Phytochemical Analysis, *In silico* Molecular Docking Study, and Evaluation of Antibacterial and Radical Scavenging Activities of Constituents of the Roots Extracts of *Gomphocarpus purpurascens* A. Richs.

By: Chalshisa Geba Begna

A Thesis Submitted to the Department of Applied Chemistry

College of Applied Natural Science

Presented in partial fulfillment of the requirements for the MSc Degree in Applied Chemistry (Medicinal Chemistry)

College of Postgraduate Studies

Adama Science and Technology University

Advisor: Professor Yadessa Melaku (PhD)

February, 2025
Adama, Ethiopia

Phytochemical Analysis, *in silico* Molecular Docking Study, and Evaluation of Antibacterial and Radical Scavenging Activities of Constituents of the Root Extracts of *Gomphocarpus purpurascens* A. Richs.

By: Chalshisa Geba Begna

A Thesis Submitted to the Department of Applied Chemistry
College of Applied Natural Science

Presented in partial fulfillment of the requirements for the MSc Degree in Applied Chemistry (Medicinal Chemistry)

College of Postgraduate Studies
Adama Science and Technology University

Advisor: Professor Yadessa Melaku (PhD)

February, 2025
Adama, Ethiop

Declaration

I declare that this thesis entitled "**Phytochemical Analysis, *in silico* Molecular Docking Study, and Evaluation of Antibacterial and Radical Scavenging Activities of Constituents of the Root Extracts of *Gomphocarpus purpurascens* A. Richs.**

" is my own work and has not been submitted to any university for similar purpose. The references used in this proposal are duly recognized by proper citations.

Chalshisa Geba Begna _____ _____

Name of student Signature Date

Recommendation of Advisors/ Supervisors

As the advisor for the thesis, I have thoroughly read the paper titled “Phytochemical Analysis, *in silico* Molecular Docking Study, and Evaluation of Antibacterial and Radical Scavenging Activities of Constituents of the Root Extracts of *Gomphocarpus purpurascens* A. Richs “prepared by Chalshisa Geba under my guidance. Hence, I recommended the submission of the thesis to the department for defense

_____	_____	_____
Advisor/Supervisor	Signature	Date

Approval Page for Thesis

I the advisor of this thesis, hereby certify that I have read the revised version of the thesis entitled "Phytochemical Analysis, *in silico* Molecular Docking Study, and Evaluation of Antibacterial and Radical Scavenging Activities of Constituents of the Root Extracts of *Gomphocarpus purpurascens* A. Richs.)" by Chalshisa Geba Begna. 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 Chemistry (Medicinal Chemistry). Therefore, I recommend the submission of revised version of the thesis to the department following the applicable procedures.

_____	_____	_____
Advisor	Signature	Date

Approval of Board of Reviewers

We, the undersigned, members of the Board of Reviewers of the thesis open defense by **Chalshisa Geba Begna** have read and evaluated the thesis entitled "Phytochemical Analysis, *in silico* Molecular Docking Study, and Evaluation of Antibacterial and Radical Scavenging Activities of Constituents of the Root Extracts of *Gomphocarpus purpurascens* A. Richs. " and assessed the understanding of the candidate about the thesis research. This is, therefore, to certify that the thesis is accepted and we recommend the implementation of the thesis

Chairperson	Signature	Date
Reviewer 1	Signature	Date
Reviewer 2	Signature	Date

Final approval and acceptance of the thesis are contingent upon the 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

ACKNOWLEDGEMENT

First, I would like to acknowledge the grace and blessings of God, which have been source of my strength, comfort, and guidance throughout my journey. I would like to express my deepest appreciation to my advisor, Professor Yadessa Melaku for his unwavering contributions, guidance, support, and encouragement throughout developing my thesis. I also thank Professor Aman Dekebo and Dr. Tolessa Duguma for their cooperation and material support. I am also grateful to School of applied natural sciences, for providing me with the opportunity to pursue my academic and allow me my thesis goal. In addition I would like to great Department of biology for allowing me to conduct biological activities. I express my gratitude to …… project for chemical and solvent support. I would like to thank Mr. Guta Amenu, Mr.Girma Amenu, Mr. Kebede Shenkute, Mr. Urgessa Ensermu, Mr. Muktar Ahmed, and Ms. Bontu for their support in doing antibacterial, antioxidant, GC-MS, and providing me with some laboratory materials. Finally, I would like to thank my families, classmate students and friends all have positive feedback with me.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

APPENDEXES

LIST OF ABBREVIATIONS

ABTS: - (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid))

AMR: - Anti-Microbial Resistance

CC: - Column Chromatography

$CDCl_3$:- Deuterated Chloroform

^{13}C NMR: - Carbon 13-Nuclear Magnetic Resonance

DCM: - Dichloromethane

DMSO: -Dimethyl Sulfoxide

DPPH: - 1-1- diphenyl-2-picrylhydrazyl

FTIR: - Fourier Transform-Infra-Red

GC-MS: - Gas-Chromatography-Mass Spectrometer

^{1}H NMR: - Proton-Nuclear Magnetic Resonance

IC50:- Half maximal Inhibitory Concentration

IZ: - Inhibition Zone

MHA: - Muller Hinton Agar

PDA: - Potato Dextrose Agar

PKM2: - Pyruvate Kinase M2

PKM2-IN-1: - Pyruvate kinase M2 Inhibitor 1

PqsA: - Pseudomonas Quinolone Signal A

POWO: - Plants of the World Online

Rf: - Retention factor

ROS: - Reactive Oxygen Species

TLC: - Thin Layer Chromatography

UV-Vis: - Ultraviolet-Visible

WHO: - World Health Organization

ABSTRACT

*The notable increase in emerging infectious diseases (EIDs) and re-emerging infectious diseases (REIDs), coupled with the rise of antimicrobial resistance in healthcare environments, has become a significant global health issue. Natural products derived from plants origin and their derivatives continue to play a key role in the development of antimicrobial drugs. In Ethiopia, there are cultural diversity which allow varies use of medicinal plants. G. purpurascens is an endemic plant to Ethiopia which widely practiced as a medicinal plant to treat different human ailment such as Pneumonia, itching, mental illness, ring worm, wound, abdominal pain, and hemorrhoid. Despite of it's widely uses, to the best of our knowledge, there are no scientific reports on the isolation of bioactive molecules, and evaluation of biological activities of the plant. This study aims to perform a phytochemical analysis and assess the in-vitro antibacterial and antioxidant activities along with molecular docking study of phytoconstituents of root extract of Gomphocarpus purpurascens. Hydrodistillation and maceration extraction techniques using DCM/MeOH were employed in this study where silica gel column chromatography used for separation of compounds. The isolated compounds were characterized by determining their physical properties and spectroscopic techniques (GC-MS, UV, FT-IR, ^{1}H NMR and ^{13}C NMR). The biological activity was evaluated in vitro for its anti-bacterial activities by using the disc diffusion method and radical scavenging activity was assessed by DPPH assay. The phytochemical screening indicated the possible presence of flavonoids, coumarins, saponins, tannins, steroids, anthraquinones, glycosides, phenolics, and terpenoids. Hydrodistillation yielded (0.028% w/w) essential oils and its GC-MS analysis furnished 51 different compounds with major constituent of benzoic esters, fatty acid and its derivatives, phenols and steroids. Silica gel column chromatography has led to the isolation of five compounds namely: β-sitosterol (**32**), ethyl palmitate (**37**), oleanolic acid (**38**) ursolic acid (**39**) and Isorhamnetin 3-O-rhamnoside (**40**).The crude extract and isolated compounds exhibited promising biological activity. DCM/MeOH extract showed inhibition zone 16.14±0.58 mm, and 15.00±0.44 mm against (E. coli), and (S. aureus) at a concentration of 200 mg/mL. Compound **38** and **39** displayed highest activity against P. aeruginosa and E. coli with inhibition zone of 17.22±0.58mm and 17.00±0.22 mm respectively at concentration of 200 mg/mL. This was validated by the binding affinity recorded (-8.2 kcal/mol) against Pseudomonas quinolone signal A and (-8.1 kcal/mol) E. coli DNA Gyrase B in docking studies. The highest radical scavenging activity was observed by isorhamnetin 3-O-rhamnoside with IC_{50} 8.63 (μg/mL) followed by Oleanolic acid and ursolic IC_{50} =11.21 (μg/mL. Generally the*

study showed the plant is enrich of phytochemicals and showed anti-bacterial and radical scavenging activities

Keywords: *Gomphocarpus purpurascens*, Phytochemistry, Biological activity, Molecular docking

1. INTRODUCTION

1.1. Background of Study

Infectious diseases can caused in the presence of certain microorganisms, such as bacteria, viruses, fungi, and parasites that affect wide range of health problems, from mild to severe illness and even death. It has long been consistently emphasized as a major contributor to global health burden by leading cause of health loss globally (Rahamouz-Haghighi *et* al., 2022). Moreover, it has been reported that, 7·7 million of the world peoples deaths annually associated with the infections caused by 33 bacterial pathogens (Ikuta *et* al., 2022). Even though different antimicrobial drugs have been used to treat different human ailment, the emergence of antimicrobial resistance (AMR) is the major challenge health setting (Doyle *et* al., 2013). High rate increment of morbidity, mortality, and costs of medication, in developing country particularly with sub-Saharan Africa having the highest globally, is a major concern for World health organization (WHO) for investigation of effective and safe drugs, and instigating national action plans to overcome AMR (Godman *et* al., 2022).

Disturbance of human health biological system as well as oxidation in foods yield radicals. Free radicals can be generated as a fall out of normal in situ cell metabolism or from various external sources like radiation, medication, etc. (Suresh & Sah, 2013, Tadeg *et* al., 2005). The environmental consequences of climate change such as sea-level rise, increasing temperatures, more extreme weather events, increased droughts, flooding, wildfires, and political instability highly generate stress in human being that impact their health and lives (Rocque *et* al., 2021). Moreover, external factors such as exposure to pollutants, radiation, and certain lifestyle choices like smoking and excessive alcohol consumption can also increase the production of free radicals. In the case of biological systems, oxidative stress, an imbalance between reactive oxygen species and defenses and repair antioxidant systems, has been shown to be involved in the development of degenerative diseases (Sánchez-Moreno, 2002). Consequently,

inflammatory cells, such as neutrophils and macrophages, produce an excess of free radicals, including reactive oxygen species (ROS), as part of their defense mechanisms. ROS can directly damage tissues and contribute to the propagation of inflammation. ROS-induced changes make individuals more susceptible to conditions such as heart disease; cancer, respiratory diseases, and diabetes which are among the top seven leading causes of death in the United States (Zuo *et al., 2019)*

Plants have traditionally been used as a source of medicine since long time ago to the present day to control various ailments including infectious and noninfectious disease that afflicting humans and their livestock. WHO estimates that 35,000 – 70,000 species of plants are used for medicinal purposes around the world (WHO, 2013). According to the World Health Organization, 70-80% of the African population uses some form of traditional medicine, with herbal medicines standing out in particular (WHO, 2002). In Ethiopia, there is cultural diversity with various patterns of using the flora. A recent work indicated that the number of higher plants was over 7000 species from which around 15 % are probably endemic and about 887 species are used as medicinal plants (Azamal, H; Mishra, K; Semwal, 2012). *G. purpurascens* is one of widely practiced medicinal plant in Ethiopia to treat different human ailment. It is an endemic plant to the Ethiopia and has different vernacular names; Ari-Yuyo in Oromia, Tseba dimu in Tigray, Tefringe in Amharic and Mexxino in South Ethiopia.

The root and leaf part of the plant has been widely used in traditional medicine of Ethiopia to treat diverse medical conditions. The roots are fumigated boiled and drunken to treat pneumonia (Amare & Getachew, 2019). It also crushed with seeds of *Allium sativum* and leaves of *Ruta chalepensis* then provided to prevent bleeding after delivery (D'Avigdor *et* al., 2014, Region, 2017). The root and leaf part of the plant also widely used to treat mental illnesses (Ayalew *et* al., 2022). The leaf, latex and fruit of the plant has traditionally used to treat ringworm and Rhesus factors (Seble *et* al., 2018). In southern part of Ethiopia, the pound

fresh/dry root bark with water is taken as a drink for febrile illness (Mesfin *et* al., 2009). In Tigray region, the root of the plant is used for treatment of abdominal pain, toothache, and wound (livestock,) and the leaf part used to treat febrile illness (michi) whereas whole of the plant used for hemorrhoids (Demir, 2020).

Based on our current information, there is no investigated bioactive compound from Ethiopian indigenous plant *Gomphocarpus purpurascens,* despite its traditional use for the treatment of different disease. Lack of scientifically investigated secondary metabolites from the plant as well as warranted evaluation of antimicrobial beyond crud, further on fractionation and isolated compounds initiate this research. Moreover, there are no scientific studies on behalf of the potential oxidative stress resulting from the imbalance between free radicals and antioxidants can trigger and perpetuate inflammation. Therefore, this study aims to investigate phytochemical constituents of root of *Gomphocarpus purpurascens* as well as evaluate their antibacterial and radical scavenging activities

1.2. Statement of the problems

The current dramatically increased emerging infectious diseases (EIDs) and re-emerging infectious diseases (REIDs) as well as developed antimicrobial resistance in health care setting posed world health threats (Dharsan *et* al., 2020). In 2019, approximately 4.95 million individuals lost their lives due to infections that were resistant to drugs and out of these deaths around 1.27 million were directly caused by antimicrobial resistance (AMR) in the world. The report indicated that, in Ethiopia in 2019, there were 21,200 deaths attributable to AMR and 85,300 deaths associated with AMR (Institute for Health Metrics and Evaluation & University of Oxford, 2023). Most microbes developed resistance to antimicrobial drugs in fastest and complex way today. For example, *Staphylococcus aureus* is a potent gram-positive bacterium which causes wide range of diseases starting from soft-tissue infections to mild skin to bacteremia or pneumonia, chronic osteomyelitis, and life-threatening endocarditis, is one of the

common strain that developed resistance. The development of antibiotic resistance in *Staphylococcus aureus* bacteria, which are potentially harmful, has shown that when these bacteria were already resistant to one type of antibiotic, they developed resistance to a second antibiotic almost three times faster compared to bacteria that were fully susceptible to antibiotics (Siddhardha *et* al., 2020). Reactive oxygen species (ROS), free radicals are either directly damage the cells or change to make individuals more susceptible to conditions such as heart disease; cancer, respiratory diseases, and diabetes which are among the top seven leading causes of death in the United States (Zuo *et al.*, 2019).

Natural products and their derivatives continue to play a dominant role in the development of antimicrobial drugs because of,; in synthetically derived antibiotics there was minimal diversity (Pancu *et* al., 2021). *Gomphocarpus purpurascens* is an endemic plant to Ethiopia used for the treatment of various arrays of diseases including *Pneumonia, itching, mental illness, ring worm, wound, abdominal pain, and hemorrhoid*. The roots are fumigated, boiled and drunken to treat pneumonia (Amare & Getachew, 2019). Pneumoniae is a general term for disease caused by different species of gram negative and gram positive bacteria like *S. Pneumoniae, L. pneumoniae, M. pneumoniae P. aeruginosa, S. aureus Streptococcus* group B and so far (Poovieng et al., 2022). The same study reported pneumonia commonly caused by gram-negative pathogenic bacteria accounted for 60.5% whereas *P. aeruginosa* was a the most frequent gram-negative pathogen (Poovieng et al., 2022). In Tigray region, the root of the plant is used for treatment of abdominal pain, toothache, and wound: the leaf part used to treat febrile illness (michi): whole of the plant used for treatment of hemorrhoids (Demir, 2020). Despite of it's widely uses, the plant has not been intensively studied to determine their biological activities and phytochemical constituents. For example, a few reports were found in the literature on the experimental-based biological activity. Regarding to isolated compound of the plant, to our knowledge, only one cardenolide-type compound named calotropin was reported

from the leaves (Mohammed, 2024) and no reported work from roots of the plant. On the other hand, the affinity binding capacity of the isolated compound, calotropin, was studied by docking against the only *P. aeruginosa* PqsA (5OE3) Therefore; this study focuses on the extraction and isolation of compounds, as well as to evaluate antibacterial and radical scavenging activities of extracts and isolated compounds from the roots of the compounds. Additionally, it incorporates an *in silico* molecular docking analysis of these compounds to provide further insight into their potential mechanisms of action on two gram positive and two gram negative strain of bacteria.

1.3. Significance of the study

This study contributes to the phytochemical knowledge of the endemic medicinal plant, evaluation of anti-bacterial, and radical scavenging activity, isolation of compounds, and insilco molecular docking studies on the isolated compounds from root part of *Gomphocarpus purpurascens*. It was confirmed that this endemic plant possesses phytochemicals, as well as antioxidant and antimicrobial properties. Given the global rise in antibiotic resistance and the prevalence of oxidative stress-related diseases, the characterization of these compounds not only enriches the existing phytochemical database but also opens new pathways for research and application in health and nutrition. These results underline the potential for *G. purpurascens*. to serve as a sustainable source of bioactive compounds, contributing to both scientific advancement and public health initiatives.

Hopefully, this was highly significant in terms of its potential to arrive at finding leadlike compounds for drug discovery. Additionally, the study was offered foundational data that can be utilized by researchers interested in pursuing subsequent pharmacological investigations in the field as basic reference on the plant. This study also awarded degree in masters of Science to the author.

2. OBJECTIVES

2.1. General objective

- ❖ To conduct phytochemical investigation and evaluate biological activities on the root extracts of *Gomphocarpus purpurascens* and study the interaction of the isolated compounds with protein target using *in silico* molecular docking

2.2. Specific objectives

- To extract the root of *Gomphocarpus purpurascens* through maceration using dichloromethane to methanol (DCM/MeOH (1:1))
- To isolate compounds from DCM/MeOH root extract of *Gomphocarpus purpurascens*
- To extract essential oils from fresh roots of *Gomphocarpus purpurascens* by hydrodistilation and analyze by using GC-MS.
- To elucidate structure of isolated compound from root of *Gomphocarpus purpurascens* using UV-Vis, FT-IR, ^{1}HNMR, ^{13}CNMR and comparing with literature data
- To evaluate antibacterial activities of the extracts and isolated compounds from the root parts of *Gomphocarpus purpurascens* against Gram negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa,* and Gram positive *bacteria including Staphylococcus aureus,* and *Streptococcus pyogenes* using agar disc diffusion method.
- To determine the radical scavenging activity of the extracts and isolated compounds from the root parts of *Gomphocarpus purpurascens* using DPPH radical assay method
- To conduct *in silico* molecular docking analysis of the isolated compounds by using AutoDock Vina plugin-Pymol against *E. coli* DNA gyrase B (PDB ID: 7P2M), Pseudomonas quinolone signal A (PqsA, PDB ID: 5OE4), pyruvate kinase M2 (PKM2; PDB ID: 4G1N), and human topoisomerase IIβ (PDB ID: 3QX3).
- To compute the drug-like properties of the isolated compounds using the SwissADME online tool.
- To evaluate the toxicities of the isolated compounds utilizing the ProTox 3.0 online tool.

3. LITERATURE REVIEW

3.1. The Family Apocynaceae

The family of Apocynaceae includes subfamily of Periplocoideae, Secamonoideae, and Asclepiadoideae, which were previously considered a separate family called Asclepiadaceae. These subfamilies encompass approximately 230 genera and 3400 species (Goyder, 2006). Subfamily Asclepiadoideae (180 genera, 3000 spp.) are distributed worldwide, with three main centers of generic diversity – tropical Central and South America with 47 genera, eastern and southern Africa with 86 genera, and tropical Asia with 38 genera (Goyder, 2006).

The family Apocynaceae is renowned for its remarkable diversity of medicinal plants, making it one of the most pharmacologically valuable families in the plant kingdom. It serves as a abundant reservoir for drugs that have been utilized in both traditional and modern medicine (Islam & Lucky, 2019). Apocynaceae species have long been employed in traditional medicine for the treatment of various conditions such as gastrointestinal disorders, fever, malaria, antimicrobial, pain, diabetes, antioxidant as well as skin and ecto-parasitic diseases. Its species also have been documented to exhibit anti-cancer and anti-malarial properties. Notably, certain species such as *Catharanthus roseus*, *Nerium oleander*, *Plumeria rubra*, and *Ichnocarpus frutescens* have demonstrated cytotoxic activity. Among them, *Catharanthus roseus* holds significant medicinal importance within this family, primarily due to its utilization in the treatment of various forms of cancer (Islam & Lucky, 2019)

3.2. Taxonomic classification:

Kingdom: Plantae

Phylum: Streptophyta

Class: Equisetopsida

Subclass: Magnoliidae

Order: Gentianales

Family: Apocynaceae

Genus: *Gomphocarpus*

Species: *Gomphocarpus purpurascens* (Nazar et al., 2013)

3.3. The Genus of *Gomphocarpus*

Gomphocarpus R. Br. (Apocynaceae : Asclepiadoideae) is a genus of mostly shrubby herbs with non- tuberous rootstocks native to Africa (Br & Asclepiadeae, 2001). The botanical

exploration in tropical Africa started much later than temperate, Southern part of the continent. In his enumeration Ethiopian flora, Richard (1851) described three new species in *Gomphocarpus* following Decaisne's circumscription of genus of two of them, *G. purpurascens* A. Rich., *G. semilunatus* A. Rich. and *G. robestus* A. Richard (Br & Asclepiadeae, 2001). Later, *G. robestus* A. Rich fall under species of *pachycarpus* (Botanical *et* al., 1991). The genus is restricted to putatively monophyletic assemblage of more than 20 species (Table 1) in several complexes (Br & Asclepiadeae, 2001). Among the Flora of Ethiopia and Eritrea contains seven species of *Gomphocarpus* (*G. semilunatus, G. phillipsiae, G. abyssinicus, G. purpurascens G. fruticosus, G. stenophyllus, and G. integer*) (Botanical *et* al., 1991).

Table 1: Lists of *Gomphocarpus* species

Name of *Gomphocarpus* species	Location	Name of *Gomphocarpus* species	Location	References
G. abyssinicus	Ethiopia	*G. praticola*	Angola	(Gardens, 2001)
G. cancellatus	South Africa	*G. purpurascens*	Ethiopia	
G. filiformis	SW Africa	*G. rivularis*	South Africa	
G. glaucophyllus	South Africa	*G. semiamplectens*	Tropical Africa	
G. fruticosus	South Africa	*G. semilunatus*	Ethiopia	
G. integer	SE Africa	*G. sinaicus*	Sinai Peninsula	
G. kaessneri	Kenya	*G.stenophyllus*	Tropical Africa	
G. munonquensis	Angola	*G.swynnertonii*	Mozambique, Zimbabwe	
G. peltiger	South Africa	*G. tenuifolius*	Zimbabwe	
G. phillipsiae	SE Africa	*G. tomentosus*	South Africa	
G. physocarpus	South Africa; China, N + S America			

The genus of *Gomphocarpus* is used in the traditional medicine of tropical Africa to treat malaria, diabetes asthma, bronchitis, cancer, cardiac palpitations, as a diuretic, infections, wounds and other livestock disease (Belayneh & Bussa, 2014). Some studies have shown that

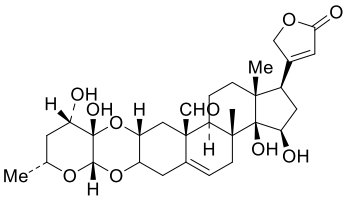
certain *Gomphocarpus* species possess antimicrobial properties, exhibiting inhibitory effects against certain bacteria and fungi. A report indicated that aerial part of *G. fruticosus* extract have shown significant anti-oxidant activity and anti-microbial activity on different bacterial strain (Yusuf Sukman, 2017).

Gomphocarpus species are known to contain mainly cardenolide glycosides. Moreover, recent findings indicated the discovery of two novel cardenolide glycosides from *Gomphocarpus sinaicus*, specifically from the chloroform extract of its stems. One of these glycosides, named 15β-hydroxy-5, 6-dehydrocalotropin, (Table 2) possesses a sugar molecule that is doubly linked, while the other glycoside, known as coroglaucigenin-3-(6-deoxy-β-D-allopyranoside)-19-acetate, contains a sugar molecule that is singly linked. These compounds represent new additions to the existing knowledge of chemical constituents found within *Gomphocarpus sinaicus* (El-askary, 2018a).

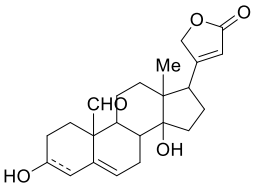
Table 2: Chemical constituents isolated from the genus of *Gomphocarpus*

Structure number	Isolated compounds	Species	References
	Cardenolides		
1	Cardenolide glycoside- 15 β -hydroxyl-5, 6-dehydrocalotropin	*G. sinaicus*	(El-Askary *et* al., 1993)
2	3,4,5,6-dehydrocalotropin	*G. sinaicus*	(Abbassy *et* al., 2012)
3	calotropin	*G. sinaicus*, *G.purpuracsens*	(El-askary, 2018b, Mohammed, 2024)
4	5, 6-dehydro-calotropin	*G. sinaicus*	(El-askary, 2018b)
5	3'-Epiafroside	*G. sinaicus*	(El-askary, 2018b)
6	15β-hydroxy-calotropin	*G. sinaicus*	(El-askary, 2018b)
7	14β 17α-Epoxy- 5,6-dehydro calotropin	*G. sinaicus*	(El-askary, 2018b)
8	cardenolide glycosides calotropin	*G. fruticosus*	(Marzouk *et* al., 2015a)
9	cardenolide glycosides gomphoside	*G. fruticosus*	(Marzouk *et* al., 2015a)
10	gomphotoxin	*G. fruticosus*	(Komissarenko K.e*t*

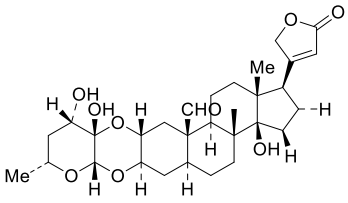
			al 1996)
11	gomphotin	*G. fruticosus*	(Komissarenko K.e*t* al 1996)
12	3’-Epiafroside	*G. fruticosus*	(Komissarenko K.e*t* al 1996)
13	Uzarigenin	*G. sinaicus*	(El-askary, 2018b)
14	Xysmalogenin (5,6-Dehydrouzarigenin)	*G. sinaicus*	(El-askary, 2018b)
	Terpenoids		
15	Betulinic acid	*G. fruticosus*	(Marzouk *et* al., 2015b)
16	Triterpenoids 3β-taraxerol acetate	*G. fruticosus*	(Marzouk *et* al., 2015b)
17	13α-methyl, 27-norolean-14-en, 3β-ol (3β-taraxerol)	*G. fruticosus*	(Marzouk *et* al., 2015b)
	Flavonoids		
18	3- O- Rhamnoglucoside	*G. sinaicus*	(EI Batran A. *et* al 2005)
19	Luteolin-7-O-glucoside 3-O-Rhamnoside	*G. sinaicus*	*(*EI Batran A. *et* al 2005)
20	Rutin	*G. sinaicus*	(EI Batran A. *et* al 2005)
21	Rutin-7-O-Rhamnoside	*G. sinaicus*	(EI Batran A. *et* al 2005)



1



2



3

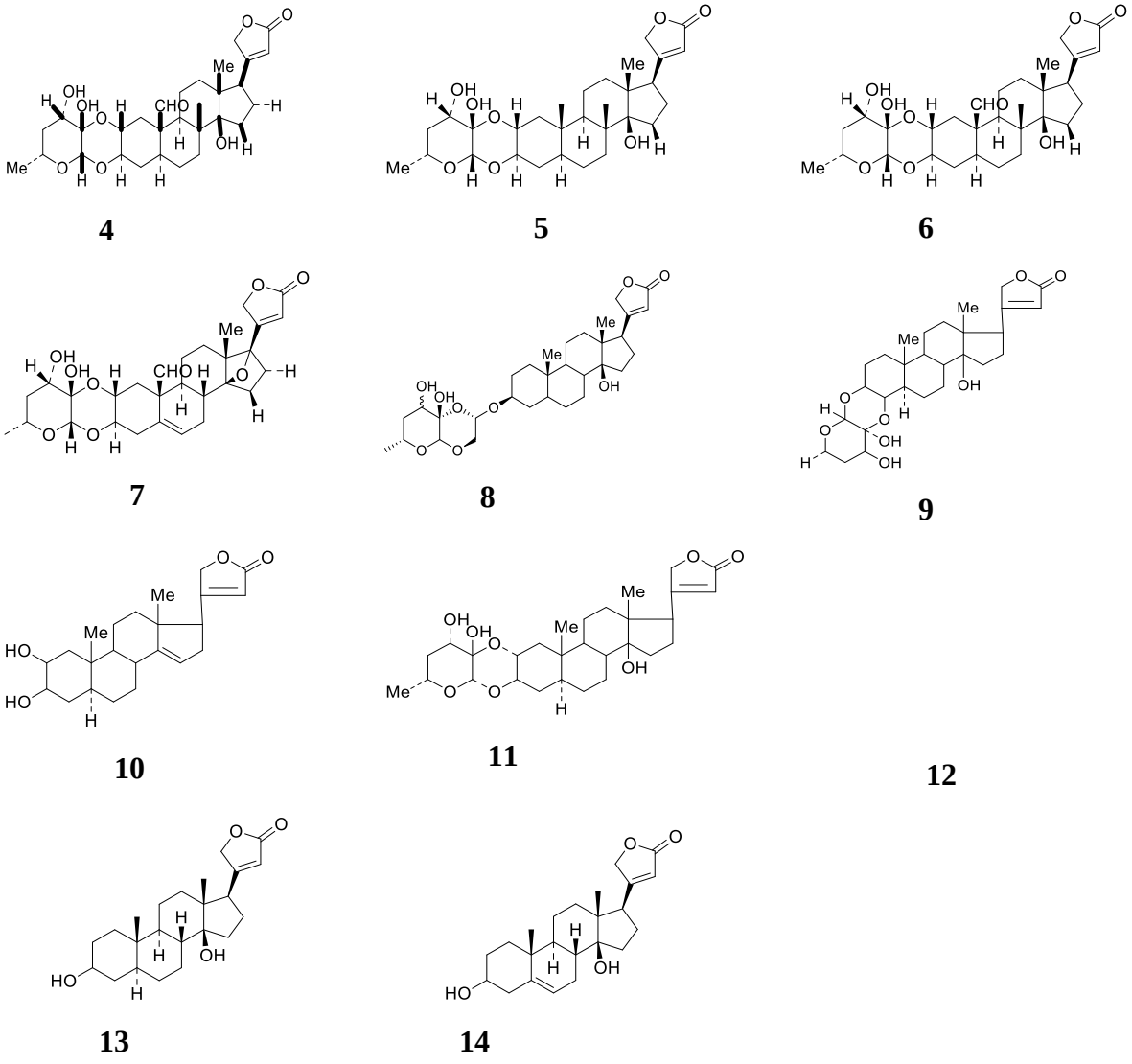


Figure 1: Cardenolide from the genus of *Gomphocarpus*

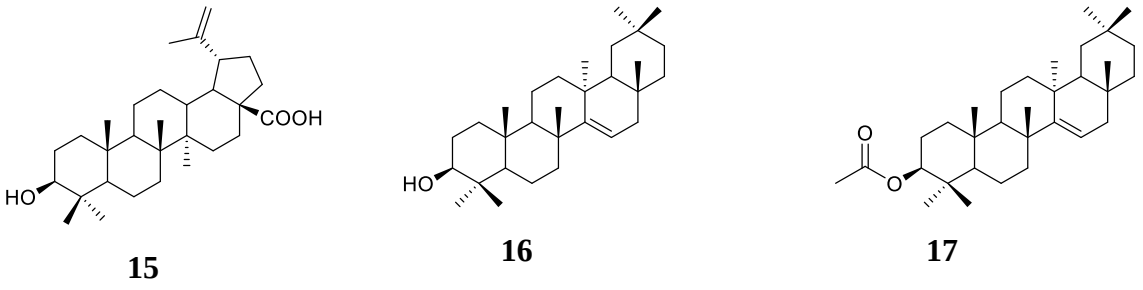
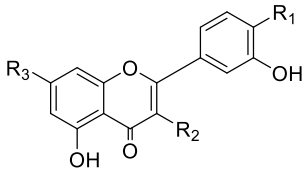


Figure 2: Terpenoids from the genus of *Gomphocarpus*



1**8.** R1=OCH3, R2 =O- Rhamnosyl (1 6), R3= OH

19. R1= O- Rhamnosyl (1 6), R2=H, R3 =O- glucose

20. R1=OH, R2=O-Rhamnosyl (1 6) glucose R3=OH,

21. R1= OH, R2= O-Rhamnosyl (1 6) glucose, R3= O Glucose

Figure 3: Flavonoid glycosides from genus of *Gomphocarpus*

3.4. *Gomphocarpus purpurascens*

3.4.1. Botanical Description of *Gomphocarpus purpurascens*

A *Gomphocarpus purpurascens* A. Rich. was enumerated in 1951 by Richard with other species called as *Gomphocarpus semilunatus* A. Rich. as part of Ethiopian flora *(*Br & Asclepiadeae, 2001*). G. purpurascens* is an annually grown remain an endemic plant to Ethiopia and it is a shrubby perennial herb 0.6–2 m tall arising from a tap root; stems erect, much branched from base, woody below, densely spreading-pubescent or tomentose. Leaves paired; sessile or with petiole to 2 mm long; lamina 5–15×0.1–0.5 cm, flowering throughout the year in open rocky ground and disturbed areas; altitude of 1500–2500 m . The fruiting pedicel is twisted, while the follicles are ovoid in shape, measuring between 4 and 8 centimeters in length and 1.5 to 2.5 cm in width. The follicles gradually narrow into a slender beak and are inflated, covered in dense pubescence or tomentum, and adorned with filiform processes that are 5 to 8 mm long. The seeds are approximately 3.5 mm long and 1.5 mm wide, ovate in shape, with one convex and one concave face, and have a rough texture (Gardens, 2001). It has different Vernacular name Ari-Yuyo in Afaan Oromo, Tseba dimu in Tigrigna, Tefringe in Amharic and Mexxino in South Ethiopia **(**Figure 4). Leaf, root, root bark, fruit and latex part of the plant have been used for treatment of different human and livestock ailments. The root and leaf part have been widely used whereas high curative evidence was noticed in wound management and wart treatment, where people used Tifrena (local name) as a medicament (Adugna *et* al., 2023).

