Phytochemical Analysis and Antimicrobial and Antioxidant Activity Evaluation of *Aloe debrana* Leaf Latex Extracts against Selected Human Pathogenic Bacteria and Fungi

Alemitu Chalesa

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the degree of Master of Science in Applied Biology (Biotechnology)

Office of Graduate Studies

Adama Science and Technology University

February,2024

Adama, Ethiopia

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DECLARATION

I hereby declare that this Master Thesis entitled **"Phytochemical Analysis and Antimicrobial and Antioxidant Activity Evaluation of *Aloe debrana* Leaf Latex Extracts against Selected Human Pathogenic Bacteria and Fungi"** is my original work. That is, it has not been submitted for the award of any academic degree, diploma, or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

Name of student	Signature	Date

RECOMMENDATION

we, the advisor(s) of this thesis, hereby certify that we have read the revised version of the thesis entitled **"Phytochemical Analysis and Antimicrobial and Antioxidant Activity Evaluation of *Aloe debrana* Leaf Latex Extracts against Selected Human Pathogenic Bacteria and Fungi"** prepared under our guidance by **Alemitu Chalesa** submitted in partial fulfillment of the requirements for the degree of Master of Science in Applied Biotechnology. Therefore, we recommend the submission of a revised version of the thesis to the department following the applicable procedures.

Major Advisor	Signature	Date
Co-advisor	Signature	Date

APPROVAL PAGE

We, the advisors of the thesis entitled **"Phytochemical Analysis and Antimicrobial and Antioxidant Activity Evaluation of *Aloe debrana* Leaf Latex Extracts against Selected Human Pathogenic Bacteria and Fungi"** and developed by **Alemitu Chalesa**; hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Major Advisor	Signature	Date
Co-advisor	Signature	Date

APPROVAL OF BOARD OF REVIEWERS

We, the undersigned, members of the Board of Examiners of the thesis by **Alemitu Chalesa** have read and evaluated the thesis entitled **"Phytochemical Analysis and Antimicrobial and Antioxidant Activity Evaluation of *Aloe debrana* Leaf Latex Extracts against Selected Human Pathogenic Bacteria and Fungi"** and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied Biotechnology.

Chairperson	Signature	Date
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Final approval and acceptance of the thesis is contingent upon submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC).

Department Head	Signature	Date
School Dean	Signature	Date
Office of Postgraduate Studies, Dean	Signature	Date

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LIST OF ACRONYMS/ABBREVIATIONS

CC	Column Chromatography
CFU	Colony Forming Unity
CLSI	Clinical and Laboratory Standards Institute
^{13}C-NMR	NMR Carbon-13 Nuclear Magnetic Resonance
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethyl sulfoxide
DPPH	2, 2-diphenyl-1-picrylhydrazyl
EO	Essential oil
GC-MS	Gas Chromatography and Mass Spectrometry
MBC	Minimum Bactericidal Concentration
MFC	Minimum Fungicidal Concentration
MHB	Muller Hinton Broth
MBA	Muller Hinton Agar
MIC	Minimum Inhibitory Concentration
NMR	Nuclear Magnetic Resonance
^{1}H NMR	Proton Nuclear Magnetic Resonance
SDA	Sabouraud Dextrose Agar
SDB	Sabouraud Dextrose Broth
SDA	Simultaneous Distillation Extraction
TLC	Thin Layer Chromatography
UV	Ultraviolet

ABSTRACT

Aloe debrana (Family: Liliaceae) is a stem-less evergreen endemic medicinal plant of Ethiopia, which is traditionally used to treat eye infection, wounds, and malaria. This study aimed to analyze phytochemicals and evaluate the bioactivity of A. debrana leaf latex crude extracts against human pathogens. To obtain crude extracts, various solvents were employed through the maceration process. Qualitative phytochemical analysis was conducted using standard techniques, and the essential oil extracted from A. debrana was obtained through hydro-distillation using a Clevenger apparatus. The antimicrobial evaluation was carried out using the disk diffusion method. And, the minimum inhibitory concentration (MIC) of both crude extracts and isolated compounds was determined through the broth dilution method. Among the solvents used for extraction, the methanol extract displayed the highest crude yield 34.6%*. Phytochemical analysis revealed the presence of saponins, flavonoids, tannins, terpenoids, phenols, and* steroids*. The essential oil extracted from A. debrana revealed the presence of 20 chemical components. The ethyl acetate was subjected to column chromatography to isolate pure compounds. Silica gel column chromatography separation of the ethyl acetate extract affords two compounds (Coded as compound* ***1*** *and compound* ***2****). Compound* ***1*** *was identified to be* flavonol skeleton and *Compound* ***2*** *to be* 2-4-dimethoxy chrysophanol *based on spectral data (NMR). Regarding antimicrobial activity, the crude extracts, compounds****,*** *exhibited good inhibitory effects against selected human pathogenic bacteria and fungi. The methanol crude extract displayed a maximum zone of inhibition of 18±1.63 against Escherichia coli* (ATCC 25922) *at 200 mg/ml. At the same time, compound* ***1*** *and* ***c****ompound* ***2*** *showed good inhibition zones of 15.66±1.24 and 14.66±0.47 at 10 mg/ml against Escherichia coli* (ATCC 25922)*, respectively. Crude extract and isolated compounds also showed inhibitory effects against C. albicans* (ATCC 10231)*. MIC values ranged from 25 to 100 mg/ml for crude extracts and 2.5 to 10 mg/ml for isolated compounds, with positive results for minimum bacterial concentration and minimum fungal concentration. The study assessed antioxidant activity, revealing high Radical scavenging activity for ethyl acetate extract and compound* ***1*** *(IC_{50} values 42.01 and 5.03), respectively. The diverse phytochemical profile and promising biological activities suggest that A. debrana could be a valuable plant for future drug development.*

Keywords: *Aloe debrana*, Antimicrobial activity, Antioxidant activity, Essential oil

1. INTRODUCTION

1.1. Background

The increasing resistance rate to antimicrobial drugs reduces the number of reliably effective drugs used to treat infectious diseases (Grover *et al*., 2011). The main contributing factors to the problem of antibiotic resistance are the misuse of antibiotics by humans and the employment of antibiotics in veterinary practices (Moshirfar *et al*.,2006). Prolonged use of antimicrobial agents led to microbial adaptation, resulting in resistance in microorganisms. The consequent failure of antibiotic therapy has led to hundreds of thousands of deaths annually (Palmer & Kishony, 2013). These developments and increasingly resistant microbial infections intensified the search for new, safer, and more efficacious agents to combat serious microbial infections (Vandeputte *et al*.,2012).

Globally, the widespread resistant pathogenic organisms that are accompanied by increased morbidity and mortality include *E. coli*, *S. aureus*, methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *S. aureus*, vancomycin-resistant *enterococci*, *Enterococcus species, Klebsiella pneumoniae (K. pneumoniae), Pseudomonas aeruginosa, Streptococcus pneumonia, Mycobacterium tuberculosis, Salmonella species, Acinetobacter,* and *Neisseria gonorrhoeae* (Akinde & Taiwo, 2017; Pendleton *et al*., 2013).Fungi species that belong to *Candida, Cryptococcus, Pneumocystis,* and *Aspergillus* are the leading causes of illness and death (Brown *et al*., 2012). Mainly, *Candida* species are among the leading causes of superficial and severe life-threatening systemic infections, especially for people living with HIV/AIDS (Mayer *et al*., 2013).

Most of the world population trusted to use traditional medicine (ethno-pharmacognosy) for their vital healthcare and to cure chronic as well as infectious diseases. Mostly societies, believe that certain herbal products are active and safe with important biological activity such as anticancer, antimicrobial, antioxidant, antidiarrheal, painkilling and wound healing action. The Scientific examination helps to evaluate carefully pharmacokinetics, bioavailability, effectiveness, toxicology and both immediate and long-term side effects of bioactive extracts of the plant before application (Sasidharan *et al*., 2011). Plants have a wide range of scientific and economic value. They are considerably valuable in agricultural, food,

cosmetics, medical, and pharmaceutical industries. They are an essential source of bioactive compounds that can be used to cure or treat diseases. Medicinal plants are an important element of the system in Ethiopia as well as in other countries (Rajasekharan, 2004).

Genus *Aloe* is a well-known herb that has received particular attention from researchers. It is one of the oldest herbs with a history that dates back to traditional medicine thousands of years ago. Additionally, the gel in *Aloe* leaves contains dry matter, which has more than 75 biologically active ingredients. These compounds exhibit medicinal effects, offering valuable therapeutic applications in treating diseases (Desalegn & Ahmed, 2020). In this line, studies on the medicinal benefits of *Alo*e have been conducted on both humans and several animal models. These include anti-inflammatory, immune-modulatory, wound healing, antibacterial, antiviral, antifungal, antidiabetic, antioxidant, and various enzyme inhibitor activities (Stanić, 2007; Pandey & Mishra, 2010). *Aloe* gel is one of the widely accessible and excessively abundant herbal extracts in Africa that are expected to produce desirable results. Today, people prefer aloe products with physiological benefits beyond essential nutrition to reduce their chance of developing chronic diseases (Gadzirayi *et al* ., 2005). There is a proliferation of these value-added products to maintain one's health and treat various ailments ranging from heart diseases to cancer (Keweye & Emire, 2017).

Aloe debrana is one of the indigenous aloe species of Ethiopia, which is traditionally used medicinally by herbalists in the central and northern parts of Ethiopia to cure various infectious diseases (Desalegn & Ahmed 2020). The *in-vivo* antimalarial activities of *A. debrana* were examined, and positive results were found (Deressa *et al* ., 2010). *A. debrana* was also investigated for its potential thickening agent for printing polyester and cotton with disperse dyes (Awoke *et al* ., 2013). *In vivo,* the anti-plasmodial and toxicological effect of *A. debrana* was previously evaluated (Muluye *et al* .,2021). The chemical composition and biological activity of essential oils from *A. debrana* roots were also previously studied by Gemechu *et al.* (2014). To determine the impact of *A. debrana* and *A. pulcherrima* leaf gel infusions on the prevention of sporulation of mixed Eimeria species oocysts obtained from naturally infected hens, an *in vitro* experiment was conducted (Desalegn & Ahmed 2020a). These endemic Aloes are essential for the nation's economic growth and development. There were limited phytochemical investigations and solvents crude extract analysis on the latex part of *Aloe*

debrana. Therefore, this study is expected to fill the knowledge gap, add essential facts about the phytochemical components of latex parts of *A. debrana,* and show their antimicrobial and antioxidant activity.

1.2. Statement of Problem

There is much biomedical knowledge on preventing, treating, and controlling various infectious diseases. However, it remained a public health problem, mainly because of the rapid development of microbes with resistance to the available first-line drugs. This has motivated researchers to look for novel antimicrobial sources, mostly from medicinal plants. In addition to therapeutic substances, medicinal plants are a great source of knowledge for various chemical components that could be created as medications with precise selectivity. Ethiopia is remarkably rich in its biological, ecological, and landscape diversity and is home to outstanding natural bio-resources of medicinal plants. The genus *Aloe* has a high degree of endemism (89%) in the Flora of Ethiopia; among these plants, *A. debrana* (family: Aloaceae) is an indigenous medicinal plant of Ethiopia, which is evergreen and stemless. *A. debrana* is one of the endemic species that grows mainly in Showa, the central part of Ethiopia, and is rarely found in other parts of the country (Demissew and Gilbert 1997). In Ethiopian traditional medicine, the leaf latex of *A. debrana* is used to treat several diseases, including malaria. Additionally, farmers treat their oxen's nape injuries from plowing with the leaf latex of *A. debrana.* It also treats wounds, stops blood flow, and cleans accidentally injured eyes (Gemechu *et al*., 2014). *A. debrana* species is a source of some compounds and exhibits several biological activities. Thus, this plant has attracted the attention of researchers who want to determine its bioactivity. However, the latex part of *A. debrana* shows different biological activities in traditional remedies. This conventional application hindered the standardization and development of this herb and made its recognition, acceptance, and utilization remain locally restricted. Understanding the chemical composition and antimicrobial activity of *A. debrana* latex is essential for their practical application. Despite its medicinal use, no phytochemical screening test or isolation of compounds has been reported. Therefore, this study was done to analyze the phytochemical constituents and to evaluate the antimicrobial and antioxidant activity of the latex extracts of *A. debrana.*

1.3. Objectives

1.3.1. General Objective

To investigate the phytochemical composition and evaluate *in vitro* antimicrobial and antioxidant activity of the crude extracts and isolated compounds of the leaf latex of *Aloe debrana*.

1.3.2. Specific Objectives

- To screen the phytochemical constituents of the *A. debrana* leaf latex crude extracts.
- To extract the essential oils of the plant using hydro- distillation technique and analyze its constituents using GC-MS.
- To evaluate the antimicrobial activity of *A. debrana* leaf latex crude extracts and isolated compounds against selected bacteria and fungi.
- To evaluate the antioxidant activity of *A. debrana* leaf latex crude extracts, essential oil and isolated compounds using DPPH assay.
- To characterize the bioactive compounds obtained from leaf latex of *A. debrana* the using NMR.

1.4. Justification of the Research

Several researchers reported the biological activity of the crude extracts of the genus *Aloe* and the pure isolated compounds. However, to our knowledge, there is no prior detailed phytochemical study and biological activities of the latex of *A. debrana,* which is native to Ethiopia. Therefore, phytochemical screening, biological activity, and antioxidant activity tests on the latex extracts and comprehensive column chromatographic separation are required.

1.5. Significance of the study

This research is expected to:

- Provide antimicrobial activity information of *A. debrana* leaf latex extracts and bioactive compounds against selected microbial strains to provide scientific support.
- To provide scientific proof for claims of traditional uses of the plants to treat infectious diseases.
- Provide information on essential oils composition of *A. debrana* leaf latex.
- Used as reference for future related scientific studies on this plant.

2. LITERATURE REVIEW

2.1. Medicinal plants in Ethiopia

Traditional medicine has been practiced in Ethiopia for many years. Even today, plants remain the source of health problems for most people in developing countries. Knowledge, mainly oral, has been transferred from one generation to the next via experienced traditional practitioners and knowledgeable elders (Aschale *et al*.,2021). Ethiopia is believed to be home to approximately 6500 species of higher plants, of which 12% are endemic, making the country among the most diverse floristic regions of the world. Since Ethiopia is home to numerous beliefs, cultures, and languages, several traditional knowledge and uses of medicinal plant practices are highly expected. Complementary and alternative medicines offer a wide variety of plants that can be used to alleviate human pathologies. Approximately 60% of the world's population, including Ethiopia, depends on traditional medicine for infection treatment (Aschale *et al*., 2021).

2.2. Importance of natural product

Nowadays, there is increased awareness about sources of components in consumer products. Many consumers prefer natural ingredients to artificially synthesized products because organic products are safer and more eco-friendly. Besides that, the consumers are conscious of the sustainability and environmental friendliness of the manufacturing processes. Therefore, manufacturers are incorporating more green and organic elements to attract consumers (Chuo *et al*., 2022).

Researchers are also continuously searching for natural compounds that replace chemically synthesized compounds. The numerous species of plants on earth are one of the significant sources of natural compounds. Plants that produce active compounds such as phenolic acids, flavonoids, alkaloids, polysaccharides, and volatile oils depend on their growth environments. These active compounds have been applied in many industries, including pharmaceuticals, nutraceuticals, cosmetics, food processing, and pesticides(Mgbeahuruike *et al*., 2017; Hosseini *et al*., 2018). The ability to synthesize a range of secondary metabolites, which may repel or attract other organisms, is one way of the survival strategy. Flower color, pollination, preventive

properties in plants, and chemical defense may explain some of the essential activities of secondary metabolites for the survival of an organism (Abreham, 2021).

2.3. Antibacterial activity

Antibacterial activity is the action by which bacterial growth is inhibited or destroyed. Bacteria are classified into Gram-positive and Gram-negative depending on whether they give positive or negative results on Gram's stain test. That is, while those that retain the initial pink color of crystal violet stain, by their thick peptidoglycan layer in their cell walls are named Gram-positive, those that are decolorized and are stained red with carbol fuchsin or safranin owing to their relatively thinner peptidoglycan layer in their cell walls are Gram-negative. The zone of inhibition or clearance zone is where no bacteria growth is observed due to the antibacterial action of the applied test nanocrystals suspended in an appropriate solvent such as dimethyl sulfoxide, which doesn't kill bacteria itself. Standard antibiotics such as ampicillin and chloramphenicol are used as a positive control against which the inhibition zone of test solutions is compared (Weldegebrieal, 2020).

2.4. Antioxidant activity

Free radicals are atoms or molecules (groups of atoms) with unpaired electrons; free radicals are also very reactive; radicals can be stabilized by taking electrons from other molecules and starting chain reactions or extending chain bonds that can damage tissues. In the metabolic process, free radicals are formed when food is converted into energy. Free radicals such as superoxide anions, hydroxyl, and others are easily formed because electron leakage often occurs in the metabolic process. In addition, compounds such as hydrogen peroxide (H_2O_2), ozone, and others are not free radical compounds but are easily transformed into free radicals. As a result, these free radicals can cause degenerative diseases such as cancer, diabetes, and others. Chemically, antioxidant compounds are electron-donating compounds (electron donors). We need compounds that can reduce free radicals, namely antioxidants (Noviyanti *et al*., 2022).

