were used in the experiment.

3.1.3. Instrument

Digital plethysmometer, hot plate and electronic balance (Orchid Scientific, India), rotary evaporator (Heidolph, Germany), UV spectrophotometer, nuclear magnetic resonance spectrometer (Bruker Avance DMx400 FT-NMR spectrometer), drying oven, desiccator, tongs, spatula, burette, fume hood, pipette, conical flask, volumetric flask, glass cuvette, test tubes,

beaker, soxhlet, extraction chamber, crucible holder boiling chips , centrifuge, digester, defatted cotton, rack , tecator rack, vortex mixer were used in this experiment.

3.1.4. Experimental Animals

Healthy Swiss albino mice (6–8 weeks old) of both sexes weighing 25–35 g and Wistar albino rats (6–8 weeks old) of both sexes weighing 200–250 g were used for the experiment. Animals were housed under standard environmental conditions in plastic cages at room temperature with a 12-h light-dark cycle and had unlimited access to a standard pelleted diet and water. To minimize stress, animals were acclimated to laboratory conditions before the start of the experiment. Animals used in this study were handled according to internationally accepted standard guidelines for the use of laboratory animals (Couto & Cates, 2019).

3.2. Methods

3.2.1. Preparation of plant material

The roots of *G. schweinfurthii* were cleansed and air-dried for two weeks at room temperature, then crushed into a coarse powder. The powdered root (400 g) was macerated with 80% methanol (3 lt) for 72 h with occasional stirring. Upon completion of the extraction, the extract was filtered through a filter paper. The marc was re-macerated twice with the same solvent, and the filtrates were combined and concentrated *in vacuo*.

G. schweinfurthii fruits were also cleansed and air-dried at room temperature, then milled with an electric mill, The powder was sieved through 100-mesh sieves, and stored in polyethylene bag until use

3.2.2. Preliminary phytochemical screening

The 80% methanol root extract of *G. schweinfurthii* was subjected to phytochemical screening to determine the presence or absence of secondary metabolites such as cardiac glycosides, tannins, saponins, terpenoids, flavonoids, anthraquinones, and steroids using standard procedures.

3.2.3. Isolation of compound

In the column chromatographic process, the column was initially packed with $CHCl_3$-silica gel slurry. The adsorbed sample was prepared by mixing the root extract (2 g) and silica gel (2 g) in MeOH. The mixture was then allowed to dry using a Rotary evaporator. The adsorbed sample was placed on the upper part of the column packing. The adsorbed sample (root extract plus silica gel) was subjected to a silica gel column chromatography and eluted with a mixture of $CHCl_3$ and MeOH gradients, resulting 120 fractions (each 10 ml). The fractions were collected as follows: 1–10 Frs, $CHCl_3$ (100%); 11–20 Frs, $CHCl_3$-MeOH (95:5); 21–30 Frs, $CHCl_3$-MeOH (90:10); 31–40 Frs, $CHCl_3$-MeOH (85:15); 41–50 Frs, $CHCl_3$-MeOH (80:20); 51–60 Frs, $CHCl_3$-MeOH (75:25); 61–70 Frs, $CHCl_3$- MeOH (70:30); 71–80 Frs, $CHCl_3$-MeOH (65:35); 81–90 Frs, $CHCl_3$-MeOH (60:40); 91–100 Frs, $CHCl_3$-MeOH (55:45); 101–110 Frs, $CHCl_3$-MeOH (1:1); 110–120 Frs, MeOH (100%). The purity of all fractions was monitored using analytical silica gel TLC and detected under UV light of 254 nm and 366 nm. Fractions 21–30, which displayed a single spot-on TLC ($CHCl_3$: MeOH (6:1)), were combined to yield a amorphous crystalline substance, coded AL-3, which was weighed, transferred to an amber-colored vial, and kept in a refrigerator at 4 °C until used.

3.2.4. Spectroscopic techniques

NMR spectra were recorded on a Bruker Avance DMX 400 FT-NMR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The region from 0 to 12 ppm for ^{1}H and 0 to 205 ppm for

^{13}C was scanned. Signals were referred to as internal standard tetramethylsilane (TMS). Chemical shifts are reported in d (ppm) and coupling constants (J) in Hz. Multiplicities of ^{1}H NMR signals are indicated as s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets) and m (multiplet).

3.3. Acute oral toxicity test

Acute toxicity testing was performed for the crude root extract (single dose of 2000 mg/kg) according to OECD guidelines. A limit dose of 2000 mg/kg was administered to a fasting mouse on the first day, and four additional mice were treated sequentially based on the results observed in the first animal. The animals were monitored closely for signs of poisoning, such as weight loss, diarrhea, tremors, lethargy, and paralysis, during the first four hours of the 24-hour period and then observed daily for 14 days see if there is any mortality. Three dose levels were selected based on data from the acute toxicity tests. The median dose was 200 mg, which was one-tenth of the maximum dose reached in the acute toxicity study. The low dose was half the median dose, or 100 mg, and the high dose (400 mg) was twice the median dose (Couto & Cates, 2019).

3.4. Animal grouping and dosing

Wistar albino rats weighing 200–300 g (for anti-inflammatory activities) and Swiss albino mice of either sex weighing 25–35 g (for analgesic activities) was randomly divided into five groups of six animals each. Group I was used as a negative control and received distilled water. Group II served as positive control and received standard drugs such as aspirin (150 mg/kg) for acetic acid-induced writhing test, morphine (10 mg/kg) for the hot plate test and indomethacin (10 mg/kg) for carrageenan-induced paw edema. The other three groups (test groups) received various doses (100, 200, and 400 mg/kg) of the 80% methanol extract orally. The pure

compound at concentrations of 10, 20, and 40 mg/kg was used for carrageenan-induced paw edema (antinflammatory activities).

3.5. Evaluation of analgesic activities of the extract

3.5.1. Acetic acid-Induced writhing test

The method described, by Ayanaw *et al* (2023) was used to determine the peripheral analgesic properties of the extract. Mice were randomly divided into five groups (six mice per group), each with free access to water and fasted overnight. One hour before intraperitoneal injection of acetic acid (0.6% v/v) (10 ml/kg), mice of both sexes received 100, 200, or 400 mg/kg of crude extract, distilled water (negative control), and aspirin 150 mg/kg (positive control) orally. Five minutes after the injection of acetic acid, the analgesic effect of the extract (extent of writhing), measured based on the contraction of abdominal muscles and extension of hind limbs for 20 minutes. The analgesic potential of the extract was demonstrated by a reduction in the number of writhes compared to that of the control group, and expressed as a percentage inhibition of writhing as follows.

$$\%\text{inhibition} = \frac{\text{mean writhing count (control group} - \text{treated group)}}{\text{mean writhing count control group}} \text{ x } 100$$

3.5.2. Hot plate method

This test was performed to determine if the extract of *G. schweinfurthii* has the potential as a central analgesic. In this experiment, each mouse was placed in an open-ended cylindrical chamber with a bottom made of a metal plate maintained at a constant temperature of 55°C. Two behavioral responses elicited by the hotness of this plate, paw licking and jumping, are considered supra-spinally integrated responses and can be evaluated by their response times. After one hour of oral administration of the conventional drug, distilled water, and 100, 200, and 400 mg/kg of the extract, the reaction times of the animals were recorded. To avoid burns on the

paws, the animals were placed on a hot plate with a cut-off time of 15 seconds. The time required to lick the paw or jump off the hot plate was used to evaluate analgesic activity. Response times were noted after 0, 30, 60, 90, and 120 minutes (Ashagrie, 2023).