Figure 4: *Gomphocarpus purpurascens*

(Photo was taken by Chalshisa Geba on 07/ December 2023, Dengego, East Hararge Zone, Oromia Region, Ethiopia)

Synonyms

Homotypic Synonym is a name that designates the same type specimen as another name according to the formal guidelines set by the International Code of Nomenclature (ICN) whereas heterotypic Synonyms is a name derived from different type specimens from the point of views of taxonomists (Schoch, 2020). *Gomphocarpus purpurascens* is known as *Asclepias pubiseta* N.E.Br (Homotypic Synonym) and, *Asclepias albida* N.E.Br and *Gomphocarpus fruticosus* var. *purpureus Schweinf (*heterotypic Synonyms https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:94568-1.

3.4.2. Geographical Distribution of *Gomphocarpus purpurascens*

Gomphocarpus purpurascens is an endemic plant to Ethiopia which widely distributed in different regions of the country (Table 3).

Table 3: Local distribution of *G. purpurascens* and its medicinal uses in Ethiopia

Botanical name	Geographical distribution	Local name	Traditional medicinal uses	References
	Chiro district, Western Hararge Ethiopia	Ari-Yuyo A/Oromo)	Pneumonia	(Amare & Getachew, 2019)
	Dengego valleys, eastern Ethiopia	Ari-Yuyo (A/Oromo)	Mental illness	(Wubetu et al., 2018)
Gomphocarpus purpurascens	SNNP Ethiopia	Mexxino (Gediyigna)	Topically for itching	(Mesfin et al., 2009)
	Furi mount	Kinchib	For affected body part	(Length et al.,

Southwest Oromia, Ethiopia	(A/ Oromo)		2022)
Fiche, Salale Ethiopia	Tefrindo (Amharic)	Evil eye, bleeding	(Miseganaw & Mhiret, 2019)
East Gojjam, Ethiopia	Tifrena (Amharic)	Bleeding after delivery, Evil eye	(Region, 2017)
Menz Gera Midir District, Ethiopia	Tifrindo (Amharic)	Ring worm, Rh factors	(Seble et al., 2018)
Kilte Awulaelo, Tigray, Ethiopia	Tseba dimu (Tigrigna)	Abdominal pain, wound, Haemorrhoids, michi, toothache, babesia	(Demir, 2020)

3.4.3. Ethno-medicinal Uses

Gomphocarpus purpurascens is widely used in Ethiopian traditional healing practices, utilizing it as local flora for various health remedies across different districts. In the Chiro district of Western Hararge, Oromia, the community utilizes the root of *G. purpurascens* by boiling it and used orally for pneumonia (Amare & Getachew, 2019). In Amhara region, peoples have used the root *G. purpurascens* to stop bleeding after delivery, and to clear evil eye (Yihenew *et* al., 2017). In Oromia, near Furi Mountain in the Hawas-Sebata area, people apply the leaves of the plant topically to the affected areas for treatment of skin disease (Beselam *et* al.,., 2022). In Eastern part of Ethiopia in west Hararge zone around Dengago valley, peoples use the leaf plant part roasting and make powdered, then mix with oil & use as ointment for itching skin. They have also taken a cup of infusion orally & smoke bath with dry leaf to treat evil eye (Belayneh & Bussa, 2014). This plant reviewed in Ethiopia as one of the herbal medicine that widely practiced for treating mental disorder (Wubetu *et* al., 2018).In the north part of the country Tigray region, it is widely practiced to treat human and livestock disease like; abdominal pain, hemorrhoid's, michi, toothache, and babesia and wound(livestock) (Demir, 2020). Central to north around Menz Gera District Amhara region, peoples use the plant for ring worms and Rh factor (Seble et al., 2018).

3.4.4. Biological Activity of *Gomphocarpus purpurascens*

G. purpurascens have not been intensively studied to determine its biological properties. The study that have been performed to evaluate the *in vitro* antibacterial activity of crude leaf and root bark extractions of *G. purpurascens* at 150, 300 and 600 mg/ml doses, against clinically isolated bacterial pathogens such as *E. coli*, *P. aeruginosa,* and *S. aureus* with compared to standard strains of *E. coli, S. aureus*, and *S. pneumonia* as well as antifungal activity against *C. albicans* by disc diffusion method as previously reported (Miseganaw & Mhiret, 2019). It was

observed that, root and leaf of *Gomphocarpus purpurascens* extracts posses' potential antibacterial and antifungal activity against all tested organisms. Report indicated that the highest antibacterial activity was 14.51 mm (in acetone extract) in standard *S. aureus* and least activity was recorded in standard *E. coli* measured 6.09 mm (in ethanol extract) in the lowest concentration (150 mg/mL) (Miseganaw & Mhiret, 2019). A recent report indicated, n-hexane chloroform, chloroform: methanol (1:1), ethanol, and methanol leaves extracts and calotropin from root showed good antibacterial activities at studied doses (Mohammed, 2024).

Study have been conducted to detect the peripheral analgesic activity and anti-inflammatory activity of the 80% methanol extract of *Gomphocarpus purpurascens* on healthy adult Swiss Albino mice of either sex (20–35 g, and 6–8 weeks of age) through acetic acid-induced writhing test and hot plate method for analgesic activity as well as carrageenan-induced paw edema and formalin-induced pedal edema models of anti-inflammatory activity by Meaza Adugn and her coworkers (Adugna *et* al., 2023) . It was reported that at triplet testing doses (100 mg/kg, 200 mg/kg, 400 mg/kg), the extract significantly ($P < 0.001$) reduced the number of writhes in mice as compared to the negative control whereas the higher (400 mg/kg) dose of the extract showed a significant ($p < 0.001$) difference compared to the lower and middle (100 mg/kg and 200 mg/kg) doses of the extract in the acetic acid-induced writhing method for analgesic activity (Adugna *et* al., 2023). In spite of significant activity have been observed on scientific study performed at crude level, no much study conducted further to isolate bioactive compounds and evaluate its activity as compared to the crude one. The previously conducted study warranted researchers as further study is needed to isolate lead compounds and evaluate their biological activity of *Gomphocarpus purpurascens* extracts (Miseganaw & Mhiret, 2019).

3.4.5. Phytochemical composition

Study indicated that phytochemical screening through qualitative color changes based prediction with test reagents of 80% methanol extracts from the roots and leaves of *G. purpurascens* was conducted to identify the presence or absence of various phytoconstituents. It had been reported the potential presence of saponins, triterpenes, glycosides, phenols, steroids, tannins, anthraquinones, and alkaloids, while flavonoids were not detected in the root samples (Adugna *et* al., 2023). The finding was based solely on qualitative assessments and do not confirm isolation. Regarding to isolated compound of the plant, to our knowledge, only one cardenolide-type compound named calotropin was reported from the leaves (Mohammed, 2024) and no reported work from roots of the plant. Therefor further research is necessary to isolate and identify compounds from the roots of the plant

4. MATERIAL AND METHODS

4.1. Apparatus and instruments

The apparatus and instruments used in this study include: mortar and pestle, balance, measuring cylinder, jar, Whitman No.1filter paper, Rota vapor, round flask, beakers, pipettes, TLC plate, UV lamp, separatory funnel, silica gel (mesh size 60-120), Column Chromatography, UV/Vis, (JASCO V-770 UV-Vis, and UV-Vis spectrophotometer (Shimadzu, Model 1800, Japan), FT-IR, (iS50ABX) ^{1}H NMR,(Bruker Avance 400, 500 MHz spectrometer) ^{13}C NMR (Bruker Avance 101, 126 MHz spectrometer) 6 mm filter paper disks, autoclave, incubator, sterile petri dishes, and agar plate bacterial cultures.

4.2. Chemicals and reagents

The chemicals and reagents used in the study were solvents (petroleum ether, n-hexane (99% AR Loba Chemie) chloroform, (alpha chemica) ethyl acetate, (Loba Chemie PVT. LTD., 99.5%), dichloromethane (for HPLC and UV spectroscopy Loba Chemie and methanol (99.8% HPLC grade Loba Chemie PVT)) p-Anisaldehyde/sulfuric acid, ascorbic acid, lead acetate solution, Fehling solution A and B, α-naphtol, acetic anhydride, Iodine, KI, Ferric chloride solution, Conc. HCl, Ammonium thiocynate, Ammonium solution, glacial acetic acid, Mayer's reagent, PDA, MHA, DPPH, deuterated chloroform ($CDCl_3$) and DMSO (Srlchem, 99.5%).

4.3. 4.3. Plant Collection and Preparation

The root part of *G. purpurascens* was collected on December 07/ 2023 from Dengego valley, Genda Ware Kebele, Kersa Woreda; Dire Dawa located East Hararge Zone, Oromia region, Ethiopia (Figure 6) which is 462 km far from Addis Ababa**.** The area is defined by the coordinates of 9°39′N latitude and 42°20′E longitude along with annual mean temperature 25.5 °C (Fazzini *et* al., 2015). Its elevation varies from 950 to 2,260 m. The collected plant was packed with plastic sheets to avoid contamination and sun exposure during transportation. Then authentication of the plant specimen and voucher specimen identification (CG-02) was done at the National Herbarium of Ethiopia, Addis Ababa University, Ethiopia by Mr. Melaku Wendaferash and was stored for future references. The fresh root part of the plant was washed with distilled water (Figure 5) which subjected to hydrodistillation for essential oil extraction and the rest was dried by air at room temperature under shade**.**

Figure 5: Routine procedures of the experiment

The dried plant was ground to coarsely fine powder by using metal made mortar and pestle then weighed for readily prepared maceration extraction.

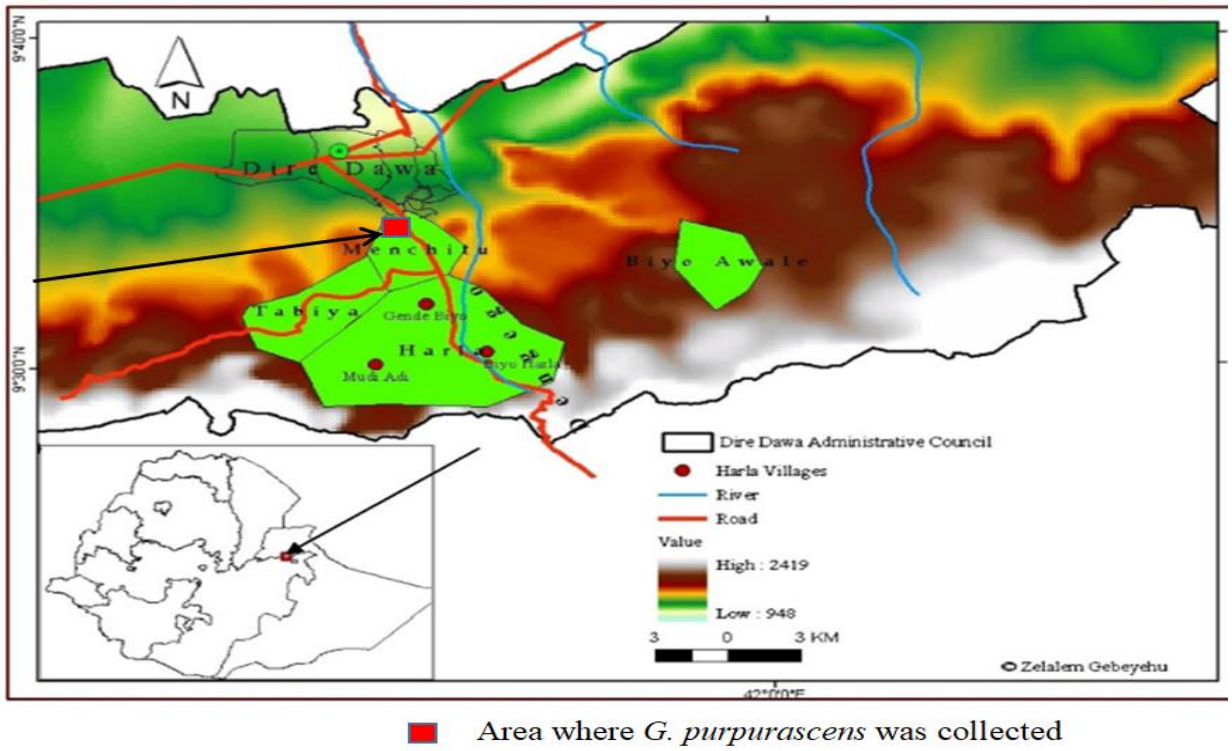


Figure 6: Map area where *G. purpurascens* was collected for this study

Source : Map area of ethnomedicinal plants of Harla and Dengego mountains/valleys complex (Belayneh & Bussa, 2014).

4.4. Extraction

4.4.1. Hydro-distillation

Hydro-distillation method of extraction was used for obtaining essential oils from the root *G. purpurascens* following literature report (Akdağ *et* al., 2019). The fresh roots of the *G. purpurascens* (100 g) were chopped using a metal mortar and pestle, then placed in a flask containing 1 liter of distilled water. This mixture was then subjected to hydro distillation in a Clevenger apparatus for three hrs. The distillates were extracted using chloroform, dried over anhydrous Na_2SO_4, and the organic solvent was then evaporated by Rotavaporator at 34 $^{\circ}$C to obtain the oil. After the yield was determined by the following equation (Equ...1), the oil was stored in a refrigerator at 4 $^{\circ}$C for future use

$$\text{Percentage Yeild}\,(\%) = [\frac{\text{wheight extract}\ \ (\text{w})}{\text{wheight of sample}\,(\text{w})}]100 \quad \text{.....Equ-1}$$

4.4.2. Maceration

The conventional method of extraction, the maceration extraction technique was employed in this study following procedure optimized by Gori (Gori *et al.*, 2021). Successfully, the dried

and ground plant material was extracted with dichloromethane/methanol (1:1) at room temperature by frequently stirring for 72 hrs. and was filtered using Whitman No. 1 filter paper. This was performed in triplicate to ensure the efficiency of the extraction. The filtrate was concentrated using rotary evaporator at 37 ℃ to afford the extract. The extract was then subjected to successive fractionation using n-hexane, chloroform, ethyl acetate and methanol following literature reported by Nuurul & Mohammad, (2016). The fractions were then analyzed using TLC. Then each fractionation was dried and their yield was determined.

4.5. Phytochemical screening

The phytochemical screening test was conducted following the procedures that has been previously documented by Packirisamy and Moorthy, (2014). Thus, the dichloromethane/methanol (1:1) root extract root *G. purpurascens* was examined for the presence of flavonoids, alkaloids, coumarins, saponins, tannins, steroids, anthraquinones, glycosides, phenolics, and terpenoids.

Tests for alkaloids:

Tests for alkaloids (Wagner's test): The prepared extract (10 mg) was mixed with 1% HCl (0.5 mL) in a water bath for 5 min and then filtered into a test tube. Two drops of Wagner's reagent (iodine in potassium iodide) were added, and the appearance of a brown or reddish precipitate indicates the presence of alkaloids (Knv & Tabassum, 2017).

Test for anthraquinones:

The plant extract (20 mg) was placed in a dry test tube, followed by the addition of 5 mL of chloroform, and the mixture was shaken for 5 min. The extract solution was then filtered, and the filtrate was mixed with 5 mL of 10% v/v ammonia solution. The development of a pink, violet, or red color in the ammonia layer indicates the presence of anthraquinones (Duval *et* **al**., 2016).

Test for Coumarins:

The plant extract (10 mg) was dissolved in 5 mL of ethanol, followed by the addition of alcoholic KOH. The development of a yellow color, which disappears upon the addition of a few drops of concentrated HCl, indicates the presence of coumarins (Packirisamy and Moorthy, 2014).

Test for Flavonoids (Ferric Chloride Test):

The extract (10 mg) was dissolved in 5 mL of methanol. The solution was heated, and magnesium ribbon was added. Then, 2-3 drops of concentrated hydrochloric acid were

introduced. The development of a pink, scarlet, crimson red or sometimes green to blue color after a few minutes indicates the presence of flavonoids (Patil & Deshmukh, 2016).

Test for Glycosides (Keller-Killiani Test):

The plant extract (10 mg) was dissolved in 3 mL of glacial acetic acid with 1 drop of ferric chloride solution. This mixture was then layered with 1 mL of concentrated sulfuric acid. The presence of a brown ring at the interface indicates the presence of deoxysugar, which is characteristic of cardenolides. A violet ring may appear below this ring, while a greenish ring may form in the acetic acid layer (Packirisamy and Moorthy, 2014).

Test for Phenols: The prepared extract (10 mg) was dissolved in 5 mL of distilled water. A few drops of a 5% ferric chloride solution were then added. The appearance of a dark green color indicated the presence of phenolic compounds (Priyanthi, 2021).

Test for Saponins (Foam test):

The extract (10 mg) was dissolved with 6 mL of distilled water in a test tube. The mixture was shaken vigorously while being heated to a boil. The development of persistent foam indicates the presence of saponins (Shaikh & Patil, 2020).

Test for steroids (**Libermann Burchard Reaction**):

Ten milligrams of the prepared extract was mixed with 5 mL of chloroform. Then, 2 mL of acetic anhydride and 2 drops of concentrated sulfuric acid were added from the side of the test tube. The appearance of red, followed by blue and finally green colors indicates the presence of steroids (Shaikh & Patil, 2020)

Test for Tannins:

Ten milligrams of the extracts were dissolved in distilled water, and 2 mL of 5% ferric chloride solution was added to this mixture. The formation of a blue-green color indicates the presence of tannins (Packirisamy and Moorthy, 2014).

Test for Terpenoids (**Salkowski's Test**):

Ten milligram of the extract was treated with 1 mL of chloroform, followed by a few drops of concentrated H_2SO_4. The mixture was shaken thoroughly and allowed to stand for 5 min. A red color in the lower layer indicates the presence of sterols, while the formation of a yellow-colored lower layer indicates the presence of triterpenoids (Shaikh & Patil, 2020).

4.6. Isolation of compounds

The extract of the root of *G. purpurascens* (68 g) was subjected to successive solvent fractionations using *n*-hexane , dichloromethane , ethyl acetate and methanol following reported procedures in literature report (Mulatu, 2020) to afford 8, 21, 23 and 16 g of the

extracts respectively. After TLC analysis of extract and solvent fraction had conducted, the fraction of dichloromethane and ethyl acetate obtained from DCM: MeOH extract showed very close TLC profile and hence mixed together. Some part of resulting combined extract (16 g) was adsorbed onto 16 g of silica gel and subjected to silica gel (160 g) column chromatography, using *n*-hexane for packing. Elution was performed by gradually increasing the polarity using *n*-hexane, then *n*-hexane in ethyl acetate, followed by ethyl acetate finally ethyl acetate in methanol with different proportion as the eluent. A total of 320 fractions were collected initially four fractions of 100 mL each with n- hexane, followed by subsequent fractions of 10 mL—using gradient elution based on the solvent system of eluent, color bands and TLC profile and left to dry at room temperature. Different fractions were checked by TLC and the spots were visualized under UV lamp at 254 nm and 366 nm as well as spraying vanillin in H_2SO_4 heating on hotplate. These fractions showed single spot with similar retention factors (R_f) were collected to same container and coded with fraction number (Table 4). The isolated pure compounds were submitted to UV/ Vis, FT- IR and NMR analysis and the rest were stored in a refrigerator at 4 $^{\circ}$C for biological activity.

Table 4: Solvent system gradient with its fractions number result of column chromatography

Solvent system	Gradient Ratio	Fractions number	Solvent system	Gradient Ratio	Fractions number
n-Hex	100 %	F_1–F_4	n-Hex: EtOAc	55:45	F_{161}–F_{176}
n-Hex: EtOAc	95:5	F_5–F_{14}		50:50	F_{177}–F_{196}
	90:10	F_{15}–F_{33}		40:60	F_{197}–F_{210}
	90:10	F_{34}–F_{41}		30:70	F_{211}–F_{238}
	85-15	F_{42}–F_{58}		20:80	F_{239}–F_{253}
	80:20	F_{59}–F_{81}		10:90	F_{254}–F_{268}
	75:25	F_{82}–F_{91}	EtOAc	100	F_{269}–F_{284}
	75:25	F_{92}–F_{116}	EtOAc: MeOH	90:10	F_{285}–F_{298}
	70:30	F_{117}–F_{130}		80:20	F_{299}–F_{312}
	65:35	F_{131}– F_{144}		70:30	F_{313}- F_{320}
	60:40	F_{145}–F_{160}	-	-	-

TLC analysis of the collected all 21 groups of fractious was performed for identification and revealed some fractions showed single spot and were led pure compounds. For instance fraction three, **F_3 (**F_{15}-F33) which eluted in Hex/ EtOAc (90:10) yielded jelly like compound **37** (32 mg), **F_5** (F_{42}-F_{58}) eluted with a mixture of Hex/EtOAc (85:15) afforded white crystal with needle shape compound **32** (64 mg) and **F_{17} (**F254-F268) which was eluted and Hex/EtOAc (10:90) coded as compound **40** (53 mg). Fraction **F_6** (F_{59}-F_{81}) which were eluted in n- a hexane/EtOAc (80:20) and some fractions from 75:25 (n-hexane/EtOAc) (912 mg) were re-chromatographed over 40 g of silica gel, using n-hexane for packing. A gradient elution was performed by increasing the polarity of solvent system (ethyl acetate in n-hexane) with a range of deduced 10 mL of hexane within 100 ml of solvent in each step while ethyl acetate was increased. It began with n-hexane (100 mL) for the first three fractions then followed by a gradient of n-hexane/ EtOAc (90:10, 80:20, 70:30 … up to 100% ethyl acetate). Total of 180 fractions were collected. Based on TLC analysis among fraction **F_{25}** (F_{68}-F_{90}) eluted in Hex/ EtOAc 30:70 afforded compounds **38** and **39** in combined (42 mg) as white crystal.

4.7. Characterization of isolated compounds

Characterization of the isolated compounds was done using melting point and spectroscopic methods including UV-Spectroscopy, FT-IR-spectroscopy, GC-MS, and NMR analysis.

4.7.1. Melting point determination

Thiele tube method was used to measure the melting point of isolated compounds which help for identification comparing their MP values with known compounds according to procedure reported by Carey (2022). Thiele tube was clamp with a ring stand and filled with clear mineral oil up to 1cm higher than the top triangular arm. The sample was added to one side closed capillary tube which inserted into a Thiele tube. Then the thermometer was stand with in Thiele tube along with the sample held capillary. The arm of the Thiele tube was gently heated with burner and the temperature at which the compound start to melted and totally changed to liquid was carefully followed and recorded.

4.7.2. UV-Spectroscopy analysis

Isolated compounds were analyzed in UV- spectroscopy, JASCO V-770 tool for identification of interesting compounds within range of wave length 200 nm to 800 nm and absorbance 0 au to10 au. The UV-spectra were obtained on a JASCO V-770 UV-Vis spectrophotometry at Addis Ababa Science and technology (AASTU)

4.7.3. IR-spectroscopy Analysis

FT-IR experiment was conducted to identify specific functional groups of isolated compounds within a molecule based on characteristic absorption bands as reported method (ENVH 555/2008/MM., 2008) at Addis Ababa Science and technology (AASTU). The compounds were analyzed by Fourier-transform infrared (FTIR) spectrometers (iS50 ABX) with 1mm slit width, closed cell system. IR spectra was generated with a range of wave length of 400 nm to 4000 nm, resolved at 16 and scanned at 32 nm and the obtained plectra was exercised.

4.7.4. GC-MS Analysis

GC-MS analysis of the oil was carried out using an Agilent gas chromatography that had HP-5 capillary column (30 m × 0.25 mm × 0.25 µm) using method reported (Bhardwaja *et* al., 2016). This GC was coupled to a mass spectrometer with a single quadrupole detector Agilent Technologies capable of scanning m/z values from 50 to 550 at 70 eV using an EI ionization source. The temperature of GC was programmed at 40 °C for the first five min, increased at a rate of 6°C/min to 250 °C and held isothermally at 250 °C for the next twenty minutes. The GC injector was set to splitless mode at 250 °C with injecting volume of 1 µL for both series of n-alkanes (C_7-C_{30}) and oil at position number 1 and 2 respectively. In this study, gradient programmed temperature, for quantification purposes, relative percentages of peak area were used and retention index was determined by equation given below (Equ-3)

$$RIx = 100n + 100\left[\frac{tx - tn}{tn + 1 - tn}\right] \quad \text{.....Equ -2}$$

Where "x" is the our interesting compound, "tx" is the retention time of "x," "tn" is the retention time of *n*-alkane eluted just before "x," and "tn+1" is the retention time of *n*-alkane eluted just after "x.", "n" is number of n-hexane just eluted before "x," and " RIx " is retention index of our interesting compounds

4.7.5. Nuclear Magnetic Resonance (NMR) Analysis

The spectroscopic methods, including ^{1}H-NMR and ^{13}C-NMR, techniques were utilized to determine the structure of the isolated compounds. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ and DMSO using a Bruker Avance 126, 400, and 500 MHz spectrometer for different samples. The chemical shifts (δ) in ppm were determined starting from calibrated tetramethylsilane (TMS) to left side

4.8. Biological Activity Testing

4.8.1. Antibacterial activity

Antibacterial activities were performed using the susceptibility disk diffusion method with slight modification of the method originally described by Kirby- Bauer *et,* al (Susceptibility *et* al., 2018). All samples were evaluated for their antibacterial activity against two Gram-negative bacteria (*Escherichia coli* ATCC 25922 & *Pseudomonas aeruginosa* ATCC 27853) and two Gram-positive bacteria (*Staphylococcus aureus*, ATCC 25923 & *staphylococcus pyogenes* ATCC 19615) and in comparison with ciprofloxacin as standard drug and DMSO as negative control. All bacterial strains were sourced from the American Type Culture Collection (ATCC) and obtained from the Adama Public Health Research & Referral Laboratory Center. The selection of these bacteria was based on their availability and the potential to cause bacterial infections. The concentrations of the samples were prepared by dissolving the required amounts in DMSO, resulting in three sets of dilutions: 200, 100, and 50 mg/mL for the extracts, and 5, 2.5, and 1.25 mg/mL for the isolated compounds (Miseganaw & Mhiret, 2019). Similarly, the reference antibiotic (ciprofloxacin) was used at a concentration of 30µg/mL, with DMSO serving as an inert negative control. Bacterial cultures for each test organism were prepared by taking 100 µL of bacterial subcultures and inoculating it into 10 mL of sterile nutrient broth (inoculation medium), which was then incubated overnight at 37°C. After incubation, the turbidity of each culture was adjusted to the 0.5 McFarland standards using nutrient broth. The prepared Mueller-Hinton Agar (MHA) solution was aseptically poured into sterile petri dishes (approximately 20 mL) and allowed to solidify. Next, the bacterial strains were inoculated onto the pre-labeled MHA plates using a sterile cotton swab, streaking it across the entire agar surface. This procedure was repeated two more times, rotating the plates 60° each time to ensure even distribution. A duplicate plate for each organism was also prepared.

Sterile filter paper disks, each 6 mm in diameter and impregnated with the test compounds and the standard drug, was placed on each MHA plate. The plates were then incubated at 37°C for 18 hrs. Once plate is incubated, zone of inhibition appears due to antibiotic properties of samples that can inhibit bacterial growth or kills the bacteria were determined along with the standard antibiotic (ciprofloxacin). The experiment was conducted in triplicate for each bacterium, and the mean zones of inhibition were calculated, expressed as mean ± SEM of the triplicates.

4.8.2. DPPH Radical Scavenging Activities

The radical scavenging activities of the extract, fractionations and isolated compounds from root of *G. purpurascens* was assessed against the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical measuring its absorbance at 517 nm, following the method utilized by Gulcin & Alwasel, (2023).

Sample solutions were prepared in methanol, after which serial dilutions were conducted using stock solutions of 2 mg/mL for the plant extract and fractions, and 0.4 mg/mL for the isolated compounds. This process resulted in concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/mL, as well as 400, 200, 100, 50, 25, and 12.5 µg/mL, respectively. Each concentration of sample (2 mL) was placed in a clean test tube and DPPH solution (2 mL) was added to the same test tube (Sharma & Bhat, 2009). Sample solutions were prepared in methanol, after which serial dilutions were conducted using stock solutions of 2 mg/mL for the plant extract and fractions, and 0.4 mg/mL for the isolated compounds. This process resulted in concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/mL, as well as 400, 200, 100, 50, 25, and 12.5 µg/mL, respectively. Each concentration of sample (2 mL) was placed in a clean test tube and DPPH solution (2 mL) was added to the same test tube (Sharma & Bhat, 2009).