Antioxidants are compounds or chemical components that, in certain levels or amounts, can inhibit or slow down the damage caused by the oxidation process. Compounds with antioxidant activity can be found in plants, including polyphenols, vitamin C, vitamin E, carotene, and

flavonoids. These antioxidants will stimulate the body's ionic response to bind free radicals to be stable and prevent degenerative diseases such as cancer(Zhan *et al*., 2016; Jami'ah *et al*., 2018). Antioxidant compounds such as phenolic acids, polyphenols, and flavonoids can reduce or inhibit free radicals by inhibiting oxidative mechanisms. The main characteristic of antioxidants is the ability to capture or inhibit free radicals. These free radicals can oxidize nucleic acids, proteins, fats, or DNA and initiate a degenerative disease (Fidrianny *et al*., 2015).

Antioxidant activity test with the DPPH method is based on a reduction of DPPH, a stable free radical. Free radical DPPH (2.2-Diphenyl-1-Picrylhydrazyl) has an electron that can provide maximum absorption at 517 nm (purple color). When antioxidants react with DPPH, a stable free radical, they will be paired due to the presence of hydrogen donors (e.g., antioxidants) and transformed into DPPH-H later because the absorption of DPPH will be reduced. In the form of DPPH-H, the result obtained is decolorization or color change to yellow due to electron capture. Thus, the more decolorization occurs, the higher the ability to reduce free radicals. In other words, antioxidants can reduce the purple DPPH radicals into yellow diphenyl-picrylhydrazine when the fluid from the DPPH is mixed with the substance that can donate hydrogen atoms; it will occur in the form of non-radical discoloration (Diphenyl-picrylhydrazine) with a loss of purple. (Leaves & Leaves, 2014 ; Bamidele *et al*., 2017)

2.5. Essential oil

Essential oils are natural, volatile liquid, complex compounds characterized by a strong odor, rarely colored, and soluble in lipid and organic solvents. It could be synthesized by all plant organs, i.e., buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood, or bark, and are stored in secretary cells, cavities, canals, epidermic cells or glandular trichomes (Bozin *et al*., 2006). Essential oils generally have 2-3 major components at high concentrations (20- 70%) compared to other components in trace amounts. (Betts, 2001).

The major components include two groups of distinct bio-synthetic origin, which may determine the biological activity against the pathogenic microorganisms (Pichersky *et al*., 2006). Many essential oils are composed of terpenes, terpenoids, other aromatic and aliphatic constituents, all characterized by low molecular weight. Terpenes are the major group of plant natural

products characterized by various structural types and the most valuable compounds (Degenhardt *et al*., 2009).

The terpene compounds are hydrocarbons of general formula (C_5H_8) n formed from isoprene units. These compounds could be acyclic, monocyclic, bicyclic, or tricyclic (Abed, 2007). Based on the diversity in their chemical structure, they could be classified into several groups: monoterpenes (C_{10}), sesquiterpenes (C_{15}), and diterpenes (C_{20}). Many essential oils' components are monoterpenes representing approximately 90% of the essential oils. These are generally volatile in nature with pleasant odor (Bakkali *et al*., 2008).

2.6. The genus *Aloe*

2.6.1. Ethnobotanical uses of the genus *Aloe*

Aloe is one of the oldest therapeutic plants known to humans since prehistoric times. The name came from the Arabic word “alloeh,” which means “bitter,” due to the bitter liquid in the leaves. It is also referred to as “lily of the desert,” the immortality plant and a medicinal plant with the potential as an alternate medicine. *Aloe* is used as a popular folk remedy. It is present in the arid regions of India. It is believed to effectively treat stomach ailments, gastrointestinal problems, skin diseases, and constipation for radiation injury, its anti-inflammatory effect, wound healing, and burns, as an antiulcer, and diabetes. Currently, the plant is used extensively in nutraceuticals, cosmetics, and skincare. It is among the folk treatments that have been utilized for a long time. Here, we’ll examine Aloe both botanically and horticultural and briefly explore its potential medical use (Dagne *et al*., 2000).

Due to its importance in medicine and commerce, *Aloe* is a fascinating topic for research from a chemical, pharmacological, economic, and taxonomic standpoint. Two products made from commercial Aloe are Aloe gel and Aloe latex. Aloe latex, which is yellow and comes from beneath the skin of the leaves, includes hydroxyl anthracene derivatives like anthraquinone glycosides (Fozia, 2014). The translucent, jelly-like fluid called Aloe gel can be discovered in the interior portion of an Aloe leaf. The majority of the plant’s health advantages are attributed to the bioactive components (polysaccharides, soluble sugars, proteins, enzymes, amino acids,

vitamins, and anthraquinones) found in the gel of Aloe leaves, which is 99% water and composed primarily of other organic compounds (Chun-hui *et al*., 2007).

2.6.2. Ecological uses of *Aloe* species

Aloes play a significant role in Ethiopia's dry-land ecosystems. They play a significant part in resolving ecological issues. For instance, the *A. gilbertii* plant and its parts have been used for various things, including efforts to save soil and restore degraded land. The local community relocated the whole people to the areas surrounding their farms, range lands, and backyard gardens for demarcation and protection through area enclosure in the form of hedging and fences. Additionally, it has been frequently noticed that individuals of *A. gilbertii* are planted along slopes to create bunds, terracing, or ditches to prevent soil erosion and to hold onto soil while erosion occurs (Dessalegn, 2013). The existence of *A. macrocarpa* is used as a fence for home, farmland, and range land (Assefa & Abebe 2014). *A. debrana* plants are also important in soil and water conservation (Tesfaye Awas, 2009).

2.6.3. Aloe plants in industry

For producing jute sacks, the extract oils of various Aloe species, including *A. ankobernsis, A. camperi*, *A. debrana*, *A. pulcherrina*, *A. schelpei*, *A. sinana*, *A. trichosantha* and *A. weloensis*, were tested and identified. However, because other Aloe species are uncommon, the Ethiopian Biodiversity Institute has only permitted *A. debrana* and *A. trichosantha* plants and these two Aloe species' natural oils are being employed in the manufacture of jute bags for the packaging of coffee, grains, and oil seeds. Recent research indicates that extracts of Aloes can be used in different industries and contribute to the future development of various industrial developments in the country. Some *Aloes* of Ethiopia are tested for different industry inputs in the future: for example, the extract of *A. barbadensis* for antimicrobial activity in cotton fabric, i.e., controlling *S. aureus* (Jothi, 2009), the *in-vivo* antimalarial activities of *A. debrana* was tested and it showed positive results (Deressa *et al*., 2010). *A. debrana* was also examined as a good thickening agent for printing polyester and cotton with disperse dyes (Awoke e*t al*., 2013).

2.6.4. Compound reported from the genus *Aloe*.

The major secondary metabolites in many of the aloe species have been identified as anthraquinones, pre-anthraquinones, and bianthraquinoids, although alkaloids, anthrones, benzene, naphthalene, furan derivatives, coumarins, pyrans, pyrones, flavonoids, and sterols (Dagne *et al*., 2000). Some of the compounds isolated from the genus *Aloe* are given below.

Aloe vera is known to be an ornamental as well as a medicinal plant. Since Roman times, it has been used therapeutically, with its different properties ascribed to the inner colorless leaf gel and the exudates from the outer layers. Ascorbic acid (**1**) and pyrocatechol (**2**) were isolated from the leaves of *A. Vera* was reported to exhibit antimicrobial and antibacterial activities, as shown in **Figure 1** (Ombito *et al.*, 2015).

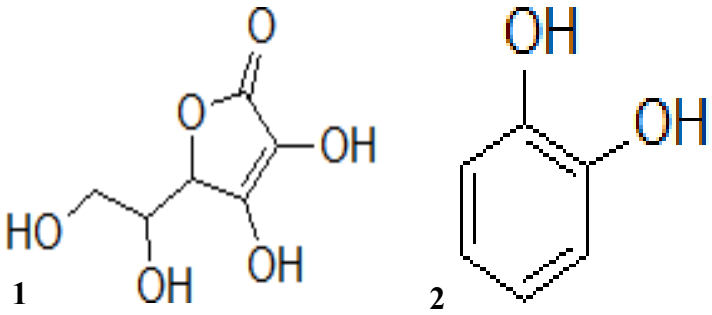


Figure 1: Compounds isolated from *A. vera*.

(Minale *et al*., 2014) investigated the leaf latex of *A. Sinana* for its antibacterial and antifungal activities. In their study, they isolated and characterized aloinoside (**3**) and microdontin (**4**), which are believed to be responsible for the antimicrobial effect of the latex. The two compounds showed broad-spectrum antibacterial activities against Gram-positive and Gram-negative bacteria (Ombito *et al.*, 2015). Some of compounds reported from rhizomes and leaves of *Aloe turkanensis* were: 3, 5, 8-Trihydroxy-2-methyl naphthalene-1,4-dione (**5**), 5,8, -D-hydroxy-3-methoxy-2-methyl naphthalene-1,4-dione **(6**), Chrysophanol (**7**), Aloesaponol (**8**), Helminthosporin (**9**), **Figure 2** as presented in (Fozia, 2014).

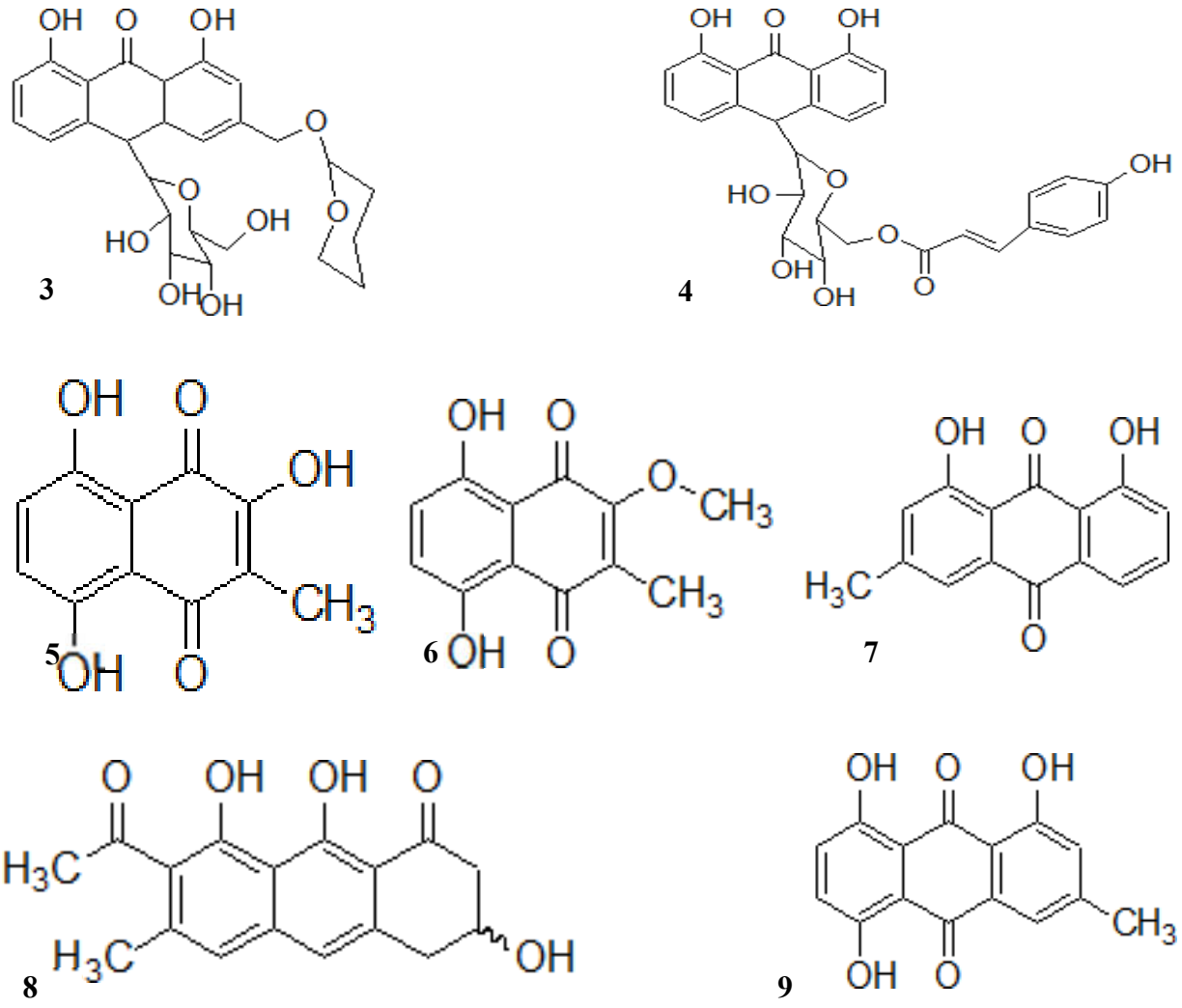


Figure 2: Compounds isolated from *A. Sinana* and *A. turkanensis*.

2.7. *Aloe debrana*

2.7.1. Botanical description

A. debrana (Family: Liliaceae) is a stem-less evergreen endemic medicinal *Aloe* plant of Ethiopia usually grows on gentle slopes between 2000 and 2700 m in Shoa, Gojam, and Wollo floristic regions. The main flowering period is in the dry season, from December to February (Sebsebe and Inger 2010). Among other species in the genus Aloe, this plant needs much less water and is resistant to diseases and insects. The *A. debrana* plant has long (up to 20 inches long and 5 inches wide) leaves. Each plant usually has 12 to 16 leaves and can be harvested every 6 to 8 weeks by removing 3 to 4 leaves per plant. The fresh parenchyma gel from the

center of the leaf is clear and shining (Dagne *et al*., 2000). The specific epithet "debrana" alludes to the plant's location and serves as the basis for describing the species (Debre Berhan in the Shewa Region). Debre Berhan, from the Amharic words for "place" and "light," means "the location of the light" (Sebsebe & Nordal, 2010) **(Figure 3)**.

Table 1:Taxonomic Classification of *A. debrana*.

Taxonomic Classification	
Kingdom	Plantae
Order	Asparagales
Division	Spermatophyte
Subdivision	Angiospermae
Class	Monocotyledoneae
Family	Liliaceae
Genus	*Aloe*
Species	*Aloe debrana*

Figure 3: Picture of *A. debrana*.

2.7.2. Traditional uses

Aloe debrana (Family: Liliaceae) is a stem-less evergreen endemic medicinal plant of Ethiopia, which is traditionally used to treat eye inflammation, wounds, excessive pain, malaria, and gastrointestinal and dermatological problems, and it is also used to stop breastfeeding (Getahun *et al*., 2020a). Its leaf latex is an anti-diabetic, laxative, and antimalarial agent. It has a parasitemia inhibition effect with no observable signs of toxicity or mortality in mice (Abera *et al*., 2019). Farmers also use the leaf latex of *A. debrana* to cure the wounds made during plowing on the nape of their oxen (Demissew & Nordal 2010). It is also used to heal wounds, clean blood, and clean accidentally injured eyes (Getahun *et al*., 2020a).

2.7.3. Economic importance

A. debrana was also examined as an excellent thickening agent for printing polyester and cotton with disperse dyes (Awoke *et al.*, 2013). *A. debrana* plants are also essential in soil and water conservation. The oil extract was checked and identified for the production of jute sacks, and the *A. debrana* plant was allowed for the company by the Ethiopian Biodiversity Institute. The plant's natural oil produces jute sacks for packing coffee, cereals, and oil seeds. The fiber company has started using naturally earned oils from *A. debrana,* especially in manufacturing soaks for coffee export*, a*s reported (Oda & Anbessa Erena 2017). According to (Awoke *et al*., 2013), the *A. debrana* plant keeps control of land conservation, and the gel from this plant can be used for several purposes, like dyeing fabrics.

2.7.4. Biological activity

The EO of *A. debrana* roots obtained by SDE demonstrated promising antibacterial activity (8.96±1.20 and 13.29±1.06 mm) against Gram-positive *S. aureus* bacterium at 0.5 and 1.0 mg/mL concentrations, respectively (Getahun *et al*., 2020b). This EO also showed promising antioxidant activity with IC_{50} values of 48.65±1.32 µg/mL in DPPH and 51.97±2.14 µg/mL in H_2O_2 (Getahun *et al.*, 2020b). An *in vivo* antiplasmodial activity test of the methanolic leaf extract of *A. debrana* showed a dose-dependent chemo-suppressive effect (Deressa *et al.*, 2010). When comparing the inhibitory potential of *A. debrana* and *A. pulcherrima* on sporulation of coccidian oocysts, *A. debrana* leaf gel prevented sporulation of Eimeria oocysts at significantly

higher inhibitory efficacy than *A. pulcherrima* gel at the tested dosages (Desalegn & Ahmed 2020a). *A. debrana in-vivo* antimalarial abilities were examined. The findings were encouraging (Deressa *et al*., 2010). In rats with STZ-induced diabetes mellitus, the ethanolic extract of the *A. debrana* plant showed considerable antidiabetic efficacy at 300 mg/kg, and the impact was comparable to that of the conventional medication glibenclamide (Suleyman *et al*., 2015).

Table 2: Biological activities of different parts of *A. debrana*.

Plant part	Activity	Solvents extract	Reference
Leaves	Antimalarial	Methanol and water	Yehuala (2013), Deressa *et al*. (2010).
Leaf Latex	Antimalarial and parasitemia	Methanol and aqueous	Muluye *et al*. (2021)
Root	Antibacterial and antioxidant	hydro- distillation	Getahun *et al*. (2020)
Leaf Gel	Antiglycemic, antidyslipidemic(antidiabetic), Anticoccidial	Ethanol, methanol, and Distilled water	Suleyman *et al*. (2015), Desalegn & Ahmed (2020)

2.8. Methods of plant extract preparation

2.8.1. Plant material.

Before conducting extraction, plant materials need to be processed so that they are in suitable form for extraction. The pretreatment usually involves grinding and drying of plant materials. Extraction of active compounds from plant materials often consists of soaking the materials in a solvent for a certain amount of time. After that, the extracts are separated for further processing (Chuo *et al*., 2022).