3.6. Anti-inflammatory activity

3.6.1. Carrageenan-induced paw edema

In vivo anti-inflammatory activity was evaluated on the basis of inhibition of carrageenan-induced rat hind paw edema as previously described by Yimer *et al*., (2020). Prior to the experiment, the rat was fasted for 12 hours but had free access to water. All eight groups (6 rats per group) received an injection of carrageenan into the rat left hind paw (1% w/v carrageenan in normal saline, 100 µl) 30 min after oral administration of crude extract (100, 200, or 400 mg/kg), distilled water, standard drug (10 mg/kg), and AL-03 compound (10, 20, 40 mg/kg). At time points 0, 1, 2, 3, 4, and 5 hours after carrageenan injection, inflammation was quantified in ml by measuring the displacement of water by edema. Percent inhibition of edema was determined compared with control rats according to the following formula

$$\%\ \text{Ihnibition of paw edema} = \frac{(\text{Vt} - \text{Vo})\text{control} - (\text{Vt} - \text{Vo})\text{treated}}{(\text{Vt} - \text{Vo})\text{control}} * 100$$

Where: Vt: is the right hind paw thickness volume (in ml) at time t

Vo: is the right hind paw thickness volume (in ml) before carrageenan injection

3.7. Analysis of proximate compositions

The proximate parameter (moisture, ash, fats, crude, fiber, crude protein) content of the air-dried fruit of *G. schweinfurthii* were determined according to the AOAC, (2016) using sub component of 930.04 (moisture), 930.05 (ash), 2003.06 (fat), 962.09 (fiber) and 984.13 (protein).

3.7.1. Moisture analysis

The moisture content of *G. schweinfurthii* was determined by AOAC method 930.04, (2016) in a triplicate analysis. Five gram of the air-dried sample was heated in oven at 105 ^{0}C for 2 h, cooled in a desiccator, weighed, and the moisture content was calculated according to the following formula.

$$\% \textbf{ Moisture} = \left(\frac{\textbf{W2}-\textbf{W3}}{\textbf{W2}-\textbf{W1}}\right) \textbf{ X 100}$$

Where, W_1= Weight of the crucible, W_2 = Weight of the sample and the crucible before drying W_3= Weight of the crucible and the sample after drying.

3.7.2. Crude protein

Crude protein by Kjeldahl method following AOAC method 984.13 (2016) in triplicate analysis. One gram of the powdered sample was digested with 20 mL of concentrated H_2SO_4 and Kjeldahl catalyst (9 parts K_2SO_4 and one-part $CuSO_4$) in a digestion chamber until it became clear. The blank test was performed without the sample. After digestion, it was distilled in a Kjeldhal distillation chamber. The evaporated ammonia was condensed and then titrated against the known concentration (0.1 N) of HCl. The nitrogen concentration was calculated using the following formula

$$\% \textbf{ Nitrogen } = \frac{\textbf{(V} - \textbf{B) x N x 14 x 100}}{\textbf{1000 x Wo}}$$

% Protein = 6.25 x % Nitrogen

Where: V- Volume of HCl consumed to the end point of titration, N- The normality of the HCl used, Wo- Sample weight on dry matter basis, 14- The molecular weight of the atomic nitrogen.

3.7.3. Crude Fat

Crude fat content was determined by AOAC method 2003.06 (2016) in triplicate analysis. The initial weight of the extraction cylinder was determined by heating in oven overnight at 105 ^{0}C followed by cooling in a desiccator. Three to five gram of the sample was extracted with petroleum ether in a Soxhlet apparatus for about 6 hours. The extracted fat was dried in a rotary evaporator, and the weight was measured.

$$\%\mathbf{Fat} = \frac{\mathbf{W2-W1}}{\mathbf{W}}\ \mathbf{X\ 100}$$

Where, W1 = weight of the extraction cylinder, W2 = weight of the extraction cylinder plus dried crude fat and W= weight of sample

3.7.4. Total Ash

Total ash was determined by AOAC method 930.05 (2016) in a triplicate analysis. Porcelain crucible was cleaned and dried in a muffle furnace at 550 ºC for 30 minutes. Then crucible was cooled in a desiccator and weighed. In the crucible, 5 g of the fruits of *G. schweinfurthii* was placed and charred on a hot plate in a fume hood until no smoke was produced. The ashing process was continued in a muffle furnace at 550ºC for 5 hours. The ash became clean and white. Finally, the ash content was determined using the following calculation.

$$\%\ Ash = \frac{\mathrm{M3-M1}}{\mathrm{M2-M1}}\ \mathrm{X}\ 100$$

Where; M1 = mass of crucible M2 = mass of crucible and sample M3 = mass of crucible and sample after ashing.

3.7.5. Crude fiber

The amount of crude fiber was determined by an official method (AOAC 2016 Method No. 962.09) in a triplicate analysis. 1 g of the dry sample was boiled with 0.25 N H_2SO_4 for 30 min,

then filtered with a gauze cloth, washed with hot water, and boiled again with 0.313 N NaOH. It was again filtered and washed with hot water followed by 0.5N H_2SO_4 and 50% ethanol. The residue was dried in an oven at 130 °C for 2 hours. Dry weight of the digested sample was taken, burned in a muffle furnace at 600 °C for 30 min, cooled in a desiccator, and the weight of the ash was measured. The crude fiber content was calculated based on 100 g of the air-dried sample using the following formula.

$$\% \text{ Crude fiber } = \frac{W3 - W2}{W2 - W1} \text{ x } 100$$

Where: W1= sample weight, W2= crucible and dried sample weightW3 = crucible and sample weight after ashing

3.7.6. Total carbohydrate

Total Carbohydrate content of the *G. schweinfurthii fruit* was determined by the difference method (i.e.by subtracting the sum of percentage of moisture, crude protein, crude fat, crude fiber and ash content from 100%)

3.7.7. Gross energy

The gross energy (GE) content was determined mathematically using the following formula.

Gross energy (Kcal) = (9 x crude fat) + (4 x crude protein) +(4 x utilizable carbohydrate)

(Adebiyi *et al*., 2015)

3.8. Analysis of Anti-nutritional factors

3.8.1. Phytate content

The phytate content of *G. schweinfurthii* fruit was determined according to the method described by Wheeler and Ferrel (1971) in triplicate analysis. 0.03g of dried fruit from a *G. schweinfurthii*

sample was extracted with 10 ml of 0.2 N HCI for 1 hour at ambient temperature. The mixture was centrifuged at 3000 rpm for 30 minutes, and 3 ml of the supernatant was mixed with 2 ml of Wade reagent (a mixture of 0.03% $FeCl_3.6H_2O$ and 0.3% sulfosalicylic acid diluted in distilled water). The mixture was shaken using a vortex mixer. absorbance of the mixture was measured at 500 nm, and the phytate content was calculated from the absorbance difference between that of the blank (3 ml of 0.2N HCI and 2 ml of Wade reagent) and that of the assayed sample. Phytate concentration was calculated using the phytic acid standard curve (5, 9, 18, 27, and 36 ppm), and results were expressed as milligrams of phytic acid per 100g dry weight. The following formula was used to calculate the phytic acid content of the samples.