The DPPH solution was prepared by dissolving 4 mg of DPPH in 100 mL of MeOH. The solution was shaken vigorously by hand and stored in a dark environment for 30 min. Afterward, the absorbance was measured using a UV-Vis spectrophotometer at 517 nm. A DPPH solution without the test sample served as the control, while methanol was used as the blank. L-ascorbic acid was utilized as the reference standard in this method. The DPPH radical scavenging rate for each sample was calculated using a formula from the literature. The results are presented as averages of three measurements

$$\text{Radical scavenging activity (\%)} = 100[\frac{Ac - As}{Ac}] \quad \text{.....Equ - 3}$$

Where Ac: represents the absorbance of the control (DPPH solution) and

AS: denotes the absorbance of the sample (extracts and compounds) or standard (L-ascorbic acid). The IC_{50} value is defined as the concentration of an antioxidant required to reduce a particular oxidative stress or free radical activity by 50%. IC_{50} values are used to assess the potency of a compound in inhibiting a specific target, such as an enzyme, receptor, or cell viability.

4.9. Molecular Docking, Pharmacokinetic and Drug likeness Prediction

Molecular docking is a computational method that predicts the binding affinity of ligands to receptor proteins, making it a powerful tool for drug development and research. It has emerged as a crucial element of *in-silico* drug development in recent years, focusing on predicting the interactions between molecules and proteins, pharmacokinetic properties, drug likeness and their toxicity at atomic level (Agu *et* al., 2023). In this study molecular dynamics, binding site estimation methods, homology modeling, and both two-dimensional and three-dimensional structure analyses, along with physicochemical properties and SwissADME assessments, were utilized for the isolated compounds (**32**, **37**, **38**, **39**, and **40**) using Gaussian 09 software and the Protox III online tool according to report reported in the literature (Muhammed & Aki-Yalcin, 2022).

4.9.1. *In silico* Pharmacokinetic and Drug likeness Prediction

The isolated compounds (**32**, **37**, **38**, **39**,and **40**) underwent computational studies to assess their drug-likeness properties by evaluating the physicochemical characteristics needed to qualify as drug-like molecules according to Lipinski's rule of five (cLog P ≤ 5, H-bond donors ≤ 5, H-bond acceptors ≤ 10, and molecular weight ≤ 500 Daltons)(Lipinski *et* al., 2012). Veber's parameters (rotatable bonds ≤ 10 and topological polar surface area) (Veber *et* al., 2002), along with pharmacokinetic parameters evaluated using SwissADME (Raju *et* al., 2021), as well as assessments of gastrointestinal absorption and blood-brain barrier (BBB) permeability, were also conducted (Martin, 2005). The toxicity of ligands, including LD_{50} values, organ toxicity profiles, and toxicological endpoints, as well as inhibitory enzyme effects, was predicted using the online program ProTox-III (Sing *et* al., 2012).

4.9.2. *In silico* Molecular Docking

The chemical structures of the isolated compounds, (**32**, **37**, **38**, **39**, and **40**) and standard ciprofloxacin were drawn using ChemDraw, then optimized by the Density Functional Theory (DFT/B3LYP) method with 6–31G(d,p) basis sets using Gaussian09 software (Muhammed & Aki-Yalcin, 2022). The energy-minimized ligands were then input into the docking procedure. The targets, *E. coli* DNA gyrase (Protein Data Bank (PDB) ID: 6F86*), S. aureus* pyruvate kinase (PDB ID: 3T07), and PqsA (UniprotKB ID: Q9l4 × 3) were retrieved from the Protein Data Bank(PDB) (Bender *et* al., 2021). After targets were selected, the protein complex's water molecules were removed, and the bound ligand was chosen to determine the characteristics of the binding site before being removed from the complex (Agu *et* al., 2023b). Redocking of the

bound ligand with the target protein was performed to confirm the binding site. The target and ligand were generated in the necessary format (pdbqt) for docking, and docking was performed by Autodock 4 (Allouche, 2012). Utilizing Biovia Discovery Studio 2020, the conformation with the lowest binding energy was selected to assess the interactions between the receptor and the ligand (Biovia, 2020).

4.10. Statistical analysis

The experimental outcomes of the study were reported as the mean ± standard deviation (SD) based on three replicates. Data was entered, coded, verified, and analyzed using IBM SPSS version 27 software. Statistical significance was assessed using one-way ANOVA, followed by the Tukey post hoc test, with $P < 0.05$ considered statistically significant for biological activity data.

5. RESULTS AND DISCUSSION

5.1. Extract Yield

The essential oils extracted from the roots of *G. purpurascens* were yellowish in color, yielding 0.028 g (0.028% w/w) while dichloromethane/methanol (1:1) extracts yielded a dark blue 68 g (6.04% w/w). The highest yield was obtained from root maceration extracted with organic solvent (dichloromethane: methanol) as compared to hydrodistilation extraction

5.2. Phytochemical Screening

The phytochemical screenings study of root extract found to contain secondary metabolites like anthraquinones, coumarins, flavonoids, glycosides, phenols, saponins, steroids, and tannins (Table 6). Previous study done on *G. purpurascens* had reported presence of alkaloids in 80% methanolic root and leaves extracts and absence of flavonoids in root of the plant extract (Adugna *et al*., 2023). In this study, the test result indicated the presence of flavonoid in DCM/MeOH extract, whereas alkaloid was absent. The variation in the production of phytochemical constituents might be attributed to difference on plant geographical location.

Table 5 : Phytochemicals screening result of the root extract of *G.Purpuracsens*

Phytochemicals tested	Tests/reagents	DCM: MeOH Extract
Alkaloids	Wagner's test	–
Anthraquinones	Ammonia Test for	+
Coumarins	Potassium Hydroxide	+
Flavonoids	Ferric Chloride test	+
Glycosides	Keller-Killiani	+
Phenols	Ferric Chloride test	+
Saponins	Froth test	+
Steroids	Libermann Burchard	+
Terpenoids	Salkowski's test	+
Tannins	Ferric Chloride	+

DCM: MeOH = dichloromethane: methanol; + = indicates presence - = indicates absence

In recent years, there has been growing recognition of secondary metabolites for their health benefits. These compounds have been shown to provide a range of biological activities including antioxidant, antimicrobial, ant-carcinogenic, and anti-inflammatory effects, as well as helping to prevent cardiovascular diseases, cancers, diabetes, and conditions related to

oxidative stress (Zhang *et* al., 2022). For example, glycosides provide incredible health benefits for various conditions including antibacterial and anticancer effects, along with anti-inflammatory, cardiovascular, and neurological properties (Riaz *et* al., 2023). Steroids and terpenoids also display a wide range of biological activities, including antimicrobial, anti-inflammatory, diuretics, immunosuppressant, progestational agents and anticancer effects (Ueno, 1978).

Generally, the result of qualitatively determined phytochemical constituents of the root of *G. purpurascens* was indicating the possible presence of secondary metabolites which may contribute for medicinal uses of the plant including; pneumonia, prevent bleeding, wound, itching skin, abdominal pain and toothache, hemorrhoid's, michi, mental disorder, cardiovascular disorder among community.

5.3. GC-MS Analysis of the Essential Oils (EOs)

The GC-MS analysis of extracts from *G. purpurascens* roots revealed 43 components of which 14 (84.6%) were identified based on their abundance and their chromatograph peaks with their chemical structures depicted in (Figure 7) and **(**Figure 8). Essential oils extracted from root of *G. purpurascens* has been found to contain several phytochemicals with the highest percent found to be benzoic esters (52.58%) followed by, fatty acid and its derivatives (12.43%), alcohols and phenolic alcohols (7.42%), steroids (4.73%) and others (Table 6). It has been reported that stigmast-5-en-3-O- β-sitosterol showed antimicrobial, anti-inflammatory, antiasthma , and diuretic activity (Mujeeb *et* al., 2014). 9-Octadecenoic acid showed anti-bacterial activity while its methyl ester displayed antimicrobial, anti-inflammatory, and anticancer activity (Rahamouz-Haghighi *et* al., 2022). The chemical structures of the major constituents of the essential oil of the roots of *G. purpurascens* are depicted in (Figure 7). Total ion chromatogram, mass spectrum and proposed mass fragmentation of the major compounds and n-alkane series were depicted in (appendix 1).

Table 6: Chemical constituents of EOs from root extracts of *G. purpurascens* analyzed using GC-MS.

			RI		
S/N	Compound name	RT	Calculated	Reported	%A
22	p - xylene	5.439	811.3	809	4.79
23	3,7-Dimethylocta-1,6-diene	6.274	855.8	857	1.00

24	P-Cresol	9.856	1049.4	1051.7	6.08
25	Benzoic acid- 1,6- dihydro methyl ester	20.116	1372.1	-	21.11
26	1-decanol	22.307	1256.4	1255	1.34
27	cyclohexadocane	28.367	1425.0	1423	1.66
28	Methyl palmitate	36.435	1920.3	1921	3.44
29	13-Octadecenoic acid, methyl ester	38.04	2117.6	2118	4.58
30	9-Octadecenoic acid (Z)-, methyl ester	38.873	2157.2	2159	1.47
31	n-Hexadecanoic acid	39.06	2166.5	2167	1.45
32	(24R)-Stigmast-5-en-3.beta.- ol	38.730	2141.7	-	3.12
33	Octadecanoic acid, methyl ester	39.096	2113.4	2112.2	1.49
34	Beta -Amyrin	41.293	2298.5	2299	1.61
35	Buthyl-9,12-octadecadienoate	42.438	1247	-	3.46
36	1,2-Benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl) ester	43.101	328.6	-	28.0

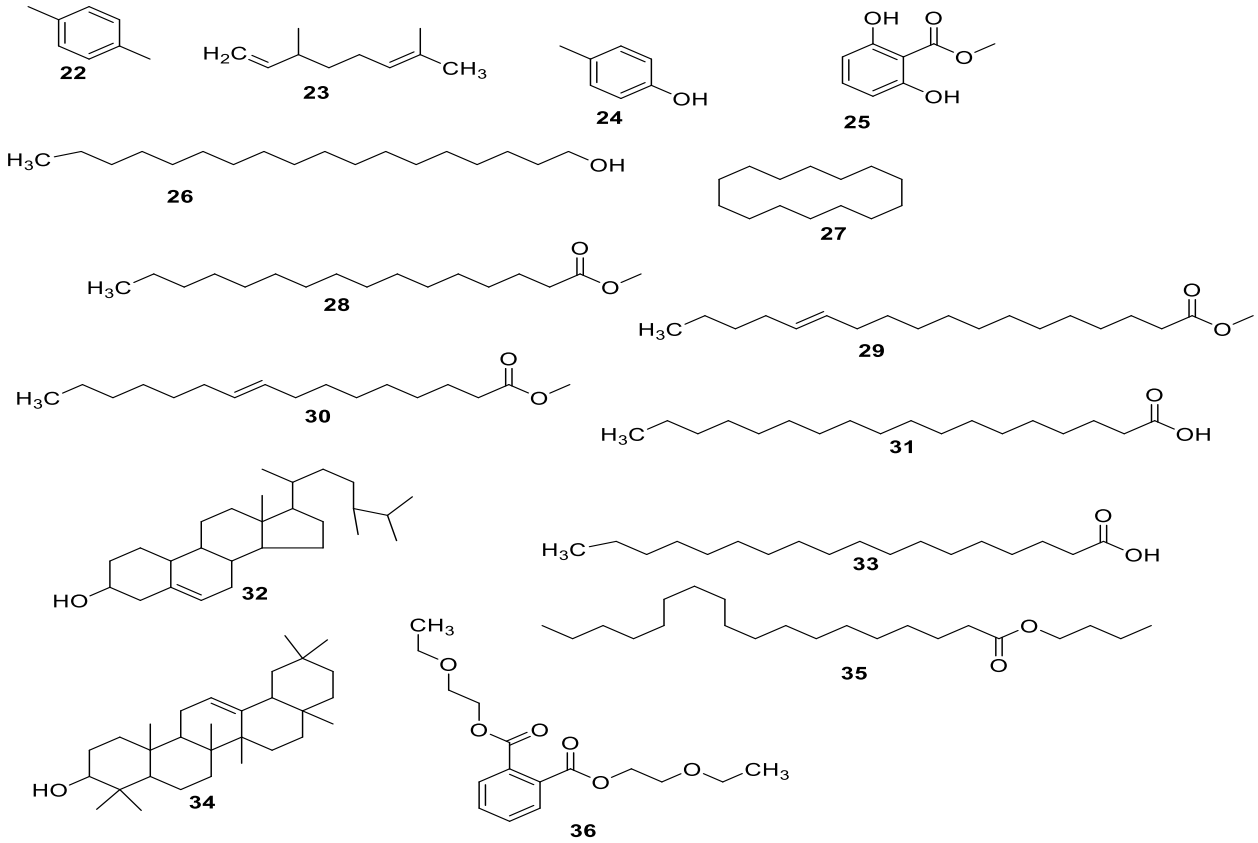


Figure 7: Chemical structure of Eos isolate from roots of *G. purpurascens*

Despite of no data are available on chemical composition of essential oils from *G. purpurascens* this study investigates various photochemical those exhibited different biological activities. Study showed benzoic acid 1, 6-dihydro methyl ester (**25**) exhibited good biological activity against *E. coli* and other bacterial strains (Friedman *et* al., 2003). Benzoic acid derivatives were used as model compounds to study their radical scavenging properties (Velika & Kron, 2012). This contribution is due to the presence of in variety group of compounds which are well known as antioxidants. It was reported compound **34** exhibited different biological activities including antimicrobial, antidiabetic, analgesic, antimalarial and anti-inflammatory (Fabiyi *et* al., 2012). Generally, the findings supported the traditional uses of this medicinal plant to treat different bacterial diseases and other ailments

5.4. Structure Elucidations of the Isolated Compounds

Fractionation of root extracts of *G. purpurascens* using silica gel column chromatography has led to the isolation of five compounds with their structures presented in (Figure 8**).** The structure elucidations of the isolated compounds were accomplished using physical and spectroscopic methods with the details presented as follows:

Compound **32** (48 mg) isolated as a white crystal from mixture of chloroform and ethyl acetate fractions of DCM/MeOH (1:1) root extract of *G. purpurascens*. TLC showed spot at an R_f value of 0.64 using *n*-hexane/ethyl acetate (9:1) as a mobile phase (Appendix 2a). The melting point of the compound was found to be between 136 - 137 °C. The FT-IR spectrum revealed the presence of a hydroxyl group at 3363 cm^{-1}, along with C-H stretching vibrations at 2917 cm^{-1} and 2865cm^{-1}. Furthermore, the presence of a peak at 1600 cm^{-1}, along with another at 1500 cm^{-1}, suggests the existence of a double bond in the molecular structure. In addition, the pronounced peak observed at 1050.13 cm^{-1} is indicative of oxygenated carbon compounds.

The ^{1}H NMR spectrum of compound **32** (Table 7, Appendix 5) displayed an olefinic proton signal at δ_H 5.35 (t, 1H, t, J = 11.2, Hz) and an oxygenated proton at δ_H 3.53 (m, 1H). The characteristic signal of protons associated with the steroid nucleus at carbon C-3 was observed at a chemical shift of δ_H 3.52. Additionally, proton signals were observed at δ_H 0.69 (s, 3H), 1.01 (s, 3H) 0.92 (d, 6.6 Hz, 3H), 0.84 (d, 7.5 Hz, 3H), 0.81 (d, 6.9 Hz, 3H) and 0.86 (t, 7.2 Hz, 3H,) which were attributed to six methyl groups.

The ^{13}C NMR spectrum (Table 7, Appendix 6) revealed signals corresponding to 28 carbon atoms. Among these, 27 signals exhibited similar intensities, while one signal was observed to be twice as intense as the others. This indicates the presence of overlapping signals due to two carbon atoms, resulting in a total of 29 distinct carbons in the compound. There are two olefinic carbons (δC 140.7 and δC 121.7) and carbon attached to oxygen (δC 71.8). These findings support our assertion of a steroid skeleton featuring one double bond and an oxygenated carbon at C-3.The others are available as range of aliphatic carbons in which the most upfield signals at δ_C 19.8, 19.4, 19.1, 18.8, 11.9 and 11.8 are due to methyl groups. Based on UV-Vis, FT-IR, ^{1}H NMR and ^{13} NMR along with literature report, the structure of the compound was proposed as ***β*-sitosterol** (Figure 9, compound **32**) (Duguma & Rentsch, 2023). All carbon NMR spectra are in agreement with proposed structure.

Table 7: ^{1}H NMR and ^{13}C NMR (400 MHz, $CDCl_3$) spectral data for compound **32**

Carbon No.	Compound 32 δ_H (m, J in Hz)	δ_C in ppm	Reported (Duguma & Rentsch, 2023).	Appearance
1.	1.44 (m, 2H)	37.4	37.4	CH_2
2	1.84 (m, 2H)	31.8	31.8	CH_2
3	3.53 (m, 1H)	72	72.0	CH
4	2.27 (m, 2H)	42.5	42.4	CH_2
5		140.9	140.9	C

6	5.35 (t, 1H)	121.9	121.9	CH
7	1.95 (m, 2H)	32.1	32.0	CH_2
8	1.44 (m, 1H)	32.1	32.0	CH
9		50.3	50.3	CH
10		36.7	36.6	C
11		21.2	21.2	CH_2
12		39.9	39.9	CH_2
13		42.4	42.5	C
14	1.29 (m, 1H)	56.2	56.9	CH
15		24.6	24.4	CH_2
16		28.4	28.4	CH_2
17		56.9	56.2	CH
18	0.68 (s, 3H)	12.1	12.0	CH_3
19	1.01 (s, 3H)	19.5	19.5	CH_3
20		36.3	36.3	CH
21	0.92 (d, J = 6.6, 3H)	18.9	18.9	CH_3
22	1.25 (m, 2H)	34.1	34.1	CH2
23	1.01 (m, 2H)	26.2	26.2	CH2
24	1.15 (m, 2H)	46.0	46.0	CH
25	1.67 (m 1H)	29.3	29.3	CH
26	0.84 (d, J = 7.5 Hz, 3H)	20.0	20.0	CH_3
27	0.81 (d, J = 6.9 Hz, 3H)	19.8	19.2	CH_3
28	1.25 (m, 2H)	23.2	23.2	CH_2
29	0.86 (t, J= 7.2 Hz, 3H)	12.0	12.1	CH_3

β-Sitosterol is a bioactive phytosterol that widely available naturally in the cell membranes of larger plants, including the family of the plants like root and root bark of *Carissa carandas*, seeds of *Acokanthera oppositifolia*, vegetable oils; avocado oil (76 mg/100 g) (Babu & Jayaraman, 2020).

It has been reported that β-Sitosterol showed biological activity hypoglycemic activity, metabolic activities, antidiabetic activity, cortisol lowering activity and antiproliferative activity, wound healing activity anti-bacterial activity, anti-fungal and anti-oxidant activity (Islam & Lucky, 2019). This may attributes the medical importance of the plant among community for abdominal pain, wound, Haemorrhoids, itching and so far.

Compound **37** (32 mg) obtained colorless jelly like from the mixture of chloroform and ethyl acetate soluble portion of the DCM/MeOH (1:1) extract of the roots of *G. purpurascens.* The compound (F_{23}) was eluted from column chromatography on silica gel columns with a mobile phase consisting of *n*-hexane/ethyl acetate (9:1). TLC analysis showed a single spot at R_f value of 0.78 using 1: 4 ethyl acetate in n-hexane (Appendix 2b.) The observation of a band in the

UV-Vis spectrum at around 220 nm suggests that the compound lacks conjugated systems (Appendix 3b). The FT-IR spectrum showed bands between 2942 cm^{-1} and 2816 cm^{-1}, indicating the presence of C-H bonds (methyl and methylene). Additionally, the spectrum exhibited bands around 1718 cm^{-1} and 1050 cm^{-1}, which are characteristic of carbonyl carbon and oxygenated carbon, respectively (Appendix 4e).

^{1}H NMR spectrum (400 MHz, $CDCl_3$) displayed a peak around δ 3.32 (2 H) as a multiplet indicating protons near an electronegative atom, most likely oxygen. A peak at 2.1 ppm is due to protons on a carbon attached to a carbonyl group. Additionally, a peak at 0.8 ppm (24 H) indicated a collection of protons from long open-chain aliphatic groups. The most upfield signal δ 0.67, (2 H, t) indicate terminal methyl (Table 8, Appendix 7).

The ^{13}C NMR (101 MHz, $CDCl_3$) showed signals for 17 carbons. Diagnostics signal due to ester carbonyl appeared at δ174.5 and signal at δ60.7 ppm for carbon attached to electronegative oxygen. The carbon signals observed around δ29 ppm represent aliphatic carbon most likely that of fatty acid (Table 8, Appendix 8). Based on its physical properties and spectroscopy data information along with comparing literature (Tesfamicael & Ele, 2016)), compound **37** is identified as **ethyl palmitate**(Figure 9, compound **37**). All spectral data align with deduced structure.

Table 8 : ^{1}H NMR and ^{13}C NMR (400 MHz, $CDCl_3$) spectral data for compound **37**

No.	Compound 37 δH(m, J in Hz)	δC in ppm	Reported (Tesfamicael & Ele, 2016)	Appearance
1.		174.54	173.4	ΔC(O)
2	2.28-25 (m, 2H)	33.70	33.9	CH_2
3	1	32.6	31.7	CH_2
4		31.4	29.4	CH_2
5	1.2 (m, 2H)	29.1	29.4	CH_2
6		28.9	29.3	CH2
7		28.8	29.1	CH_2
8				CH_2
9		28.6	29.1	CH_2
10	1.4-12 (m, 2H)			CH_2
11		28.4	28.8	CH_2
12		25.5		CH_2
13		22.13		
14	1.6 -1.5 (m, 2H)	24.5	24.9	CH_2
15		22.2	22.5	
16	0.80 (t, t, J = 7.0	14.0	14.4	CH_3

	Hz, 3H)			
1’	3.32 (s, 3H)	60.7	60.10	O-CH_2
2’		16.0	14.6	CH_3

Hexadecanoic acid, ethyl ester (Palmitic acid ethyl ester) is an ester of fatty acid which found in various natural sources, primarily in the form oils; *Plantago lanceolata* root, Virgin olive oils (Palagano *et* al., 2020). It can be also available in synthetic form derived from hexadecanioc acid by esterification process (Tesfamicael & Ele, 2016). It has been reported that ethyl palmitate showed varies biological activity such as antioxidant, hemolytic, antipsychotic, antifungal, antitumor, antibacterial (Rahamouz-Haghighi *et* al., 2022) and ant-inflammatory activity (Saeed *et* al., 2012).This support the traditional uses of the plant for varies infections and non-infectious diseases like: mental illness, wounds, itching, bleeding and tonsillitis.

Compound **38** and **39** (42 mg) were co-isolated as a white solid from the mixture of chloroform and Ethyl acetate partition of DCM/MeOH (1:1) extract of the root of *G. purpurascens*. The compound sub-fraction (F_{25}) was collected from column chromatography, eluting on silica gel columns with a mobile phase consisting of *n*-hexane/ethyl acetate mixture in a ratio of 70:30. TLC analysis revealed an R_f value of 0.81 mm using 40% ethyl acetate in *n*-hexane a mobile phase. The spot was visualized after spraying with vanillin/H_2SO_4 and heating on a hot plate and observed as pink with broad size (Appendix 2c). The compound was melted at 293-297 °C.

UV-Vis spectrum of compound 38 and 39 showed λ_{max} at 220 nm to 250 nm indicates presence of carbonyl carbon and double bond (appendix 3e). The FT-IR spectrum revealed a broad band at 3380 cm^{-1}, indicating the presence of a hydroxyl group. A peak at 1713 cm^{-1} confirmed the presence of a carbonyl group. Additionally, the spectrum around 1623 cm^{-1} indicated the presence of olefinic carbons, and an intense peak at 1012 cm^{-1} was associated with oxygenated carbon (Appendix 4).

The ^{1}H NMR spectrum revealed signals at δ_H 11.98 ppm correspond to an OH group, indicating an acidic functional group. Additionally, two olefinic protons are observed at δH 5.15 and 4.30 ppm (Table 9). The most upfield protons, with chemical shifts at δ 0.90 (s, 3H), δ 0.90 (s, 3H), δ 0.67 (s, 3H), δ 1.09 (s, 3H), and δ 0.79 (s, 3H), correspond to methyl protons as well as δ 11.99 (s, 1H), oxygenated protons (Appendix 9).

The ^{13}C NMR spectrum (Table 9, Appendix 9) displayed signals for 60 carbon atoms, with different signal intensity. The most deshielded carbons δ C178.3 and 181.0 indicated two carbonyl carbons available nearly with equal chemical shift. The spectrum also appeared

having four olefinic carbons with chemical shift of, δC 143.8, 138.2, 124.6, and 121.5. Two overlapping signals at δC 76.8 likely correspond to two equivalent oxygenated carbons. Based on the total number of carbon signals, their intensities, overlaps, and chemical shifts, the signals of each carbon were categorized into two groups, each containing 30 signals in the ^{13}C NMR spectrum (Table 9).

Based on spectral data along with literature report, we identified the structure of these compounds **oleanolic acid** (**38**) and **ursolic acid** (**39**) figure 9 compound **38** & **39**. There is a literature report that point out factors for the co-existence of these compounds, along with betulinic acid, and made the issues of researchers to separate them during experiments. These compounds are biosynthetically derived from the same carbon skeleton (C_{30}). They also have nearly similar polarity. Basically we able to identify the proportional co-existence of these compounds based on the nature of their ^{1}H NMR on carbon 19 which made them unique. The report indicated separation of three triterpenic acids—ursolic acid, oleanolic acid, and betulinic acid can be achieved high-performance thin-layer chromatography (HPTLC), C_2 reverse phase (RP) and C_{18} RP and TLC silica gel 60 (Naumoska *et* al., 2013)

Oleanolic acid is a triterpenoids that occurs in nature either as a free acid or as an aglycone of triterpenoids saponins. It is commonly found alongside its isomer, Ursolic acid (Ayeleso *et* al., 2017). The triterpenoids betulinic acid, 3β-taraxerol acetate, and 13α-methyl, 27-norolean-14-en, 3β-ol (3β-taraxerol) were previously reported from the same genus species of *G. sinaicus* (Marzouk *et* al., 2015b). Moreover, these compounds along with betulinic acid isolated from hairy root of *Ocimum basilicum* (Marzouk, 2009)**.** The report indicated that these triterpenic acids (Oleanolic acid and Ursolic acid) demonstrated a variety of activities, including antitumor, anti-inflammatory, anti-HIV-1, antimicrobial, anti-ulcer, ant oxidative, anti-atherosclerotic, hepatoprotective, gastroprotective, hypoglycemic, hypolipidemic, cardiovascular, cardiotonic, antidysrhythmic, and chemopreventive effects (Naumoska *et* al., 2013). The Isolated compounds along with their previous biological activity reported supports the traditional use of the plant among the community to treat different ailments.

9:^{1}H NMR and ^{13}C NMR (500 MHz) spectral data for compound **38** and **39** (in DMSO- d6)

	Compound **38** δH(m, J in Hz)	δC in ppm	Reported (Marzouk, 2009)	Compound **39** δH(m, J in Hz)	δ ^{13}C in ppm	Reported (Marzouk, 2009)	Appearance
1.	1.44 (t, 2H)	38.4	38.5	δ 1.44 (t, 2H)	38.1	38.8	CH_2
2	1.72 (m, 2H)	28.3	27.4	δ1.72 (m, 2H)	27.5	27.3	CH_2
3	2.74 (d, *J* = 13.7 Hz ,1H), 3.00 (s, 1H)	77.3	78.7	δ 2.74 (d, *J* = 13.7 Hz ,1H), 3.00 (s, 1H)	76.8	78.8	CH
4	-	38.4	38.7		38.2	38.8	C
5	-	55.2	55.2		54.8	55.4	C
6	1.86 – 1.76 (m, 4H)	16.9	18.3	δ 1.86 – 1.76 (m, 4H)	18.0	18.4	CH_2
7	-	36.5	32.6		32.4	33.0	CH_2
8	-	38.9	39.3		40.8	39.6	C
9	1.91 (m, 1H)	46.8	47.6	δ 1.91 (m, 1H)	47.0	47.5	CH
10	2.13 (m, 2H)	38.4	37.0	δ 2.13 (m, 2H)	38.1	37.0	C
11	-	21.1	23.1		22.6	23.3	CH_2
12	4.30 (d, J = 15.5 Hz,1H)	121.5	122.1	δ 5.14 (d, J = 15.5 Hz,1H)	124.6	125.5	ΔCH
13	-	143.8	143.4		138.2	138.0	ΔC
14	-	41.7	41.6		40.8	42.0	C

15	1.44-1.61 (m, 4H)	28.2	27.7	δ 1.44-1.61 (m, 4H)	30.2	28.2	CH_2
16	-	23.4	23.4		24..6	24.3	CH_2
17	-	45.5	46.6		47.5	48.1	C
18	1.86 (m, 1H)	41.3	41.3	δ 1.86 (m, 1H)	52.8	52.8	CH
19	1.01 (d, *J* = 29.9 Hz, 2H), 1.47(dd, 1H)	45.6	45.8	δ 1.01 (d, *J* = 29.9 Hz, 2H),	39.0	39.1	CH_2
20	-	30.4	30.6		38.9	38.8	C
21	1.65 – 1.55 (m, 4H)	36.6	33.8	δ 1.65 – 1.55 (m, 4H)	32.1	30.7	CH_2
22	-	32.9	32.3		36.3	36.7	CH2
23	0.90 (s, 3H)	28.2	28.1	δ 0.90 (s, 3H)	29.0	28.2	CH_3
24	0.90 (s 3H)	15,.2	15.6	δ 0.90 (s 3H)	15.2	15.5	CH_3
25	0.67 (s, 3H).	15.1	15.3	δ 0.67 (s, 3H).	16.9	15.7	CH_3
26	1.09 (s, 3H)	16.1	16.8	δ 1.09 (s, 3H)	17.0	16.9	CH_3
27	0.79 (s,3H)	28.2	26.0	0.79 (s,3H)	22.9	23.6	CH_3
28	11.99 (s, 1H)	179.1	181.0	δ 11.99 (s, 1H)	178.3	179.5	ΔCO
29	0.91 (s, 3H),	23.3	23.1	δ 0.91 (s, 3H),	23.3	23.5	CH_3
30	0.81(s, 3H)	25.6	23.6	δ 0.81(s, 3H)	22.1	21.2	CH_3

Oleanolic acid is a triterpenoids that occurs in nature either as a free acid or as an aglycone of triterpenoids saponins. It is commonly found alongside its isomer, Ursolic acid (Ayeleso *et* al., 2017). The triterpenoids betulinic acid, 3β-taraxerol acetate, and 13α-methyl, 27-norolean-14-en, 3β-ol (3β-taraxerol) were previously reported from the same genus species of *G. sinaicus* (Marzouk *et* al., 2015b). Moreover, these compounds along with betulinic acid isolated from hairy root of *Ocimum basilicum* (Marzouk, 2009)

The report indicated that these triterpenic acids (Oleanolic acid and Ursolic acid) demonstrated a variety of activities, including antitumor, anti-inflammatory, anti-HIV-1, antimicrobial, anti-ulcer, ant oxidative, anti-atherosclerotic, hepatoprotective, gastroprotective, hypoglycemic, hypolipidemic, cardiovascular, cardiotonic, antidysrhythmic, and chemopreventive effects (Naumoska *et* al., 2013). The Isolated compounds along with their previous biological activity reported supports the traditional use of the plant among the community to treat different ailments.