2.8.2. Selection of extraction methods

Plant's active compounds are usually contained inside the plant matrixes; thus, appropriate extraction methods are needed to extract the active compounds. Before extraction, pretreatments such as drying and grinding are usually conducted to increase efficiency. After extraction, further processing may be required to isolate or purify the desired components. Conventional extraction methods to obtain active compounds from plant materials are based on solid-liquid extraction techniques. Some commonly used traditional methods are maceration, Soxhlet extraction, and hydro distillation. Although conventional extraction methods are easy to operate, they are frequently criticized for large solvent consumption and long extraction time. Extracts obtained using different extraction methods may have different compositions and properties (Abuduwaili *et al.*, 2019). Therefore, the extraction methods must be chosen carefully to get the desired extracts. Optimization of extraction parameters should be conducted to increase extraction efficiency, reduce solvent and energy consumption, and save costs (Chemat *et al*., 2019).

2.8.3. Choice of Solvents

The successful isolation of biologically active compounds from plant materials relies heavily on selecting an appropriate solvent for the extraction process. Preparation of a suitable solvent in plant extractions will include low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, and inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extracts. The choice of solvent is influenced by what is intended with the extract. The intended use of the extract also guides the selection of a solvent. As the final product may contain residual solvent traces, the chosen solvent must be non-toxic and not interfere with the bioassay. Additionally, the choice of solvent depends on the specific compounds targeted for extraction (Ijaiya *et al*., 2014).

2.9. Chromatographic techniques and structure determination

Since plant extracts usually occur as a mixture of different bioactive compounds or phytochemicals with different polarities, their separation remains a significant challenge for bioactive compound identification and characterization. Isolating these bioactive compounds using various separation methods, such as TLC column chromatography, is a common practice to obtain pure compounds. The pure compounds are then used to determine structure and biological activity. Fourier-transform infrared spectroscopy (FTIR) can also be used to achieve and promote the detection of bioactive compounds in addition to this phytochemical screening assay (Priya *et al.*, 2020).

2.9.1. Thin-layer chromatography (TLC)

TLC is a simple, easy, and inexpensive procedure that quickly responds to the researcher on how many components are in a blend. When a compound's Rf is contrasted with the Rf of a known compound, TLC is often used to help the identification of a compound in a mixture, which has also been used to check the purity and identification of isolated substances (Shahverdi *et al*., 2007).

2.9.2. Fourier transforms infrared spectroscopy (FTIR)

Fourier-transform infrared spectroscopy is a valuable method for detecting functional groups present in the plant extract. It helps define the molecule and its structure (Eberhardt *et al*., 2007). To fingerprint herbal extracts or powders, FTIR provides a quick and non-destructive investigation.

2.9.3. Nuclear magnetic resonance spectroscopy (NMR)

Nuclear Magnetic Resonance Spectroscopy gives matter's physical, chemical, and biological properties. One-dimensional technique is routinely used, but two-dimensional NMR techniques could be used to achieve the complex structure of the molecules. Solid-state NMR spectroscopy is used to determine the molecular structure of solids. Radiolabeled ^{13}C NMR is used in the compound to distinguish the types of carbon present. ^{1} H-NMR is used to determine the proton

types present in the compound and to determine how the protons couple each other. (Ingle *et al*., 2017).

3. MATERIALS AND METHODS

3.1. Description of the study area

The leaf latex of *Aloe debrana* was collected from Chancho town, Sululta Woreda, Oromia region, Ethiopia (**Figure 4)**. Chancho is located between 9º 10‟ 60” N and 38º 45‟ E latitude and longitude, respectively. The altitude of the study area ranges between 1600 and 3340 m above sea level. The people's primary occupations is farming. According to its climatic condition, Sululta woreda is subdivided into three agro-ecological zones known as highland (Badda, in the local language) covering 71% of the area, mid-altitude (Badda Daree, in the local language) that covers 23.4% of the area and lowland (Gammojjii, in local language) that covers about 5.6% of the area. The area receives higher rainfall between July and August, with an annual average of 1440.5 mm/annum and moderate rain from March to June. The dry season extends from October to February and, to a lesser extent, to March. There are also different soil structures found in the area, including silt soil (35%), clay soil (12%), and sand soil (53%) Sululta woreda Agricultural Office (2016).

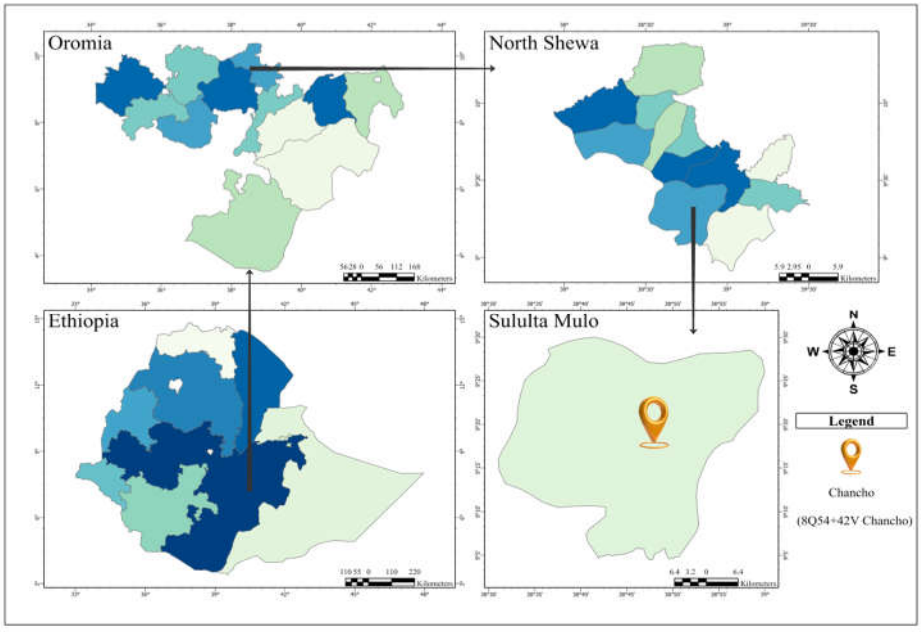


Figure 4: Map of the study area, Sululta Woreda.

3.2. Collection and identification of plant material

The leaf latex of *Aloe debrana* was collected on January 8, 2015, EC from Chancho, 40 km North of Addis Ababa, Ethiopia. Mr. Melaku Wendaferash identified the plant material, and the voucher specimen number (AC-001) was deposited at the National Herbarium of Ethiopia, Addis Ababa University, Ethiopia.

3.3. Study Design

The study was carried out in the Applied Biology laboratory of Adama Science and Technology University. Phytochemical screening tests of crude extracts were conducted following standard protocol. *A. debrana* was extracted with four organic solvents, and each extract was applied to five test pathogens in three replications. Ciprofloxacin and ketoconazole (i.e., alternative antibiotics for resistant strains) were used as positive controls for bacterial and fungal pathogens, respectively. In contrast, Dimethyl Sulphoxide (DMSO) was used as a negative control for both. The antimicrobial activities of the plant extracts against the test pathogens were determined using the disk diffusion method, and the antioxidant activities of the extracts, isolated compounds, and essential oils of *A. debrana* were conducted by the DPPH assay method. The minimum inhibitory concentrations (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) were determined for extracts against the selected pathogens using the broth dilution method. Isolation of compounds from the ethyl acetate extract of *A. debrana* was conducted using a silica gel column chromatographic technique. The obtained pure fractions were submitted to NMR spectroscopy for further analysis.

3.4. Extraction process

The leaf latex was extracted and prepared per the procedure described by (Geremedhin *et al*., 2014). The leaf latex of *A. debrana* was cut transversally near the base and then inclined in plastic to collect the yellow sap inside the leaf. Finally, the collected sap was left in the shaded open air for one week on aluminum foil to make a thin film that suites the evaporation of the sap, resulting in golden latex (Geremedhin *et al*., 2014). The dried leaf latex was ground using mortar and pestle, and the powdered leaf latex was weighed using an electrical balance. *A. debrana* powders (75 g) were extracted separately with 500 ml petroleum ether, chloroform,

ethyl acetate, and methanol solvents and macerated for 72 hr on occasional shaking. Then, the extracts were filtered from the residue with Whatman No. 1 filter paper, and the extracts were concentrated using a rotary evaporator to obtain the dried powdered extracts (**Figure 5**). Extracts were stored in closed and dark places until used for further analysis, and the percentage yield of the crude extracts was calculated.

$$Extraction\ yield\ (\%) = \frac{Mass\ of\ concentrated\ \ crude}{Dry\ mass\ of\ plant\ powder\ used}\ x\ 100$$

Figure 5: Schematic diagram for a crude extract preparation from *A. debrana*.

3.5. Essential oil extraction

The fresh leaf latex of *A. debrana* (100 ml) was hydro-distilled with 300 mL deionized water for about 3 hr using a modified Clevenger-type apparatus, following the methodology described by Esmaeili *et al*.(2018). The condensate (mixture of essential oil and water) was collected in a 100 mL separatory funnel. The essential oil was consecutively separated from the aqueous layer, and the separated EOs were then dried and kept in a sealed glass vial in the refrigerator before analysis.

3.6. Gas chromatography/mass spectrometry (GC-MS) analysis of the essential oils.

The components of EOs were identified by GC- MS analysis using a GC-MS/ Triple Quad (Thermo-Fisher, USA), which was equipped with BP-5MS (30 m x 0.25 mm i.d., 0.25 µm film thickness) capillary separation column. The EOs was diluted in n-hexane (1:10), and 1 µL quantity was used for analysis. The oven temperature was raised from 50 to 250℃ at a rate of 5 ℃/min, and the injector temperature was maintained at 220°C. Helium was used as a carrier gas with a constant 1.0 mL/min flow rate. The ionization of different components of the EOs was performed in electron impact (70 eV) mode in the m/z range of 50-650. The components of the EOs were identified from the generated chromatograms based on their elution time mass fragmentation patterns and by comparison with the spectral data available in the literature (Getahun *et al.*, 2020a) and NIST library. The relative percentage amount of each compound was calculated from the electronic integrations by comparing its average peak area to the total area. The peaks were integrated using Hewlett Packard Chem-Station software (G1701BA Version B.01.00) to quantify the peaks.

3.7. Isolation of Bioactive Compounds

3.7.1. Thin Layer Chromatography (TLC) Analysis of the Extracts

Analytical thin-layer chromatograms were run on a readymade 0.2 mm thick layer of silica gel GF254 (Merck) coated on an aluminum plate. The TLC plates were used to observe the separation of individual compounds as a single spot was trimmed, and a straight line marked the position of the origin (Priya *et al*., 2020).

Retention factor (RF) = Distance traveled by substance / Distance traveled by the solvent front

Two solvent systems with differing polarities were used to analyze 2mg/ml of the *A. debrana* leaf latex extract placed in a band of 1cm by thin-layer chromatography. These solvents were n-hexane: ethyl acetate (3.5:1.5). Visible bands were marked under ultraviolet light (254 nm and 360 nm wavelengths).

3.7.2. Column chromatography

The appropriate solvent was selected for elution for column chromatography after carrying out the TLC profile of the extracts in variable combinations of solvents. The solvent system that showed good resolution of the components of the crude extract on TLC was selected to isolate compounds in column chromatography. The ethyl acetate crude extract that showed good TLC profile was subjected to silica gel column chromatography. Briefly, silica gel (150 g) was mixed with 200 mL of petroleum ether to form a homogenous suspension/slurry and stirred using a glass-stirring rod to remove bubbles. The silica gel slurry was then poured into a glass column, and the column was packed. The ethyl acetate extract (2 g) was adsorbed with silica gel (2 g) and subjected to silica gel column chromatography. Elution of the extract was done with increasing gradient of solvent systems (increasing gradient of ethyl acetate in petroleum ether followed by methanol in ethyl acetate). The column was first eluted with petroleum ether as the mobile phase, with the polarity increasing by 10 % increments of ethyl acetate. After getting to 100 % ethyl acetate, the polarity was further increased by 10 % increments of methanol. Silica gel was used as the stationary phase, while varying solvent combinations were used as the mobile phase. One hundred four (104) fractions, each 100 mL **(Table 3)** were collected. Each fraction was collected and labeled, and the purity of all fractions was analyzed with TLC. Fraction 14 afforded Compound **1** (26 mg), a yellowish solid compound with single spot-on TLC (eluted with 70% petroleum ether in EtOAc). The Rf value was found to be 0.66.

Table 3: Summary of fractionation of ethyl acetate extract.

Solvent System	Ratio	Fraction	Solvent System	Ratio	Fraction
Petroleum Ether	10	F1-F3	EtOAc: MeOH	9:1	F47-F52
Petroleum: EtOAc	9:1	F4-F5	"	8:2	F53-F59
"	8:2	F6-F10	"	7:3	F60-F65
"	7:3	F11-F14	"	6:4	F66-F72
"	6:4	F15-F17	"	5:5	F73-F75
"	5:5	F18-F21	"	4:6	F76-F80
"	4:6	F22-F25	"	3:7	F81-F87
"	3:7	F26-F29	"	2:8	F88-F95
"	2:8	F30-F35	"	1:9	F96-F99
"	1:9	F36-F41	MeOH	100:0	F100-F104
EtOAc	10:0	F43-F46			

Fractions (30-39) eluted with petroleum ether: ethyl acetate (9:1) showed four spots in the solvent system petroleum ether: ethyl acetate (9:1). Fraction (30-39) was re-chromatographer on silica gel column (25 g, 2 cm x 100 cm), elution was carried out beginning with petroleum ether: ethyl acetate. The polarity of the eluting solvent mixture was increased gradually with successive addition of ethyl acetate. At 2% addition of ethyl, 42 fractions (20 mL each) were collected, and fraction 27 yielded compound **2** (32 mg/ml). The solvent evaporated, and the fractions were monitored by TLC (60%petroleum ether in ethyl acetate), and the Rf value was found to be 0.42. The color of the compound was found to be light yellow. The isolated pure compounds were subjected to NMR spectral analysis.

Table 4: Summary of ethyl acetate extract fractionation re-chromatographed.

Solvent System	Ratio	Fractions
Petroleum Ether	10	F1-F7
Petroleum: EtOAc	9:1	F8-F15
"	8:2	F16-F23
"	7:3	F24-F29
"	6:4	F30-F36
"	5:5	F37-F42

3.7.3. Characterization of compounds

The NMR spectroscopic technique (^{1}H NMR, ^{13}C NMR, and DEPT-135) spectra were employed to elucidate the structure of compound **1**.

3.8. Phytochemical screening of crude extract

The preliminary screening of phytochemical constituents of crude extract of the *A. debrana* was done for the investigation of secondary metabolites compounds such as alkaloids, flavonoids, saponins, tannins, phenols, and steroids by using the standard procedures (Trease & Evans, 1989; Christen, 2000; Young & Woodside, 2001; MacNee, 2005).

3.8.1. Test for Alkaloids (Wagner's Test)

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). The formation of a brown/reddish precipitate indicates the presence of alkaloids (Sunil & Vinod 2019).

3.8.2. Test for Flavonoids (Cyanidine test)

One-half gram (0.5g) of the *A. debrana* crude extract was dissolved in methanol, and 2 ml of concentrated hydrochloric acid was added. A spatula full of magnesium turnings was added, and the mixture was observed. An observed brick-red coloration indicates the presence of flavonoids (Christen, 2000).

3.8.3. Test for Phenols

The latex crude extract was treated with 3-4 drops of ferric chloride solution. The formation of a bluish-black color indicates the presence of phenols (Sha'a *et al*., 2019).

3.8.4. Test for Tannins (Ferric chloride test)

A 0.5g of the latex crude extract was dissolved, added to a tube containing 20ml of boiling distilled water, and then boiled for an hour. A few drops of ferric chloride were added and allowed to stand for proper color development. A blue-black coloration indicates the presence of tannins (Alebiosu & Yusuf, 2015).

3.8.5. Test for Saponins (Frothing test)

Saponins were tested by dissolving about 0.5g of the latex crude extract in a test tube containing 3 ml of hot distilled water. Then, the mixture was shaken vigorously for one min and persistent foaming indicated the presence of saponins (Solanki *et al*., 2019).

3.8.6. Test for Steroids (Lieberman-Burchard test)

A 0.5 g of the *A. debrana* crude extract was dissolved in 0.5 ml dichloromethane to give a dilute solution. Then, 0.5 ml of acetic anhydride was added, followed by three drops of concentrated sulphuric acid. A blue-green coloration indicates the presence of steroids (MacNee, 2005).

3.8.7. Test for Terpenoid (Salkowski test)

A 0.5 g of the extract was mixed with 2 mL of chloroform. Then, 3 mL of concentrated sulphuric acid was added to form a layer. A reddish-brown coloration at the interface indicated the presence of terpenoids (Takaidza *et al*., 2018).

3.9. Antimicrobial activity assay

3.9.1. Test strain collection

A total of four types of bacterial species were subjected to antibiotic assay. These are *S. aureus* (ATCC 25923) (Gram-positive), *P. aeruginosa* (ATCC 27823) (Gam-negative), *S. pyogenes* (ATCC 11778) (Gram-positive), *E. coli* (ATCC 25922) (Gram-negative) and one fungi *C. albicans* (ATCC 10231). All these strains were obtained from the Oromia regional laboratory, Adama. The bacterial strains were maintained in Muller Hinton Broth (MHB, pH 7.2) at 37℃, and fungi were held in Sabouraud dextrose Broth (SDB, pH 5.4) at 25℃. The stock culture slants were maintained in the refrigerator until use.