Phytic acid(µg/g) =[([(AB-SA)- intercept]X 10)/ (Slope x W x 3)]

Where; **AB**: absorbance of blank, **SA**: absorbance of sample, **W**: weight of sample

3.8.2. Tannin content

Tannins were determined using the method explained by Price *et al.* (1987) in triplicate analysis. 0.25 g of *G. schweinfurthii* fruit was added to a screw-capped test tube containing 10 ml of 1% HCl in methanol and kept in a mechanical shaker at 150 rv/min for 24 hours at room temperature. The content of the tubes was centrifuged for 5 minutes. One ml of the clear supernatant was taken and mixed with 5 ml of vanillin HCl reagent in another test tube and allowed to stand for 20 minutes to complete the reaction, followed by reading the absorbance at 500nm. The concentration of tannins was calculated using a standard curve drawn for D-catechin, and the result was expressed as D-catechin equivalent in mg per 100g dry weight.

$$\text{Tannin } \left(\frac{\mathbf{mg}}{\mathbf{g}}\right) = \frac{(\mathbf{As}-\mathbf{Ab})-\mathbf{int}\times\mathbf{10}}{\mathbf{S}\times\mathbf{D}\times\mathbf{W}}$$

Where; **As**: is the sample absorbance, **Ab**: is the blank absorbance, **S**: slope of the absorbance equation **D**: is the density of the solution (0.791 g/ml), **W**: is the weight of the sample in gram, and 10 is the aliquot.

3.8.3. Oxalate analysis

Oxalate was determined using AOAC [2005] method in triplicate analysis. The procedure involves three steps digestion, precipitation and permanganate titration.

3.9. Statistical Analysis

All data obtained were expressed as mean ± SEM (standard error of the mean) and mean ± SD (standard deviation). One-way ANOVA was used in the statistical analysis of the data, and the Tukey Post Hoc Test for multiple comparisons was used to compare the results between groups. At a significance level of $p<0.05$, the results were considered statistically significant. Data were processed using SPSS version 27 software.

4. Result and Discussion

4.1. Acute toxicity

The acute toxicity study finding showed that a dose of 2000 mg/kg of *G. schweinfurthii* root extract did not cause any deaths within the first 24 hours and also in the following 14 days. No obvious symptoms of acute toxicity, such as lethargy, tremor, fatigue, paralysis, autonomic changes, or behavioral changes, were observed in the physical or behavioral characteristics of the experimental mice. Data suggest that LD_{50} of the root extract is greater than 2000 mg/kg.

4.2. Preliminary phytochemical screening

On qualitative phytochemical screening, 80% methanol root extract of *G. schweinfurthii* displayed positive results for tannins, terpenoids, flavonoids, saponins and steroids, while it showed negative results for cardiac glycoside and anthraquinones (**Table 2).**

Table 2: Qualitative phytochemical screening of 80% methanol root extract of *G. schweinfurthii*.

Secondary metabolite Results	Methanol extract
Cardiac glycoside	–
Tannins	+
Saponins	+
Terpeniods	+
Flavonoids	+
Anthraquinones.	–
Steroids	+

4.3. TLC analysis of the isolated compound

Further phytochemical investigation of the root extracts of *G. schweinfurthii* over silica gel column chromatography led to the isolation of one compound (AL-03). Figures below depict the TLC chromatograms of the compound under both UV light of 254 nm and 366 nm.

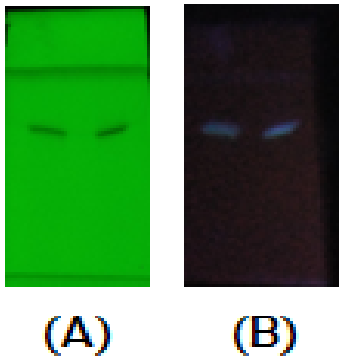

Figure 3: TLC chromatogram of compound AL-03 using $CHCl_{3:}$ MeOH (6:1) as a solvent system, and viewed under UV 254 nm (A) and 366 nm (B).

4.4. Structural elucidation compound AL-03

Compound AL-03 was isolated as a white amorphous, with an R_f-value of 0.7 using $CHCl_{3:}$ MeOH (6:1) as a solvent system. Analysis of the ^{1}H-NMR spectral data of AL-03 indicated the presence of two nearby methylene groups, resonating at δ 4.60 (2H, *t*, H-2, *J* = 8 Hz) and δ 4.44 (2H, *t*, H-3, *J* = 8 Hz). Additionally, the ^{1}H-NMR spectrum of AL-03 showed two sets of equivalent *ortho*-coupling aromatic protons, assignable to H-2'/H-6' (δ7.59, 2H, *d*, *J* = 8.56 Hz) and H-3'/H-5' (δ 7.37, 2H, *d*, *J* = 8.56 Hz), as well as a singlet signal resonating at δ 7.80 (1H, *s*, H-5). The ^{1}H NMR spectrum also displayed a singlet signal at δ 1.32 and integrated for six protons, indicating two equivalent CH_3 groups (δ 1.32, 6H, *s*). Furthermore, a doublet signal resonating at δ 2.47 suggested the presence of two equivalent CH_3 groups (6H, *d*, isopropyl) adjacent to a CH group (δ 1.9, 1H, *s*, isopropyl).