Compound **40** (53 mg) was isolated as a pale pink color from the mixture of chloroform fraction and Ethyl acetate fraction of DCM/MeOH (1:1) extract of the root of *G. purpurascens*. The compound (F_{298}) was collected from column chromatography, eluting on silica gel columns with a mobile phase consisting of a 90% Ethyl acetate and in n-hexane. TLC analysis revealed a distinct spot at *Rf* = 0.80 using ethyl acetate: hexane (9.5: 0.5) as mobile phase. The spot was visualized after spraying with vanillin/H_2SO_4 and heating on a hot plate (Appendix 2d.) The compound was melted at 303 to 304 °C. The UV/Vis spectrum in MeOH showed λ_{max} at 260 to 350 to 350 nm accounted to the presence of flavonoid skeleton in the structure (Appendix 3d). The FT-IR spectrum showed the presence of a hydroxyl group around 3423 cm^{-1}, acidic proton, as well as peaks at 2997 cm^{-1} and 2854 cm^{-1}, for C-H and a prominent peak at 1720 cm^{-1}, along with several bands ranging from 1671 cm^{-1} to 1371 cm^{-1} for carbonyl carbon and double bond (Appendix 3b). Additionally, peaks observed between 1150 and 1012 cm^{-1} suggested the presence of oxygenated carbons.

The ^1H NMR (400 MHz, DMSO-d6) spectrum of compound **40** displayed a signal δ 11.25 which is diagnostic for the presence of oxygenated hydrogen. The spectrum displayed a signals 6.26 (d, J =2.1, 1H) and 6.77 (d, J =2.1, 1H) indicated Meta coupled protons with one another. The absence of proton signals on the rest of ring A indicated the ring is tetra-substituted. 7.67 (dd, J =7.6, 1.9 1H) indicated Orto and Meta coupled protons on ring C along with their chemical shift justify a distinct signal of flavonoids. The spectral data displayed at δ 5.34 ppm correspond to proton of anomeric carbon. A peak observed 3.32 ppm for 3H just indicator of methoxy protons. The signals in the range

between δ 3.71 ppm to 4.07 ppm along with the anomeric proton signal indicated the presence of sugar moiety in the structure. The most upfield proton 2.12 ppm appeared as doublets for three protons may for methyl protons (Table 10, Appendix 11).

The ^{13}C NMR (126 MHz, DMSO-d6) spectrum displayed peaks corresponding to 22 carbons. The most deshielded peak at *δ* 177.6 ppm likely represents a carbonyl carbon. Signals due to oxygenated carbons were evident at *δ* 166, 161.9, 159, 151.4, 149, 148.5, and 148.9 ppm (Table 10, Appendix 11). Diagnostic signals at *δ* 74.6, 72.1, 71.0, 68.6, and 55.0 along with a peak at *δ* 100.3 indicate the presence of sugar moiety. There is also a single upfield peak at a chemical shift of 15.4 ppm, corresponding to an aliphatic carbon (Table 10). Based on spectral data along with literature report, compound **40** is identified as **isorhamnetin 3-O-rhamnoside** (Figure 9 compound **40**)

Table10: ^{1}H NMR and ^{13}C NMR (400 MHz, DMSO-d6) spectral data for compound **40**

Carbons no.	Compound **40** δH(m, J in Hz)	δC in ppm	Reported (Abdel Elatif *et* al., 2020)	Appearance
2	-	151.4	156.4	Aro- C-OH
3	-	131.3	133	Aro-C-O-
4	-	177.6	177.6	Aro-C=O
5	11.25 (s, 1H)	161.9	161.1	Aro-C-OH
6	6.26 (d, J =2.1, 1H)	100.3	98.9	Aro-CH-
7		166.5	164.5	Aro-C-OCH_3
8	6.77 (d, J =2.1, 1H)	81.9	93.7	Aro-CH- meta to –O-
9		159.6	156.4	Aro-C- Orto to –O-
10		106.3	104.1	Aro- C-
1’		119.2	121.0	Aro-CH
2’	7.16 (d, J =1.9, 1H)	114.9	115.3	Aro-C-OH
3’		148.5	149.5	Aro-C-OH
4’		146.9	146.9	Aro-CH
5’	7.63(d, J =7.6, 1H)	110.8	113.5	Aro-C-

6’	7.67 (dd, J =7.6, 1.9 1H)	122.7	122.0	CH_2
	3.32 (s, 3H)	55.0	55.6	OCH_3
1’’	5.34 (dd, 1H)	103.1	103.9	O-CH-O-
2’’	3.74 (d, 1H)	72.21	74.69	CH-OH
3’’	3.71 (d, 1H)	72.15	71.08	CHC-OH
4’’	3.76(dd, 1H)	71.06	70.21	CH-OH
5’’	4.07 (d, 1H)	68.66	68.35	CH
6’’	2.12 (d, 3H)	15.46.	17.98	CH_3

Isorhamnetin 3-O-rhamnoside was previously reported from *Caesalpinia gilliesi* flower (Mag *et* al., 2016), *Hippophae rhamnoides* seeds (Sławińska *et* al., 2024) *Ginkgo biloba* L., (Wang *et* al., 2023), *Cryptocarya alba* (Номера, 2016) *Apis mellifera* (Sobral *et* al., 2017). It was also synthesized through a highly efficient cascade involving three enzymes-rhamnosyltransferase, glycine max sucrose synthase, and uridine diphosphate (UDP)-rhamnose synthase-utilizing a UDP-rhamnose regeneration system (Rhamnosyltransferase *et* al., 2019). It exhibited varies biological activity against bacteria (Rhamnosyltransferase *et* al., 2019), cytotoxic activity (Abdel Elatif *et* al., 2020), and antidiabetic activity and anti-oxidant activity (Sławińska *et* al., 2024). Flavonoid glycosides are versatile compounds with a wide range of health benefits, primarily due to their antioxidant, anti-inflammatory, and antimicrobial properties. Their complex structures, which include a sugar moiety attached to a flavonoid backbone, enhance their solubility and bioavailability, further contributing to their biological activities. The biological activity of aglycone part, (isorhamnetin) was reported as it had antimicrobial activity like anti-tuberculosis *E. coli, K. pneumoniae, S. aureus* antioxidant activity, anticancer, neuroprotective, hepatoprotective and so far (Dong *et* al., 2022)

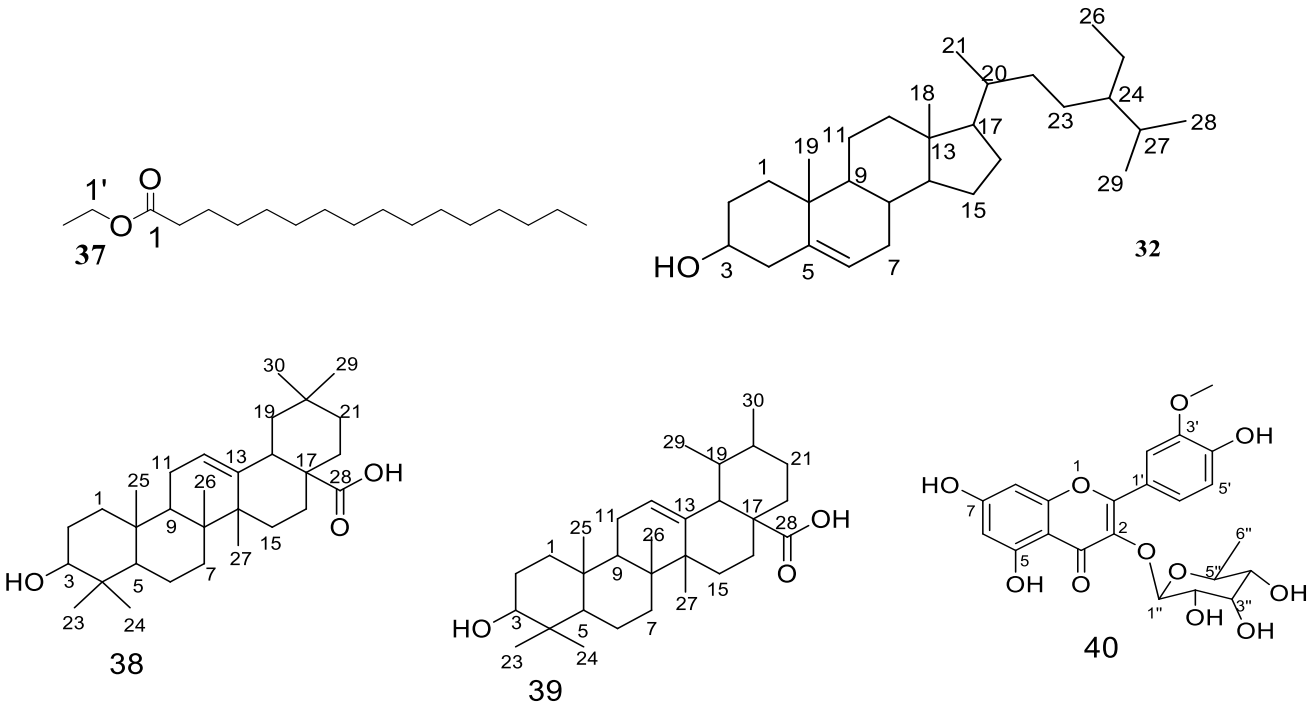


Figure 8: Structure of column isolated compounds from roots of *G. purpurascens*

5.5. Biological activity of the *G. purpurascens.*

5.5.1. Anti-bacterial Activities

The root extract and isolated compounds **32, 37, 38, 39 and 40** of *G. purpurascens* were tested for their *in vitro* antibacterial activity against Gram-negative bacteria (*Escherichia coli* & *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus*& *Staphylococcus pyogenes*) at different concentrations and compared with ciprofloxacin as positive control and DMSO as negative control. Results from the *in vitro* antibacterial activity test indicated that the extract and all the fractions exhibited variable growth inhibition activities against both Gram-positive and Gram-negative bacteria in a dose-dependent manner.

The extract (DCM/MeOH) of the root was generally the most effective against all tested organisms, showing inhibition zones of 16.14±0.58 mm, (*E. coli*), 15.00±0.44 mm (*S. aureus*) 12.33±0.42 mm, (*S. pyogenes*) and 11.08±0.58 mm (*P. aeruginosa*) at a concentration of 200 mg/mL. The Ethyl acetate fraction also showed significant activity against *S. aureus,* and *E. coli*

with an inhibition diameter of 14.23±0.81 and 15.20±0.52 mm at 200 mg/mL, but it was less effective against *P. aeruginosa*. The growth level of all strain of bacteria was found to be significantly inhibited ($P < 0.01$) in concentration 200 mg/ mL of EtOAc fraction in comparison to ciprofloxacin (Table 11). The MeOH fraction displayed notable activity against all strain of bacteria: *S. aureus, S. pyogenes, P. aeruginosa, and E. coli,* with inhibition zones of 12.34±0.22 mm, 11.13±0.21 mm, 9.42±0.00 mm and 13.32±0.17 mm respectively. Hence the extract exhibited significantly inhibits *S .aurous* and *E. coli* growth at concentration 100 mg/mL and 200 mg/mL growth of content compared to the ciprofloxacin ($p < 0.001$) 30 μg/Ml. It has been reported that in earlier study of *in vitro* antimicrobial activity was observed with 99.5% methanol, 97% ethanol, and 99.5% acetone crude extracts from the roots of the plant against clinical isolates of bacterial pathogens such as *E. coli*, *P. aeruginosa*, and *S. aureus*, as well as standard strains of *E. coli, S. aureus, and S. pneumoniae* (Miseganaw & Mhiret, 2019). Test doses of 150, 300, and 600 mg/mL showed significant antimicrobial activity ($p < 0.005$), with the maximum zone of inhibition recorded at 17.66 mm for the methanol extract against clinical *S. aureus*, followed closely by 17.45 mm for the acetone extract against the standard strain of *S. aureus* (Miseganaw & Mhiret, 2019). Despite of different concentration and solvent extraction were used, this study consistence with the report.

Table 11: Antibacterial activity of root extract and solvent fractions of *G. purpurascens*

Sample Standard	**Concentration (mg/mL)**	**Inhibition Diameter mm (mean ± SEM)**			
		S. aureus	***S. pyogenes***	***P. aeruginosa***	***E. coli***
DCM/MeOH (1:1) extract	200	15.00±0.44[c]	12.33±0.42	11.08±0.58[a]	16.14±0.58[c]
	100	14.00±0.52[c]	12.22±0.23[a]	9.33±0.33	14.17±0.65[b]
	50	10.06±0.29	8.33±0.13	7.67±0.23	13.22±0.22[b]
Hex fraction	200	7.00±0.51	4.10±0.32	5.33±0.12	6.14±0.12
	100	6.22±0.52	4.00±0.18	4.60±0.88	5.06±0.00
	50	5.04±0.55	3.33±0.62	4.08±0.18	5.00±0.14
EtOAc fraction	200	14.23±0.81[b]	13.06±0.58[b]	11.66±0.16[b]	15,20±0.52[b]
	100	12.00±0.58	12.33±0.33[a]	10.00±0.58	13.33±0.20[a]
	50	11.36±0.81	10.00±0.58	9.00±0.16	12.00±0.52
MeOH fraction	200	12.34±0.22[a]	11. 13±0.21[a]	9.42±0.00	13.32±0.17[b]
	100	11.56±0.14	10.43±0.20	9.00±0.14	12.42±0.23[a]
	50	10.34±0.12	10.00±0.08	8.16±0.16	9.33±0.00[a]
Ciprofloxacin (μg/mL)	30 μg/mL	18.32±0.15	19.33±0.80	21.33±0.16	22.67±0.20

Data are expressed as mean ± SEM; n=6 and analyzed by one way ANOVA followed by

tuckey post hoc test against: *S. aureus = Staphylococcus aureus, S. Pyogenes = Streptococcus pyogenes, P. aeruginosa = Pseudomonas aeruginosa, E. coli = Escherichia coli;* [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$.

Results from the *in vitro* antibacterial activity indicated that all the compounds except compound **37** displayed growth inhibition against both Gram-positive and Gram-negative bacteria in a dose-dependent manner. Compound **37** (ethyl palmitate) did not showed inhibition against growth of gram positive (*S. aureus* and *S. Pyogenes*) and exhibited minor inhibition on gram negative bacteria's (*E. coli* and *P. aeruginosa*). Each compounds showed varies effectively against different strains of bacteria. Among the compounds isolated from the root of *G. purpurascens*, compound **38** and **39** exhibited the most significant antibacterial activity which showed inhibition zone 17.22±0.58 mm, against *P. aeruginosa* at concentration of 5 mg/mL.

The disk diffusion *in vitro* antimicrobial test indicated that compound **38** and **39** exhibited anti-bacterial activity against *E. coli* and *P. aeruginosa* at all concentration (5 mg/Ml, 2.5 mg/mL and 1.25 mg/mL) significantly ($p<0.01$) and ($p<0.001$) as compared to positive control, ciprofloxacin. It is in agreement with literature report indicated these compounds (oleanolic acid and ursolic aced) displayed synergistic activity on methicillin resistance *S. aureus* and *P. aeruginosa* (Catteau *et* al., 2017). Jesus and his coworkers also reported incontestable biological activity of oleanolic acid and ursolic acid against different strain of bacteria and they showed (MIC of 8 μg /mL and 64 μg /mL (MIC of 8 μg /mL and 64 μg /mL, against *Staphylococcus aureus* (Jesus *et al*., 2015) respectively.

Compound **40** displayed antibacterial activities against all strain of bacteria at all concentration and greatest activity was observed against gram positive *S. aureus* and gram negative *P. aeruginosa* with inhibition zone 16.13±0.48 mm and 16.54±0.46 mm at concentration 5 mg/ mL respectively. Earlier studies on the *E. coli, S. aureus* and other *K. pneumoniae, Proteus* reported significant activity of the compound as a class of flavonoids (Gong *et* al., 2020). Previous anti-bacterial investigation conducted aglycone part of flavonoids using the agar well diffusion method against selected human pathogens, including *E. coli, S. aureus,* and others showed 11mm to 19 mm at different concentration which was consistent with this study

(Habtamu & Melaku, 2018).

Compound **32** also showed moderate activity against all strains of bacteria among it inhibited significantly ($p<0.05$) the growth of *E. coli* at concentration 5 mg/mL as compared to positive control. This result also consistence with previous report regarding to antibacterial activity of the compound with the same doses and methods (Abera *et* al., 2024). This study indicated that compounds **38** and **39** along with **40** had highest zones of inhibition against all tested organisms, and compound **32** had moderate activity whereas compound **33** had lowest zone of inhibition. The isolated compounds from the *G. purpurascens* displayed comparable significant inhibitory effects at lower concentrations compared to the higher concentration of extract and fractionations. On the other hand it suggests the antibacterial activities observed with the fraction from the compounds isolated may be due to the isolated compound and other minor bioactive components.

The moderate antibacterial activities of ursolic acid against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* was reported (Do Nascimento et al., 2014). Similar pharmacological activities, including hepatoprotective, anti-inflammatory, antioxidant, and anticancer effects, have been reported for both oleanolic acid and ursolic acid (Jesus et al., 2015). More over in this study compound **38** and **39** were co-isolated in 2:1 proportion and demonstrated representative synergistic effect which showed highest effect against *P. aeruginosa* and *E. coli.* It was reported isorhamnetin significantly enhances survival and treated lung tissue of *E. coli*-infected mice(Collins & Charles, 1987). This study also suggest the glycoside compound showed effective biological activities against both gram positive and gram negative bacterial strain which is parallel with reported literatures.

Antibacterial and antifungal activities along with other biological activities of ethyl palmitate was reported (Rahamouz-Haghighi *et* al., 2022). However this compound showed no activities against *Streptococcus aureus* and *Streptococcus pyogenes* and showed minor activities against *P. aeruginosa* and *E. coli.*

This justifies the various classes of phytochemicals of plant attribute different biological activities on different bacterial strains with different mechanism of action. Example flavonoids can inhibit nucleic acid synthesis, disrupt cytoplasmic membrane function, and interfere with energy metabolism (Shamsudin *et* al., 2022). Terpenoids act through changes in membrane permeability of *S. aureus and E. coli* without cell lysis (Oliveira E Nogueira *et al*., 2021).

Generally the zones of inhibition of isolated compounds against four strains of bacteria were summarized in (Table 12).

Table 12: Antibacterial activity of the isolated compounds from root extract of *G. purpurascens*

Sample/ Standard	Concentration (mg/mL)	Inhibition Diameter mm (mean ± SEM)			
		S. aureus	***S. pyogenes***	***P. aeruginosa***	***E. coli***
32	5	7.55±0.52	6.22±0.74	8.008±0.58	12.00±0.12 [a]
	2.5	6.00±0.44	5.00±0.22	9.33±0.36	11. 67±0.33
	1.25	5.60±0.29	5.00±0.24	7.67±0.31	9.67±0.34
37	5	0.00	0.00	5.40±0.46	6.00±0.00
	2.5	0.00	0.00	5.00±0.80	6.00±0.00
	1.25	0.00	0.00	4.00±0.55	6.00±0.00
38 and **39**	5	14.33±0.88 [b]	13.00±0.58 [b]	17.22±0.58 [c]	17.00±0.22 [c]
	2.5	12,00±0.52	12.33±0.31	15.30±0.23	16.00±0.20 [a]
	1.25	9.60±0.33	9.00±0.00	14.11±0.18	13..65±0.28
40	5	16.13±0.48 [c]	14.33±0.88 [c]	16.54±0.46 [b]	11.33±0.88 [a]
	2.5	14.36±0.82	14.33±0.88	16.33±0.88 [c]	10.23±0.24
	1.25	14.12±0.18	14.33±0.88	14.33±0.88 [b]	10.36±0.12
Ciprofloxacin (μg/mL)	30 μg/mL	18.32±0.15	19.33±0.80	21.33±0.16	22.67±0.20

Data are expressed as mean ± SEM; n=6 and analyzed by one way ANOVA followed by tuckey post hoc test against: *S. aureus = Staphylococcus aureus, S. Pyogenes = Streptococcus pyogenes, P. aeruginosa = Pseudomonas aeruginosa, E. coli = Escherichia coli;* [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$.

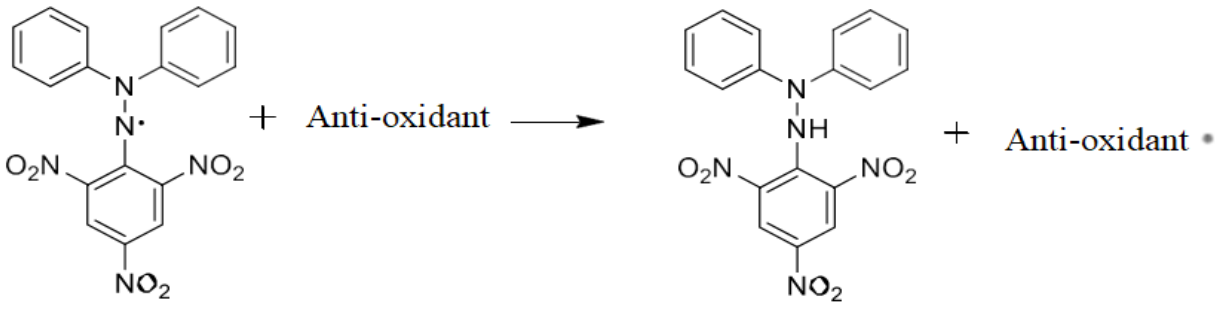
Overall, the antibacterial activity results of the plant extracts and isolated compounds support the traditional use of *G. purpurascens* for treating various pneumonia, skin infections, wound healing, abdominal pain, hemorrhoids, mental illness, bleeding and so far. The antibacterial effects observed against *E. coli, P. aeruginosa, S. aureus* and *S. pyogenes* are especially

significant, given that these pathogens represent serious public health issues globally, leading to increased morbidity and mortality rates and drug resistance. They are among the primary causes of skin and soft tissue infections, while *S. aureus* is the most common bacteria known as methicillin resistance *S. aureus* (MARS) due to developed drug resistance.

5.5.2. Radical Scavenging Activity

The radical scavenging activity was assessed using the DPPH assay for extract, and its fractions as well as isolated compounds (**32**, **37**, **38**, **39** and **40**). DPPH assay relies on the reaction of DPPH, a stable free radical with a deep violet color. Any substance that can donate a hydrogen atom to DPPH converts it into a stable, non-magnetic molecule. The single electron from the nitrogen atom in DPPH is reduced to the corresponding hydrazine by accepting a hydrogen atom from antioxidants. The DPPH· radical exhibits a notably stable and bright intense color (Gulcin & Alwasel, 2023).

It has also reported that electron paramagnetic resonance (EPR) measures the spin densities at the two hydrazyl nitrogen atoms, which are significant and nearly equal and the two distinct bands observed in the UV-vis spectrum of DPPH· result from π–π* transitions, with the unpaired electron playing a major role in the band within the visible region (Kawai & Shibuya, 2002). These two characteristics of the radical have led to its widespread use in anti-oxidant test. A compound or sample that can effectively donate a proton exhibits strong DPPH scavenging activity. The radical scavenging activity of the samples was assessed by measuring the decrease in absorbance at 517 nm with a UV-Visible spectrophotometer. The IC_{50} value represents the concentration of a sample responsible to scavenge 50%. A lower IC_{50} value signifies a higher potential for scavenging activity.



DPPH radical (purple) DPPH-H (Yellow)

Figure 9: The structure of DPPH radical and Protonated DPPH

In this study, the DPPH radical scavenging activities of extract, fraction and compounds from the root of *G. purpurascens* were assessed, using ascorbic acid as a positive control. The IC_{50} values for all samples were determined, with the results presented in (Tables 13 and 14) as well as Figures 14 and 15. Overall, the results demonstrated that the scavenging activity increased with higher concentrations of the samples. Radical scavenging activity of the extract and fractions was range from 92.15% to 34.54%. The extract demonstrated the highest percentage of DPPH radical inhibition compared to the fractions. It achieved 92.15 % and 81.00% inhibition at concentrations of 1000 µg/mL and 31.25µg/mL, respectively. The concentration of this extract was determined to achieve 50% inhibition of the DPPH radical (IC_{50}) was 14. 43 µg/mL whereas ascorbic acid was found IC_{50} of 0.62µg/mL. The DPPH assay also showed different levels of antioxidant activity among the fractions, with IC_{50} values ranging from 14.89 µg/mL (MeOH) fractions to 52.47 µg/mL (hexane) fraction. Among fractions, hexane fraction had the least radical scavenging activity 34.54% to 70.17% whereas the ethyl acetate fraction was found moderate to high scavenging potential depends on concentration, i.e. 31.25 µg/mL (71.74%) and 1000 µg/mL (91.21%).Generally the DCM/MeOH extract and methanol fraction has been observed nearly similar measuring IC_{50} 14. 43 µg/mL and 14.89 µg/mL receptively.

Table 13 : DPPH radical scavenging activities (%) of *G. purpurascens* root extract and solvent fractionated

Concentration of Samp.	**Control**	**DCM/MeOH Extract**		**Ethyl acetate fraction**		**MeOH Fraction**		**Hex fraction**		**Ascorbic acid**	
	Control	A	% Rsa	A	% Rsa	A	% Rsa	A	% Rsa	A	% Rsa
1000	1.274	0.100	92.15	0.112	91.21	0.104	91.84	0.380	70.17	0.064	94.98
500	1.274	0.108	91.52	0. 184	85.56	0.106	91.68	0.444	65.15	0.067	94.74
250	1.274	0.110	91.37	0.230	81.95	0.111	91.29	0.468	63.27	0.072	94.3

											5
125	1.274	0.112	91.21	0.255	79.98	0.114	91.05	0.548	56.99	0.075	94.11
62.5	1.274	0.116	90.89	0.315	75.27	0.118	90.74	0.664	47.88	0.078	93.88
31.25	1.274	0.242	81.00	0.360	71.74	0.122	90.42	0.834	34.54	0.080	93.72
IC_{50} **(**µg/mL)		14.43		25.55		14.89		52.47		0.62	

A = Absorbance, % Rsa= Percent of radical scavenging activity, IC_{50} = half-maximal inhibitory concentration

All compounds at tested concentration showed the percentage of DPPH radical scavenging activities ranging from 48.59-90.27%. The IC_{50}, determined for these compounds range from 42.66 to 2.63 **(**µg/mL) while that of ascorbic acid was 0.62 µg/mL. The lowest IC_{50}, 2.63 **(**µg/mL) was observed by compound **40** which has been considered the most effective on DPPH radical scavenging activity among all isolated compounds.

The highest inhibition (92.31%) was observed with compound **40**, followed by compound **38** and **39** (90.89%), while the lowest inhibition (48.59%) was achieved by compound **37** with an inhibition value of 73.78% at 400 µg/mL and an IC_{50} value of 42.66µg/mL. compound **37** exhibited the lowest radical scavenging activity,. Overall the scavenging capacity of all tested compounds along with IC_{50} values depicted in Table 10 Figure 11: The concentration of this compound that resulted in 50% inhibition of the DPPH radical (IC_{50}) was 6.21µg/mL. The effectiveness of radical scavenging activities of compounds influenced by their structural characteristics, which allow them to create stable radicals through resonance stabilization after donating a hydrogen atom to a radical (Charlton *et* al., 2023) For instance Oleanolic acid and ursolic acid showed radical scavenging activity through neutralizing reactive oxygen species (ROS), preventing lipid peroxidation, and enhancing cellular antioxidant defenses having hydroxyl of acidic functional group readily donate proton (De Stefani *et* al., 2022). It has been reported hat isorhamnetin 3-O-β-D-glucopyranoside (**40**) demonstrated dose-dependent scavenging activities against DPPH, hydroxyl, and carbon-centered radicals, as well as reduced intracellular ROS levels (Gong *et* al., 2020). This contribution is resonance structural feature and

hydroxyl substituent nature of flavonoids which leads to the formation of a stable phenolic compound after hydrogen donation and made it wide range of pharmacological (Gong *et* al., 2020). Additionally, compound **40** demonstrated the highest inhibition of DPPH radicals, followed by compounds **38** and **39**, while compound **37** exhibited the lowest scavenging activity. The disparity in DPPH radical scavenging between compounds **40** and **37** may be attributed to the greater number of hydroxyl groups in compound **40**, indicating that phenolic compounds possess significant DPPH radical scavenging abilities (Gil et al., 2000). The DPPH free radical is reduced by the abstraction of hydrogen from antioxidant compounds (Blois, 1958). The effectiveness of antioxidant compounds in reducing this radical is influenced by their structural properties, which allow for the formation of stable radicals through resonance stabilization after donating a hydrogen atom (Charlton et al., 2023). In this context, compound **40** displays strong antioxidant properties due to the presence of an ortho-hydroxyl donating group in its structure. This feature enhances the delocalization of its free radical. In contrast, compound **37**, which has the lowest antioxidant activity, lacks the ability to delocalize its free radical as effectively as compound **40**. In principle, a drug with the lowest IC_{50} value can be taken as the most effective as showing lower toxicity when administered to the patients (Shamsudin *et* al., 2022). In this regard, compound **38** and **39** with medium activities IC_{50} value (3.21µg/mL) can be). The antioxidant activity of the tested compounds demonstrated a significant capacity to scavenge free radicals, reduce oxidative stress, and protect cellular components from damage, indicating their potential health benefits in preventing oxidative-related diseases.