3.9.2. Standardization of inoculum

The active culture bacteria for the test were prepared by taking a loop full of bacterial cells from stock cultures and streaking them on Mueller Hinton Agar, and it was incubated at 37℃ for 24 hrs. The cell suspension of the above four bacteria was freshly prepared by transferring the isolated colony, which is 24 hrs old, into a test tube containing 10 ml of MHB and incubating it overnight. Then, fresh cultures were diluted by adding sterile saline solution and vortexed thoroughly. The turbidity of the suspension was matched with that of 0.5 McFarland standards, equivalent to 1.5×10^8 CFU/ml for the sensitivity test as described by NCCLS (Isitua *et al*., 2016).

Similarly, the fungus (*C. albicans*) was sub-cultured on SDA and incubated for 48 hrs at 27℃. The inoculum suspension was prepared by picking two up to three colonies of the fresh cultures of SDA with a sterile inoculating loop and transferred into a test tube containing 10 ml of SDB and incubated overnight. Then, new cultures were diluted with a saline solution and vortexed thoroughly. Finally, the fungal isolate was matched with 0.5 McFarland standards corresponding to 1×10^6 cells for fungal species.

3.9.3. Preparation of McFarland standard

McFarland number 0.5 standard was prepared by mixing 9.95 ml 1% H_2SO_4 in distilled water and 0.05 ml 1% $BaCl_2$ in distilled water to estimate bacterial concentration, and the preparation was kept in a flask and used for contrast of bacterial suspension (Saeed & Tariq 2007).

3.9.4. Disk Diffusion Method

The *In vitro,* disc diffusion method was conducted to evaluate the antimicrobial activities of *A. debrana* leaf latex crude extracts and isolated compounds (Isitua *et al*., 2016). The antibacterial activities were determined against four pathogenic bacterial and one fungus. For this experiment, Muller-Hinton agar (MHA) and Sabouraud dextrose agar (SDA) media for bacteria and fungus were prepared and autoclaved at 121℃ for 15 psi. After autoclaving, media was poured into sterile petri plates up to a uniform thickness of approximately 4 mm and allowed to solidify. As mentioned above, the microbial inoculum was subcultured into the MHB for bacteria and SDB for fungus and incubated overnight for fresh culture. Then, these inoculums were diluted to adjust with 0.5 McFarland standard. A sterile cotton swab was inserted into the culture suspension to test bacteria and fungi. The swab was then streaked on the surface of the Muller-Hinton agar and SDA plate for bacterial strain and *C. albicans,* respectively. The swab was streaked three times over the entire plate surface to ensure a uniform, confluent growth. A 200 mg/ml of stock solution of each crude extract was prepared by dissolving 0.4 g of methanol, ethyl acetate, chloroform, and petroleum ether in 2 ml of 10% DMSO. Each extract was formed for disc diffusion assay by two-fold serial dilution (200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml). Similarly, a 10 mg/ml of stock solution of isolated compound **1** and compound **2** was prepared by dissolving 0.02 g of isolated compound **1** and compound **2** in 2 ml of 10% DMSO. Two- fold serial dilution (10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml) were formed from this stock solution. The sterilized filter paper (Whatman No. 1, diameter = 6 mm) disc was dipped in the extracts, compound **1** and compound **2**. Then, the extracts and compounds was left to diffuse in the disc for 30 min and placed on the inoculated agar (Xu *et al*., 2019; Elbashiti *et al*., 2011). Sterilized paper discs soaked in 10 µg/ml ciprofloxacin and ketoconazole standardized antibiotic were used as a positive control. At the same time, a disc impregnated with DMSO was used as a negative control. The plates were incubated overnight at 37℃ for 24 hr for bacterial and fungal at 25℃ for 48 hr. Finally, the microbes' inhibition zone by microbial agents

was measured using ruler in mm scale. The test was done in triplicate and the results were presented as an arithmetic average mean (mean ±SD).

3.9.5. Determination of Minimum Inhibitory Concentration

The MIC of the crude extracts, compound **1** and compound **2** was determined using the broth dilution method (Nagalakshmi *et al*.,2019)i. For bacterial and fungal MIC determination, the initially adjusted microbial suspension was diluted to concentrations of 1.5×10^8 CFU/ml and 1×10^6 CFU/ml, respectively. The extracts were diluted in 1 ml of 10% DMSO to achieve the concentrations of (200, 100, 50, and 25 mg/ml) in vials. Similarly, compound **1** and compound **2** were diluted in 1 ml of 10% DMSO to achieve the concentrations of (10, 5, 2.5, and 1.25 mg/ml). Using a micropipette, 100 µl of each crude extract and isolated compound were transferred into test tubes containing 5 ml of Mueller Hinton Broth (MHB) and Sabouraud dextrose Broth (SDB), each marked with their respective concentrations. Subsequently, 10 µl of inoculum adjusted to 0.5 McFarland of selected pathogenic microbes (*S. aureus, S. pyogenes, P. aeruginosa, E. coli, and C. albicans*) were added to each marked test tube. Two test tubes served as negative controls (broth with inoculum) and positive controls (broth only) for each tested microbe. Finally, the all test tubes were incubated at 37^0C for 24 hr for bacteria and 27 ^{0}C for 48hr for fungus to observe their growth. After incubation, the lowest concentration of which inhibited the microbial growth was recorded as MIC value determinaion by visual observation for each crude extracts and isolated compounds .The minimum concentration with low micribial turbidity were taken as MIC value.

3.9.6. Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

From each of the test tubes in the MIC determination that did not show any visible growth, 50µl of the broth was aseptically inoculated onto a sterile Mueller Hinton agar (MHA) surface for bacteria and Sabouraud dextrose agar (SDA) surface for fungus and gently spread all over the surfaces with a sterile bent glass rod. The inoculated plates were incubated for 24 hr at 37°C for bacteria and at 25°C for 48 hr for fungus. After incubation, the MBC and MFC were regarded as the dilution at which the plate had no visible growth (De & Ifeoma, 2002; Rotimi *et al*., 1988).

3.10. Antioxidant activity

Antioxidant activity of the crude extract and isolated compounds were measured based on their free radical scavenging activity, which was determined by the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) method. By this method, it is possible to decide on the radical scavenging power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm.

DPPH solution (0.1 mM) was prepared in methanol by dissolving 0.004 mg/ml DPPH in 100 ml methanol. The solution was kept in darkness for 30 min to complete the reaction. The crude extract of the latex was diluted in four vials containing methanol to give 1000, 500, 250, and 125 µg/ml. The same procedure was performed for the compounds and essential oils. The samples were diluted 200, 100, 50, 25µg/ml. From each concentration, 1 ml of each was mixed with 3 ml of 0.004% DPPH. 1 ml of the solution was mixed with 3 ml of 0.004% DPPH. Ascorbic acid was prepared with the same concentration of samples and used as a positive control. Sample-free DPPH solution in methanol was used as a negative control. The solution was kept in darkness for 30 min to complete the reaction. The resulting solutions were subjected to a UV-Vis spectrophotometer to record absorbance at 517 nm. The percentage of DPPH inhibition was calculated according to the following formula:

$$\% RSA = Ao - A1 / Ao \times 100\%$$

Where, %RSA is percent radical scavenging activity, A0 is the absorbance of the blank DPPH and A1 is the absorbance of sample. The IC_{50} value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using a log dose inhibition curve.

3.11. Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data was subjected to SPSS 16 to one-way analysis of variance (ANOVA), and the results were presented using tables and graphs. P values less than 0.05 were considered statistically significant. Microsoft Excel 2010 statistical package was used for all analyses.

4.RESULTS AND DISCUSSION

4.1. Extract yield

The extraction yield measures the solvent efficiency to extract specific components from the original material. In the present study, the petroleum ether extract yielded a deep yellow of 7%, the chloroform extract yielded an orange crude extract of 8.9%, EtOAc yielded a light red crude extract of 13.3%, and the MeOH extract yielded a black crude extract 34.6%. The percentage yield of crude extract in respective solvent was tabulated in (**Table 5)**. The methanol extract yielded the maximum, while the petroleum ether extract result was a minimum yield**.**

Table 5: The percentage crude extract yield of A. debrana leaf latex using different solvent.

Crude Extract	**Mass of Crude**	**Yield in (%)**	**Color of crude**
Petroleum ether	5.3g	7%	Yellow green
Chloroform	6.7g	8.9%	Yellow
Methanol	26g	34.6%	Brown
Ethyl acetate	10g	13.3%	Red

The efficiency of the extraction is strongly affected by the extraction method, temperature, extraction time, the composition of phytochemicals, and the solvent (Turkmen *et al*., 2006; Ngo *et al*., 2017). According to the results of these authors, under the same extraction conditions, solvent is recognized as one of the most critical parameters. The current results illustrated that the methanol extract of *A. debrana* latex had the highest amount of crude extract, followed by the ethyl acetate extract, with the lowest yield from the petroleum ether extracts. Better extraction with methanol might be linked to the fact that methanol has a better penetration efficacy across the plasma membranes that facilitate immediate liberation of cellular contents into the solvent and hence, results in net higher yield of crude extract. Our findings on better yield and subsequent bio- activities of methanolic extract are augmented by previous reports on bioassays in differential solvent systems (Mehmood *et al*., 2022). Similarly, Kankamol *et al*.(2021) reported that the methanol extract of *A. vera* rind had the highest amount of crude extract, followed by the ethyl acetate extract, with the lowest yield from the n-hexane extract.

4.2. Phytochemical screening results

In the present study, the qualitative analysis of the phytochemical constituents of air-dried latex samples of the *A. debrana* plant in the Petroleum ether, methanol, chloroform, and ethyl acetate crude extracts was carried out (**Table 6)**. Phytochemical constituents are responsible for the medicinal activity of plant species. Hence, each latex extracts of *A. debrana* were tested for the presence of various secondary metabolites (phytochemical) such as terpenoids, flavonoids, tannins, alkaloids, steroids, saponins, and phenols. Among these, flavonoids, tannins, steroids, terpenoids, saponins, and phenol compounds were revealed to be present in latex extracts of the *A. debrana* plant. At the same time, alkaloids did not appear in all extracts.

Steroids are absent in petroleum ether and chloroform extracts. On the one hand, methanol showed the presence of all the six phytochemicals listed in **(Table 6)**. The ethyl acetate extract contains all phytochemicals except flavonoids. Accordingly, a study done by Jha1 *et al*. (2018) shows that the ethanolic extracts of *A. vera* contain active compounds such as alkaloids, tannins, saponins, terpenoids, phenols and flavonoids which is similar with the present investigation, However, alkaloids were absent in present study. This study's results were also compatible with research conducted by (Arunkumar & Muthuselvam, 2009), who reported the presence of bioactive secondary metabolites in the leaf extracts of *A. vera*.

Furthermore, a study conducted by (Asmerom *et al*., 2020) showed that the qualitative phytochemical screening of the leaf exudate of *Aloe megalacantha* contains active compounds similar to those reported in the current study except alkaloids. A previous study by (Sbhatu *et al*., 2020) also reported that methanol extracts of *A. elegans* yielded positive results for anthraquinones, flavonoids, saponins, and tannins. The potency of the plant extracts depends on the solvent used for extraction. This may be due to the degree of solubility of the bioactive constituents. It has been documented that different solvents have diverse solubility capacities for other phytochemicals (Takazawa *et al*. 1982; Nenaah and Ahmed 2011). Variations in the composition of the secondary metabolites might be due to the difference in their genotypes, climatic conditions in the respective locality where the plants were grown, and also to the plant parts processed and extracted (Onuminya *et al*., 2017; Dewijanti *et al*., 2019). Similarly based

on the presence or absence of colour change indicate positive and negative results are indicated (**Appendix1**).

Table 6: Phytochemical screening results of *A. debrana* leaf latex crude extract.

Phytochemicals	**Extraction solvents**			
	Petroleum ether	**Chloroform**	**Methanol**	**Ethyl acetate**
Alkaloids	-	-	-	-
Flavonoids	+	+	+	-
Phenols	+	+	+	+
Steroids	-	-	+	+
Tannins	+	+	+	+
Saponins	+	+	+	+
Terpenoids	+	+	+	+

Key: + present, - absent

4.3. Characterization of the compounds

4.3.1. Thin-layer Chromatography profile

The isolated compound **1** has a yellowish color with R f value of 0.66 in petroleum ether: ethyl acetate (70:30) solvent system. The TLC analysis result of this compound showed yellowish when viewed under UV lamp 365 **(Figure 6)**. Similar procedures were repeated for combined fractions 30-39 and 32 mg yellowish compound (labeled as compound **2**) and its Rf values were determined and found to be 0.42 in petroleum ether (60:40) solvent system.

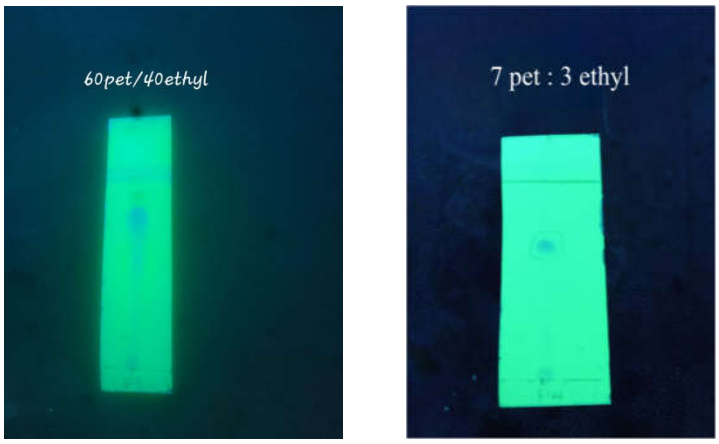


Figure 6: TLC profile of isolated compounds obtained observed under 365nm.

4.3.2. Compound 1

Compound **1** (**Figure 6)** was obtained as an amorphous yellow powder. The ^{13}C NMR **(Figure 9)**, DEPT 135 (**Figure 10),** and DEPT 90 spectroscopic data obtained by 600 MHz NMR in CDO_3D indicated that Compound **1** has 26 carbons, including five aromatic CH; nine quaternary carbons, four methylene, four methyl and one methoxy, groups) (**Table 7)**. The ^{1}H-NMR data (**Table 7 and Figure 8)** showed characteristic proton signals in the aromatic region at 6.01-6.33 ppm. Proton signals at 4.11 m and 3.55 s indicated the presence of oxymethylene and methoxy groups, respectively. These NMR spectral data are characteristic signals indicating the compound has a flavanol skeleton. Based on the NMR spectral data obtained and comparison with literature reports, the compound was proposed to have the following flavone structure.

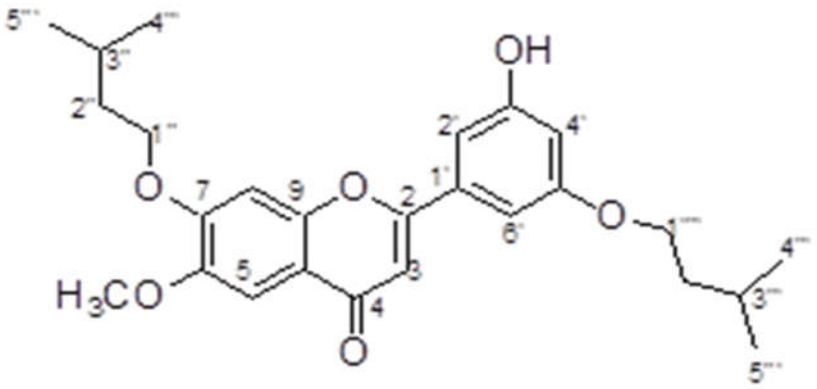


Figure 7: Structure of proposed compound **1**.

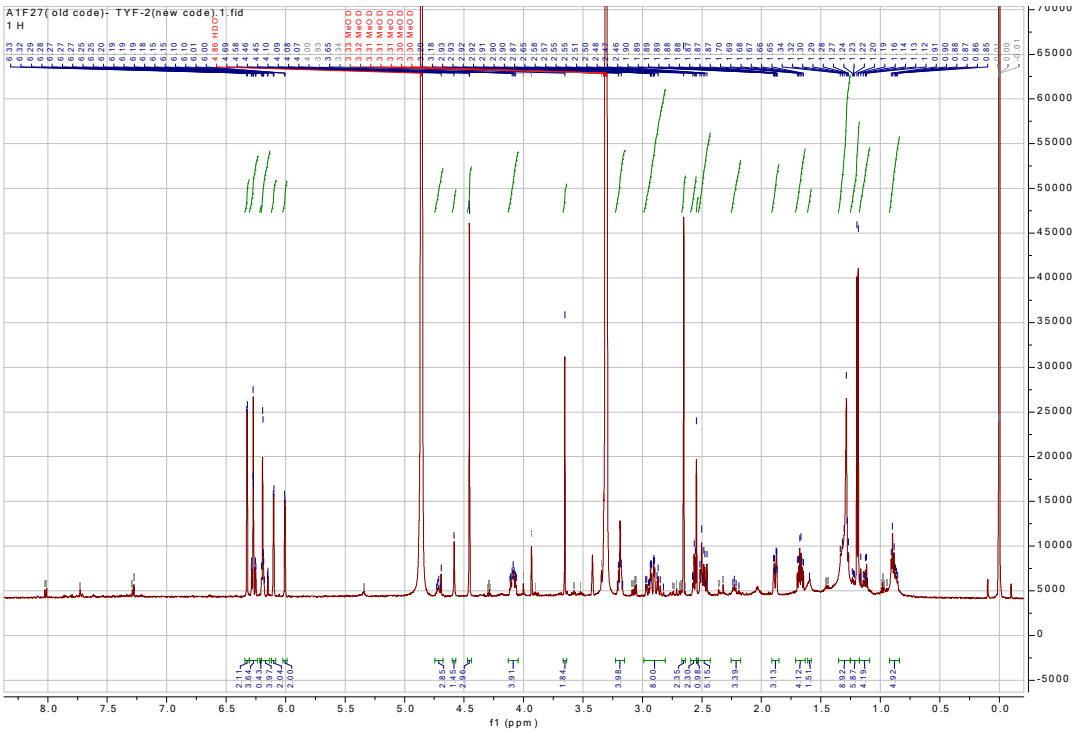


Figure 8: ^{1}H NMR spectrum of Compound **1**.