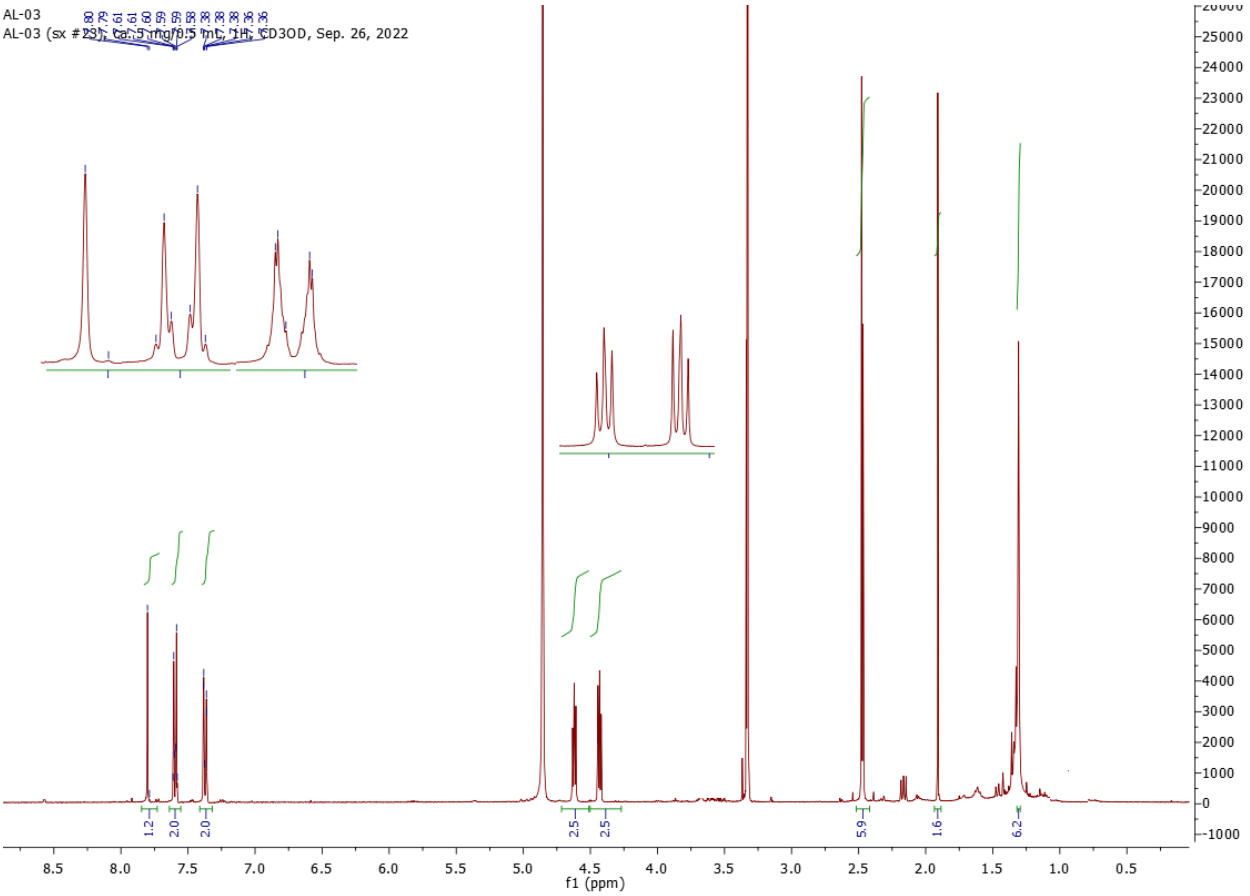

Figure 4: ^{1}H NMR spectrum of compound AL-03

The ^{13}C-NMR spectral data of AL-03 was in a good agreement with the findings from the ^{1}H-NMR spectrum. The presence of a total of sixteen carbons atoms was evident from the ^{13}C-NMR and HSQC spectral data of AL-03, corresponding to 4CH_3 (δ 12.73 (2x), 20.20 (2x)), 2CH_2 (δ 67.8, 44.93), 6CH (δ 29.34, 127.35 (2x), 129.86 (2x), 131.41) and 4 quaternary carbons (δ 31.58, 131.91, 145.72 151.52). A complete list of proton and carbon assignments is presented in **Table 4**.

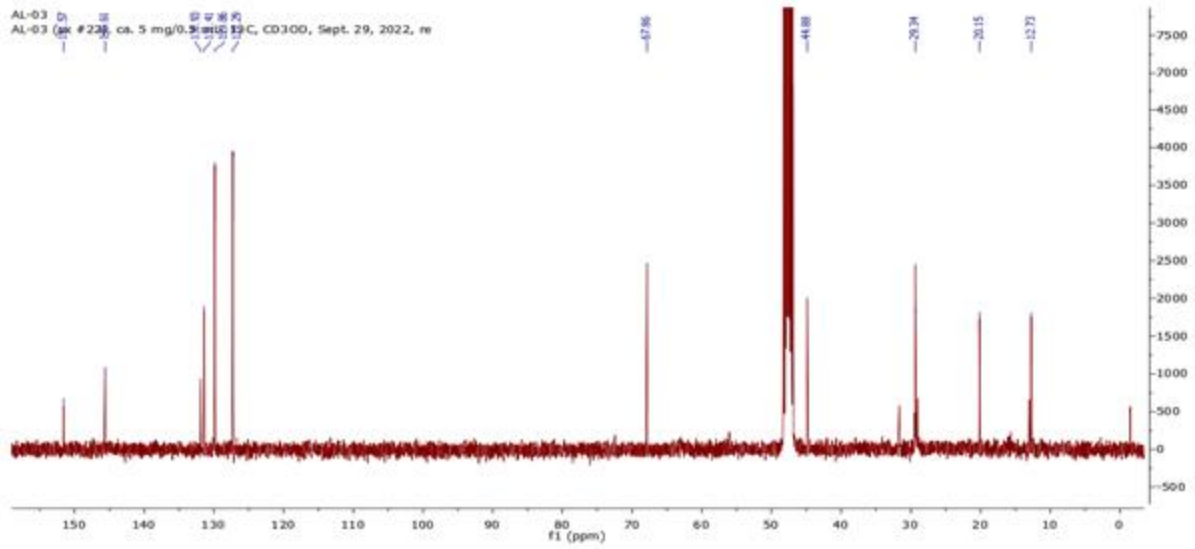

Figure 5: ^{13}C NMR spectrum of compound AL-03

Table 3: ^{1}H NMR and ^{13}C NMR chemical shifts for AL-03

C/H numbering	^{1}H (δ, ppm)	^{13}C (δ, ppm)
2	4.60 *t* (*J* = 8.0 Hz)	67.8
3	4.44 *t* (*J* = 8.0 Hz)	44.93
5	7.80 *s*	131.12
4	-	131.91
1'	-	151.57
4'	-	145.72
2' & 6'	7.59 *d* (*J* = 8.5 Hz)	127.35
3' & 5'	7.37 *d* (*J* = 8.5 Hz)	129.86
1" & 3"	1.32 *s*	29.53
2"	-	32.23
2CH_3 (Isopropyl)	2.47 *d*	12.73, 20.20
CH (Isopropyl)	1.9 *s*	30.03

Note: s-singlet, d-doublet, t-triplet, m-multiplet

From the ^{1}H, ^{13}C-NMR and HSQC spectral data (**Figure 6**), the structure of compound AL-03 was tentatively characterized as 4-(2"-(4'-isopropylphenyl) propan-2"-yl)-2,3-dihydrofuran.