This study is very consistence with previously reported anti-oxidant activities of isolated compounds which enhance the traditional uses of the plant for different human ailments

Table 14: DPPH radical scavenging activities (%) of compounds from root extract of *G. purpurascens*

Concentration of Compounds (µg/mL)	**Control**	**Compound 32**		**Compound 37**		**Compound 38 and 39**		**Compound 40**		**Ascorbic acid**	
	Control	A	% Rsa	A	% Rsa	A	% Rsa	A	% Rsa	A	%Rsa
400	1.274	0.268	78.96	0.334	73.78	0.116	90.89	0.098	92.31	0.064	94.98
200	1.274	0.345	72.92	0.422	66.88	0.178	86.03	0.102	91.99	0.067	94.74
100	1.274	0.462	63.74	0.445	65.07	0.228	82.10	0.116	90.89	0.072	94.35
50	1.274	0.488	61.70	0.512	59.81	0.260	79.59	0.118	90.74	0.075	94.11
25	1.274	0.520	59.18	0.655	48.59	0.300	76.45	0.124	90.27	0.078	93.88
12.5	1.274	0.598	53.06	0.834	34.54	0.368	71.11	0.129	89.87	0.080	93.72
IC_{50} (µg/mL)		21.55		42.66		3.21		2.63		0.62	

A = Absorbance, % Rsa= Percent of radical scavenging activity, IC_{50} = half maximal inhibitory concentration

DPPH Assay of root extract and fractions of *G. purpurascens*

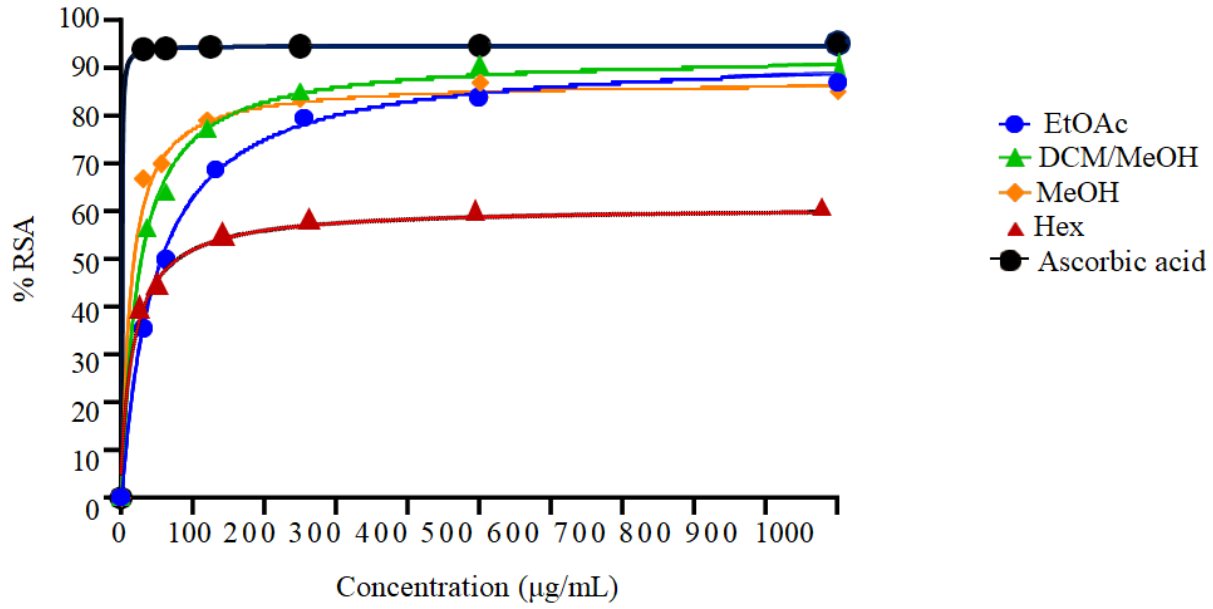


Figure 9: DPPH radical scavenging activities (%) of the extract and fraction

DPPH Assay of compounds isolated from root of *G. purpurascens*

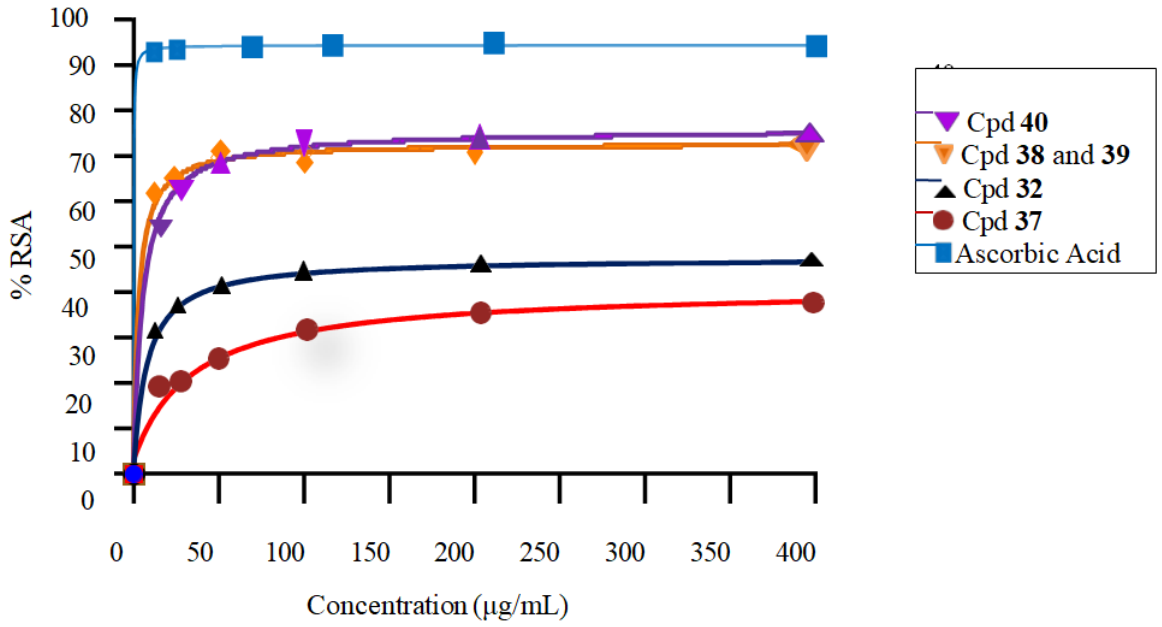


Figure 10: DPPH radical scavenging activities (%) of compounds of *G. purpurascens*

5.6. *In silico* ADME and Drug Likeness Prediction

In silico methods are employed to predict the ADME parameters, pharmacokinetic properties, drug-likeness, and acute toxicity of the isolated compounds (**32**, **37**, **38 39** and **40**). ADME properties assess how a chemical agent behaves like a drug. While there are many parameters for assessing drug-likeness, Lipinski's (Lipinski *et* al., 2012) and Veber's rules (Veber *et* al., 2002) are the most utilized online tools. The original Rule of Five (RO5) addresses orally active compounds and specifies ranges for four key physicochemical parameters: molecular weight (MWT) ≤ 500, log P ≤ 5, hydrogen bond donors, ≤ 5 and a maximum of hydrogen bond acceptors ≤10 (Lipinski, 2004). The pharmacokinetic profile of compounds are presented in Table 16 indicated compound **32**, **38** and **39** no violate criteria of Veber's rule and did not fit one criteria of Lipinski's rule. All compounds except **40** break rule of five due to their high lipophilicity (cLogP > 5). The open chain of ethyl palmitate violet Lipinski rule of five having more than five numbers of rotatable bonds. All compound's molecular mass recorded were less than 500 Daltons (g/mol) which is favorable for oral drug uses. As compared to Veber's parameters (rotatable bonds ≤ 10 and topological polar surface area) (Veber *et* al., 2002), only compound **37** violet having 16 numbers of rotatable bonds. Out of isolated compounds, **40** has total polar surface area (TPSA) equal to 74.57 $Å^2$ which indicated the area occupied by polar atoms, primarily oxygen and their associated hydrogen atoms while compound **32** scored the least 20.23 $Å^2$.

Table 15: Drug-likeness predictions of compounds and standard, computed by Swiss ADME

Compounds	Formula	MW (g/mol)	NHD	NHA	LogP (cLogp)	Lipinski's ROF violation	NRB	TPSbA ($Å^2$)	Veber's rule violation
32	$C_{29}H_{50}O$	414.71	1	1	7.24	1	6	20.23	0
37	$C_{18}H_{36}O_2$	284.48	0	2	5.90	1	16	26.30	1
38	$C_{30}H_{48}O_3$	456.70	2	3	6.07	1	1	57.53	0
39	$C_{30}H_{48}O_3$	456.70	2	3	5.93	1	1	57.53	0
40	$C_{22}H_{22}O_{11}$	462.40	6	11	0.65	2	4	70.67	0
CPFX	$C_{17}H_{18}FN_3O_3$	331.34	2	5	1.1	0	3	74.57	0

CPFX = Ciprofloxacin, NHD = Number of Hydrogen donor, NHA = Number of Hydrogen acceptor, NRB = Number of rotatable bonds, and TPSA = total polar surface area, MW = Molecular Weight

5.6. ADME and Toxicity Prediction

Of the isolated compounds, only compounds **37** and the reference drug, CPFX, demonstrated high gastrointestinal absorption. This is may be due to smaller molecular size and its lipophilic character. Smaller molecules generally have better absorption characteristics compared to larger ones and lipophilic compounds can more easily pass through the lipid membranes of intestinal cells (Lucas *et* al., 2019). BBB permeability is a key parameter that determines how effectively molecules exert their effects in the central nervous system (CNS) and their neurotoxicity (Rashid *et* al., 2022). This *in silco* prediction revealed that among all the isolated compounds and the reference drug, only compounds **34** demonstrated permeability across the blood-brain barrier (BBB). This was confirmed by *in silco* Swiss pro-tox prediction that caused organ neurotoxicity.

Skin permeability (Kp) measures the rate at which a substance penetrates epidermis and helps quantify the transport of molecules through the outermost layer to emphasize the significance of skin absorption (Chedik *et* al., 2024). The less negative the log Kp, the higher the epidermal permeability of the molecule. Log Kp values of the isolated compounds range from -8.28 (cm/s) to -2.20 (cm/s). Among isolated compounds **32** and **37** had lower Log Kp (-2.20 (cm/s and -2.44 cm/s) which may made them favorable for dermal dosage preparation. This is might be due to their lipophilic characters. As compared to ciprofloxacilline all compounds had lesser Log Kp which allow them higher skin permeability than the control.

P-glycoprotein (P-gp) is a membrane protein transporters play a vital biological role by

expelling toxic substances from the cytosol and enabling the uptake of essential nutrients into the cell (Ahmed Juvale *et* al., 2022). P-glycoprotein is also serving at the blood–brain barrier as defense mechanism against damages of the brain by toxins appears to be even more important (Cascorbi, 2011).As a result it can limit can limit the absorption of medicines and reduce bioavailability. From the isolated compounds only compound **40** and positive control showed with P-gp substrate expression. Therefore compounds, **32**, **37**, **38**, and **39** may have higher distribution than ciprofloxacilline as they lack plasma protein binding.

CYP (Cytochrome P450) inhibitors are substances that reduce the activity of cytochrome P450 enzymes, which play a critical role in the metabolism of many drugs and other compounds in the liver and other tissues (Guengerich, 2022). Understanding CYP inhibitor interactions is essential for predicting drug metabolism and potential drug-drug interactions. The most drug are metabolizing CYP enzymes: CYP3A4 accounting for about half of drug metabolism, followed by CYP2D6, CYP2C9, CYP2C19, and CYP 2E1.The in silco SwissADME prediction displayed only compound **32** and **37** inhibit the cytochrome P450 class of enzymes (CYP 2C9 and CYP 1A2) whereas the positive control inhibit CYP 3A4. This indicated compound **38**, **39** and **40** are possible to administer with other drugs those metabolized by these enzymes because less likely to cause drug-drug interactions that could lead to increased toxicity.

Table 16: Pharmacokinetic properties of compounds computed by Swiss ADM

Compounds	**GIABS**	**BBB Permeability**	**Log Kp (cm/s)**	**P-gp substrate**	**CYP Inhibitor Interaction (SwissADME/PreADMET)**					
					CYP 1A2	**CYP 2C19**	**CYP 2C9**	**CYP 2D6**	**CYP 3A4**	**CYP 2E1**
32	Low	No	-2.20	No	No	No	Yes	No	No	No
37	High	No	-2.44	No	Yes	No	No	No	No	No
38	Low	Yes	-3.77	No	No	No	No	No	No	No
39	Low	No	-3.87	No	No	No	No	No	No	No
40	Low	No	-8.28	Yes	No	No	No	No	No	No
CPFX	High	No	-9.09	Yes	No	No	No	No	Yes	No

BBB = Blood-Brain Barrier, CYP= Cytochrome P450, GIABS = Gastrointestinal Absorption P-gp= P-glycoprotein Log Kp = logarithm permeability coefficient

5.7. Toxicity studies

The aim of toxicity testing is to identify any potential harmful effects of chemicals by assessing its safety. It also provides essential information about the compound's absorption, distribution, metabolism, and excretion (ADME) within the human body (Trisciuzzi *et* al., 2018). The

toxicity assessment is based on the LD_{50} (mg/kg) value, which indicates the dose required to kill 50% of the test animals (Morris-Schaffer & McCoy, 2021). The level of toxicity is rated on a scale from 1 to 6, where a smaller represents a higher level of toxicity and greater number represent lower level of toxicity. In this study LD_{50}, organ toxicity and toxicity endpoint were determined for the isolated compounds. Most of the compounds exhibited class four toxicity; whereas compound **37** and **40** were grouped class five toxicity. None of the compounds showed cytotoxicity. Compound **32** displayed neurotoxicity along with the control. From the isolated compounds only **39** showed mutagenicity as compared to ciprofloxacilline (Table 18).

Table 17: Toxicity prediction of isolated compounds and standard, computed by Pro-Tox 3.0

Compound	**LD_{50} (mg/kg)**	**Toxicity Class**	**Organ Toxicity**				**Toxicity end points**			
			Hepatotoxicity	Neurotoxicity	Nephrotoxicity	Cardio toxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
32	890	4	No	No	No	No	No	Yes	No	No
37	5000	5	No	No	No	No	Yes	No	No	No
38	2000	4	Yes	Yes	No	Yes	Yes	Yes	No	No
39	900	4	Yes	No	No	Yes	Yes	No	Yes	No
40	5000	5	No	No	yes	Yes	No	Yes	No	No
CPFX	2000	4	No	Yes	Yes	No	No	No	Yes	No

LD50 = Lethal Dose 50; CPFX = ciprofloxacilline

5.8. *In silico* Molecular Docking study of isolated compounds

The molecular docking studies performed on the target proteins, *E. coli* DNA gyrase B, PqsA, and *S. aureus* pyruvate kinase, were utilized to validate the evaluation of *in vitro* antibacterial activities.

5.8.1. Molecular Docking with *E. coli* DNA Gyrase

Gyrase is a molecular motor that utilizes the energy released from ATP hydrolysis to exert mechanical force on DNA whereas DNA gyrase is a topoisomerase enzyme vital for bacterial DNA replication and transcription, as it preserves the topology and integrity of the DNA (Micha, 2017). Currently, bacterial DNA gyrase, a type II topoisomerase belonging to the GHKL (gyrase, HSP90, histidine kinase, MutL) enzyme family, is one of the most studied and validated targets for the development of new antibacterial agents (Medapi *et* al., 2015). GyrB is a functional subunit of heterodimer structure DNA gyrase that plays a major role in ATPase

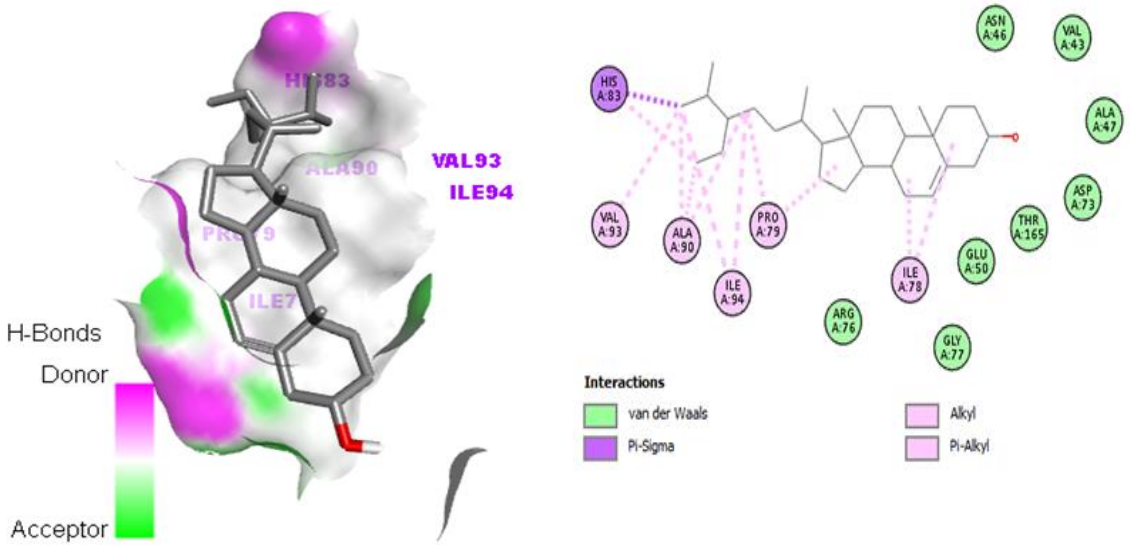
activity (Figure 12).

In this study, *E. coli* DNA gyrase B (PDB ID: 6F86) was selected as the target, and ciprofloxacin served as the reference control for docking studies. The binding affinities of the isolated compounds varied from -8.1 to -5.0 kcal/mol, in comparison to the binding affinity of the positive control, ciprofloxacin, which was -7.4 kcal/mol. The binding affinities and interactions with different amino acids are presented in Table 18. The 3D and 2D binding interactions are illustrated in Figure. Compound **39** and exhibited the highest binding affinity (-8.1 kcal/mol) followed closely by compound by compound **32** (-8.0 kcal/mol). The lowest binding affinity observed was -5.0 kcal/mol that of compound **37**. The dominant *in vitro* activity of compound **38** and **39** demonstrated against *E. coli* might be due to compound **39** which align with docking prediction further supports its significant potential against *E. coli.*

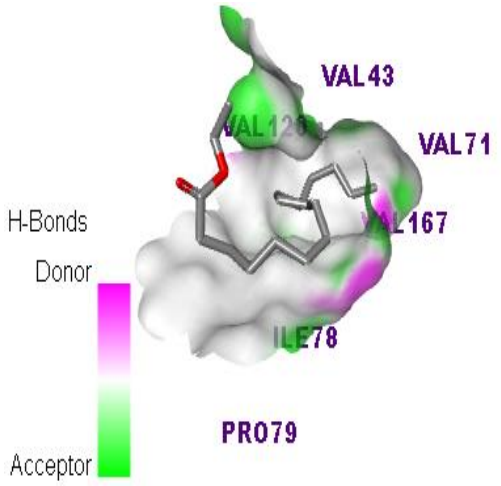
CPFX formed hydrogen bond with Arg-76 whereas compound **40** interacted with these two amino acids Arg-76 and Asn-46. Other tested compounds exhibited electrostatic and hydrophobic interaction with alkyl and Pi-alkyl while compound **38** bind only through carbon hydrogen bond interaction (Pro-79).All of the compounds exhibited an interaction with amino acids through different van der Waals force. Compound 40 and standard (ciprofloxacilline,) showed an interaction Val-167 and Val-120. More over different binding affinity among the compounds was noted at different amino acids on target receptor.

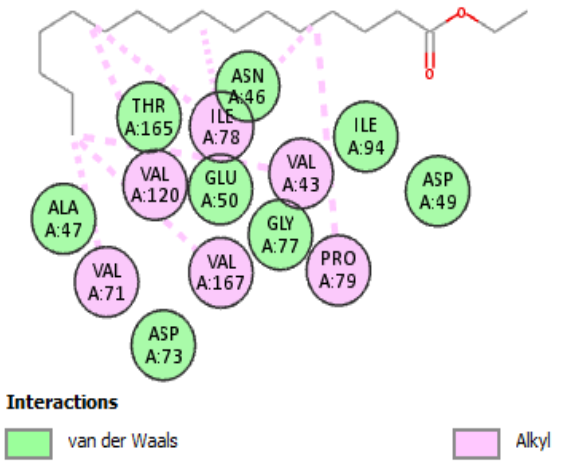
Table 18: Molecular docking binding interactions (3D & 2D) of isolated compounds and standard with the against *E. coli* DNA Gyrase B

Target	Ligand	Binding affinity (kcal/mol)	H-bonding	Hydrophobic and Electrostatic	Van der Waals
E. coli DNA gyrase	**32**	-8.0	-	Pi-sigma: His-83; Alkyl and Pi-alkyl: His-83, Val-93, Ala-	Arg-76, Gly-77, Glu-50Thr-165, Asp-73, Ala-47, Val-43, Asn-46
	37	-5.0	-	Val-167, Val-120, Pro-79, Ile-78, Val-71, Val-43	Thr-165, Ile-94, Gly-77, Asp-73, Glu-50, Asp-49, Ala-47, Asn-46

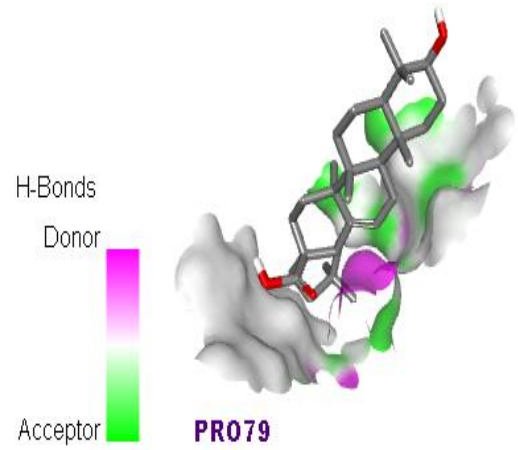
	38	-6.5		Carbon hydrogen bond:Pro-79	Ile-94,Ile-78, Arg-76, Ala-53, Glu-50,Asp-49, Asn-46
	39	-8.1	-	Alkyl: Ile-78	Ala-73, Asp-49, 73, Arg-76, Glu-50, Asn-46, Thr-165, Val-120, Pro-79, Ile-94
	40	-7.1	Arg-76, Asn-46	Pi sigma:Thr-165,Alkyl and Pi-alkyl Pro-79, Ile-78, Carbon hydrogen bond:Gly-179 Pi-	Val-167,120,71,43, Met-95, Ile-94Asp-73, Ala-53, 47,
	CPFX	-7.4	Arg-76	Carbon hydrogen bond: Asp-73; Halogen: Asp-73; Amide pi-stacked: Gly-77; Alkyl and pi-alkyl: Pro-79, Ile-	Glu-50, Thr-165, Val-120, 167, 43, Asn-46, Ala-47
32					

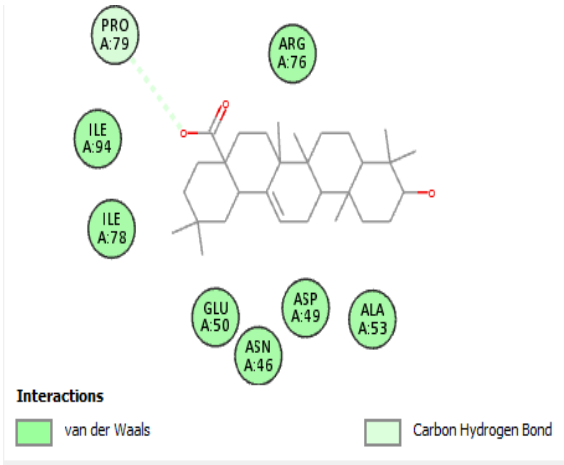
37


VAL43
VAL71
VAL167
ILE78
PRO79
H-Bonds
Donor
Acceptor

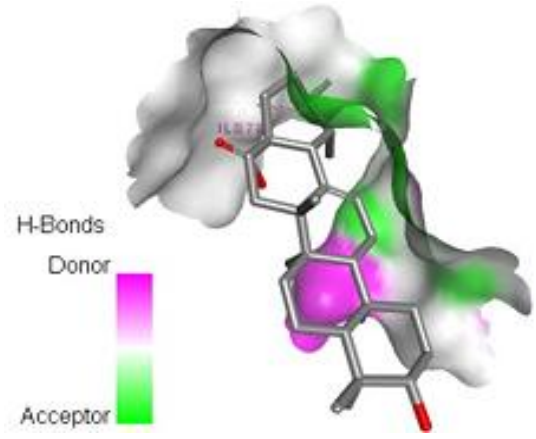

ASN A:46
THR A:165
ILE A:78
ILE A:94
VAL A:120
GLU A:50
VAL A:43
ASP A:49
ALA A:47
GLY A:77
VAL A:71
VAL A:167
PRO A:79
ASP A:73
Interactions
van der Waals
Alkyl

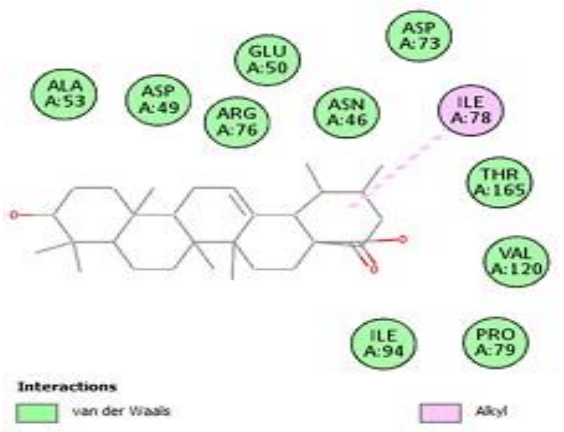
38


H-Bonds
Donor
Acceptor
PRO79


PRO A:79
ARG A:76
ILE A:94
ILE A:78
GLU A:50
ASP A:49
ALA A:53
ASN A:46
Interactions
van der Waals
Carbon Hydrogen Bond

39


H-Bonds
Donor
Acceptor


ASP A:73
GLU A:50
ALA A:53
ASP A:49
ARG A:76
ASN A:46
ILE A:78
THR A:165
VAL A:120
ILE A:94
PRO A:79
Interactions
van der Waals
Alkyl

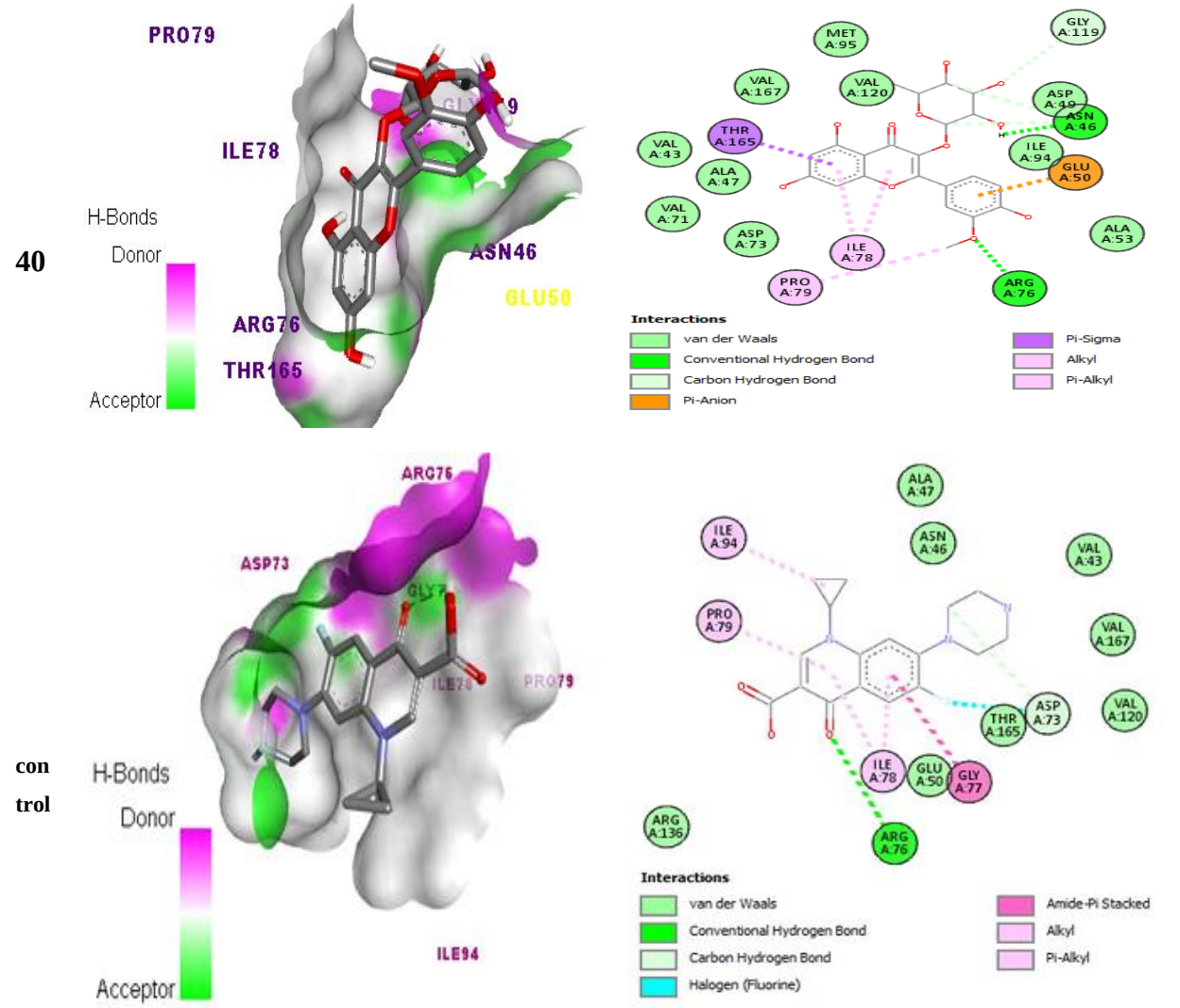


Figure 11: Molecular docking results of isolated compounds and Ciprofloxacin against *E. coli* DNA Gyrase B

5.8.2. Molecular docking with *P. aeruginosa* the target PqsA

Pseudomonas aeruginosa utilizes chemical signals to facilitate communication between its cells through a process known as quorum sensing (QS). *Pseudomonas aeruginosa* possesses three primary quorum sensing systems (rhl, las, and pqs) that facilitate cell-to-cell communication and regulate the production and release of virulence factors (Dickson *et* al., 2012). The pqs system employs two signal molecules: namely, *Pseudomonas quinolone* signal (PQS) and its biosynthetic precursor 2-heptyl- 4-hydroxyquinoline (HHQ). Even though either PQS or HHQ molecules bind to the PqsR for transcriptional activator, PQS is 100 fold potent than HHQ (Xiao *et* al., 2006). The enzyme PqsA is responsible for the production of PQS signals. Inhibiting the PqsA enzyme can interfere with the biosynthesis of the PQS signal

molecule, which in turn disrupts PqsR-dependent gene regulation and ultimately affects biofilm formation (Shaker *et* al., 2020).

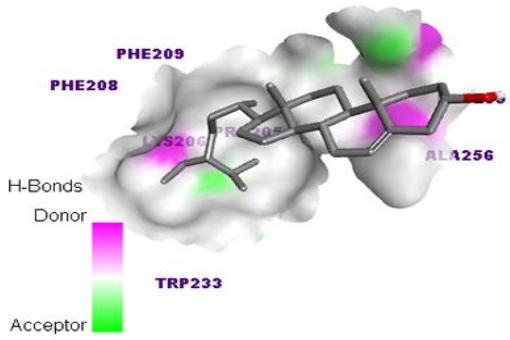
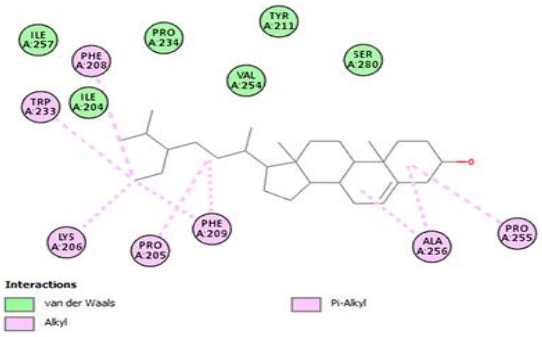
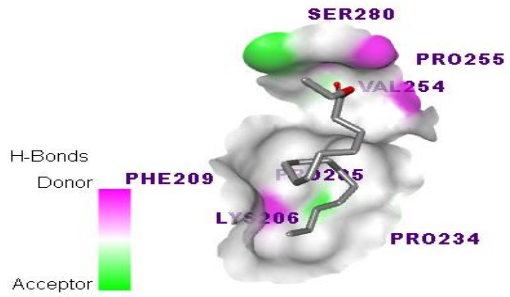
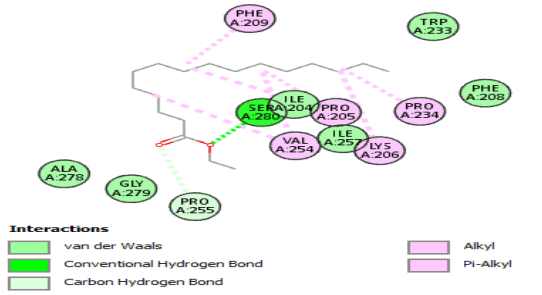
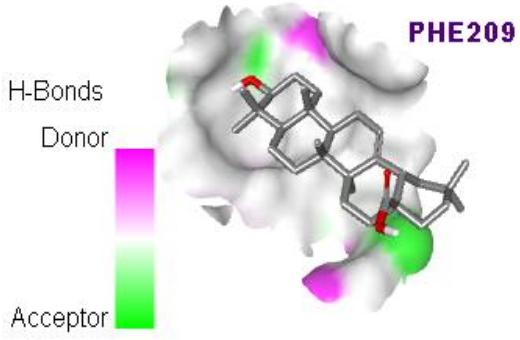
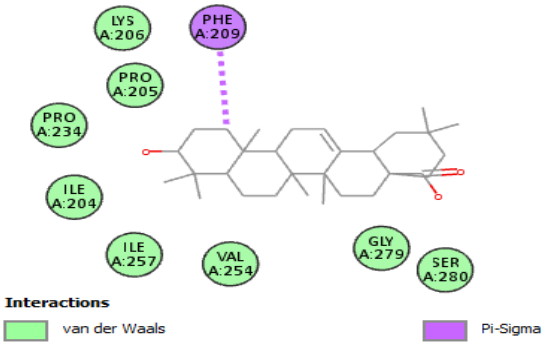
In this study PqsA enzyme structure has been selected to subject protein sequence of *P. aeruginosa* PqsA (UniprotKB ID: Q9l4 × 3) to homology molecular docking modeling. For PqsA, the binding affinity of the isolated compounds varied from -8.7 to -4.6 kcal/mol whereas the binding affinity for ciprofloxacin was -7.1 kcal/mol. The highest binding affinity of -8.7 kcal/mol was shown by compound **40**, followed by compound **32** with a binding affinity of -8.2 kcal/mol (Table 19)

Among isolated compounds, Isorhamnetin 3-O-rhamnoside (**40**) showed equivalent H-bond interaction (Glu-305 and Thr-304) as compared to with control (Arg-397 and Gly-302) followed by Ethyl palmitate (**37**) which was showed H- bonding with (Ser-280). For hydrophobic and electrostatic interaction with PqsA, compound **38** (oleanolic acid)display an interaction only through Pi- sigma with Phe- 209 while compound **39** (ursolic acid) exhibited interaction carbon hydrogen bond (Gly-279) (Figure13). Other isolated compounds showed different binding affinity by Pi-alkyl and alkyl hydrophobic interaction. Generally all compounds exhibit interaction with more than six amino acid residues of the targets through Van der Waals binding.