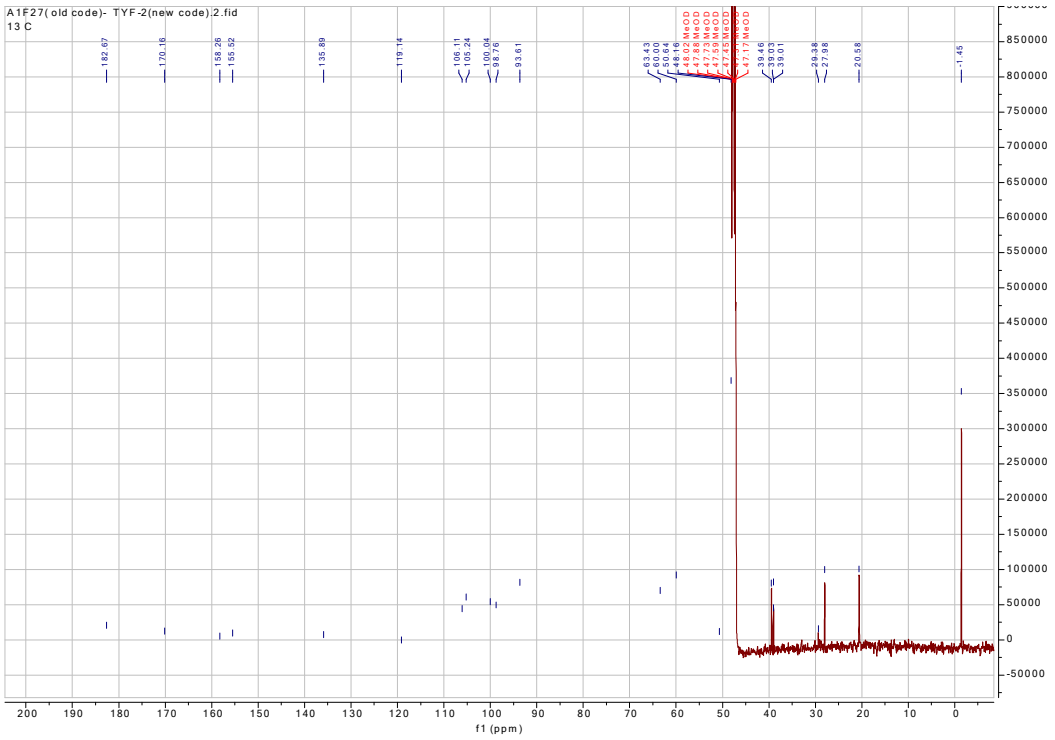


Figure 9: ^{13}C NMR spectrum of Compound **1**.

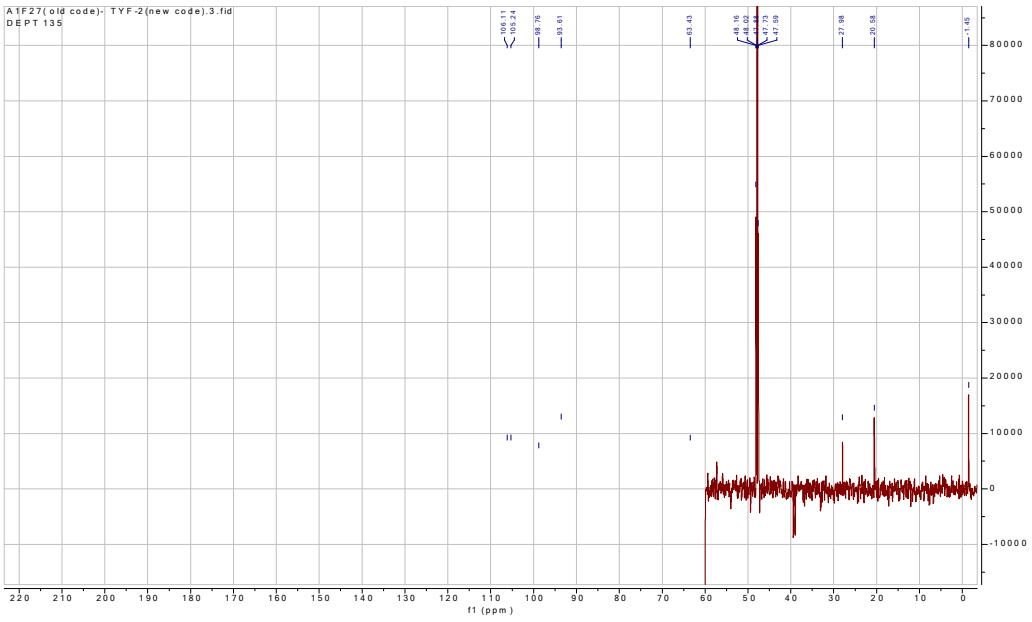


Figure 10: DEPT 135 spectrum of Compound **1**

Table 7: ^{1}H NMR (400 MHz),^{13}C NMR (100 MHz) DEPT-135 and DEPT 90 NMR data of compounds **1**.

compound 1			
C/H	δ^{1}H	δ^{13}C	DEPT-135 and DEPT-90
2	-	170.1	Q
3	6.19 (d)	93.6	=CH-
4	-	182.6	Q
5	6.32 (d)	106.1.	=CH-
6	-	135.4	Q
7	-	158.2	Q
8	6.27 (s)	95.7	=CH-
9	-	155.4	Q
10	-	119.4	Q
1’	-	135.4	Q
2’	6.15 (d)	106.1	=CH-
3’	-	158.2	Q
4’	6.06 (brd)	100.0	=CH-
5’	-	158.2	Q
6’	6.15 (d)	105.3	=CH-
1”	4.11 (t)	60.0	-CH_2O-
2”	1.55-1.99 (m)	39.4	-CH_2
3”	1.18 (d)	27.9	-CH-
4”	1.18 (d)	20.6	-CH-
5”	1.18 (d)	20.6	-CH-
1”’	4.11 (t)	60.0	-CH_2O-
2”’	1.55-1.99 (m)	39.4	-CH_2
3”’	1.18 (d)	27.9	-CH-
4”’	1.18 (d)	20.6	-CH-
5”’	1.18 (d)	20.6	-CH-

Chemical shift indicating: DEPT= Distortionless Enhancement by Polarization Transfer, *δ(= delta)*, m= multiplet, t= triplet, d= doublet, s= singlet.

4.3.3. Compound 2

Compound **2 (Figure 11)** was isolated as alight-yellow powder. The ^{1}H NMR spectrum (**Figure 12)** showed three aromatic methyl and two methoxy protons. Three mutually coupled aromatic protons at δH 7.76 (*d*, *J* = 7.6 Hz), 7.81 (*t*, *J* = 7.8 Hz), and 7.33 (*d*, *J* = 8.2 Hz) with ABX spin system were assigned to H-5, H-6, and H-7 of mono-substituted ring C of a 1,8-dihydroxyanthraquinone, respectively. The compound has two methoxy protons at δH 3.32 (s) and 3.34 (s) due to MeO-2 and MeO-4 and aromatic methyl (δH 1.90) **(Table 8)**. The unusual upfilled aromatic methyl proton from that of chrysophanol is due to the electron donation of methoxy groups at C-2 and C-4 of ring A. Based on the ^{1}H NMR data and comparison of the ^{1}H NMR data of chrysophanol (Abdissa *et al*., 2017), compound **2** is proposed to be 2-4-dimethoxy chrysophanol, which has the following structure.

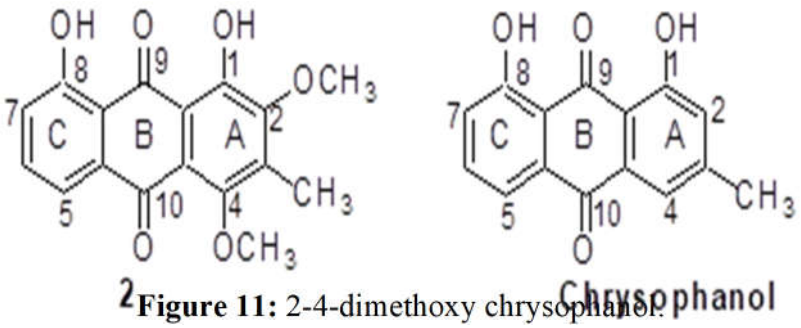


Figure 11: 2-4-dimethoxy chrysophanol.

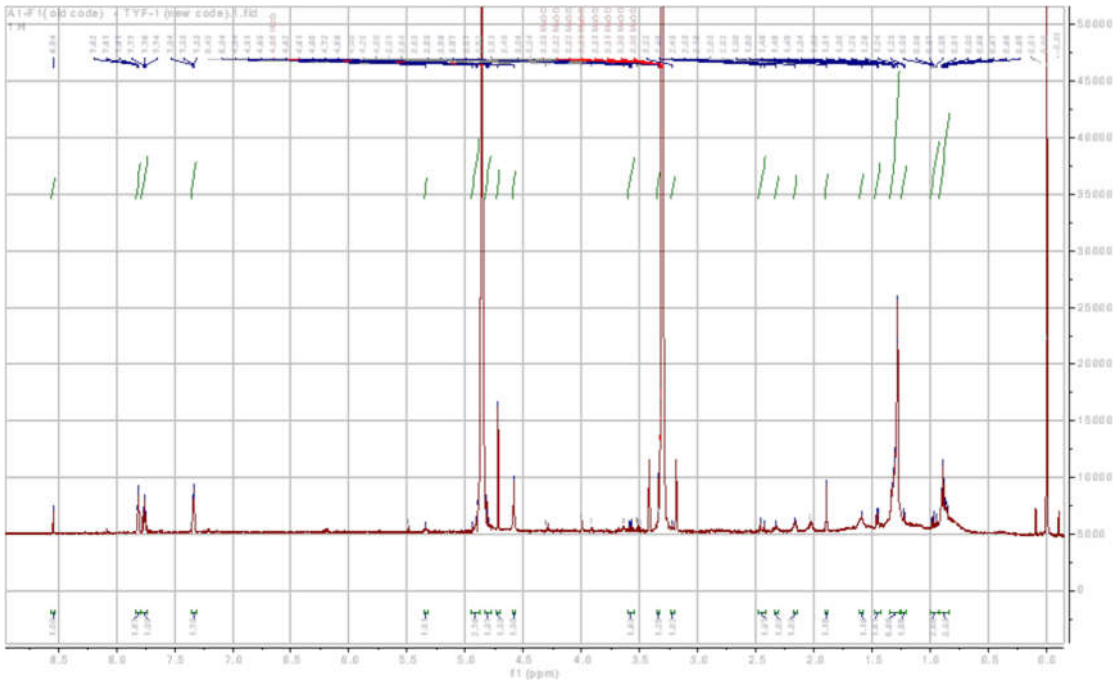


Figure 12 : ^{1}H NMR spectrum of Compound **2**.

Table 8:[1] H NMR (400 MHz) data of compounds **2**.

	Compound 2		(Abdissa *et al*., 2017)	
C/H	δ^1H	δ^{13}C	δ^1H	δ^{13}C
1				164.8
1a				116.9
2			7.21 (s, 2.4, 1H)	127.3
3				152.2
4			7.55 (d, 2.4, 1H)	123.6
4a				136.2
5	7.76 (d, 7.6, 1H)		7.70 (d, 7.6, 1H)	122.4
5a				136.4
6	7.81 (t, 7.8, 1H)		7.80 (t, 7.8, 1H)	140.4
7	7.33 (d, 8.2, 1H)		7.37 (d, 8.2, 1H)	127.6
8				164.6
8a				119.0
9				194.4
10				184.6
CH_3	2.44 (s, 3H)		2.44 (s, 3H)	
2-OCH_3	3.22 (s, 3H)			
4-OCH_3	2.34 (s, 3H)			
1-OH			12.13 (s, 1H)	
8-OH			12.02 (s, 1H)	

Chemical shift indicating: DEPT= Distortionless Enhancement by Polarization Transfer, *δ (= delta)*, m= multiplet, t= triplet, d= doublet, s= singlet.

4.4. GC-MS analysis of essential oils

The *A. debrana* leaf latex has a specific aromatic odor. GC-MS analysis of essential oils from the leaf latex of *A. debrana* revealed 20 chemical components. The compounds with their retention time (RT), molecular formula, and percentage area were presented in (**Table 9)**.

Table 9: GC MS analysis result of Essential oil of *A. debrana.*

No	Compound Name	RT (min)	Molecular Formula	%
1	Hexadecanoic (palmitic) acid, methyl ester	10.918	$C_{17}H_{34}O_2$	9.43
2	Phytol	4.762	$C_{20}H_{40}O$	12.94
3	Naphthalene	12.68	$C_{10}H_8$	4.89
4	Octadecanoic (stearic) acid, methyl ester	15.573	$C_{19}H_{38}O$	17.54
5	9,12,15- Octadecatrienoic acid methyl ester, (ZZZ)	21.175	$C_{19}H_{32}O_2$	6.62
6	Decanoic (capric) acid, methyl ester	10.803	$C_{11}H_{22}O_2$	6.11
7	Dodecanoic (lauric) acid, methyl ester	9.226	$C_{13}H_{26}O_2$	6.56
8	Benzene, 2-ethyl-1,4-dimethyl-	11.802	$C_{10}H_{14}$	5.27
9	Cyclohexadecane	31.432	$C_{16}H_{32}$	4.87
10	(E)-2-nonadecene	27.436	$C_{19}H_{38}$	4.66
11	Carvacrol	15.833	$C_{10}H_{14}O$	3.54
12	Benzene, diethyl-	9.036	$C_{10}H_{14}$	2.68
13	Propyl benzene	8.302	C_9H_{12}	1.95
14	Decane	7.528	$C_{10}H_{22}$	1.84
15	Benzene, 1-methyl-2-(2-propenyl)-	11.438	$C_{10}H_{12}$	1.79
16	Dibutyl phthalate	30.901	$C_{16}H_{22}O_4$	1.75
17	Benzene, 1,3-diethyl-5-methyl-	11.582	$C_{11}H_{16}$	1.70
18	Cyclopentanol, 1-methyl-	6.824	$C_6H_{12}O$	2.99
19	Benzene, (1-methylpropyl)-	9.463	$C_{10}H_{14}$	1.65
20	1-Sec-butyl-4-methylbenzene	12.16	$C_{11}H_{16}$	1.58

As indicated in **(Figure 13** and **Figure 14),** the major constitutes octadecanoic (stearic) acid, methyl ester (17.54 %), phytol (12.94 %), hexadecanoic (palmitic) acid, methyl ester (9.43 %), 9,12,15- octadecatrienoic acid methyl ester, (ZZZ)(6.62 %), dodecanoic (lauric) acid, methyl ester (6.56%), decanoic (capric) acid, methyl ester (6.11 %), benzene, 2-ethyl-1,4-dimethyl- (5.27 %), naphthalene (4.89 %), cyclohexadecane (4.87 %),(E)-2-nonadecene (4.66 %) and carvacrol (3.54 %). The remaining constituents ranged from 1.58 % to 2.68 %.

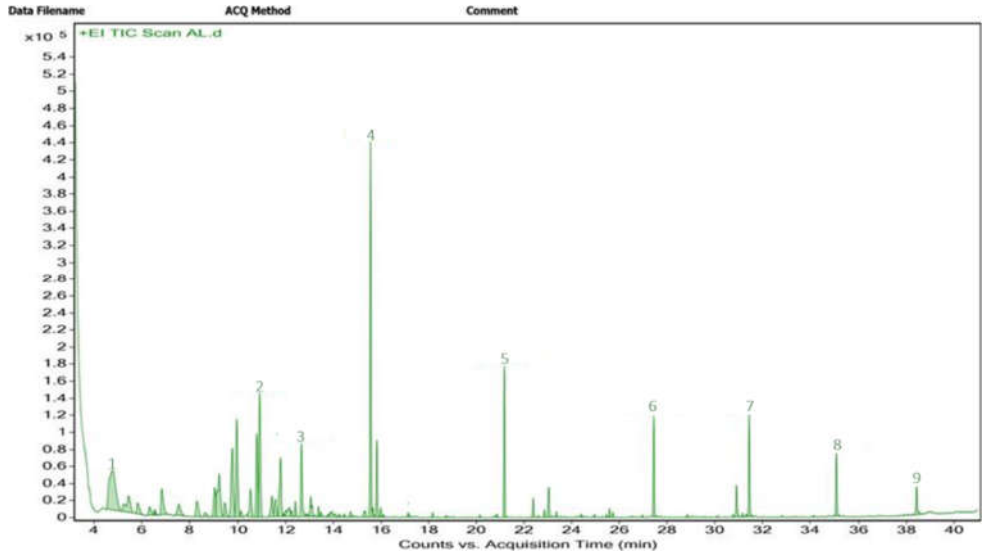
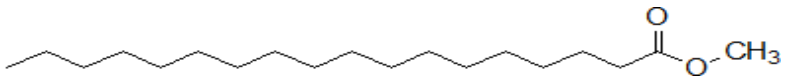
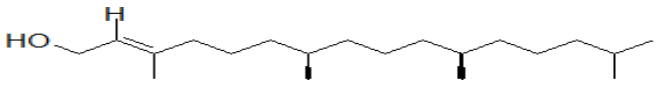


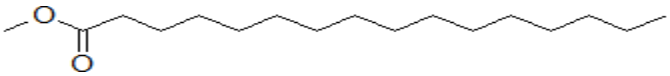
Figure 13: GC of A. debrana latex essential oil. 4: Octadecanoic 17.5 %; 2: phytol 12.94 %; 1: Hexadecanoic (palmitic) acid 9.435 %; 5: 9, 12, 15- octadecatrienoic acid methyl ester, (ZZZ) 6.62%; 7: dodecanoic (lauric) acid, 6.56%; 6: decanoic (capric) acid 6.11%; 8: benzene, 2-ethyl-1,4-dimethyl-5.27 %;3: P2714.89%;9: cyclohexadecane 4.87 %.