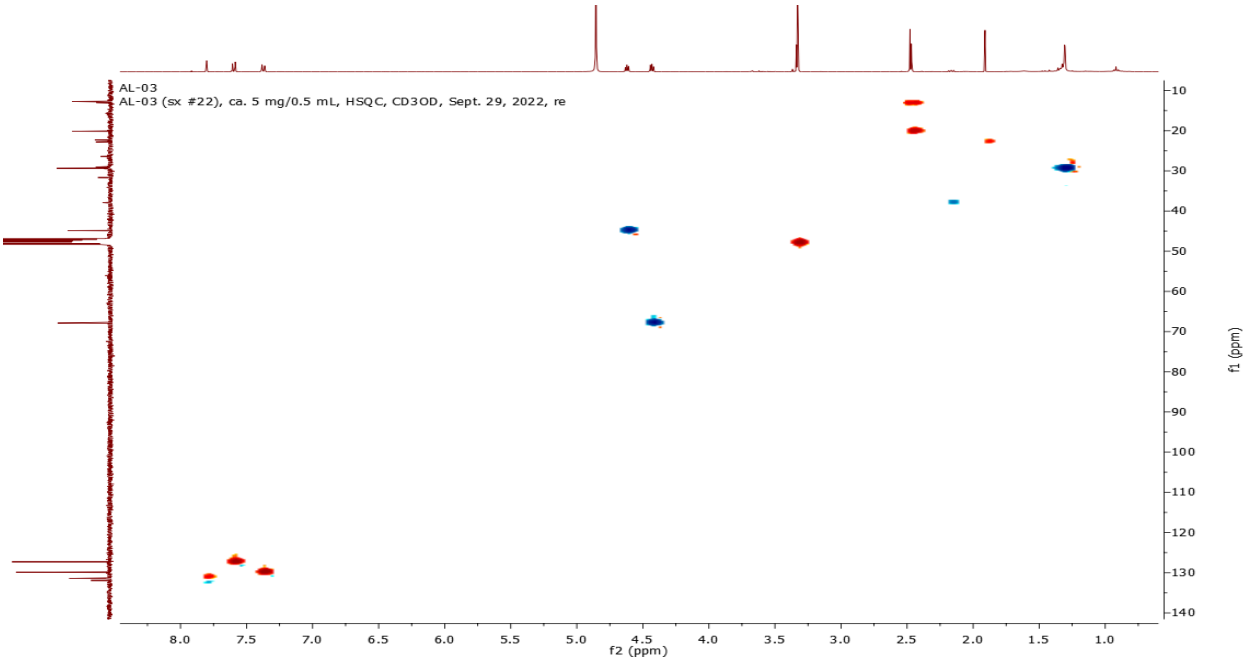

Figure 6: HSQC spectrum of compound AL-03

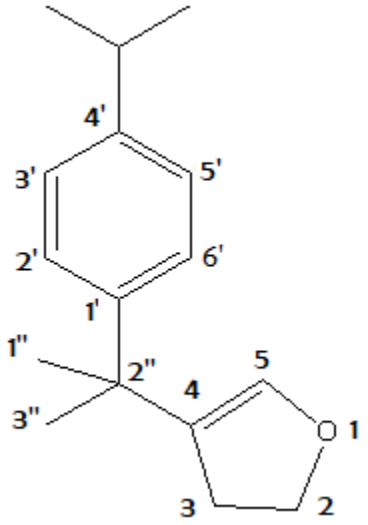

Figure 7 : Tentative structure of AL-03, 4-(2"-(4'-isopropylphenyl) propan-2"-yl)-2,3 dihydrofuran

4.5. Analgesic Activity of the root extract

4.5.1. Analgesic activity of root extract using acetic acid-induced writhing method

The peripheral analgesic properties of the root extract was determined using the acetic acid-induced writhing test (Kundu *et al*., 2022 ; Karthik *et al*., 2022), a model highly recommended for evaluating the natural products' ability to alleviate pain in the peripheral system due to its high sensitivity (Yimer *et al*., 2020). The test stimulates the peritoneal cavity, leading to the release of inflammatory mediators such as histamine, serotonin, and bradykinin that stimulate sensory nerve endings. Additionally, it has been associated with increased levels of PGE2, PGF2, and lipoxygenase products in peritoneal fluid (Karthik *et al*., 2022). During the test, writhing is often characterized by the contraction of the abdominal muscles, extension of the forelegs, and lengthening of the body.

The root extract demonstrated a significant reduction ($p < 0.001$) in the number of writhes in mice at doses of 200 mg/kg and 400 mg/kg when compared to the negative control. Additionally, the higher dose (400 mg/kg) of the extract exhibited a significant difference ($p < 0.001$) in reduction of the number of writhes as compared to the lower and medium doses (100 mg/kg and 200 mg/kg). Conversely, acetylsalicylic acid demonstrated a significant difference ($p < 0.001$) between the 100 mg/kg and 200 mg/kg doses of the extract, but no significant difference was observed at the 400 mg/kg dose of the extract (**Table 4)**.

It is also interesting to note that the present findings match with those of a previous study elsewhere, that both the 400 mg/kg of methanolic and aqueous extracts of *G. asiatica* significantly inhibited twitching ($p < 0.01$), but it did not show any significant peripheral analgesic activities ($p > 0.05$) at lower doses of the extracts (Paviaya *et al*., 2013). The results of the present study suggest that chemical constituents of *G. schweinfurthii*, such as tannins,

saponins, terpenoids, flavonoids and/or steroids, may be responsible for the analgesic effects and could reduce prostaglandin synthesis by inhibiting lipoxygenase and/or cyclooxygenase pathways (Tamrat *et al*., 2017).

Table 4: Effect of 80% methanolic root extracts of *G. schweinfurthii* on acetic acid induced writhing in mice

Group	Number of writhing (mean)	% of Inhibition
DW	25.17 ± 1.815	
Aspirin (150mg/ kg)	4.67 ± 0.422^{a1b1c1}	80.77
GS (100mg / kg)	21.67± 0.76	13.90
GS (200mg / kg)	10.87 ± 0.477^{a1b1}	56.81
GS (400mg / kg)	6.17 ± 0.477^{a1b1c2}	75.48

Note: Values are expressed as Mean ± SEM (N=6); analysis was performed with One-Way ANOVA followed by Tukey post hoc test; [a] against the control, [b]against 100mg, [c] against 200mg; [1] P<0.001, [2] P<0.05. **Abbreviation**: DW-distilled water 10ml/kg; GS100 – *G. schweinfurthii* extract 100mg/kg; GS200 – *G. schweinfurthii* extract 200mg/kg; GS400 – *G. schweinfurthii* extract 400mg/kg.

4.5.2. Analgesic activity of root extract using hot plate method

The hot plate method was employed to measure the central analgesic activity of *G. schweinfurthii* extract in mice. This method was chosen for its sensitivity to strong analgesics, low tissue damage, accuracy of data, and reduced processing time (Yimer *et al*., 2020). In this method, while a plate heated to 55 °C, it elicits two behavioral components, paw licking and jumping, which can be quantified by their reaction times. Both responses are assumed to be supraspinal integrated (Geremew *et al.*, 2015).

The root extract (400 mg/kg, $p<0.05$) and morphine (10 mg/kg, $p<0.001$) demonstrated significant analgesic activities at various time points (30, 60, 90, and 120 min) compared to the negative control. Nevertheless, the root extract at 100 mg/kg did not exhibit significant analgesic activity over time (Table 5). Additionally, at doses of 200 mg/kg and 400 mg/kg, the root extract

significantly increased the pain threshold by prolonging the reaction time, but it required more time to take full effect, which was 120 minutes for all doses. Overall, the increased concentration of active metabolites could be responsible for the much higher activity of 400 mg/kg throughout the observation period.

This result aligns with previous findings on *G. asiatica*, where both the methanolic and aqueous extracts at 400 mg/kg showed a significant ($p < 0.01$, $p < 0.05$) increase in reaction time (Paviaya *et al*., 2013). The proposed mechanism of action of the central analgesic effects of the extract could be inhibition of synthesis of PG, leukotriene, and other endogenous compounds essential for the transmission of central pain (Yimer *et al*., 2020).