Table 19: Molecular docking binding interactions (3D & 2D) of isolated compounds and standard with the target PqsA (UniprotKB ID: Q9l4 × 3

Target	Ligand	Binding	H-bonding	Hydrophobic and Electrostatic	Van der Waals
PqsA	**32**	-6.7	-	Alkyl and Pi-alkyl: Phe-208, Trp-233, Lys-206, Pro-205, Phe-209, Phe-209, Ala-256,	Ile-257, Ile-204, Pro-234, Val-254, Tyr-211, Ser-250
	37	-4.6	Ser-280	Carbon hydrogen bond:Pro-255, Alkyl and Pi-alkyl: Val-254, Pro-234, Phe-209, Lys-206, Pro- 205	Gly-279, Ala-278,Trp-233,Ile- 257, Phe-208
	38	-7.3	-	Pi- sigma: Phe- 209	Lly-279, Ser-280, Ile-257, Lay-254, Pro-234, Pro-205, Lys- 206

	39	-8.2	-	Carbon hydrogen bond: Gly-279	Ser-280, Pro-234, 205, Ile-204, Lys-206, Phe-209, Val-254
	40	-8.7	Glu-305, Thr-304	Amide Pi-stacked:Gly-302; Pi-alkyl: Ala-278; Carbon hydrogen bond: Asp-382,Gly-279, Try-231,Thr-164	Arg-397,Arg-372,Try-378,Ala-303, Val-309, His-308, Gly-307, Ser-280, His-394
	Ciproflo xacin	-7.1	Arg-397, Gly-302, Asp-299	Carbon hydrogen bond: Gly-300, Thr-323; Halogen: Gly-302; Pi-cation and anion: Arg-	Ala-278, Leu-282, Phe-384, Pro-281, Ser-280

Comp	3D	2D
32		
37		
38		

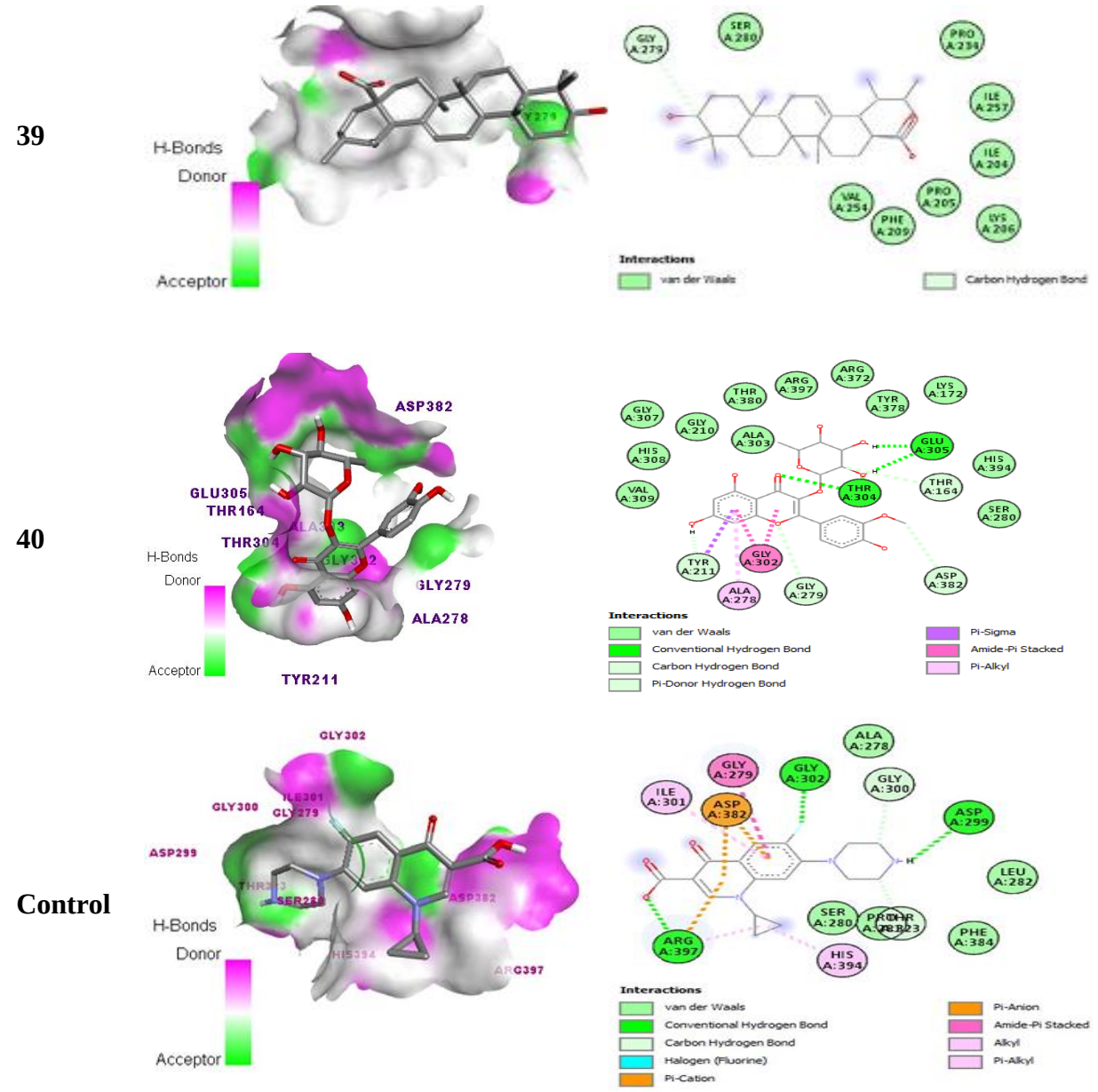


Figure 12: Molecular docking results of isolated compounds and Ciprofloxacin against *Pseudomonas quinolone* signal A (PqsA).

5.8.3. Molecular Docking with *S. aureus* Pyruvate Kinase

Pyruvate kinase (PK) is a key enzyme in *Staphylococcus* that plays a significant role in regulating growth, antibiotic resistance, and biofilm formation. It is structurally different from its human homologs, which are located on Chromosome 15 in humans, making it a promising target for the development of new antimicrobial agents (Brokowski C, 2019). For the docking

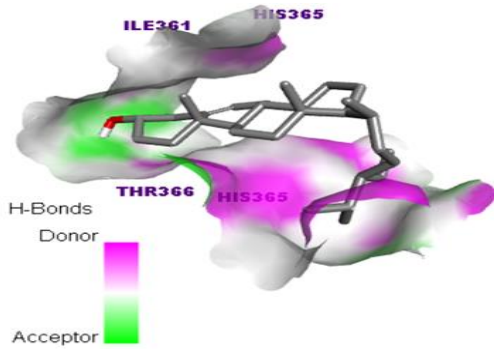
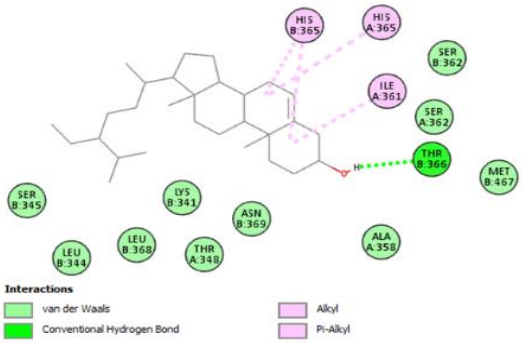
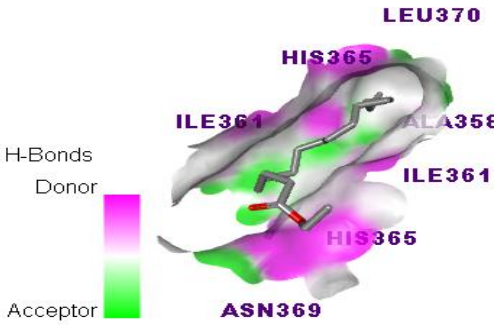
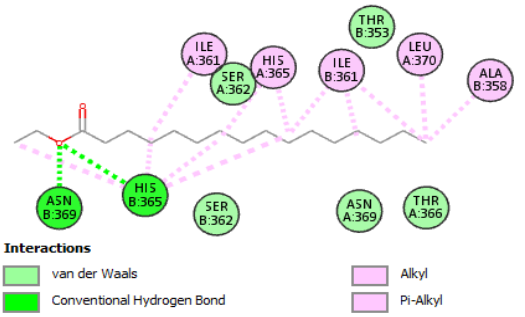
studies, *Staphylococcus aureus* pyruvate kinase (PDB ID: 3T07) was selected as the target, while ciprofloxacin (CPFX) was used as the reference control.

The binding affinity of compound with pyruvate kinase (PDB ID: 3T07) was ranged from (-8.7 kcal/mol) to (-5.0 kcal/mol).The binding affinities and interactions with different amino acids are summarized in the (table 20) and the 3D and 2D binding interactions are illustrated in the accompanying (figure 18)**.** Isorhamnetin-3-O-rhamnoside (**40**) exhibited the highest binding affinity (-8.7 kcal/mol) whereas ethyl palmitate showed least affinity (-5.0 kcal/mol) as compared to ciprofloxacin (-8.1kcal/mol). The binding scores found for compounds **39** was -6.9 kcal/mol whereas **32** and **38** were equally interact with the enzyme by -6.6 kcal/mol. From all compounds, compound **40** showed greater hydrogen bonding interactions with Asn-369, Thr-366, and His-365B, followed by compound **37** (Asn-369 and His-365) as compared to that of CPFX (Asn-369 and His-365). The other compounds (**32**, **38** and **39**) demonstrated hydrogen bonding interactions with one of these amino acids. All compounds along with ciprofloxacilline showed residual interactions with Asn-169 and Thr- amino acid on different chain (Figure 14).

Table 20: Molecular docking results of isolated compounds against *S. aureus* pyruvate kinase

Target	**Ligand**	**Binding affinity (kcal/mol)**	**H-bonding**	**Hydrophobic and Electrostatic**	**Van der Waals**
S. a. pyruvate kinase.	**32**	-6.6	Thr-B:366	Alkyl and Pi-Alkyl: His- A365, B-365,Ile-361	Met-467, Asn-369, Leu-368, 344, Ala-358, Ser-A:362A, 362B, 345, Thr-348, Lys-341
	37	-5.0	Asn-369, His-365,	Alkyl and Pi-alkyl:Leu-370,His-365,Ile-361A, Ile-361B, Ala-358	Asn-369, Thr-366A, 353B, Ser-362A, 362B,
	38	-6.6	His-365	-	Asn369A, 369B,Il3-361A, 361B, Thr-353A, 353B, 348A,348B, Lys-341B

	39	-6.9	Asn-369B		Asn369A, Ile-361A, 361B, Thr-353A, 353B, 348A,348B, His-365A, 365B
	40	-8.7	Asn-369,Thr-366,His-365B,	Pi-sigma:Ile-361A, 361B, Pi-Cation:His-365A	Asn-369, Thr-348A, 348B,366B,353A, Ser-362A, 362B
	CPFX	-8.1	Thr-366A, Ser-362A, 362B,	Pi-Cation: His-365A, 365B, Pi-sigma:Ile-361, Carbon hydrogen bond:Thr-353A,	Met-467A, Ile-361B, Thr-366

Comp	3D	2D
32		
37		
38		

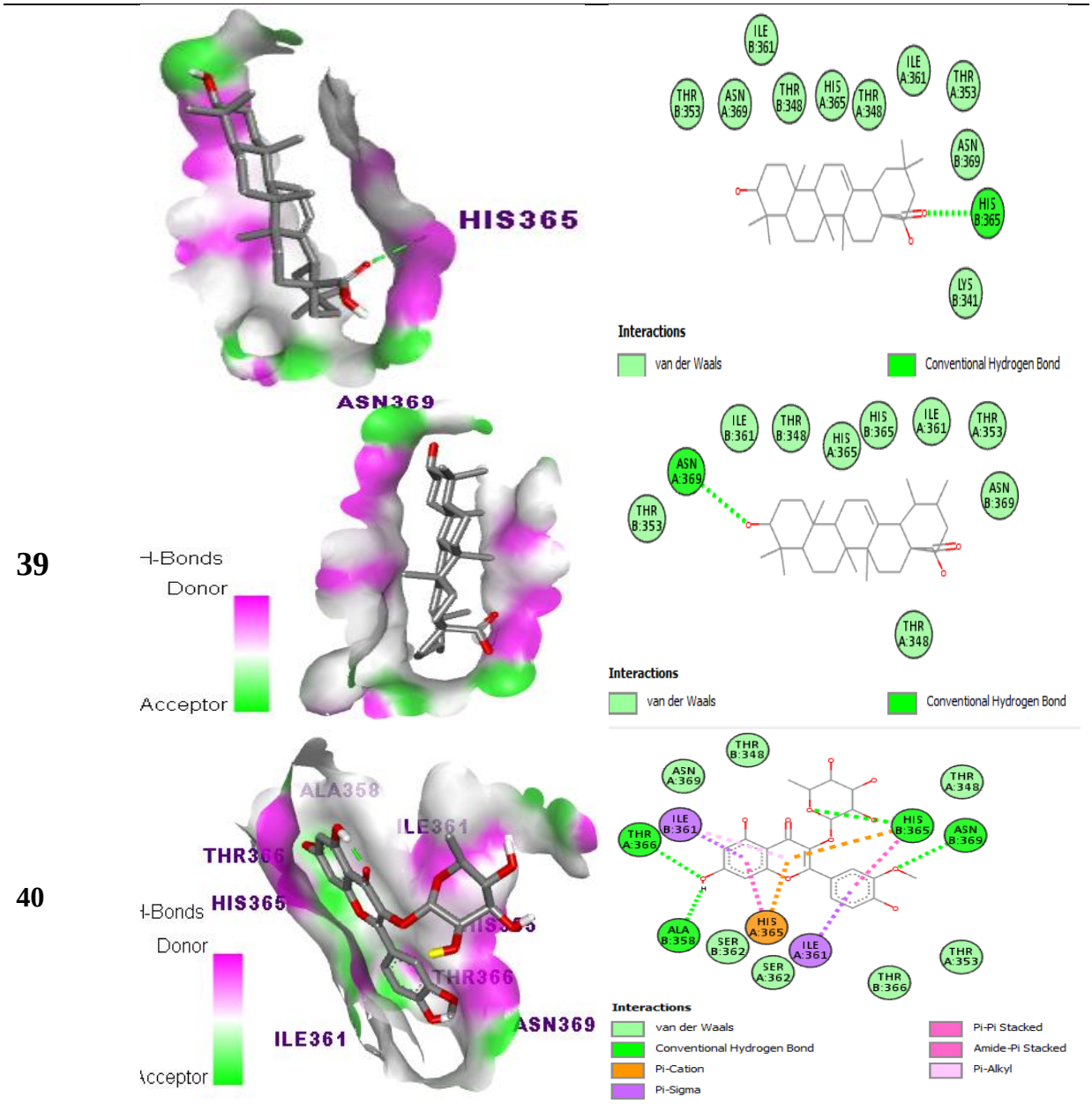


Figure 14: Molecular docking results of isolated compounds against *S. aureus* pyruvate kinase.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The phytochemical screening of root extracts from *G. purpurascens* identified the presence of anthraquinones, steroids, terpenoids, flavonoids, glycosides, coumarins, phenolics, and saponins, indicating that the plant is a rich source of secondary metabolites. For the first time essential oils extracted from root of *G. purpurascens* and it has been found to contain several biologically active phytochemicals including benzoic esters, (52.58%), fatty acid and its derivatives (12.43%) alcohols and phenolic alcohols (7.42%), steroids (4.73%) as major class of compounds. Column chromatographic using chloroform and ethyl acetate fractions from the dichloromethane/methanol extract yielded five compounds: β-sitosterol (**32**), ethyl palmitate (**37**), oleanolic acid (**38**), ursolic acid (**39**), and a flavonoid glycoside (**40**).

In vitro antibacterial activity tests showed DCM/MeOH extract and ethyl acetate fraction had significant activities. Among the isolated compounds, compound **40** exhibited good antibacterial activities against *E. coli, P. aeruginosa, S. aureus*, and, *S. pyogenes* while compounds **38** and **39** demonstrated promising activity against *E. coli* and *P. aeruginosa*. Additionally, **32** showed significant antibacterial effects against *P. aeruginosa* and *S. pyogenes*, and **37** was identified as the least active compound against all strain of bacteria. Overall the report suggest that *G. purpurascens* has potential for treating bacterial infections. The highest percent inhibition of DPPH radical was observed with extract of DCM/ MeOH soluble portion, and compound **40.** Compounds isolated from the root of *G. purpurascens* exhibited pronounced radical scavenging activities with **40** identified to be the most potent compound effective at lower doses.

The *in silico* molecular docking confirmed the biological activity of the isolated compounds is promising. Ursolic acid and oleanolic acid are exhibited high binding affinity to target PqsA (UniprotKB ID: Q9l4 × 3) than standard or ciprofloxacilline while ursolic acid and β-sitosterol showed high affinity against *E. coli* DNA Gyrase B. Compound **40** also showed highest interaction against *S. aureus* pyruvate an kinase target PqsA (UniprotKB ID: Q9l4 × 3) than ciprofloxacilline. Most of the isolated compounds behave drug likeness properties, comply with Lipinski's rules of five, and have good permeability and solubility in the human body when ingested orally. These compounds predicted as less organ toxicity and no fatality was observed during *in silico* simulation analysis. Over all this study support the traditional medicinal uses of the plant to treat different human ailments.

6.2. Recommendation

In this study, *in vitro* anti-bacterial study through disk diffusion methods was utilized. Therefore, we recommend further *in vitro* and *in vivo* antimicrobial activity tests should be conducted on the compounds employing Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) to address the issue of antimicrobial resistance and other strain of bacteria. Future researchers should prioritize studying the mechanisms of action, target identification, and *in vivo* effects of the plant we have investigated, as this will provide critical insights into its traditional uses. By elucidating the biochemical pathways and molecular targets, researchers can validate its therapeutic potential and contribute to the scientific understanding of its efficacy. Additionally, *in vivo* studies are essential for assessing the plant's safety and effectiveness in biological systems as well as other medical purposes based on traditional claim. The phytochemical screening study result also showed the plant is enriching of secondary metabolites. As a result it is essential to conduct further research to address on under-investigated classes of compounds, such as glycosides, coumarins, and phenolic. This comprehensive approach will bridge traditional knowledge with modern scientific inquiry, paving the way for the development of new therapeutic applications.

7. REFERENCES

Abbassy, M. A., Kadous, E. A., & Marei, G. I. K. (2012). Isolation and Identification of Cardenolide Compounds of *Gomphocarpus sinaicus* and Their Fungicidal Activity Against Soil Borne and Post Harvest Fungi. *Journal of Life Sciences 6*, 985–994.

Abdel Elatif, R., Shabana, M., Ibrahim, L. F., Mansour, R., Awad, H. M., & Sharaf, M. (2020). Chemical composition and biological activity of *Salicornia fruticosa* L. *Egyptian Journal of Chemistry*, *63*(5), 1713–1721. https://doi.org/10.21608/ejchem.2019.18470.2139

Abera, B., Melaku, Y., Shenkute, K., Dekebo, A., Abdissa, N., Endale, M., Negassa, T., Woldemariam, M., & Hunsen, M. (2024). *In vitro* antibacterial, antioxidant, *in silico* molecular docking and ADEMT analysis of chemical constituents from the roots of *Acokanthera schimperi* and Rhus glutinosa. *Applied Biological Chemistry*, *67*(1). https://doi.org/10.1186/s13765-024-00930-6

Abouzid, S. F., Wahba, H. M., Elshamy, A., Cos, P., Maes, L., Apers, S., Pieters, L., & Shahat, A. A. (2013). Antimicrobial activity of some *Clerodendrum* species from Egypt. *Natural Product Research*, *27*(11), 1032–1036. https://doi.org/10.1080/14786419.2012.686905

Adugna, M., Yesuf, J. S., & Birru, E. M. (2023). Evaluation of Analgesic and Anti-inflammatory Activities of Methanolic Leaf and Root Extracts of *Gomphocarpus purpurascens* A . Rich (Asclepiadaceae) in Mice. *Journal of Experimental Pharmacology 1*(-), 1-12 https://doi.org/10.2147/JEP.S361194

Agu, P. C., Afiukwa, C. A., Orji, O. U., Ezeh, E. M., Ofoke, I. H., Ogbu, C. O., Ugwuja, E. I., & Aja, P. M. (2023a). Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. *Scientific Reports*, *13*(1),1-18. https://doi.org/10.1038/s41598-023-40160-2

Agu, P. C., Afiukwa, C. A., Orji, O. U., Ezeh, E. M., Ofoke, I. H., Ogbu, C. O., Ugwuja, E. I., & Aja, P. M. (2023b). Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. *Scientific Reports*, *13*(1), 1–18. https://doi.org/10.1038/s41598-023-40160-2

Ahmed Juvale, I. I., Abdul Hamid, A. A., Abd Halim, K. B., & Che Has, A. T. (2022). P-glycoprotein: new insights into structure, physiological function, regulation and alterations in disease. *Heliyon*, *8*(6), e09777. https://doi.org/10.1016/j.heliyon.2022.e09777

Akdağ, A., Dergisi, E. Ö.-S. Ü. F. F. F., & 2019, U. (2019). Distillation methods of essential oils. *S.Ü. FenFakültesi FenDergis*, *45*(1), 22–31. https://dergipark.org.tr/en/pub/sufefd/issue/44924/461058

Allouche, A. (2012). Software News and Updates Gabedit — A Graphical User Interface for Computational Chemistry Softwares. *Journal of Computational Chemistry*, *32 (*1) 174–182. https://doi.org/10.1002/jcc

Amare, F., & Getachew, G. (2019). An ethnobotanical study of medicinal plants in chiro district, West Hararghe, Ethiopia. *African Journal of Plant Science*, *13*(11), 309–323. https://doi.org/10.5897/ajps2019.1911

Ayeleso, T. B., Matumba, M. G., & Mukwevho, E. (2017). Oleanolic acid and its derivatives: Biological activities and therapeutic potential in chronic diseases. *Molecules*, *22*(11). https://doi.org/10.3390/molecules22111915

Azamal, H; Mishra, K; Semwal, D. (2012). Biodiversity status in Ethiopia and challenges.In A.pawn Kumer(Ed.). In Environmental polution and biodiversity(first). *New Delhi,India:1-43*

Babu, S., & Jayaraman, S. (2020). An update on *β*-sitosterol: A potential herbal nutraceutical for diabetic management. *Biomedicine and Pharmacotherapy*, *131*, (-), 110702. https://doi.org/10.1016/j.biopha.2020.110702

Belayneh, A., & Bussa, N. F. (2014). Ethnomedicinal plants used to treat human ailments in the prehistoric place of Harla and Dengego valleys , eastern Ethiopia. *Journal of Ethnobiology and Ethnomedicine, 10* (1), 1-17. https:// dio.org/ 10.1186/1746-4269-10-18

Bender, B. J., Gahbauer, S., Luttens, A., Lyu, J., Webb, C. M., Stein, R. M., Fink, E. A., Balius, T. E., Carlsson, J., Irwin, J. J., & Shoichet, B. K. (2021). A practical guide to large-scale docking. *Nature Protocols*, *16*(10), 4799–4832. https://doi.org/10.1038/s41596-021-00597-z

Beselam A. B. , Gemedo Dalle, and P. Natarajan (2022) Ethnobotanical aspects of medicinal plants of Mount Furi Forest, Sebeta Awas District, Southwest Oromia, Ethiopia(2022). *Journal of Equity in science and Sustainable development.5* (2), 17-30: DOI: https: //doi.org/10.20372/mwu.jessd.2022.1534

Bhardwaja, S. K., Dwivedia, K., & Agarwal, D. D. (2016). A Review: GC Method Development and validation Santosh. *International Journal of Analytical and Bioanalytical Chemistry*, *6*(1), 1–7.

Biovia. (2020). Biovia Discovery Studio® 2020: Comprehensive Modeling and Simulations for Life Sciences Datasheet. *Dassault Systemes: The 3D Experience Company*.

Botanical, M., Press, G., & Garden, M. B. (1991). The Niger and the Nile : Botanical Exploration Around Two African Rivers : *Missouri Botanical Garden,78* ,(1) , 81–86.

Br, G. R., & Asclepiadeae, A. (2001). *A revision of Gomphocarpus R. Br. (Apocynaceae: Asclepiadeae).Royal Botanical Garden*, 769-837

Brokowski C, A. M. (2019). Pyruvate kinase M2: A simple Molecule With Complex Functions.*Physiology & Behavior*,*176* (5),139–148.

Bryant Zez Aakash Basu1, & Angelica C. Parente, and (2017). Structural dynamics and mechanochemical coupling in DNA gyrase Aakash. *Physiology & Behavior*, *176*(1), 100–106.

Carey, F. A. (2022). Organic Chemistry Organic Chemistry. *Organic Chemistry Frontiers*, *1261*(6), 1261. http://dx.doi.org/10.1039/d1qo00478f

Cascorbi, I. (2011). P-glycoprotein: Tissue distribution, substrates, and functional consequences of genetic variations. *Handbook of Experimental Pharmacology*, *201*, 261–283. https://doi.org/10.1007/978-3-642-14541-4_6

Catteau, L., Reichmann, N. T., Olson, J., Pinho, M. G., Nizet, V., Van Bambeke, F., & Quetin-Leclercq, J. (2017). Synergy between ursolic and oleanolic acids from *Vitellaria paradoxa* leaf extract and *β*-lactams against methicillin-resistant *Staphylococcus aureus*: *In vitro* and *in vivo* activity and underlying mechanisms. *Molecules*, *22*(12), 1-17. https://doi.org/10.3390/molecules22122245

Charlton, N. C., Mastyugin, M., Török, B., & Török, M. (2023). Structural Features of Small Molecule Antioxidants and Strategic Modifications to Improve Potential Bioactivity. *Molecules*, *28*(3). https://doi.org/10.3390/molecules28031057

Chedik, L., Baybekov, S., Cosnier, F., Marcou, G., Varnek, A., & Champmartin, C. (2024). An update of skin permeability data based on a systematic review of recent research. *Scientific Data*, *11*(1), 1–14. https://doi.org/10.1038/s41597-024-03026-4

Collins, M. A., & Charles, H. P. (1987). Antimicrobial activity of Carnosol and Ursolic acid: two anti-oxidant constituents of *Rosmarinus officinalis* L. *Food Microbiology*, *4*(4), 311–315. https://doi.org/10.1016/S0740-0020(87)80005-9

De Stefani, C., Vasarri, M., Salvatici, M. C., Grifoni, L., Quintela, J. C., Bilia, A. R., Degl'Innocenti, D., & Bergonzi, M. C. (2022). Microemulsions Enhance the *In Vitro* Antioxidant Activity of Oleanolic Acid in RAW 264.7 Cells. *Pharmaceutics*, *14*(10). https://doi.org/10.3390/pharmaceutics14102232

Demir, İ. (2020). An ethnobotanical study of medicinal plants used in Hizan district (Bitlis-Turkey). *Yuzuncu Yil University Journal of Agricultural Sciences*, *30*(4), 732–741. https://doi.org/10.29133/yyutbd.730202

Dharsan, R., Geetha, R. V., & Lakshmi, T. (2020). Emerging and re-emerging virus. *Indian Journal of Forensic Medicine and Toxicology*, *14*(4), 4515–4521. https://doi.org/10.37506/ijfmt.v14i4.12353

Dickson, C. J., Rosso, L., Betz, R. M., Walker, R. C., & Gould, I. R. (2012). Gafflipid: A General Amber Force Field for the accurate molecular dynamics simulation of phospholipid. *Soft Matter*, *8*(37), 9617–9627. https://doi.org/10.1039/c2sm26007g

Do Nascimento, P. G. G., Lemos, T. L. G., Bizerra, A. M. C., Arriaga, A. M. C., Ferreira, D. A., Santiago, G. M. P., Braz-Filho, R., & Costa, J. G. M. (2014). Antibacterial and antioxidant activities of ursolic acid and derivatives. *Molecules*, *19*(1), 1317–1327. https://doi.org/10.3390/molecules19011317

Dong, X., Li, J. J., Ma, N., Liu, A. Z., & Liu, J. W. (2022). Anti-Inflammatory and Anti-Oxidative Effects of Isorhamnetin for Protection Against Lung Injury in a *Rat* Model of Heatstroke in a Dry-Heat Environment. *Medical Science Monitor*, *28*, 1–9. https://doi.org/10.12659/MSM.935426

Doyle, M. P., Loneragan, G. H., Scott, H. M., & Singer, R. S. (2013). Antimicrobial resistance: Challenges and perspectives. *Comprehensive Reviews in Food Science and Food Safety*, *12*(2), 234–248. https://doi.org/10.1111/1541-4337.12008

Duguma, T., & Rentsch, D. (2023). *In vitro* antibacterial activities, DPPH radical scavenging, and molecular simulation of isolated compounds from the stem bark of *Grewia ferruginea*. *Journal of Discover Chemistry*" 1-39

Duval, J., Pecher, V., Poujol, M., & Lesellier, E. (2016). Research advances for the extraction, analysis and uses of anthraquinones: A review. *Industrial Crops and Products*, *94*, 812–833. https://doi.org/10.1016/j.indcrop.2016.09.056

El-Askary, H., Hölzl, J., Hilal, S., & El-Kashoury, E. (1993). Cardenolide glycosides with doubly linked sugars from *Gomphocarpus sinaicu*s. *Phytochemistry*, *34*(5), 1399–1402. https://doi.org/10.1016/0031-9422(91)80037-2

El-askary, H. I. (2018a). Cardenolide glycosides from *Gomphocarpus sinaicus*. *Phytochemistry*, *9422* (3), 943- 948. https://doi.org/10.1016/0031-9422(94)00606-T

El-askary, H. I. (2018b). Cardenolide glycosides with doubly linked sugars from *Gomphocarpus sinaicus*. *Phytochemistry*, *9422*(7), 4–8. https://doi.org/10.1016/0031-9422(91)80037-2

ENVH 555. (2008). Infrared Spectroscopic Methods. *Lab/Field IR Measurements*, 1, 512- 534.