Octadecanoic (stearic) acid, methyl ester



Phytol



Hexadecanoic (palmitic) acid, methyl ester

Figure 14: Major components of essential oil from *A. debrana* leaf latex.

A previous study by Choike *et al*. (2017) on ethanol extraction and GC-MS analysis of *A. vera* oil reported 26 bioactive compounds. The result revealed that 9, 12, 15- Octodecatrienoic acid, methyl ester (Z, Z, Z) (11.36 %), and Phytol (10.01%) are the major components. In our sample, Phytol was 12.9%, but in essential oil analyzed by Derwick and co-workers, phytol was 10.01% (Derwich *et al*., 2009). (Uraku *et al.*, 2020) reported that carvacrol (4.2%) was one of the components from GC-MS analysis of simultaneous distillation extraction (SDE) from *A. debrana* roots. This result is compatible with the present study.

GC-MS analysis of phytoconstituents of *A. debrana* leaf latex highlights the pharmaceutical importance of the plant. The identified bioactive compounds occupy many biological properties. The presence of fatty acid esters such as hexadecenoic acid methyl ester and 9,12-octadecanoic acid methyl ester has various bioactivities, including anti-fungal, antioxidant, pesticide, hemolytic, and anti-microbial activity (Parimalakrishnan *et al*., 2015). A similar result was observed in the study by Simon *et al*. (2009) from *A. elegans* gel. The 9,12-octadecanoic acid methyl ester also has potential cancer-preventive, anti-inflammatory, and anti-arthritic activities (Ramya, 2022).

4.5. Antibacterial and antifungal activities assay

The antibacterial and antifungal potential of the four *A. debrana* extracts (petroleum ether, chloroform, MeOH, EtOAc), and compound **1** and compound **2** were examined using the disk diffusion assay. The effectiveness of the results, specifically the zone of inhibition, was compared with the activity of standard substances ciprofloxacin (10 µg/mL) for bacteria and ketoconazole (10 µg/mL) for fungus. DMSO was employed as a negative control.

Among the four extracts tested, methanol extract exhibited the maximum inhibitory activity 18±1.63 mm against *E. coli and S. pyogenes* at 200 mg/mL while the ethyl acetate extract showed maximum inhibition zone 15.33±0.47 mm against *S. aureus* at 200 mg/mL**.** The chloroform extract showed antibacterial activity against *E. coli* with an inhibition zone of 13.33±1.69mm at 200 mg/mL and petroleum ether extract showed a promising zone of inhibition (15.0±0.81 mm) against *P. aeruginosa* at 200 mg/mL **(Figure15)**. In general, the inhibitory activity of the extracts was found to be dose-dependent, where the zone of inhibition increased with increasing concentrations. This trend was observed across different extracts and bacterial strains. The results were quantified by measurements of the zone of inhibitions in mm and presented in (**Table 10).**

The statistical analysis showed that all concentrations of the tested *A. debrana* leaf latex crude extracts significantly inhibited all bacterial strain as compared to the ciprofloxacin ($P < 0:05$); however, there was no significant difference ($P > 0:05$) among 200mg/ml and 100mg/ml as well as there was no significant difference ($P > 0:05$) among 50mg/ml and 25mg/ml *A. debrana* leaf latex concentrations against bacterial strain. As indicated in (**Table 10)**, Petroleum ether leaf latex extracts of *A. debrana* show that the mean zone of inhibition was significantly different ($P < 0.05$) for leaf latex at 200 mg/ml and 25 mg/mL, compared to that at 100 mg/ml and 50 mg/mL against *p. aeruginosa;* however, there was no significant difference ($P > 0:05$) among 200mg/ml and 100 mg/ml against *P. aeruginosa.*

Table 10: Antibacterial activity of *A. debrana* crude extracts.

Extract type	Conc.(mg/mL)	Inhibition Diameter (mm) ±SD			
		E. coli	***S. aureus***	***P. aeruginosa***	***S. pyogenes***
Methanol	200	18±1.63^{b}	15.66±1.24^{c}	14±0.81^{b}	17±2.16^{b}
	100	16.33±0.47^{b}	12.33±1.24^{bc}	11.33±1.24^{b}	14.33±0.47^{b}
	50	11±1.63^{a}	9±0.81^{ab}	7.33±1.24^{a}	10.33±0.47^{a}
	25	8.33±1.24^{a}	7±0.81^{a}	6.66±0.47^{a}	7±0.81^{a}
Ciprofloxacin	10µg/mL	26.66±0.94^{c}	25.33±1.24^{d}	25.66±0.94^{c}	25.33±0.94^{c}
DMSO	10µg/mL	-	-	-	-
Ethyl acetate	200	13.33±1.24^{c}	15.33±0.47^{c}	13±0.81^{c}	14.66±0.47^{c}
	100	10.66±0.94^{bc}	11.33±2.05^{bc}	11.33±1.24^{bc}	12.33±1.24^{bc}
	50	8.66±1.69^{b}	9.66±1.69^{b}	8.33±1.88^{ab}	9.66±2.05^{ab}
	25	6.66±0.94^{a}	7.33±1.24^{a}	6.33±0.47^{a}	7±0.81^{a}
Ciprofloxacin	10µg/mL	25.66±0.47^{d}	26±0.81^{d}	24.66±0.47^{d}	25±0.81^{d}
DMSO	10µg/mL	-	-	-	-
Chloroform	200	13.33±1.69^{b}	10.66±0.94^{b}	12.33±0.47^{b}	11±1.41^{b}
	100	9.66±0.94^{ab}	9.66±1.24^{ab}	10±1.63^{ab}	9.33±0.47^{ab}
	50	9±0.81^{a}	8±0.81^{ab}	8.66±0.94^{a}	8.66±0.94^{ab}
	25	6.33±0.47^{a}	7.33±0.47^{a}	7±0.81^{a}	7±0.81^{a}
Ciprofloxacin	10µg/mL	25.66±1.69^{c}	24.33±0.47^{c}	25±0.81^{c}	22.66±0.94^{c}
DMSO	10µg/mL	-	-	-	-
Petroleum ether	200	13.33±1.24^{c}	13.66±1.24^{c}	15±0.81^{c}	12±0.81^{b}
	100	10.66±1.24^{bc}	11.33±0.94^{bc}	11.33±0.47^{b}	10±0.81^{ab}
	50	9±0.81^{ab}	10±0.81^{b}	10.33±0.47^{b}	9±1.41^{ab}
	25	7.33±0.47^{a}	6.33±0.47^{a}	7.33±0.94^{a}	7.66±0.47^{a}
Ciprofloxacin	10µg/mL	25.33±0.47^{d}	25.33±1.24^{d}	26.33±0.94^{d}	26±0.81^{c}
DMSO	10µg/mL	-	-	-	-

Values are expressed as mean ± SD and analysis was carried out with one-way ANOVA followed by post hoc Tukey test. a, b, c, d values in a column followed by the same superscripts

are not significantly different (P > 0:05) while different superscripts indicate statistical difference (P < 0:05), (-) means that the DMSO has not shown antibacterial activity.

Overall, *A. debrana* crude extracts have inhibitory effects on the tested bacteria, varying effectiveness depending on the solvent used and its concentration**.** Ciprofloxacin, a common antibiotic, is used as a positive control and generally shows good inhibition, providing a reference for comparison.

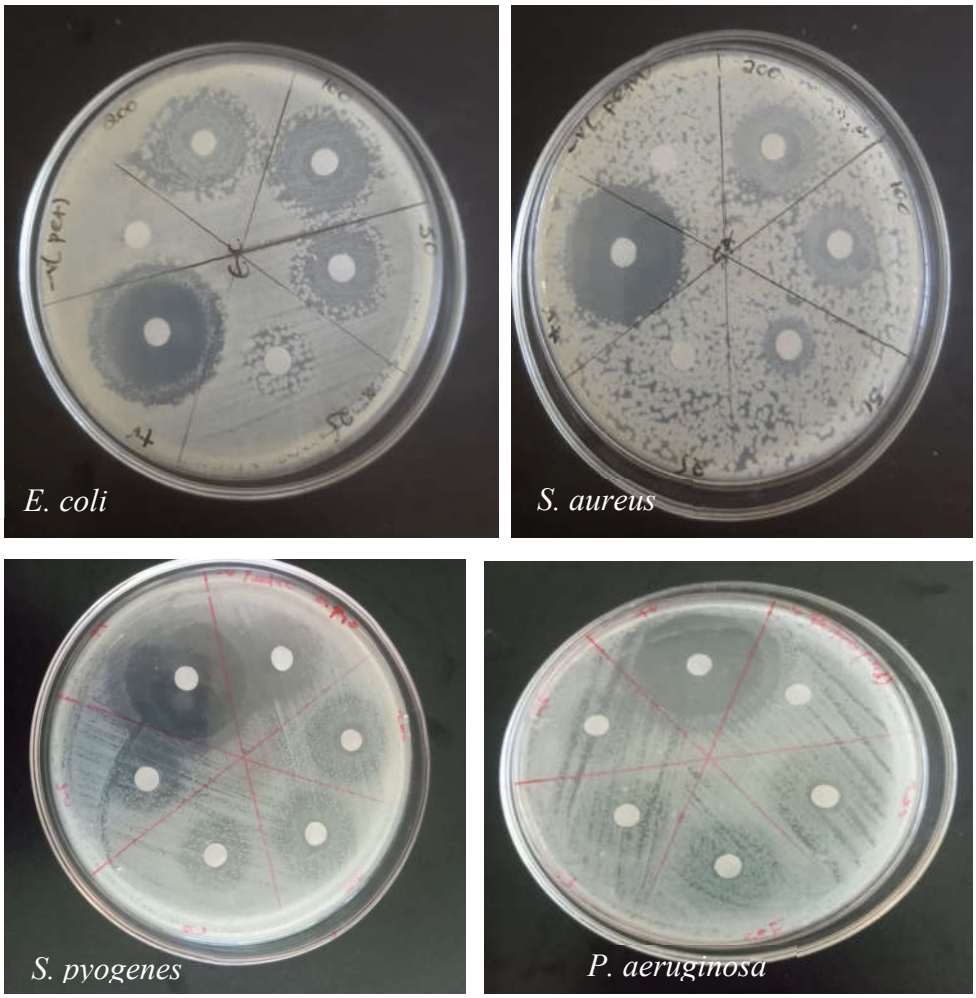


Figure15: Inhibition zones of *A. debrana* leaf latex crude extracts against bacterial strain in different concentrations.

The study revealed that various crude extracts obtained from the leaf latex of *A. debrana* demonstrated significant antimicrobial effects against the tested microbial pathogens, including *S. pyogenes, S. aureus, E. coli, and P. aeruginosa* (**Table 9 and Figure15)**. All the crude extracts exhibited good activity against the mentioned bacterial strains. Moreover, the methanol extract displayed the most potent antimicrobial activity, showing a maximum inhibition zone of 18±1.63 mm against *E. coli* at 200 mg/ml and a minimum inhibition zone of 6.66±0.47 mm against *P. aeruginosa* at 25 mg/ml. Similarly, other Ethiopian *Aloe* species, such as *A. trichosantha*, *A. sinana*, and *A. trigonantha,* have shown significant antibacterial activity against various pathogens, as reported by (Megeressa *et al*., 2015) and (Minale *et al*., 2014b).

The phytochemical analysis indicates that the methanol extract of *A. debrana* contains a rich combination of bioactive compounds, each contributing to its antibacterial activity through various mechanisms. The synergistic effects of these phytochemicals may enhance the overall efficacy of the plant extract as a potential antimicrobial agent. The plant extracts might have a wide variety of phytochemicals with different mechanisms of action for their antimicrobial activity (Adeonipekun *et al*., 2014). Phenolic compounds, specifically phenols and hydroxylated phenols, are known for inhibiting bacterial development. The mechanism involves interference with enzymes and oxidizing compounds, possibly through reactions with sulfhydryl groups or nonspecific interactions with proteins. This finding aligns with previous research highlighting the role of phenolic compounds in antimicrobial activities (Bouarab-Chibane *et al*., 2019). Flavonoids, another group of phytochemicals identified in the analysis, exhibit antimicrobial effects through various mechanisms. Their ability to bind to extracellular and soluble proteins and bacterial cell walls inactivate enzymes and disrupt microbial membranes, contributing to their antimicrobial properties (Takó *et al*., 2020). Tannins function as antimicrobials by binding to adhesions, inhibiting enzymes, depriving bacteria of their food, forming a complex with the cell wall, disrupting membranes, and complexing metal ions (Vu *et al*., 2017). Terpenoids show antimicrobial activity by membrane disruption.

The polar components were more active in our investigation than non-polar fractions. The antibacterial effects correlated with increasing polarity, suggesting that the active constituents of the extract are predominantly concentrated in the polar portions. The different antibacterial

activities of various plant extracts were more likely due to the presence of various bioactive compounds in test plants, the extraction capacity of the solvents, the sensitivity of the bacterial strains used, and the combined effect of these conditions. Previous studies also confirm these differences in antibacterial activity by different plant extracts (Ahmed *et al*., 2013; Singh *et al*., 2017).

Similarly, the antifungal activity of *A. debrana* leaf latex crude extracts against *C. albicans* was evaluated. As indicated in (**Table 11 and Figure 16),** varying diameters of inhibition zones have been observed. The methanol extract exhibited a diameter of 19.0±0.81 mm, ethyl acetate extracts 17.66±1.24 mm, petroleum ether extract 16±1.63 mm, and chloroform extract 16.66±0.94 mm at 200 mg/mL, compared to ketoconazole 24.66±1.24 mm, 23.33±1.2 mm, 25.33±0.47 mm, and 24.66±1.69 mm, respectively at 10 µg/mL.

Table 11: Antifungal activities of crude extracts from *A. debrana*.

Extract type	**Conc. (mg/mL)**	**Inhibition Diameter (mm)±SD**	**Ketoconazole (10 µg/ mL)**	**DMSO**
		C.albicans		
Petroleum ether	200	16 ± 1.63^{c}	25.33 ± 0.47^{d}	–
	100	14 ± 0.81^{b}		
	50	10.33 ± 1.24^{a}		
	25	8.66 ± 0.47^{a}		
Chloroform	200	16.66 ± 0.94^{c}	24.66 ± 1.69^{d}	–
	100	13.66 ± 0.47^{bc}		
	50	11 ± 0.81^{ab}		
	25	8.66 ± 0.47^{a}		
Ethyl acetate	200	17.66 ± 1.24^{b}	23.33 ± 1.24^{c}	–
	100	15.33 ± 1.24^{b}		
	50	11.33 ± 0.94^{a}		
	25	8.33 ± 0.47^{a}		
Methanol	200	19 ± 0.81^{b}	24.66 ± 1.24^{c}	–
	100	17.33 ± 1.69^{b}		

	50	11.66±0.47[a]
	25	9±0.81[a]

Values are expressed as mean ± SD and analysis was carried out with one-way ANOVA followed by post hoc Tukey test. a, b, c, d values in a column followed by the same superscripts are not significantly different (P > 0:05) while different superscripts indicate statistical difference (P < 0:05), (-) means that the DMSO has not shown antibacterial activity.