Table 5: Analgesic effect of 80% methanolic root extracts of *G. schweinfurthii* using hot plate method

Group	Latency (Sec) ± SEM				
	0 min	30 min	60 min	90 min	120 min
DW	4.67 ± 0.49	4.67 ± 0.67	5.33 ± 0.71	5.00 ± 0.68	4.50 ± 0.43
MO (10mg/kg)	6.67 ± 0.42	12.50 ± 0.43[a1]	12.50 ± 0.42[a1]	12.50 ± 0.43[a1]	10.83 ± 0.60[a1]
GS (100mg/kg)	5.17 ± 0.30	5.83 ± 0.60	6.50 ± 0.43	6.00 ± 0.57	6.00 ± 0.365
GS (200mg/kg)	4.83 ± 0.30	7.33 ± 0.33[a2]	6.50 ± 0.76[a2]	8.00 ± 0.57[a2]	6.83 ± 0.60
GS (400mg/kg)	5.67 ± 0.33	8.83 ± 0.30[a2]	8.33 ± 0.80[a2]	8.50 ± 0.95[a2]	9.17 ± 1.01[a2]

Note: The value expressed as Mean ± SEM (N=6); analysis was performed with One-Way ANOVA followed by Tukey post hoc test; [a] against the control, P<0.001, [2] P<0.05. **Abbreviation**: DW- distilled water 10ml/kg; MO (10mg/kg) – morphine 10mg/kg; GS100 – *G. schweinfurthii* extract 100mg/kg; GS200 – *G. schweinfurthii* extract 200mg/kg; GS400 – *G. schweinfurthii* extract 400mg/kg.

4.6. Anti-inflammatory activities

4.6.1. Anti-inflammatory activities of root extract

Carrageenan-induced paw edema was performed to evaluate the anti-inflammatory potential of the extract in the acute phase of inflammation. Carrageenan induced paw edema is an often-used model to assess the anti-inflammatory effects of medicines and to research the mechanisms

underlying inflammation. The model is an appropriate *in-vivo* paradigm to research the anti-inflammatory effects of natural products since it is linked to several mediators (Geremew *et al*., 2015).

Carrageenan (1% v/v in normal saline) was injected sub planarly into the left hind feet of rats to cause acute inflammation (edema). After administration of carrageenan, local acute inflammation is induced by the sequential release of several endogenous inflammatory mediators. These endogenous mediators are released in a biphasic manner. The first phase (0 - 2.5 hours) after carrageenan induction is mediated mainly by histamine, serotonin, and bradykinin. The late phase maintained by the overproduction of COX -2 and its proinflammatory prostaglandin, with the infiltration of polymorphonuclear leukocytes (neutrophils), occurred (2.5–5 hours) after carrageenan induction. Nitric oxide (NO), superoxide anions (O^{2-}), and hydroxyl radicals (OH^{-}), i.e. oxygen-derived free radicals, are also released and play an important role in the initiation and development of acute inflammation (Ashagrie *et al.*, 2023; Karthik *et al*., 2022; Yimer *et al*., 2020).

At the fifth observation time point, the largest percentage inhibition of edema was observed by all doses of the extract, with the corresponding values being 41.3%, 62%, and 93%. These results confirm that the anti-inflammatory effect of the extract is dose-dependent and that it is very effective in reducing the release of prostaglandin. During the fifth observation time point, the edema inhibitory potential of the higher dose of the extract (400 mg/kg) was comparable to that of the reference drug (indomethacin 10 mg/kg), with respective values of 93.00% and 94.54%. Secondary metabolites found in *G. schweinfurthii* are responsible for the anti-inflammatory properties of the plant (Karthik *et al*., 2022).

This result is consistent with that of *G. asiatica*, where both the methanolic and aqueous extracts showed a significant dose-dependent reduction in paw size in both the initial and late phases of acute inflammation (Paviaya *et al*., 2013). The proposed mechanism of action is related to the inhibition of inflammatory mediators involved in carrageenan-induced inflammation, such as histamine, serotonin, bradykinins, and prostaglandins, as well as nitric oxide, hydroxyl radicals, and superoxide anions, which also contribute to the generation of an inflammatory response (Mohanty *et al*., 2015).

Table 6: Antinflammatory effect of 80% methanolic root extract *G. schweinfurthii* using carrageenan-induced paw edema in rat.

Treatment Mg/kg	Edema Volume (mL)/ Percent Edema Inhibition					
	Baseline	1hr	2hr	3hr	4hr	5hr
DW	1.09± 0.03	1.37± 0.05	1.44± 0.05	1.55 ±0.09	1.62 ± 0.09	1.67 ± 0.06
INDO	1.07± 0.01	1.16± 0.03^{a1} (68%)	1.14± 0.02^{a1} (80%)	1.15± 0.03^{a1} (83%)	1.13± 0.01^{a1} (89%)	1.11± 0.01^{a1} (95%)
GS100	1.05 ± 0.03	1.32± 0.03 (3.5%)	1.30± 0.05 (28%)	1.36± 0.01^{a2} (32%)	1.37± 0.02^{a2} (40%)	1.39± 0.01^{a1} (41%)
GS200	1.06 ±0.03	1.26± 0.03 (28%)	1.24± 0.02^{a2} (48%)	1.26± 0.00^{a2} (56%)	1.27± 0.01^{a1} (60%)	1.28± 0.00^{a1} (62%)
GS400	1.10 ± 0.02	1.18± 0.01^{a2} (71%)	1.18± 0.01^{a1} (71%)	1.17± 0.03^{a1} (84%)	1.15± 0.01^{a1} (90%)	1.14± 0.03^{a1} (93%)

Note: Values are expressed as Mean ± SEM (N=6); analysis was performed with One-Way ANOVA followed by Tukey post hoc test; ^{a} against the control, ^{1}P<0.001, ^{2} P<0.05. **Abbreviation**: DW- distilled water 10ml/kg; INDO – indomethacin 10mg/kg; GS100 – *G. schweinfurthii* 100mg/kg; GS200 – *G. schweinfurthii* 200mg/kg; GS400 – *G. schweinfurthii* 400mg/kg.

4.6.2. Antinflammatory activity of the isolated compound

The anti-inflammatory potential of the isolated compound was investigated using paw edema induced by carrageenan in rats in the acute inflammatory phase. Beginning with the first hour of measurement of the mean increase in paw volume up to 5 hours, both the standard drug and 40 mg/kg of the isolated compound have a statistically significant inhibitory effect ($p < 0.001$ and $p < 0.05$, respectively) compared to the negative control. At the 4-hour and 5-hour measurement of

paw edema, all isolated drug doses (10, 20, and 40 mg/kg) and the standard drug had a significant inhibitory effect ($p < 0.001$) compared with the negative control **Table 7.**

Table 7: Antinflammatory effect of the compound isolated from *G. schweinfurthii* on carrageenan-induced paw edema in rat.

Treatment Mg/kg	Edema Volume (mL)/ Percent Edema Inhibition					
	Baseline	1hr	2hr	3hr	4hr	5hr
DW	1.09 ± 0.03	1.37±0.05	1.44±0.05	1.55±0.09	1.62±0.09	1.67±0.06
INDO	1.07±0.00	1.16±0.03[a1] (68%)	1.14±0.02[a1] (80%)	1.15±0.03[a1] (82%)	1.13±0.01[a1] (88%)	1.11±0.01[a1] (95%)
CX10 mg	1.05±0.02	1.29±0.02 (31%)	1.23±0.02[a2] (35%)	1.28±0.02[a2] (50%)	1.29±0.03[a1] (54%)	1.28±0.04[a1] (60%)
CX20 mg	1.06±0.02	1.27±0.01 (40%)	1.21±0.02[a2] (46%)	1.26±0.02[a2] (56%)	1.27±0.02[a1] (60%)	1.23±0.03[a1] (70%)
CX40 mg	1.06±0.02	1.25±0.01[a2] (45%)	1.20±0.01[a1] (50%)	1.24±0.01[a1] (61%)	1.25±0.02[a1] (64%)	1.21±0.03[a1] (75%)

Note: The value expressed as Mean ± SEM (N=6); analysis was performed with One-Way ANOVA followed by Tukey post hoc test; [a] against the control, [1]P<0.001, [2] P<0.05. **Abbreviation**: DW- distilled water 10ml/kg; INDO – indomethacin 10mg/kg; CX10mg – AL-03 10mg/kg; CX20mg – AL-03 20mg/kg; CX40mg – AL-03 40mg/kg.