Fabiyi, O. A., Olubunmi, A., Adeyemi, O. S., & Olatunji, G. A. (2012). Antioxidant and Cytotoxicity of β-Amyrin acetate fraction from *Bridelia ferruginea* Leaves. *Asian Pacific Journal of Tropical Biomedicine*, *2*(2),S981-S984. https://doi.org/10.1016/S2221-1691(12)60347-5

Fazzini, M., Bisci, C., & Billi, P. (2015). The Climate of Ethiopia. *World Geomorphological Landscapes*, *(10)*, 65–87. https://doi.org/10.1007/978-94-017-8026-1_3

Seham A. EI Batran, Nahla S. Abdel Azim, Khaled Abdel Shafeek. Abd- Ellatty A. Shahat & Moustafa M. Ei Miissiry (*2005).* Flavonoids of *Gomphocarpus sinaicus*. *Natural Product Sciences 11(4), 233-239*

Friedman, M., Henika, P. R., & Mandrell, R. E. (2003). Antibacterial Activities of Phenolic Benzaldehydes and Benzoic Acids against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *Journal of Food Protection*, *66*(10), 1811–1821. https://doi.org/10.4315/0362-028X-66.10.1811

Gardens, R. B. (2001). A Revision of *Gomphocarpus R* . Br . (Apocynaceae : Asclepiadeae) *: Springer on behalf of Royal Botanic Gardens , 56*(4), 769–836.

Godman, B., Egwuenu, A., Wesangula, E., Schellack, N., Kalungia, A. C., Tiroyakgosi, C., Kgatlwane, J., Mwita, J. C., Patrick, O., Niba, L. L., Amu, A. A., Oguntade, R. T., Alabi, M. E., Ncube, N. B. Q., Sefah, I. A., Acolatse, J., Incoom, R., Guantai, A. N., Oluka, M.,

… Brink, A. J. (2022). Tackling antimicrobial resistance across sub-Saharan Africa: current challenges and implications for the future. *Expert Opinion on Drug Safety*, *21*(8), 1089–1111. https://doi.org/10.1080/14740338.2022.2106368

Gong, G., Guan, Y. Y., Zhang, Z. L., Rahman, K., Wang, S. J., Zhou, S., Luan, X., & Zhang, H. (2020). Isorhamnetin: A review of pharmacological effects. *Biomedicine and Pharmacotherapy*, *128*(April). https://doi.org/10.1016/j.biopha.2020.110301

Gori, A., Boucherle, B., Rey, A., Rome, M., Fuzzati, N., & Peuchmaur, M. (2021). Development of an innovative maceration technique to optimize extraction and phase partition of natural products.*Fitoterapia*,*148*(11).

Goyder, D. J. (2006). An overview of Asclepiad biogeography. *Taxonomy and Ecology of African Plants, Their Conservation and Sustainable Use*, *206* (1), 205–214.

Guengerich, F. P. (2022). Inhibition of Cytochrome P450 Enzymes by Drugs—Molecular Basis and Practical Applications. *Biomolecules and Therapeutics*, *30*(1), 1–18.

Gulcin, İ., & Alwasel, S. H. (2023). DPPH Radical Scavenging Assay. *Processes*, *11*(8).

Habtamu, A., & Melaku, Y. (2018). Antibacterial and Antioxidant Compounds from the Flower Extracts of *Vernonia amygdalina* . *Advances in Pharmacological Sciences*, *2018*, 1–6. https://doi.org/10.1155/2018/4083736

Номера, Т. (2016). Flavonols from *Cryptocarya alba*. *Journal of Pharmacology and Toxicology 6*(2), 7–23.

Ikuta, K. S., Swetschinski, L. R., Aguilar, G. R., Sharara, F., Mestrovic, T., Gray, A. P., Weaver, N. D., Wool, E. E., Han, C., Hayoon, A. G., Aali, A., Abate, S. M., Abbasi-Kangevari, M., Abbasi-Kangevari, Z., Abd-Elsalam, S., Abebe, G., Abedi, A., Abhari, A. P., Abidi, H., … Naghavi, M. (2022). Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*, *400*(10369), 2221–2248. https://doi.org/10.1016/S0140-6736(22)02185-7

Institute for Health Metrics and Evaluation, & University of Oxford. (2023). The burden of antimicrobial resistance (AMR) in Ecuador. *Institute for Health Metrics and Evaluation*, 1–4.

Islam, M. S., & Lucky, R. A. (2019). a Study on Different Plants of Apocynaceae Family and Their Medicinal Uses. *Universal Journal of Pharmaceutical Research*, *March 2019*, 1–6.

https://doi.org/10.22270/ujpr.v4i1.235

Japir, A. A. W., Salih, N., & Salimon, J. (2021). Synthesis and characterization of biodegradable palm palmitic acid based bioplastic. *Turkish Journal of Chemistry*, *45*(3), 585–599.

Jesus, J. A., Lago, J. H. G., Laurenti, M. D., Yamamoto, E. S., & Passero, L. F. D. (2015). Antimicrobial activity of oleanolic and ursolic acids: An update. *Evidence-Based Complementary and Alternative Medicine*, *2015*(Figure 1).

Kawai, A., & Shibuya, K. (2002). Energy Separation between Quartet and Doublet Spin States of Radical - Triplet Encounter Pairs ; Unusual Ferromagnetic Interaction in a 1 , 1-Diphenyl-2-picrylhydrazyl and Triplet Coronene Pair. *American Chemical Society* 12305–12314.

Knv, R., & Tabassum, B. (2017). Preliminary Phytochemical Screening of *Spinacia*. *4*(1), 8–13.

Lipinski, C. A. (2004). Lead- and drug-like compounds: The rule-of-five revolution. *Drug Discovery Today: Technologies*, *1*(4), 337–341.

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2012). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, *64*(SUPPL.), 4–17.

Lucas, A. J., Sproston, J. L., Barton, P., & Riley, R. J. (2019). Estimating human ADME properties, pharmacokinetic parameters and likely clinical dose in drug discovery. *Expert Opinion on Drug Discovery*, *14*(12), 1313–1327.

Mag, P., Osman, S. M., El-haddad, A. E., El-raey, M. A., El-khalik, S. M. A., Koheil, M. A., & Wink, M. (2016). A New Octadecenoic Acid Derivative from *Caesalpinia gilliesii* Flowers with Potent Hepatoprotective Activity. *Journal in the field of Pharmacognosy and Natural Products*. 332–336. https://doi.org/10.4103/0973-1296.185752

Martin, Y. C. (2005). A bioavailability score. *Journal of Medicinal Chemistry*, *48*(9), 3164–3170. https://doi.org/10.1021/jm0492002

Marzouk, A. M. (2009). Hepatoprotective triterpenes from hairy root cultures of *Ocimum basilicum* L. *Zeitschrift Fur Naturforschung - Section C. Journal of Biosciences*, *64*(3–4), 201–209. https://doi.org/10.1515/znc-2009-3-409

Marzouk, A. M., Osman, S., & Gohar, A. (2015a). A new pregnane glycoside from *Gomphocarpus fruticosus* growing in Egypt. *Natural Product Research, 30*(9), *1060-1067.http://dx.doi.org/10.1080/14786419.2015.1107059*

Marzouk, A. M., Osman, S. M., & Gohar, A. A. (2015b). *A new pregnane glycoside from Gomphocarpus fruticosus growing in Egypt*. *Natural Product Research, 6419*(12). https://doi.org/10.1080/14786419.2015.1107059

Medapi, B., Renuka, J., Saxena, S., Sridevi, J. P., Medishetti, R., Kulkarni, P., Yogeeswari, P., & Sriram, D. (2015). Design and synthesis of novel quinoline-aminopiperidine hybrid analogues as *Mycobacterium tuberculosis* DNA gyraseB inhibitors. *Bioorganic and Medicinal Chemistry*, *23*(9), 2062–2078. https://doi.org/10.1016/j.bmc.2015.03.004

Mesfin, F., Demissew, S., & Teklehaymanot, T. (2009). An ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, *5*, 28. https://doi.org/10.1186/1746-4269-5-28

Miseganaw, D., & Mhiret, W. N. (2019). *Invitro* antimicrobial effects of *Gomphocarpus purpurascens* A. rich against standard and clinically isolated microorganisms *Global Scientific Journals7* (6), 121-136

Mohammed, S. (2024). Biological Activities of Extracts and Isolated Calotropin from *Gomphocarpus purpurascens* Leaves : A Computational and Experimental Study. 11(4), 1377–1386.

Morris-Schaffer, K., & McCoy, M. J. (2021). A Review of the LD_{50} and Its Current Role in Hazard Communication. *ACS Chemical Health and Safety*, *28*(1), 25–33.

Muhammed, M. T., & Aki-Yalcin, E. (2022). Molecular Docking: Principles, Advances, and Its Applications in Drug Discovery. *Drug Design & Discovery*, *21*(3), 480–495.

Mujeeb, F., Bajpai, P., & Pathak, N. (2014). Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos. BioMed Research International*, *14*(7) 812-833

Mulatu, G. (2020). Antibacterial Activities of *Calpurnia aurea* against Selected. *Advances in Pharmacological and Pharmaceutical Sciences 2020* (1) 9

Naumoska, K., Simonovska, B., Albreht, A., & Vovk, I. (2013). TLC and TLC-MS screening

of ursolic, oleanolic and betulinic acids in plant extracts. *Journal of Planar Chromatography - Modern TLC*, *26*(2), 125–131.

Nazar, N., Goyder, D. J., Clarkson, J. J., Mahmood, T., & Chase, M. W. (2013). The taxonomy and systematics of Apocynaceae:. *Botanical Journal of the Linnean Society*, *171*(3), 482–490. https://doi.org/10.1111/boj.12005

Massimiliano D'Ambola, (1996). Isolation and characterization of plant bioactive compounds and assessment of their use in veterinary science. *Natural products* 3*1*(6), 694–700.

Nuurul, S., & Mohammad, H. (2016). Herbal Processing and Extraction Technologies. *Separation and Purification Reviews*, 45(4), 305-320

Oliveira E Nogueira, J., Aguiar Campolina, G., Roberto Batista, L., Alves, E., Rodrigues, A., Caetano, S., Magalhães Brandão, R., Nelson, D. L., Das, M., & Cardoso, G. (2021). Mechanism of action of various terpenes and phenylpropanoids against *Escherichia coli* and *Staphylococcus aureus* Antimicrobial properties of terpenes and phenylpropanoids. *FEMS Microbiology Letters*, *368*(9).

OMS. (2013). Traditional medicine executive board 134th session Provisional agenda item 9.1. *Eb134/24*, *2013*(13), 1–4.

Packirisamy, V., & Moorthy, V. K. (2014). Antibacterial And Phytochemical Evaluation of *Pergularia daemia* from Nagapattinam region. *International Journal of Scientific and Research Publications*, *4*(11), 1–6.

Palagano, R., Valli, E., Tura, M., Cevoli, C., Pérez-Camino, M. del C., Moreda, W., Bendini, A., & Toschi, T. G. (2020). Fatty acid ethyl esters in *Virgin olive* oils: In-House validation of a revised method. *Foods*, *9*(7), 1–11. https://doi.org/10.3390/foods9070924

Pancu, D. F., Scurtu, A., Macasoi, I. G., Marti, D., Mioc, M., Soica, C., Coricovac, D., Horhat, D., Poenaru, M., & Dehelean, C. (2021). Antibiotics: Conventional therapy and natural compounds with antibacterial activity-a pharmaco-toxicological screening. *Antibiotics*, *10*(4). https://doi.org/10.3390/antibiotics10040401

Patil, U. S., & Deshmukh, O. S. (2016). Preliminary phytochemical screening of six medicinal plants used as traditional medicine. *International Journal of Pharma and Bio Sciences*, *7*(1), P77–P81. https://doi.org/10.46501/ijmtst061019

Priyanthi, C. (2021). The Total Antioxidant Capacity and the Total Phenolic Content of *Rice*

Using Water as a Solvent. *International Journal of Food Science 2021*(3).6

Rahamouz-Haghighi, S., Bagheri, K., Mohsen-Pour, N., & Sharafi, A. (2022). *In vitro* Evaluation of Cytotoxicity and Antibacterial Activities of *Ribwort plantain* (*Plantago lanceolata* L.) Root Fractions and Phytochemical Analysis by Gas Chromatography-Mass Spectrometry.*Archives of Razi Institute*, *77*(6), 2131–2143

Raju, L., Lipin, R., & Eswaran, R. (2021). Identification, ADMET evaluation and molecular docking analysis of Phytosterols from *Banaba* (*Lagerstroemia speciosa* (L.)Pers) seed extract against breast cancer. *In Silico Pharmacology*, *9*(1), 1–9.

Rashid, M. I., Rashid, H., Andleeb, S., & Ali, A. (2022). Evaluation of Blood-Brain-Barrier Permeability, Neurotoxicity, and Potential Cognitive Impairment by *Pseudomonas aeruginosa* 's Virulence Factor Pyocyanin. *Oxidative Medicine and Cellular Longevity*, *2022*(13) 6

Yihenew Simegniew Birhan, Sintayehu Leshe Kitaw, Yihalem Abebe Alemayehu, & Nakachew Minuye Mengesha. (2017). *Ethnobotanical Study of Medicinal Plants used to treat Human Diseases in Enarj Enawga District, East Gojjam Zone, Amhara Region, Ethiopia*. *SM Journal of Medicinal Plant Studies 1*(1),1006

Zhao, L. T., Max, G., Chen, A., Gu, N., Pei, J., Su, E., Duan, X., Cao, F., & Zhao, L. (2019). Synthesis of Isorhamnetin-3- O -Rhamnoside by a Using a UDP-Rhamnose Regeneration System. *Molecules 24, 3042; doi:10.3390/molecules24173042*

Riaz, T., Akram, M., Laila, U., Zainab, R., Talha Khalil, M., Iftikhar, M., Ahmet Ozdemir, F., Sołowski, G., Altable, M., Sfera, A., Khalifa Ibrahim, H., & Parmar, P. (2023). Therapeutic applications of glycosides obtained from medicinal plants. *International Archives of Integrated Medicine*, *10*(8), 30–38.

Rocque, R. J., Beaudoin, C., Ndjaboue, R., Cameron, L., Poirier-Bergeron, L., Poulin-Rheault, R. A., Fallon, C., Tricco, A. C., & Witteman, H. O. (2021). Health effects of climate change: An overview of systematic reviews. *British Medical Journal Open*, *11*(6).

Saed, N. M., El-Demerdash, E., Abdel-Rahman, H. M., Algandaby, M. M., Al-Abbasi, F. A., & Abdel-Naim, A. B. (2012). Anti-inflammatory activity of methyl palmitate and ethyl palmitate in different experimental Rat models. *Toxicology and Applied Pharmacology*, *264*(1), 84–93. https://doi.org/10.1016/j.taap.2012.07.020

Sánchez-Moreno, C. (2002). Methods Used to Evaluate the Free Radical Scavenging Activity in Foods and Biological Systems. *Food Science and Technology International*, *8*(3), 121–137. https://doi.org/10.1106/108201302026770

Schoch, C. (2020). National Center for Biotechnology Information Taxonomy Codes of Nomenclature Treatment of Type Material in National Center for Biotechnology InformationI Taxonomy. *National Center for Biotechnology InformationTaxonomy Md*, 1–6.

Seble, W. Y., Zemede, A., & Ensermu, K. (2018). Ethnobotanical study of medicinal plants used by local people in Menz Gera Midir District, North Shewa Zone, Amhara Regional State, Ethiopia. *Journal of Medicinal Plants Research*, *12*(21), 296–314.

Seham A. EI Batran, Nahla S. Abdel Azim, Khaled Abdel Shafeek. Abd- Ellatty A. Shahat & Moustafa M. Ei Miissiry (*2005).* Flavonoids of *Gomphocarpus sinaicus*. *Natural Product Sciences 11(4), 233-239*

Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603–608.

Shaker, B., Ahmad, S., Thai, T. D., Eyun, S. Il, & Na, D. (2020). Rational Drug Design for *Pseudomonas aeruginosa* PqsA Enzyme: An *in silico* Guided Study to Block Biofilm Formation. *Frontiers in Molecular Biosciences*, *7*(10), 1–14.

Shamsudin, N. F., Ahmed, Q. U., Mahmood, S., Shah, S. A. A., Khatib, A., Mukhtar, S., Alsharif, M. A., Parveen, H., & Zakaria, Z. A. (2022). Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation. *Molecules*, *27*(4). https://doi.org/10.3390/molecules27041149

Sharma, O. P., & Bhat, T. K. (2009). DPPH antioxidant assay revisited. *Food Chemistry*, *113*(4), 1202–1205. https://doi.org/10.1016/j.foodchem.2008.08.008

Siddhardha, B., Dyavaiah, M., & Syed, A. (2020). Model Organisms for Microbial Pathogenesis, Biofilm Formation and Antimicrobial Drug Discovery. *Model Organisms for Microbial Pathogenesis, Biofilm Formation and Antimicrobial Drug Discovery* 1–684. https://doi.org/10.1007/978-981-15-1695-5

Sing, P. R. U., Arbon, A. C. C., & Acf, F. I. (2012). ProTox 3.0: a web server for the prediction of toxicity of chemicals. *Nucleic Acids Research*, *9999*(1), 1–8.

Sławińska, N., Żuchowski, J., Stochmal, A., & Olas, B. (2024). Comparative phytochemical , antioxidant , and hemostatic studies of fractions from raw and roasted Sea *Buckthorn* seeds *in vitro*. *Scientific Reports*, *0123456789*, 1–19. https://doi.org/10.1038/s41598-024-72012-y

Sobral, F., Calhelha, R. C., Barros, L., Dueñas, M., Tomás, A., Santos-Buelga, C., Vilas-Boas, M., & Ferreira, I. C. F. R. (2017). Flavonoid composition and antitumor activity of bee bread collected in Northeast Portugal. *Molecules*, *22*(2), 1–12.

TURCK , M.D, BAUER A. W. , M.D. , KIRBY W. M . M . , SHERRIS J . C ., & AXU M M.D. , (2018). Antibiotic Susceptibility Testing by A Standardized Single Disk Method. *The American Journal of Clinical Pathology* Vol. *45 (*4),493-496

Tadeg, H., Mohammed, E., Asres, K., & Gebre-Mariam, T. (2005). Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. *Journal of Ethnopharmacology*, *100*(1–2), 168–175.

Tesfamicael, F., & Ele, E. (2016). Assessment of fatty acids composition in commercially available and widely consumed edible oils in ethiopia: their relevance and health implications. *SINET: Ethiopian Journal of Science*, *39*(1), 21–33.

Trisciuzzi, D., Alberga, D., Leonetti, F., Novellino, E., Nicolotti, O., & Mangiatordi, G. F. (2018). Molecular docking for predictive toxicology. *Methods in Molecular Biology*, *1800*(3), 181–197. https://doi.org/10.1007/978-1-4939-7899-1_8

UENO, Y. (1978). Chemistry and biological activity of trichothecenes. *Mycotoxins 1978*(7), 6-14 . https://doi.org/10.2520/myco1975.1978.6

Veber, D. F., Johnson, S. R., Cheng, H. Y., Smith, B. R., Ward, K. W., & Kopple, K. D. (2002). Molecular properties that influence the oral bioavailability of drug candidates. *Journal of Medicinal Chemistry*, *45*(12), 2615–2623. https://doi.org/10.1021/jm020017n

Velika, B., & Kron, I. (2012). Antioxidant properties of benzoic acid derivatives against Superoxide radical. *Free Radicals and Antioxidants*, *2*(4), 62–67.

Wang, H., Chen, L., Yang, B., Du, J., Chen, L., & Li, Y. (2023). Structures, Sources, Identification/Quantification Methods, Health Benefits, Bioaccessibility, and Products of Isorhamnetin Glycosides as Phytonutrients. *Moolecules 15,* 1947 https://doi.org/10.3390/ nu15081947

WHO. (2002): A manual for Medical and Clinical Officers in Africa and Clinical Officers. *World Health Organisation*, 124p.

Wubetu, M., Sintayehu, M., Aeta, M. A., Reta, H., & Derebe, D. (2018). Ethnobotany of Medicinal Plants used to Treat Various Mental illnesses in Ethiopia : A Systematic review. *Asian Journal of Plant Science and Research, 8*(1), *9-33*

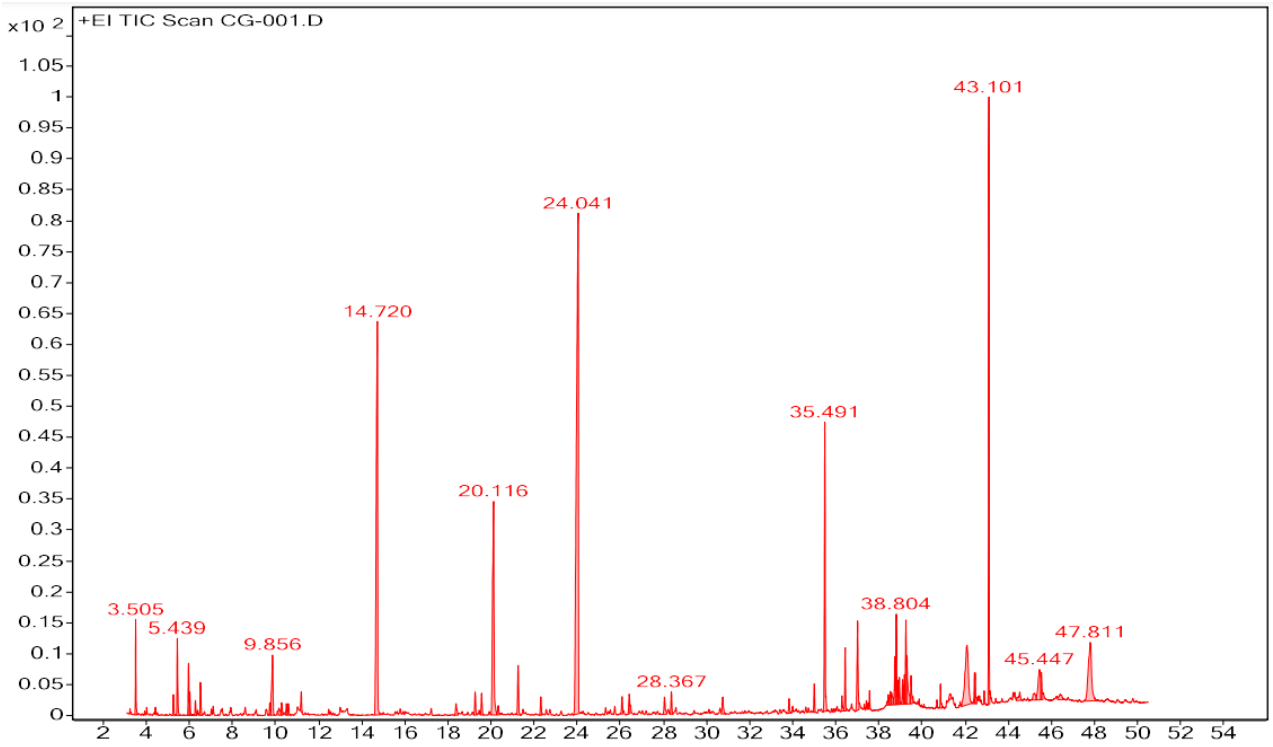
Yusuf Sukman, J. (2017). Phytochemical Analysis, Radical Scavenging and Anti-microbial Activity of the Leaves of *Gomphocarpus fruticosus. Bulletin of Roszdravnadzor .4* December 9-15

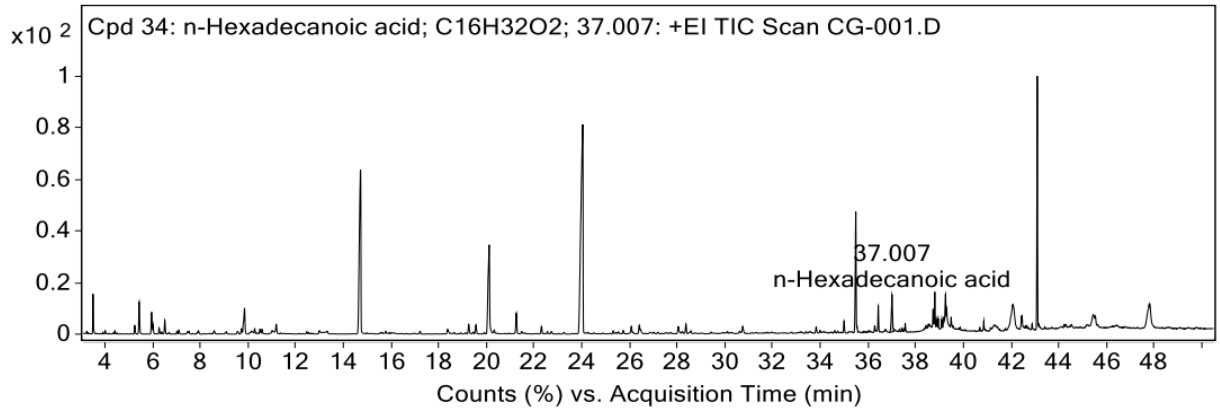
Zhang, Y., Cai, P., Cheng, G., & Zhang, Y. (2022). A Brief Review of Phenolic Compounds Identified from Plants: Their Extraction, Analysis, and Biological Activity. *Natural Product Communications*, *17*(1). https://doi.org/10.1177/1934578X211069721

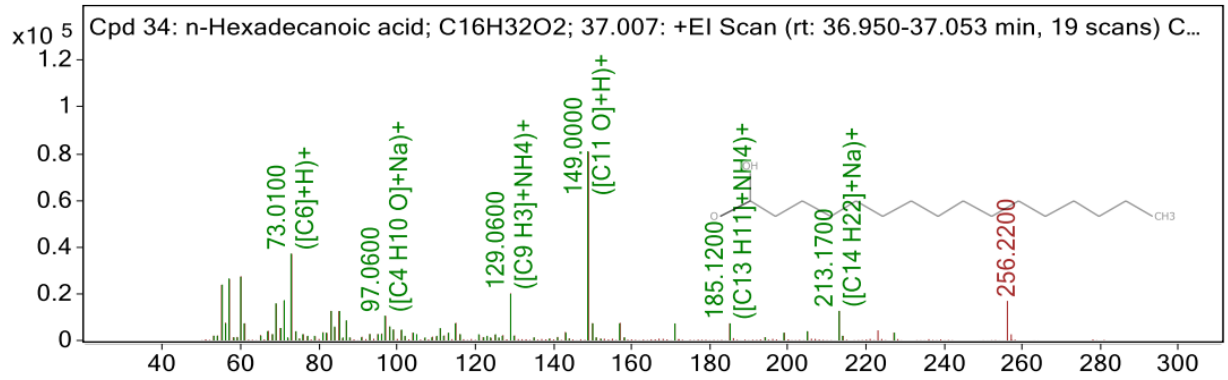
Zuo, L., Prather, E. R., Stetskiv, M., Garrison, D. E., Meade, J. R., Peace, T. I., & Zhou, T. (2019). Inflammaging and oxidative stress in human diseases: From molecular mechanisms to novel treatments. *International Journal of Molecular Sciences*, *20*(18). https://doi.org/10.3390/ijms20184472

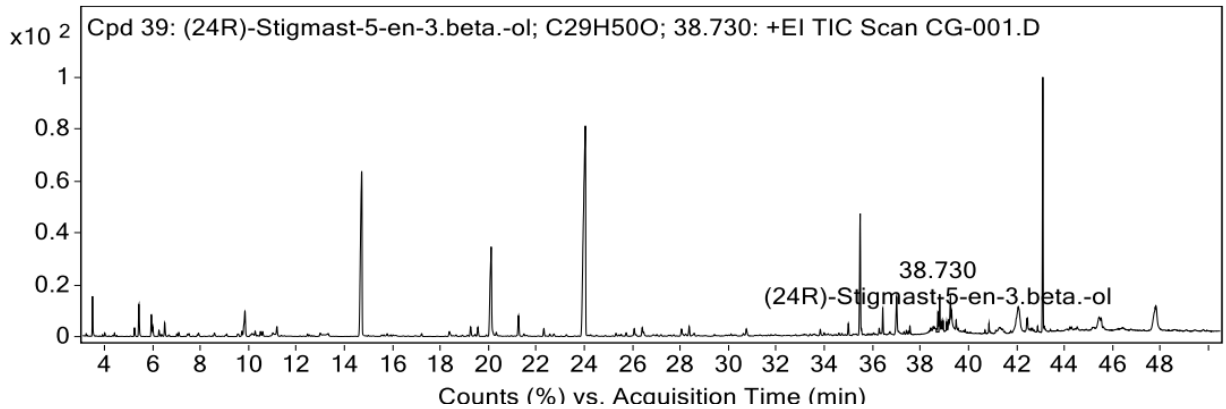
8. APPENDICES

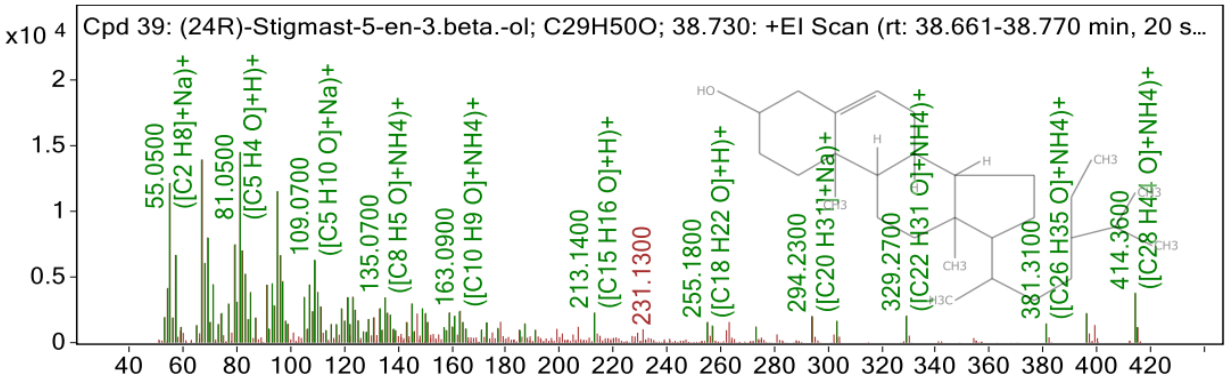
Appendix 1: GC-MS spectral data of essential oils identified from root of *G. purpurascens*