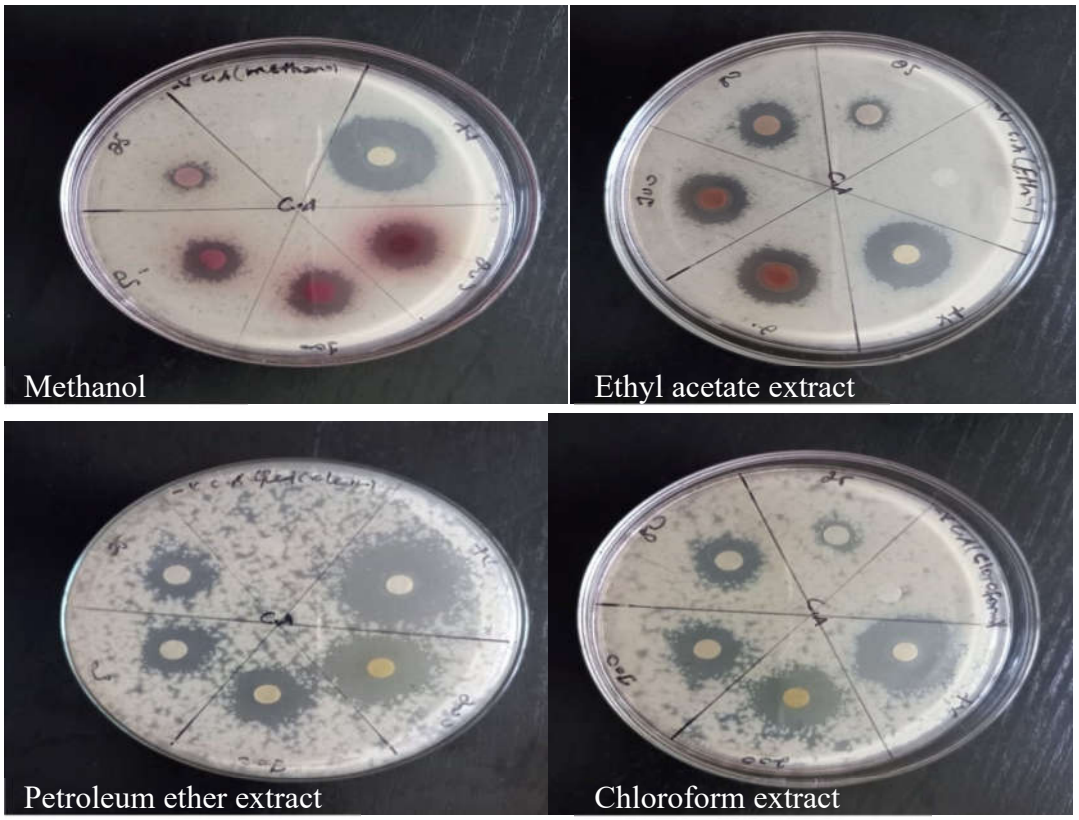


Figure 16: Inhibition zones of *A. debrana* leaf latex crude extracts against *C. albicans* in different concentrations.

The mean zone of inhibition of all concentrations of leaf latex showed statistically significant difference (P < 0.05) when compared with ketoconazole **(Figure 16).** The average zone of inhibition at 200 mg/mL for each concentration was not significantly different (P >0.05), except for Petroleum ether compared to that at 100 mg/mL against C. *albicans*. Similarly, the mean

zone of inhibition at 50 mg/mL of each concentration have no significant difference ($P > 0.05$) when compared with that at 25 as presented in **(Table 11)**.

From the result, *A. debrana* latex crude extract has antifungal activity against *C. albicans,* in which methanol extract appears to be the most potent, followed by ethyl acetate, chloroform, and petroleum ether extracts. Interestingly, the leaf latex inhibited tested *C. albicans* in a dose-dependent fashion presented in (**Table 11**). This might be because plants have various active constituents that inhibit fungal growth *(*Aqil *et al*., 2010). Most of the crude extract showed a maximum zone of inhibition against *C. albicans.* A fungal pathogen commonly found in the human gastrointestinal tract, genitourinary tracts, and oral cavity (Kabir *et al*., 2012). While *C. albicans* is typically harmless in normal microflora, immunocompromised individuals may experience various infections, ranging from mild to severe, including cutaneous, oral, vaginal, and systemic diseases (Kabir *et al*., 2012; Mayer *et al*., 2013). To counter this, the antifungal potential of *A. debrana* leaf latex was evaluated, revealing a remarkable antifungal effect.

Mainly, methanol extract showed a maximum zone of inhibition ranging from 19±0.81 at 200 mg/ml and minimum inhibition of 9±0.81 at 25 mg/ml against *c. albicans* comparable to ketoconazole. This effect is essential to remedy the global threat of antifungal resistance. In line with this investigation, Ethiopian *Aloe* species such as *A. trichosantha* and *A. elegans* exhibited suitable antifungal activities(Oumer *et al*., 2014; Habtemariam & Medhanie, 2017). This might be because *Aloe* species was the house store of various bioactive compounds with diverse biological activities (Dagne *et al*., 2000). However, in this study, crude extracts showed good antifungal activity with a large zone of inhibition compared to antibacterial activity.

Given the promising antimicrobial growth inhibitory effect of the latex crude extracts, it was further subjected to compound isolation to find a more potent antibacterial and antifungal agent. As shown in (**Table12 and Figure17)**, the isolated compounds Compound **1** and Compound **2** showed good inhibition zone at different concentrations against four bacterial strains, *E. coli, S. aureus, S. pyogenes*, and *P. aeruginosa,* than that of the latex crude extracts.

Compound **1** exhibited good antibacterial activity against *E. coli* 15.66±1.24 mm, and *P. aeruginosa* 14.33±0.47 mm at 10 mg/ml. the mean zone of inhibition of all concentrations of

compound **1** showed that statistically significant difference ($P < 0.05$) when compared with ciprofloxacin. The average zone of inhibition at 10 mg/mL for each concentration was not significantly different ($P > 0.05$) when compared to that at 5 mg/mL against all bacterial strain. Similarly, the mean zone of inhibition at 2.5 mg/mL of each concentration showed no significant difference ($P > 0.05$) when compared with that at 1.25mg/ml against all bacterial strain as presented in **(Table12)** while it have significantly different ($P < 0.05$) at 10 mg/ml and 5 mg/ml when compared with that at 2.5 mg/ml and 1.25 mg/ml.

Compound **2** showed moderate to high inhibitory activity against the tested bacterial strains notably, it displayed better activity against *E. coli* at 10 mg/ml. **Table 12** shows that the mean zone of inhibition of all concentrations of compound **2** showed statistically significant difference ($P < 0.05$) when compared with ciprofloxacin. Compound **2** average zone of inhibition at 10 mg/mL and 5 mg/mL for each concentration was significantly different ($P < 0.05$) compared to that at 2.5 mg/mL and 1.25 mg/mL against all bacterial strain. However, Compound **2** average zone of inhibition at 10 mg/ml for each concentration was not significantly different ($P > 0.05$) against all bacterial strain when compared to that at 5 mg/mL Similarly, the mean zone of inhibition at 2.5 mg/mL of each concentration showed no significant difference ($P > 0.05$) when compared with that at 1.25 as presented in **(Table12).**

Table12*:* Antimicrobial activities of compound **1** and compound **2**.

Extract type	**Conc.**	**Inhibition Diameter (mm) ±SD**			
	(mg/mL)	***E. coli***	***S. aureus***	***S. pyogenes***	***P. aeruginosa***
Compound**1**	10	15.66±1.24^{b}	13±1.41^{b}	12±1.41^{c}	14.33±0.47^{b}
	5	13.33±1.24^{b}	11.66±1.24^{ab}	10±0.81^{bc}	11.33±0.94^{ab}
	2.5	9.0±0.81^{a}	9.66±0.94^{ab}	8.66±0.47^{ab}	9.33±0.47^{ab}
	1.25	7.33±0.47^{a}	8.66±0.47^{a}	7.33±1.24^{a}	7.66±0.94^{a}
(ciprofloxacin)	**10µg/mL**	23±0.81^{c}	21±0.81^{c}	23.66±0.94^{d}	20.66±1.88^{c}
DMSO	**10µg/mL**	-	-	-	-
Compound**2**	10	14.66±0.47^{c}	13.33±0.47^{c}	12.66±1.24^{b}	13.66±0.94^{b}
	5	12.33±1.24^{bc}	11.33±0.47b^{c}	11.33±0.47^{b}	12±0.81^{b}
	2.5	10±0.81^{ab}	9.66±0.94^{ab}	8±0.81^{a}	8.66±0.47^{a}
	1.25	7.66±0.47^{a}	7.33±0.94^{a}	7.33±0.47^{a}	7.33±0.47^{a}
(ciprofloxacin)	**10µg/mL**	23±0.81^{d}	20.66±1.69^{d}	22.66±0.94^{c}	22±1.63^{c}
DMSO	**10µg/mL**	-	-	-	-

Values are expressed as mean ± SD and analysis was carried out with one-way ANOVA followed by post hoc Tukey test. a, b, c, d values in a column followed by the same superscripts are not significantly different (P > 0:05) while different superscripts indicate statistical difference (P < 0:05), (-) means that the DMSO has not shown antibacterial activity.

Moreover, the current investigation also demonstrated substantial antimicrobial efficacy of compound **1** and compound **2**, as illustrated in (**Table 12 and Figure 17)**. Remarkably, compound **1** and compound **2** exhibited markedly superior antimicrobial activity compared to the crude extracts against the tested microorganisms at different concentration. According to Lawrence *et al*.(2009) and Savoia, (2012), the location and number of hydroxyl groups in phenolic compounds may be correlated with their relative toxicity to microorganisms, and the increase in hydroxylation results in an increase in toxicity. Hence, the isolated compounds' action mechanism might be their effect on a microorganism's cell wall integrity, enzymatic activity, protein inactivation, or mechanisms yet to be determined.

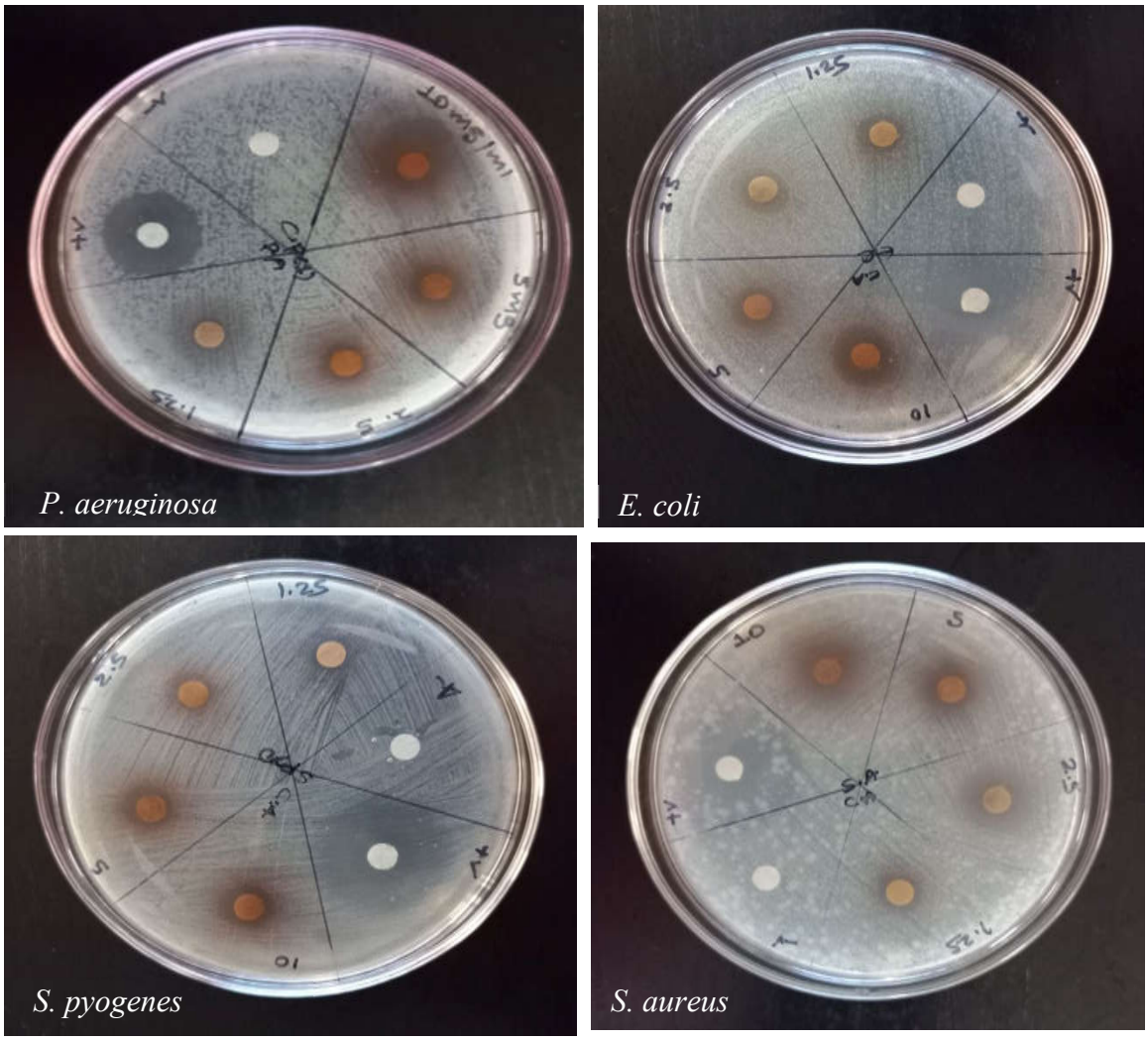


Figure 17: Inhibition zones of compound **1** of *A. debrana* leaf latex against bacterial strain in different concentrations.

In a study by (Kambizi *et al*., 2005), aloin A/B, derived from *A. ferox*, demonstrated efficacy against various Gram-negative and Gram-positive bacterial strains. The variations in morphological components between Gram-positive and Gram-negative bacteria may account for the differences in their susceptibility to antibacterial agents. The structural elements of lipopolysaccharides in the outer phospholipid membrane of Gram-negative bacteria contribute to the impermeability of their cell walls to antimicrobial substances. In contrast, Gram-positive bacteria possess an outer peptidoglycan layer that renders their cell walls more permeable to antimicrobial agents compared to the lipopolysaccharide layer in Gram-negative bacteria. Consequently, the structural complexity of the cell walls in Gram-negative bacteria surpasses that of Gram-positive bacteria. As a result, Gram-negative bacteria demonstrate lower

susceptibility to antimicrobial substances compared to Gram-positive bacteria (Chanda & Baravalia, 2010) .

However, the current investigation revealed that the leaf latex of crude extracts *A. debrana* and its compounds were much more active against the Gram-negative bacteria than the Gram-positive bacteria. This activity, therefore, could be explained from the chemical nature of the test crude extracts, which might affect the overall impermeability and integrity of the bacterial cell wall. This aligns with prior research by (Asamenew *et al.*, 2011), suggesting that *A. harlana* plant extracts are particularly effective against Gram-negative bacteria. Similar findings were reported for *A. citrina* (Girma *et al.*, 2015).

Compound **1** and Compound **2** were tested for their antifungal activity in different concentrations against *C. albicans* and compared to ketoconazole as the positive control and DMSO was used as a negative control in which activity increased in a dose dependent manner (**Figure18**). Compound **1** showed 14±0.81 inhibition zone against *C. albicans* at a concentration of 10mg/ml compared to ketoconazole 23.66±1 while compound **2** showed 12.66±0.47 inhibition zone against *C. albicans* at a concentration of 10mg/ml compared to ketoconazole 24.66±0.47 **(Table13)**.

The mean zone of inhibition of all concentrations of compound **1** and compound **2** showed statistically significant difference ($P < 0.05$) when compared with ketoconazole. however, Ketoconazole (10 µg/ mL) have not significantly different ($P > 0:05$) antifungal activity in comparison to compound **1** and compound **2** (**Figure18**). The compound **1** and compound **2** average zone of inhibition at 10 mg/mL and 5 mg/mL for each concentration was significantly different ($P < 0.05$) when compared to that at 2.5 mg/mL and 1.25 mg/ml against *C. albicans*. However, the compound **1** and compound **2** average zone of inhibition at 10 mg/ml for each concentration was not significantly different ($P > 0.05$) when compared to 5 mg/ml against *C. albicans.* Similarly, average zone of inhibition at 2.5 mg/ml for each concentration was not significantly different ($P > 0.05$) when compared to 1.25 mg/ml against *C. albicans* **(Appendix 2&3)**.

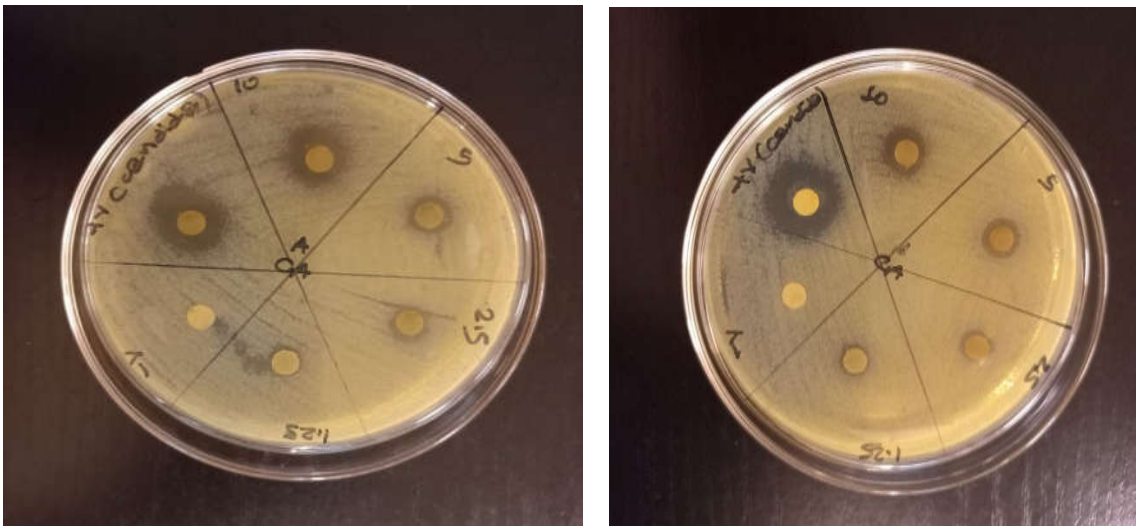


Figure18: Antifungal activity of Compound **1** and compound **2** against *C. albicans*.

Table 13: Antifungal activities of isolated compounds from *A. debrana*.