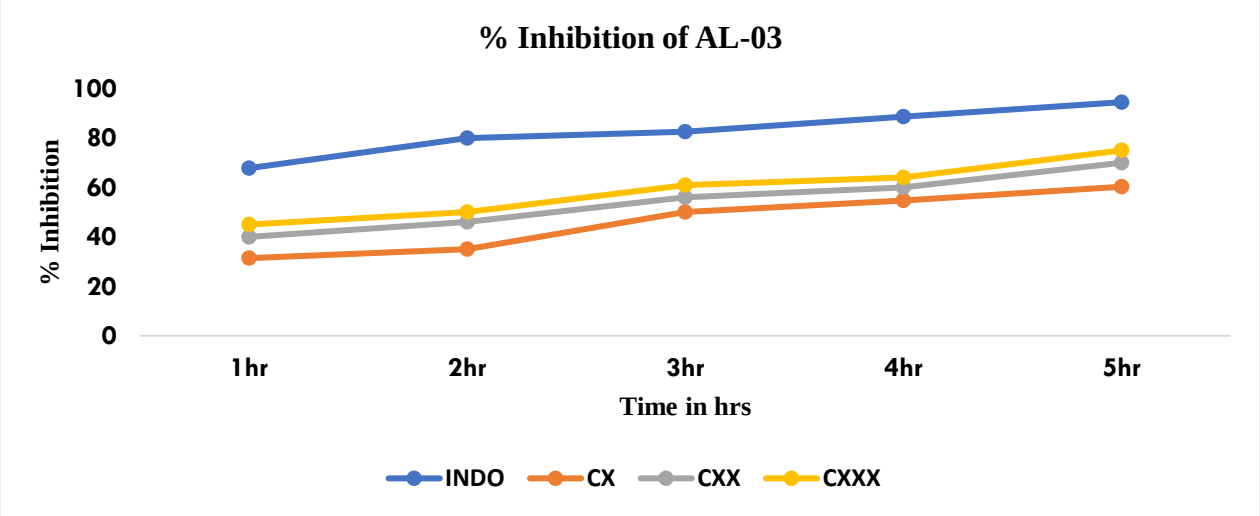

Figure 8: Comparison of percentage suppression of paw edema induced by carrageenan of the isolated compound at different doses CX (10mg/kg), CX (20mg/kg) and CX (40mg/kg) with that of the standard drug indomethacin (10mg/kg)

As shown in **Figure 5**, the maximum and minimum volume reductions were achieved at all doses of the isolated compound and the standard solution at the 5^{th} and 1^{st} h, respectively, of the study period. At the peak of activity (5 h), the percent inhibition for (10, 20, 40 mg/kg) was 60.3%, 70.6% and 75%, respectively. However, the inhibition for the standard drug was 94.5%.

4.7. Proximate composition analysis

The current work showed that *G. schweinfurthii* had a moisture content of (4.09 ± 0.10%) on dry weight basis, which was lower than the value reported by (Elhassan & Yagi, 2010) for *G. tenax* (13 ± 0.17%); *G. flavescence* (15 ± 0.19%) and *G. villosa* (14 ± 0.13%), (Sati & Fatima, 2018) for *G. mollis* (8 ± 0.23), (Tairo, 2021) for *G. forbesii* (14.14 ± 0.65%) and *G. bicolor* (13.98 ± 0.64%), (Islary *et al*., 2016) for *G. sapida* (16.25 ± 0.02%). However, these values were close to the one obtained by (Adebiyi *et al*., 2015) for *G. carpinifolia* (6.32 ± 0.48%). Moisture content affects the shelf life and storability of a food product. Therefore, the low moisture content of *G. schweinfurthii* suggests a longer shelf life and lower microbial contamination. The results of previous studies indicate that WEPs with low moisture content have a longer shelf life and less microbial contamination (Gemede *et al*., 2016).

The protein content found for *G. schweinfurthii* (11.24 ± 0.68%) on dry weight basis is higher than the value reported by (Elhassan & Yagi, 2010) (7.7±0.12 %, 8.7±0.10%, 6.7±0.06%) for *G. tenax, G. flavescence* and *G. villosa* respectively, indicating that they could be relatively good sources of protein. Although this value is lower than the value reported by (Adebiyi *et al*., 2015) for *G. carpinifolia* (18.7±0.06%).

The results of various studies on *Grewia* species revealed low crude fat contents (Elhassan & Yagi, 2010) for *G. tenax* (1.7± 0.39%); *G. flavescence* (1.3± 0.17%) and *G. villosa* (1.5±

0.13%), (Sati & Fatima, 2018), *G. mollis* (1.63 ± 0.02%), (Tairo, 2021), *G. forbesii* (1.28 ± 0.18%) and *G. bicolor* (1.37 ± 0.16%), (Islary *et al*., 2016), *G. sapida* (2.5 ± 0.26%), (Prashanth Kumar & Shiddamallayya, 2021), *G. tiliifolia* (0.81±0.02%). This finding is in agreement with the result of the current study (1.99 ± 0.01%). In general, fruits have low fat content and most of the edible wild fruits have low crude fat content, which makes them an important component of low calorie diet (Prashanth Kumar & Shiddamallayya, 2021). This could indicate their use as a diet for weight loss, as low-fat foods lower cholesterol and prevent obesity.

The ash content of *G. schweinfurthii* (5.36 ± 0.20%) is close to the values reported for *G. bicolor* (4.3%) and *G. forbesii* (3.86%) (Tairo, 2021). Pundlik (2020) reported high values of ash content (5–13 %) for some species of the genus *Grewia*.

The results of this study showed that the fruits of *G. schweinfurthii* had high crude fiber content (32.50 ± 0.50%) compared to *G. sapida* (1.17 ± 0.026%) (Islary *et al*., 2016). This indicates that the fruits of *G. schweinfurthii* are relatively rich in dietary fiber and could be used in the management of various chronic diseases (cancer, heart disease, stroke, diabetes, and arthritis), the risk of which is reduced by the consumption of high fiber diet (Adebiyi *et al*., 2015). The values obtained in this study were similar to those of *G. bicolor* (33.15%) (Tairo, 2021).

Table 8: Result for proximate composition and caloric value analysis of *G. schweinfurthii* fruit

Parameter	% Composition
Moisture	4.09 ± 0.10
Crude protein	11.24 ± 0.68
Crude fat	1.99 ± 0.01
Total ash	5.36 ± 0.20
Crude fiber	32.50 ± 0.50
Carbohydrate	44.82 ± 0.82
Total energy (calorie)	242.12 ± 1.72

Values are presented as mean ± SD of triplicate analysis.