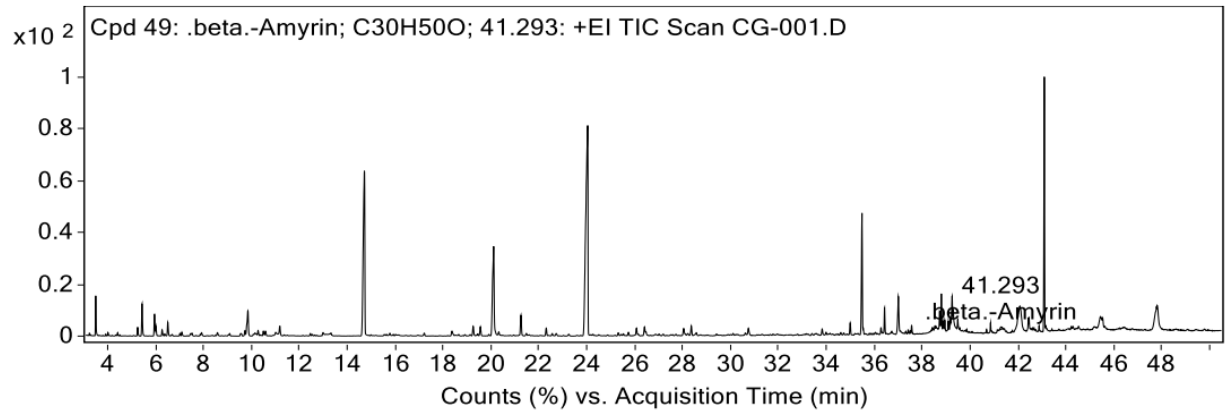


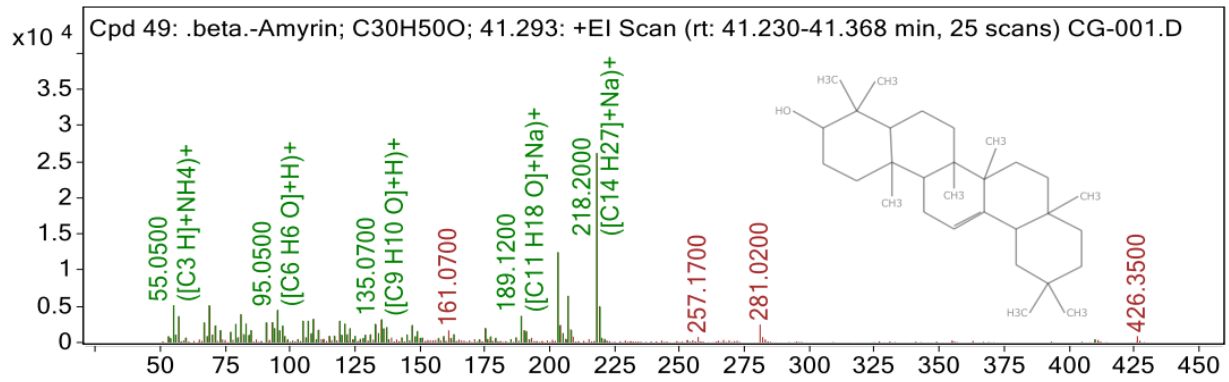


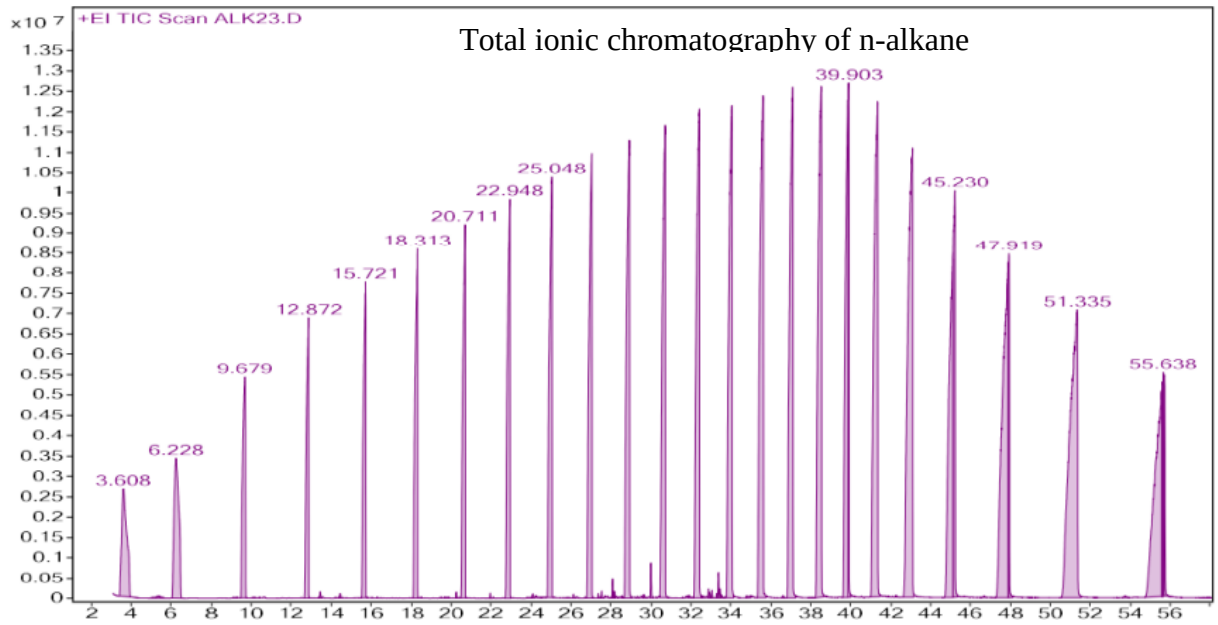



Cpd 39: (24R)-Stigmast-5-en-3.beta.-ol; C29H50O; 38.730: +EI TIC Scan CG-001.D
x10 2
38.730
(24R)-Stigmast-5-en-3.beta.-ol
Counts (%) vs. Acquisition Time (min)

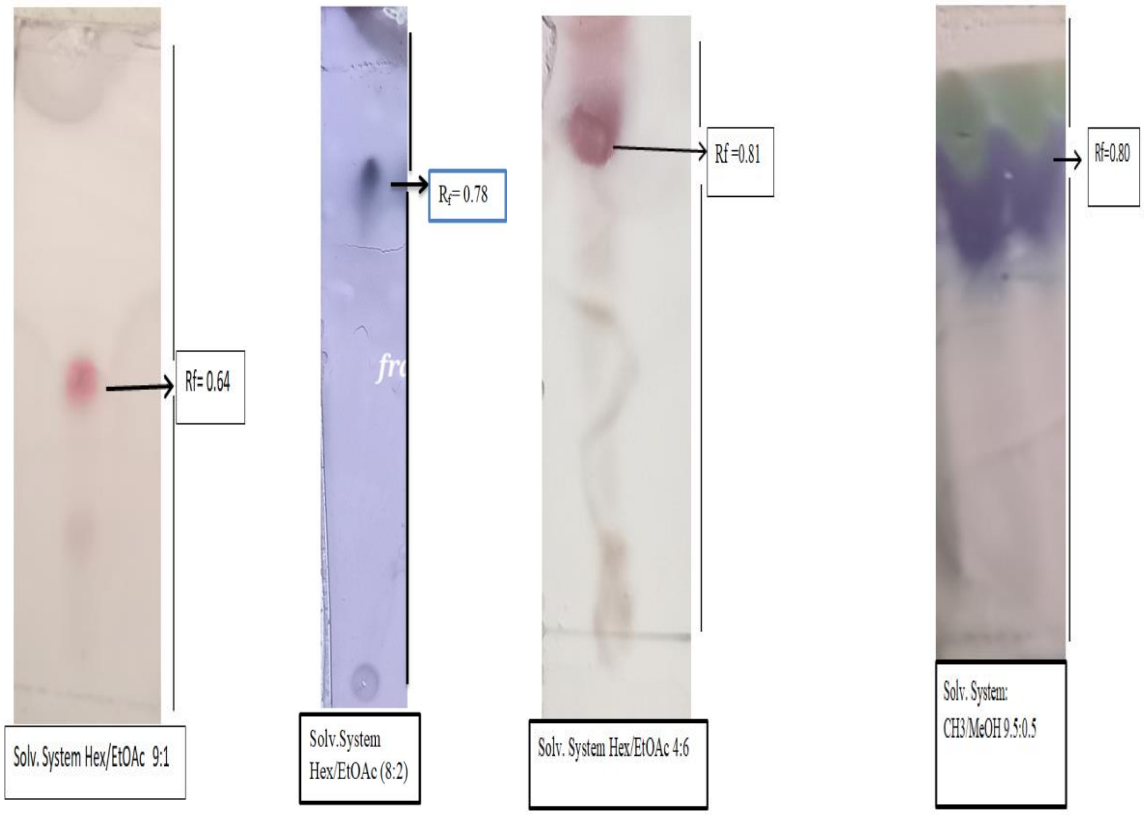

Cpd 39: (24R)-Stigmast-5-en-3.beta.-ol; C29H50O; 38.730: +EI Scan (rt: 38.661-38.770 min, 20 s...
x10 4
55.0500 ([C2 H8]+Na)+
81.0500 ([C5 H4 O]+H)+
109.0700 ([C5 H10 O]+Na)+
135.0700 ([C8 H5 O]+NH4)+
163.0900 ([C10 H9 O]+NH4)+
213.1400 ([C15 H16 O]+H)+
231.1300
255.1800 ([C18 H22 O]+H)+
294.2300 ([C20 H31]+Na)+
329.2700 ([C22 H31 O]+NH4)+
381.3100 ([C26 H35 O]+NH4)+
414.3600 ([C28 H44 O]+NH4)+


Cpd 49: .beta.-Amyrin; C30H50O; 41.293: +EI TIC Scan CG-001.D
x10 2
41.293
.beta.-Amyrin
Counts (%) vs. Acquisition Time (min)


Cpd 49: .beta.-Amyrin; C30H50O; 41.293: +EI Scan (rt: 41.230-41.368 min, 25 scans) CG-001.D
x10 4
55.0500 ([C3 H]+NH4)+
95.0500 ([C6 H6 O]+H)+
135.0700 ([C9 H10 O]+H)+
161.0700
189.1200 ([C11 H18 O]+Na)+
218.2000 ([C14 H27]+Na)+
257.1700
281.0200
426.3500

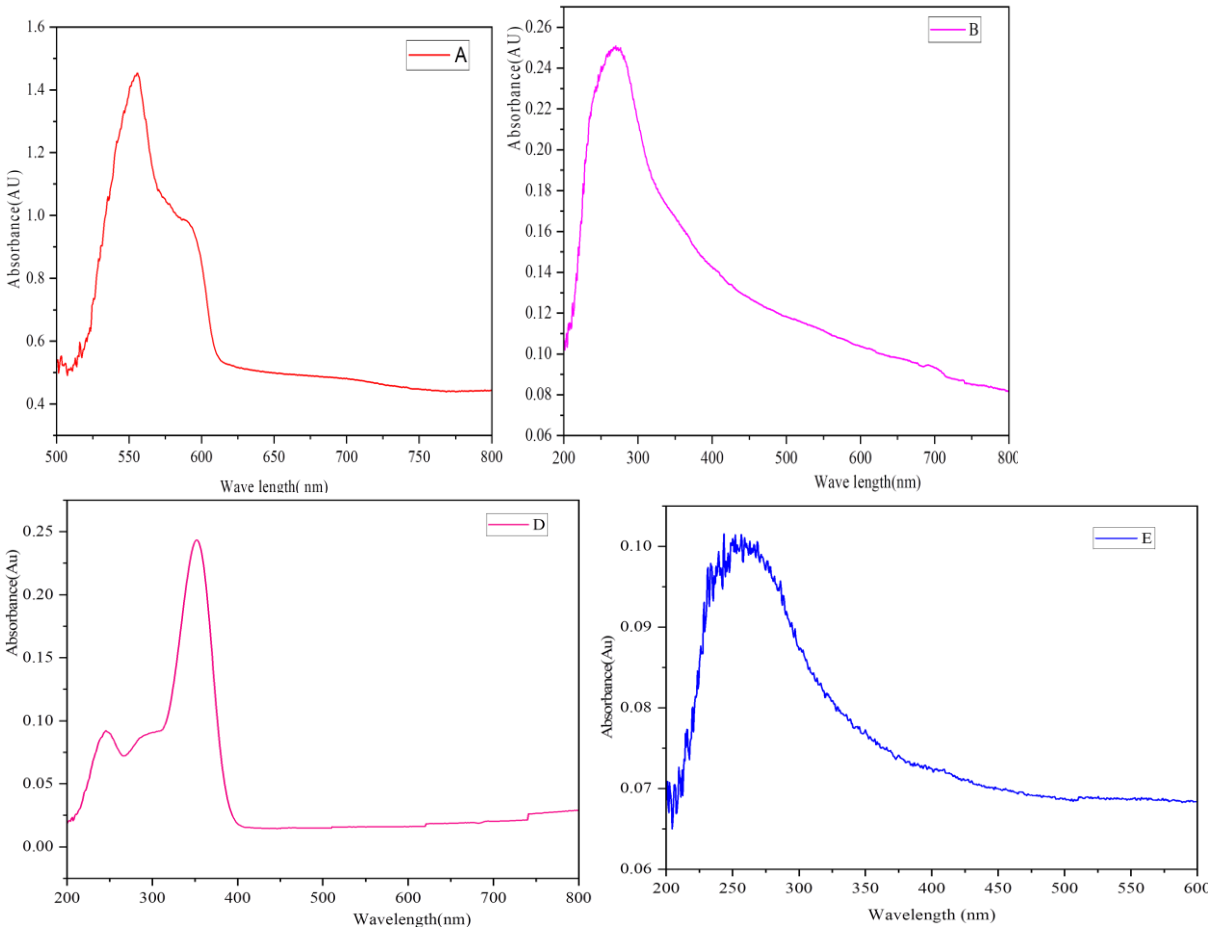


Appendix 2: TLC profile of isolated compounds from root of *G. purpurascens*



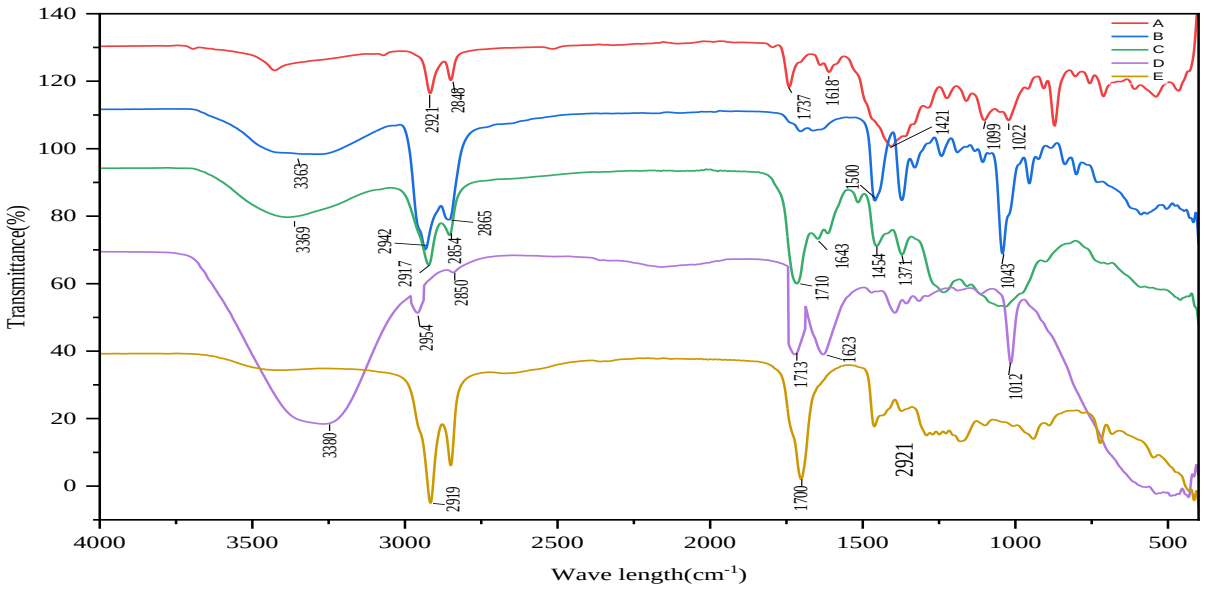
TLC of compound **32**, **37**, **38** and **39**, and **40** from left to right respectively

Appendix 3: UV/ Vis spectra of isolated compounds from root of *G. purpurascens*

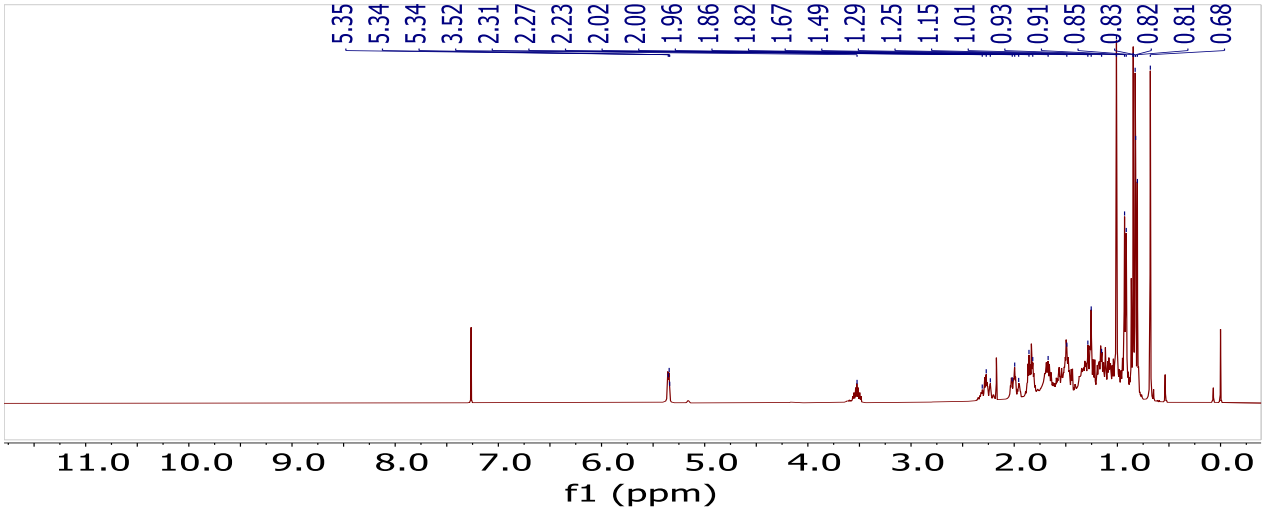


A= compound **32**, B= compound **37**, D= compound **40** and E= compound **38** and **39**

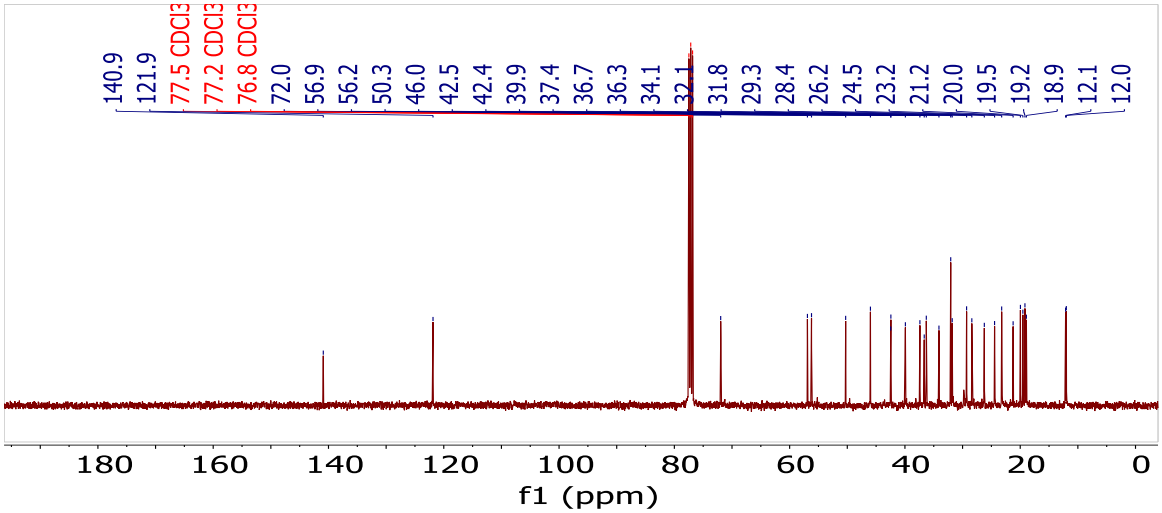
Appendix 4: FT-IR spectra of isolated compounds from root of *G. purpurascens*



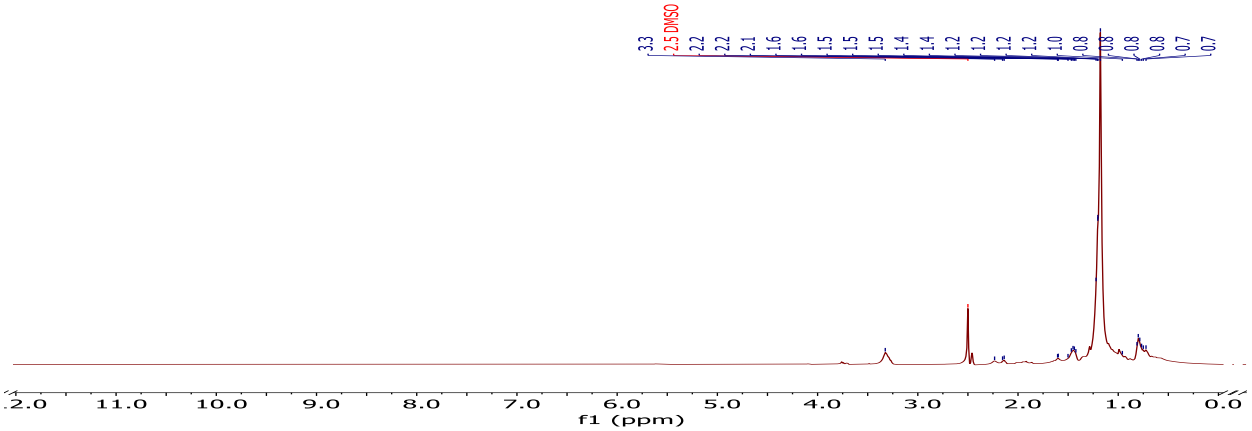
Appendix 5: ^{1}H NMR (400 MHz, CDC_{l3}) Spectra of compound **32**



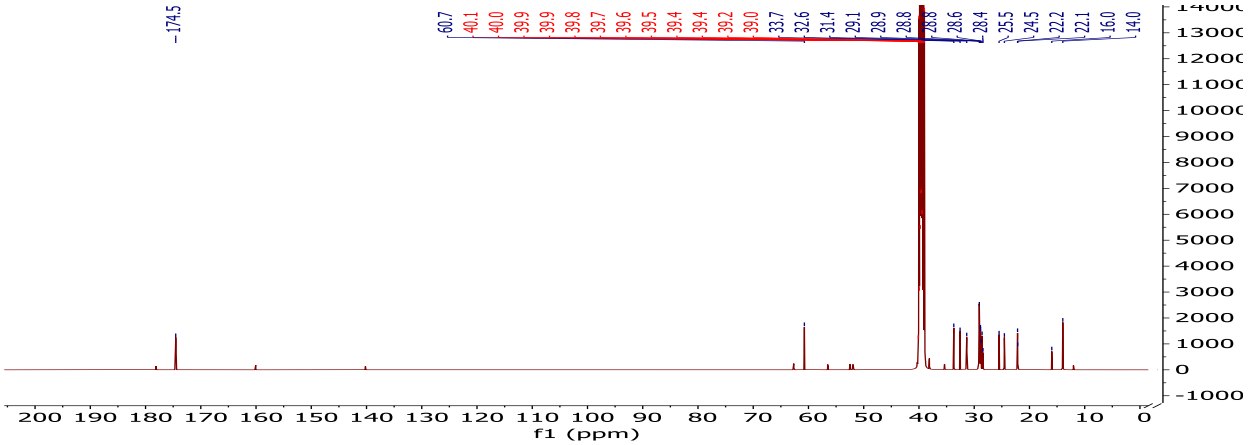
Appendix 6: ^{13}C NMR (101 MHz, CDC_{l3}) spectra of compound **32**



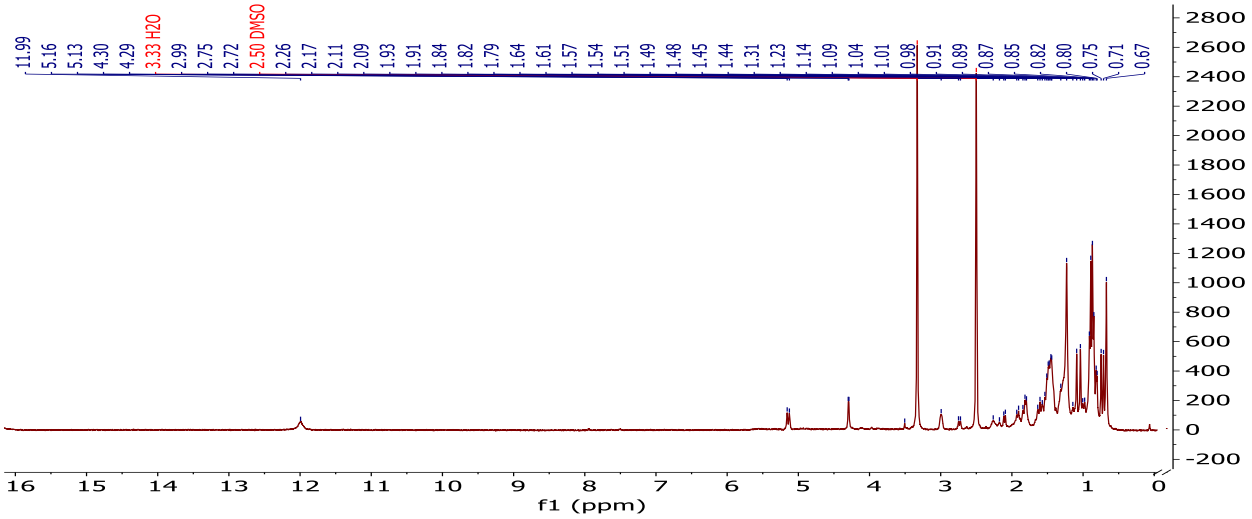
Appendix 7: ^{1}H NMR (400 MHz, CDC_{l3}) spectra of compound **37**



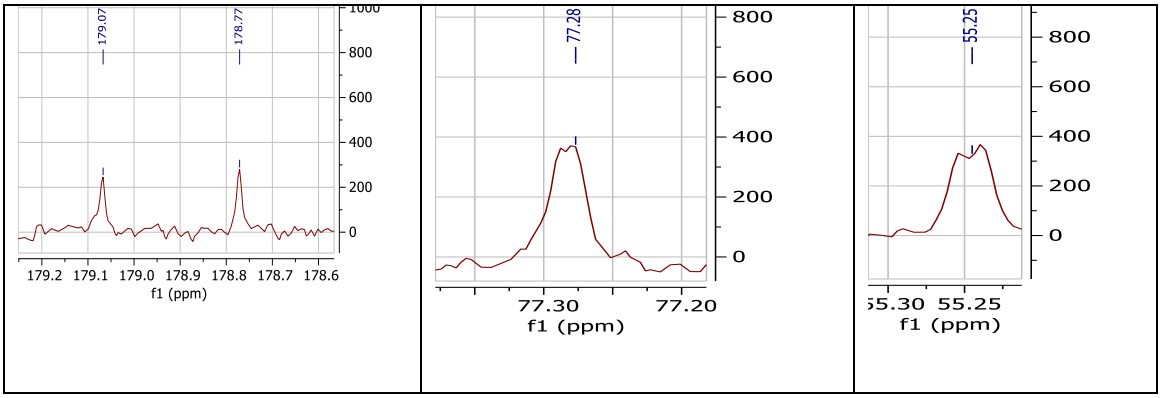
Appendix 8: ^{13}CNMR (101 MHz, CDC_{l3}) spectra of compound 37

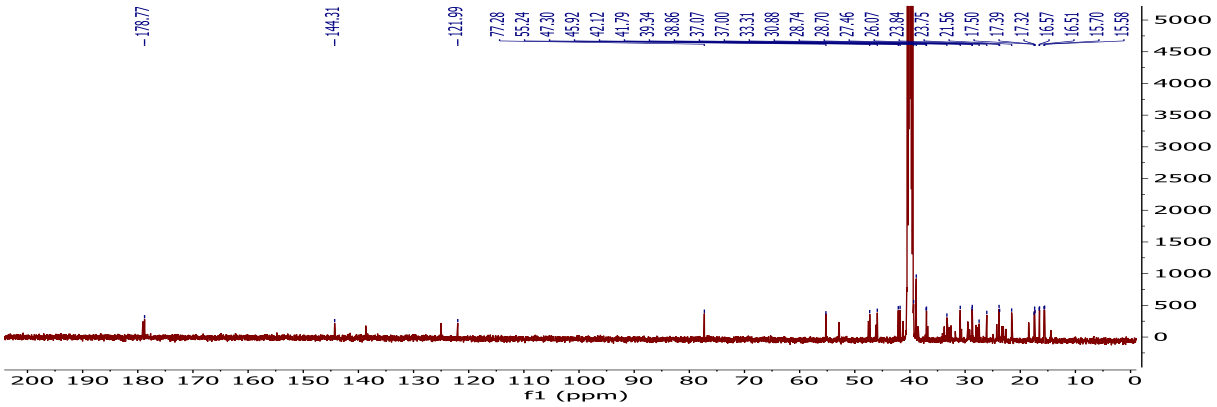


Appendix 9: ^{1}H NMR (500 MHz, DNMSO) spectra of compounds **38** and **39**

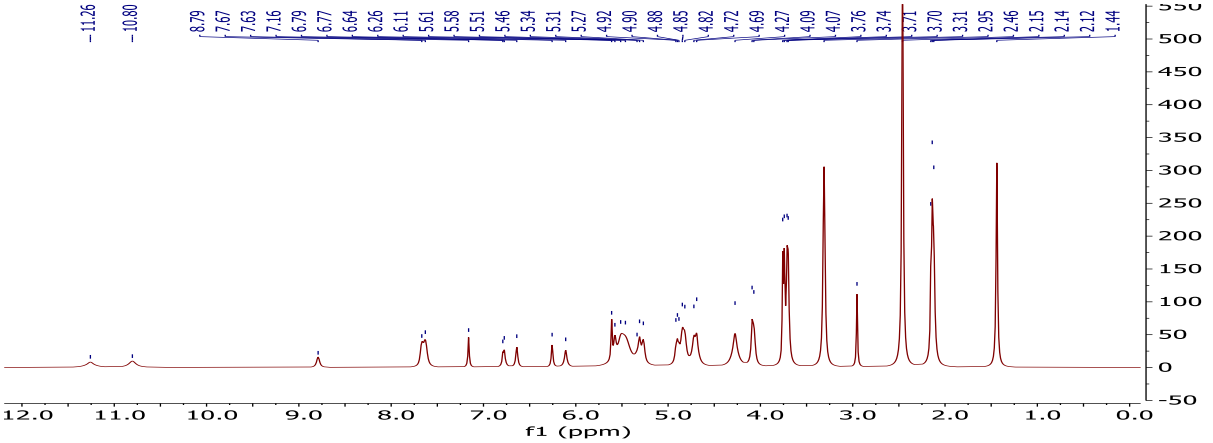


Appendix 10: ^{13}CNMR (126 MHz, DMSO) spectra of compound **38** and **39**





Appendix 11: ^{1}H NMR (400 MHz, DMSO) spectra of compound **40**



Appendix 12: ^{13}CNMR (101 MHz, DMSO) spectra of compound **40**