Extract type	**Conc. (mg/mL)**	**Inhibition Diameter (mm)±SD**	**Ketoconazole (10 µg/ mL)**	**-ve control**
		C.albicans		
Compound**1**	10	14±0.81^{c}	23.66±1.24^{d}	–
	5	11±0.81^{bc}		
	2.5	9.66±0.94^{ab}		
	1.25	7±0.81^{a}		
Compound**2**	10	12.66±0.47^{c}	24.66±0.47^{d}	–
	5	11.33±0.47^{bc}		
	2.5	9.33±1.24^{ab}		
	1.25	8±0.81^{a}		

Values are expressed as mean ± SD and analysis was carried out with one-way ANOVA followed by post hoc Tukey test. a, b, c, d values in a column followed by the same superscripts are not significantly different (P > 0:05) while different superscripts indicate statistical difference (P < 0:05), (-) means that the DMSO has not shown antibacterial activity.

This study demonstrated that compound **1** and compound **2** exhibited a significant inhibitory effect, with a zone of inhibition measuring 14±0.81 for compound **1** and 12.66±0.47 for compound **2** at a 10 mg/ml concentration against *C. albicans***.** Overall, *C. albicans* proved to be the most susceptible to these compounds among the tested organisms. These findings align well with the results reported by (Kambizi & Afolayan,2008), who reported the antifungal activity of Aloin isolated from *A. ferox* against *C. albicans*. Similarly, a study by (Asamenew *et al*., 2011) confirmed the susceptibility of *C. albicans* to latex and two isolated compounds from *A. harlana* among the tested fungal strains which agrees with current finding. Notably, there is limited existing literature on the antifungal properties of *Aloe* species.

The antibacterial and antifungal activity results of *A. debrana* crude extract, compound **1,** and compound **2** displayed good antimicrobial activity against all the tested microbial strains. The concentration of all extracts was significantly associated with the mean zone of inhibition (in mm). This study demonstrated an increase in the zone of inhibition in a dose-dependent manner with the increase in concentration, which agrees with the report of (Shetty *et al*., 2016) and Edogbanya *et al*., 2019). Finally, the results of this study elucidate the antimicrobial activities of *A. debrana* and provide evidence to support its use in folk medicine.

4.6. Minimum inhibitory concentration (MIC)

The crude extracts, compound **1** and compound **2** of *A. debrana* leaf latex, showed promising inhibition zones compared with the standard reference antibiotics, we considered continuing further tests for its bacterial activities. The broth dilution method was used to obtain the minimum concentration that can inhibit the visible growth of each bacterium. The MIC is the lowest concentration of crude extracts and Isolated compounds that inhibited visible growth of bacteria and fungus after an overnight incubation.

As shown below **(Table 14)** the MIC value were 50 mg/mL for the methanolic extract except *P. aeruginosa* and *E. coli* which were inhibited at 25 mg/ml while the MIC value were 25 mg/mL for the ethyl acetate extract except *P. aeruginosa* and *C. albicans* which were inhibited at 50 mg/ml. Petroleum ether extract couldn't inhibit the growth of bacteria below 100 mg/ml for three bacterial strains except *S. aureus* which is at 50 mg/ml .similarly chloroform extract also couldn't inhibit the growth of bacteria below 50 mg/ml for three bacteria strain except *S.*

pyogenes which is at 25 mg/ml and *C. albicans* at 100 mg/ml. Compound **1** MIC was 2.5 mg/ml except for *S. pyogenes* & *E. coli* 5 mg/ml **(Table 14)**. The MIC value of compound **2** for *E. coli and C. albicans* was 2.5 mg/ml while *P. aeruginosa* and *S. aureus was* 5 mg/ml.

Generally, *A. debrana* exhibits varying degrees of inhibitory activity against different organisms. Methanol crude extract showed relatively good inhibitory activity across all microorganisms compared to other extracts. Petroleum ether crude extract showed higher MIC values, indicating lower inhibitory activity than other extract. Similarly, compound **1** and compound **2** showed good inhibitory effects against *E. coli, P. aeruginosa,* and *C. albicans.*

Table14: The MIC value for the extracts and compounds.

Plant extract by	**Minimum inhibitory concentration (mg/ml)**				
	E. coli	***P. aeruginosa***	***S. aureus***	***S. pyogenes***	***C. albicans***
Methanol	25	25	50	50	50
Ethyl acetate	25	50	25	25	50
Petroleum ether	100	100	50	100	100
Chloroform	50	50	50	25	100
Compound **1**	5	2.5	2.5	5	2.5
Compound **2**	2.5	5	5	10	2.5

4.7. Minimum bactericidal and minimum fungicidal concentration (MBC and MFC)

The absence of bacterial and fungi growth on the MHA and SDA agar plates validated the MBC and MFC. As observed in (**Table15)**, the minimum bactericidal concentration and minimum fungicidal concentration are at high dosages in all crude extract, compound **1,** and compound **2,** especially in petroleum ether (200 mg/ml) in most bacteria and fungi. Ethyl acetate and methanol extract showed potentially bactericidal and fungicidal activity against the tested pathogenic bacteria with MBC and MFC of 50 to 100 mg/mL, except in the case of methanol the MBC for *S. pyogenes* bacteria was 200mg/mL. Chloroform extract showed potentially

bactericidal activity against the tested pathogenic bacteria with MBC of 200 mg/ml on *E coli* and 100 mg/ml on *P. aeruginosa and S. aureus* and 50 mg/ml for *S. pyogenes* while MFC at 200 mg/ml.

In the case of compound **1,** MBC activity at 10 mg/mL against *E. coli* and *S. Pyogenes* and 5mg/mL against *S. aureus* and *P. aeruginosa,* whereas MFC value at 2.5 mg/mL. Compound **2** showed MBC activity at 10 mg/ml in all tested bacteria except *E. coli.* The MFC result for compound **2** was 5 mg/ml. As observed in the present result, MFC is better than MBC value. This result supports the idea that traditional practitioners should use this plant at high dosages.

Table 15: The MBC and MFC value for the extracts, compound **1** and compound **2**.

Plant extract	**MBC (mg/ml)**				**MFC (mg/ml)**
	E. coli	***P. aeruginosa***	***S. aureus***	***S. pyogenes***	***C. albicans***
Methanol	100	50	100	200	100
Ethyl acetate	100	100	50	50	100
Petroleum ether	200	200	100	200	200
Chloroform	200	100	100	50	200
Compound **1**	10	5	5	10	2.5
Compound **2**	5	10	10	10	5

4.8. Antioxidant activity of *A. debrana*

The antioxidation potential of the crude extracts, essential oil, and isolated compounds of *A. debrana* was assayed and evaluated using different concentrations. For this purpose, the DPPH assay was conducted for each extract sample solution. The results were expressed as %RSA compared to ascorbic acid (reference standard) (**Figure 19&20)**. Additionally, all the extracts tested have displayed an increasing radical scavenging activity with an increase in concentration. The antioxidant activity test assay of the sample extracts and compounds demonstrated the effective potential of each extract to scavenge free radicals.

The DPPH radical scavenging activities (%) of methanol, ethyl acetate, and chloroform extract s were found to be 75.58%, 85.35%, and 54.78% at1000 µg/mL, respectively as 86.13%, 72.27%, and 79.20RSA were for Compound **1,** Compound **2**, and essential oil at 100 µg/mL respectively. For the various concentrations, compound **1** exhibited the highest inhibition percentage of the DPPH compared to the other extracts and isolated compounds. (Gorinstein *et al*., 2007) .The high antioxidant activity of plant extracts was due to the high phenolic, tannin, and flavonoid compounds and the polar compounds in the hydroalcoholic or alcoholic extracts. Ascorbic acid, positive control, showed maximum scavenging effect at low and high concentrations. Antioxidants can slow down or prevent the oxidation process of other molecules. Compared to standard ascorbic acid, the DPPH radical scavenging activity of *A. debrana* leaf latex extracts was lower, as indicated in (**Figure 19 & 20)**.

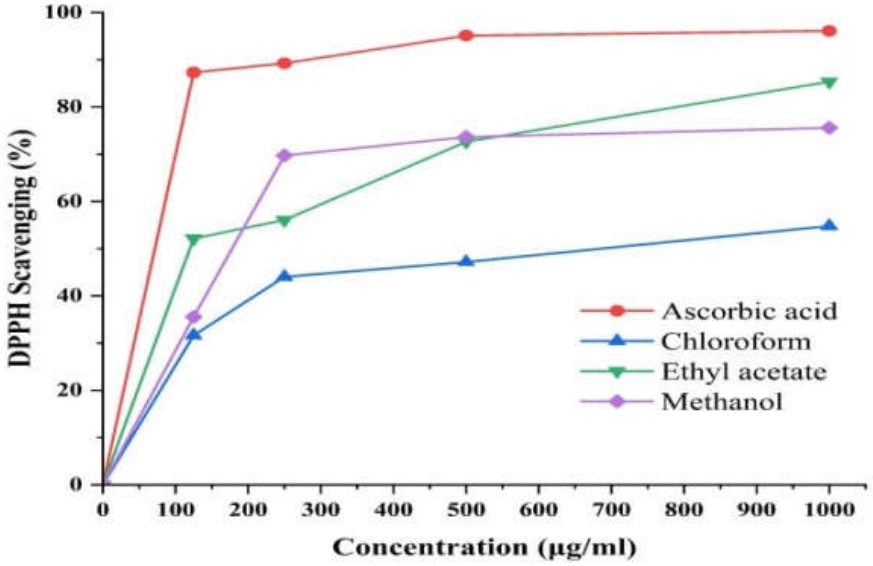


Figure 19: Graphical representation of antioxidant activities of crude extracts.

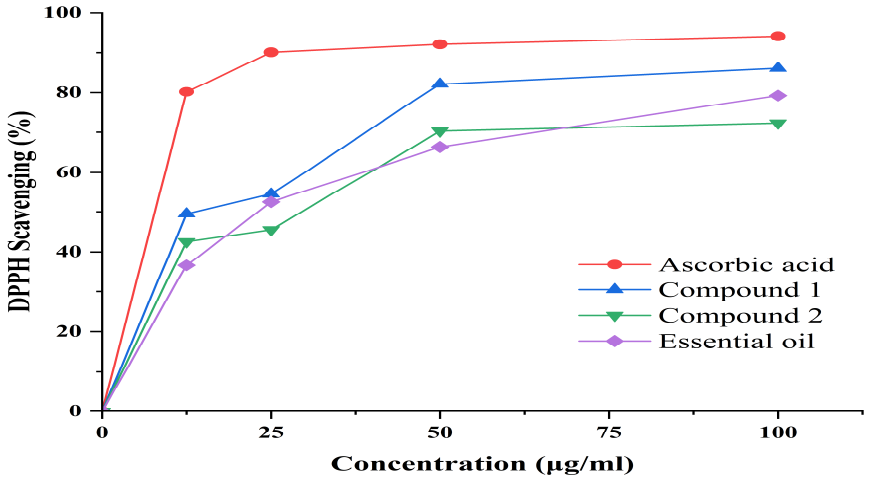


Figure 20: Graphical representation of antioxidant activities of essential oil and compounds.

The result showed that the activity of the extracts and compounds against DPPH radicals was dose-dependent, in which the DPPH scavenging activity percentage was directly increased with the increase in concentration (Elmastaş *et al*., 2006). In the present study, the chloroform extract showed relatively lower antioxidant activity than those reported in the data. This difference in the results is probably due to the methods used in extraction, the plant variety, and the methodology for evaluating the antioxidant activity.

The IC $_{50}$ value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using a log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity (Koleva *et al*.,2002). In this study, based on data in **(Figure 19)** the IC_{50} values for the ethyl acetate, chloroform, and methanol extracts were 42.01, 718.57and 60.26, respectively. Since low IC_{50} values correspond to higher antioxidant capacity, compared to chloroform and methanol extracts for their antioxidant activity, ethyl acetate extract (which is 42.01) is better. According to (Getahun *et al*., 2020a), the EO of *A. debrana* roots extract obtained by SDE also showed promising antioxidant activity with IC_{50} values of 48.65±1.32 µg/mL in DPPH, The current study agrees with this finding. Compound **1** had better radical scavenging activity among the studied samples, and its IC_{50} value was 5.03.

5.CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

In the present study, the air-dried leaf latex of *A. debrana* was extracted sequentially with petroleum ether, chloroform, methanol, and ethyl acetate. The highest crude extract yield (34.6%) was obtained from methanol extract. However, the lowest yield was obtained from petroleum ether extract. Qualitative phytochemical screening tests were done on the crude extracts of *A. debrana* leaf latex, and the results showed the presence of phenol, saponins, terpenoids, steroids, flavonoids, and tannins. The chemical components in leaf latex of *A. debrana* by GC-MS analysis clearly showed the presence of 20 compounds with other minor components. The EtOAc crude extract of the plant subjected to column chromatography yielded compounds **1** and **2**, characterized by the NMR spectroscopy technique. The extracts and isolated compounds also tested against two-Gram positive bacteria *Staphylococcus aureus* (ATCC25923) and *streptococcus pyogenes* (ATCC11778), two-Gram negative bacteria *Escherichia coli* (ATCC25922) and *P. aeruginosa* (ATCC27823) and one fungus *C. albicans* (ATCC 10231). All four crude extracts have significant antibacterial and antifungal activity on most bacteria and fungi. At the same time, methanol extract had maximum inhibition activity compared to other crude extracts. Compound **1** showed better antibacterial activity against *E. coli* (13±1.41 mm) at 10 mg/mL compared to ciprofloxacin (21±0.81 mm) at 10 µg/mL and showed potent activities against *C. albicans* (14±0.81 mm) compared to Ketoconazole (23.66±1.24 mm) at 10 mg/mL. However, the antimicrobial activities exhibited by various leaf latex extracts are less than those of the standard drugs (ciprofloxacin and Ketoconazole). The radical scavenging activities of *A. debrana* leaf latex crude extracts, essential oil, and isolated compounds were tested. DPPH radical scavenging activity revealed that compound **1** showed an IC_{50} value of 5.03, indicating the potential of *A. debrana* as an antioxidant. The findings of this study support the traditional use of the plant to treat infectious diseases caused by microbial infection.

5.2. Recommendations

Considering the above conclusions and based on the results of this study, further research work with biological activity (antifungal, anticancer, and cytotoxicity) on crude extracts, isolated compounds, and essential oil is needed in the future. Additional *in vitro* antibacterial studies are recommended on other resistant clinical isolate pathogenic bacteria and fungal organisms. A detailed study on 2D, NMR, and MS is also recommended as essential to characterize the isolated compounds' structures fully. It is recommended that further similar studies should be conducted on other parts of the plant.

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APPENDICES

Appendix 1: phytochemical test of the extracted latex sample of *A. debrana*.



Appendix 2: Significance of compound **1** against *C. albicans*.

Multiple Comparisons

Dependent Variable: Candida albicans

Tukey HSD

(I) concentration	(J) concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
10	5	3.00000	.94281	.059	-.1029	6.1029
	2.5	4.33333*	.94281	.007	1.2305	7.4362
	1.25	7.00000*	.94281	.000	3.8971	10.1029
	ketoconazole	-9.66667*	.94281	.000	-12.7695	-6.5638
5	10	-3.00000	.94281	.059	-6.1029	.1029
	2.5	1.33333	.94281	.633	-1.7695	4.4362
	1.25	4.00000*	.94281	.012	.8971	7.1029
	ketoconazole	-12.66667*	.94281	.000	-15.7695	-9.5638
2.5	10	-4.33333*	.94281	.007	-7.4362	-1.2305
	5	-1.33333	.94281	.633	-4.4362	1.7695
	1.25	2.66667	.94281	.102	-.4362	5.7695
	ketoconazole	-14.00000*	.94281	.000	-17.1029	-10.8971
1.25	10	-7.00000*	.94281	.000	-10.1029	-3.8971
	5	-4.00000*	.94281	.012	-7.1029	-.8971
	2.5	-2.66667	.94281	.102	-5.7695	.4362
	ketoconazole	-16.66667*	.94281	.000	-19.7695	-13.5638
ketoconazole	10	9.66667*	.94281	.000	6.5638	12.7695
	5	12.66667*	.94281	.000	9.5638	15.7695
	2.5	14.00000*	.94281	.000	10.8971	17.1029
	1.25	16.66667*	.94281	.000	13.5638	19.7695

*. The mean difference is significant at the 0.05 level.

Appendix 3: Significance of compound **2** against *C. albicans*.

Multiple Comparisons

Dependent Variable: Candida albicans

Tukey HSD

(I) concentration	(J) concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
10	5	1.33333	.76012	.447	-1.1683	3.8349
	2.5	3.33333*	.76012	.009	.8317	5.8349
	1.25	4.66667*	.76012	.001	2.1651	7.1683
	ketoconazole	-12.00000*	.76012	.000	-14.5016	-9.4984
5	10	-1.33333	.76012	.447	-3.8349	1.1683
	2.5	2.00000	.76012	.137	-.5016	4.5016
	1.25	3.33333*	.76012	.009	.8317	5.8349
	ketoconazole	-13.33333*	.76012	.000	-15.8349	-10.8317
2.5	10	-3.33333*	.76012	.009	-5.8349	-.8317
	5	-2.00000	.76012	.137	-4.5016	.5016
	1.25	1.33333	.76012	.447	-1.1683	3.8349
	ketoconazole	-15.33333*	.76012	.000	-17.8349	-12.8317
1.25	10	-4.66667*	.76012	.001	-7.1683	-2.1651
	5	-3.33333*	.76012	.009	-5.8349	-.8317
	2.5	-1.33333	.76012	.447	-3.8349	1.1683
	ketoconazole	-16.66667*	.76012	.000	-19.1683	-14.1651
ketoconazole	10	12.00000*	.76012	.000	9.4984	14.5016
	5	13.33333*	.76012	.000	10.8317	15.8349
	2.5	15.33333*	.76012	.000	12.8317	17.8349
	1.25	16.66667*	.76012	.000	14.1651	19.1683

*. The mean difference is significant at the 0.05 level.

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