4.8. Antinutritional composition analysis

Antinutritional factors hinder the optimal utilization of nutrients, especially proteins, vitamins, and minerals, thus reducing the nutritional value of a food by preventing optimal utilization of nutrients (Gemede *et al*., 2016; Fekadu, 2014).

Adebiyi *et al*., (2015) stated that *G. carpinifolia* had a tannin content of 0.07±0.00% which was lower than the result of the current study (3.97 ± 0.12%), while Gemede *et al*., (2016) reported that *G. forbesii* had a tannin content of (4.93 ± 9.99%) which was higher than our result. Tannins are known to negatively affect protein digestibility and non-hem iron bioavailability, resulting in poor iron and calcium absorption, and also negatively affect carbohydrates, resulting in lower energy value of a tannin-containing diet (Gemede, 2014).

The oxalate concentration in this study was 0.28 ± 0.03%, which is very low compared to the result reported by (Akwu *et al*., 2019). However, it is comparable to the result reported by (Gemede *et al*., 2016) (0.28 ± 0.28%). Oxalates can have a negative impact on human nutrition and health, especially as they hinder the body's ability to absorb calcium and promote the

formation of kidney stones. A diet high in oxalates may increase the risk of developing calcium oxalate stones in the kidneys. Since calcium oxalate stones account for the majority of urinary stones in humans, patients are advised to limit their consumption of foods with a daily oxalate intake of no more than 50–60 mg (Gemede *et al*., 2016).

Table 9: Result for antinutritional analysis of *G. schweinfurthii* fruit

Parameter	% Composition
Phytate content	62.97 ± 0.83
Tannin content	3.97 ± 0.12
Oxalate content	0.28 ± 0.03

Values are presented as mean ± SD of triplicate analysis

5. Conclusion

Based on our findings, the root extract of *G. schweinfurthii* exhibited the potential to lessen both peripheral and central pain. While both the root extract and its constituent, 4-(2"-(4'-isopropylphenyl) propan-2"-yl)-2,3-dihydrofuran demonstrated anti-inflammatory properties. Consequently, the current findings may support the traditional medicinal claim of the plant against analgesic and antinflammatory activities. Additionally, *G. schweinfurthii* fruits contain carbohydrates, lipids, proteins, and dietary fiber, and a low level of antinutritive substances (tannins, phytates and oxalates). Thus, it can be inferred that these fruits can serve to meet human nutritional necessities and can be utilized as a food supplement.

6. Recommendation

The following recommendation have been drawn from the present study.

Further investigations are required to gain a more in-depth understanding of additional bioactive compounds from the root extract. Subacute and chronic toxicity tests of the root extract are also advisable. Moreover, additional studies are also recommended concerning the fruit's mineral analysis, amino acid composition and vitamin C.

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8. Appendices

Appendices I: Traditional uses of members of the genus *Grewia*

Table 10: Traditional uses of members of the genus *Grewia*

Species	Local name	Parts plant	Indication	Country	Reference
G. bicolor Juss.	Haaroreesaa (Oromifa)	Leaf	Stomach ache (wormexpulsion)	Ethiopia	(Wondimu *et al.,* 2007)
	Esiteti(Massai)	Root	Antivenom	Kenya	(Omara, 2020)
	Mulawa(Kamba)	Bark	Antivenom	Kenya	(Omara, 2020)
		Root	Diarrhea	South Africa	(Semenya & Maroyi, 2012)
G. crenata(G.Forst.) Schinz & Guillaumin	Kamomowa (Hausa)	Leaves	Fractured bones, wound healing, and inflammatory conditions	Nigeria	(Okoye *et al.,* 2014)
G. damine Gaertn.	Ositeti (Kikamba) Harroreesaa (Oromifa)	Fruit, root & steam	Antivenom	Kenya	(Omara, 2020)
G. erythraeaSchweinfurth.	Cedayto (Afar)	Root	Furuncle diphtheria	Djibouti	(Hassan-Abdallah *et al.,* 2013)
G. ferruginea Hochst. exA. Rich.	Doqono (Oromifa)	Leaf Bark	Stomach ache and washing hair	Ethiopia	(Wondimu *et al.,* 2007) (Tura et al., 2017b)
*G. fallax*K.Schum.	Ilawa (Kikamba)	Leaves, stem Bark	Chronic joint pains, antivenom	Kenya	(Wambugu *et al.,* 2011)
*G. hexaminta*Burret	Mkone (Digo)	Root and leaves	Antimalaria	Kenya	(Nguta *et al.,* 2010)

*G. occidentalis*L.	Umnqabaza,Unvileni,Umqaqoba (Xhosa)	Twigs and leaves	Wounds	South Africa	(Appidi *et al.*, 2008)
G. optiva J.R.Drumm. ex Burret	Thamarh, Damman (Pashto) (Punjabi)	Leaves	Anthelmintic	Pakistan	(Rahman *et al.*, 2016)

Table 3 *continued…*

*G. plagiophylla*K.Schum.	Mkone (Digo)	Steam bark Leaves	Diarrhea fever	Kenya	(Gathirwa *et al.*, 2011)
G. tembensis Fresen.	Serrekto (Afar)	Root	Abscess furuncle	Djibouti	(Hassan-Abdallah *et al.*, 2013)
*G. trichocarpa*Hochst. ex A.Rich.	Cone(Digo), Lenquata (Amharic)	Leaves.	Antimalaria	Kenya	(Nguta *et al.*, 2010)
G. tenax(Forssk.) Fiori	Gudeim (Arabic), Taman (Punjab) Saarkama (Oromifa)	Leaves	Antimalaria	Pakistan	(Shah & Rahim, 2017)
	Gudeim (Arabic)	Leaves Roots Wood	Trachoma tonsillitis and infections poultice for swelling	Sudan	(Hammer, 2007)
G. villosa Willd.	Ogobdii (Oromifa)	Bark Leaf	Body swelling Stomach ache	Ethiopia	(Wondimu *et al.*, 2007)
	Olmankulai (Maa)	Root stem fruit	Food,galactagogue strength/ tonic stomachache	Kenya	(Kimondo *et al.*, 2015)
	Agewdie (Tigrigna)	Steam	Foot and mouth disease of cattle	Ethiopia	(Woreda & Southern, 2003)

Some pictures taken during laboratory work: (A) macerated root, (B) filtration, (C) evaporation solvent

A

B

C

Qualitative phytochemical screening

analgesic activities on mice

Antinflammatory activities test on rat

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Addis Ababa University

School of Pharmacy
Ethical Review Committee

ቀን Date: June 20, 2023

ቁጥር Ref. No: ERB/SOP/538/15/2023

To: **Abdi Leta**
School of Pharmacy

Re: Ethical Clearance

It is to be recalled that you submitted a research project proposal entitled **"Evaluation of Analgesic and Anti-Inflammatory Activities of Root Extract and Isolated Compounds of Grewia Schweinfurthii and Determination of Nutritional and Anti-nutritional Compositions of Its Fruit"** The committee thoroughly reviewed the proposal based on its operational guideline and found that, it fulfills the ethical requirements stipulated in the guideline. This is, therefore, to inform you that the proposal is ethically approval for implementation.

With best regards,

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

☎ 00251156 02 12 ✉ 1176 ቴሌክስ Telex: 21205 ፋክስ Fax: 00253(11)1558566 ቴሌግራም Cable: AAUNIV