

**Phytochemical Analysis and Biological Activities of Secondary Metabolites
Derived from Selected Ethiopian Medicinal Plants: An Integrated
Computational and Experimental Study**



Bihon Abera Kassa

**A Dissertation Submitted to
The Department of Applied Chemistry,
College of Applied Natural Science,**

**Presented in Partial Fulfillment of the Requirement for the Degree
of Doctor of Philosophy in Applied Chemistry (Natural Product
Chemistry)**

**Office of Graduate Studies
Adama Science and Technology University**

**November, 2024
Adama, Ethiopia**

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Major Advisor: Milkyas Endale (Ph.D, Professor)

Co-advisor: Yadessa Melaku (Ph.D, Professor)

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DECLARATION

I declare that this dissertation entitle “**Phytochemical Analysis and Biological Activities of Secondary Metabolites Derived from Selected Ethiopian Medicinal Plants: An Integrated Computational and Experimental Study**” is my work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials used for this thesis have been duly acknowledged through appropriate citations.

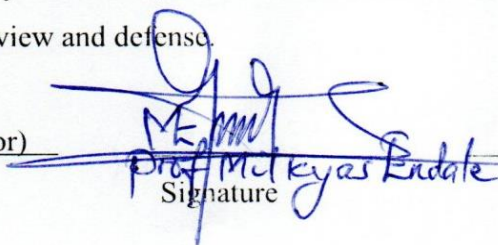
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We, the supervisors of this dissertation, hereby certify that we have read and revised the dissertation entitled **“Phytochemical Analysis and Biological Activities of Secondary Metabolites Derived from Selected Ethiopian Medicinal Plants: An Integrated Computational and Experimental Study”** prepared under our guidance by **Bihon Abera** submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Natural product Chemistry. Therefore, we recommend the submission of the dissertation to the department for further review and defense.

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I/we hereby certify that the recommendations and suggestions made by the board of examiners are appropriately incorporated into the final version of the dissertation entitled "**Phytochemical Analysis and Biological Activities of Secondary Metabolites Derived from Selected Ethiopian Medicinal Plants: An Integrated Computational and Experimental Study**" by **Bihon Abera**.

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LIST OF ABBREVIATIONS AND ACRONYMS

^{13}C NMR	Carbon-13 nuclear magnetic resonance
CC	Column chromatography
COSY	Correlation spectroscopy
CC ₅₀	50% cytotoxic concentration
DEPT	Distortionless enhancement polarization transfer
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EOs	Essential oils
FT-IR	Fourier-transform infrared spectroscopy
GC-MS	Gas chromatograph-mass spectrometry
^1H NMR	Proton nuclear magnetic resonance
HMBC	Heteronuclear multiple bond correlation
HSQC	Heteronuclear single quantum coherence spectroscopy
IC ₅₀	50% inhibited concentration
MIC	Minimum inhibition concentration
MTT	3-(4, 5dimethylthiazolyl-2)-2,5 diphenyltetrazoliumbromide
PDB	Protein data bank
PDBQT	Protein data bank, partial charge, and atom type format
RF	Retention factor
RT	Retention time
RI	Retention index
SI	Selectivity index
TLC	Thin layer chromatography
UV-Vis	Ultraviolet-visible spectroscopy
WHO	World health organization
ZI	Zone of inhibition

ABSTRACT

Plants synthesize a wide range of biologically active secondary metabolites, which serve for therapeutic agents against various human and animal diseases. Ethiopian traditional medicine has long harnessed the therapeutic properties of medicinal plants, including their potential to address various infectious diseases and cancer. By integrating modern scientific techniques with traditional knowledge, there is great potential to discover and develop new medicinal agents from Ethiopia's rich botanical heritage, thereby preserving both cultural wisdom and biodiversity. In this study, five plants were selected based on their ethnobotanical uses, reflecting their traditional significance. Silica gel column chromatographic separation of roots extracts of *W. uniflora*, *T. vogelii*, *A. schimperi*, *R. glutinosa*, and *C. spinarum* yielded four (**97-99**), seven (**97, 100-105**), five (**106-110**), eight (**97,111-115**), and two (**116, 117**) compounds, respectively. The structure of the isolated compounds was characterized using spectroscopic (IR, ^1H and ^{13}C (1D and 2D) NMR data and literature reports. GC-MS analysis of essential oils revealed that dibutyl phthalate (28.66%), cisanerolidol (41.7%), and cedrol (25.85%) as major constituents in the roots of *W. uniflora*, *T. vogelii*, and *A. schimperi*, respectively. Meanwhile, 4-hydroxy acetophenone was the principal component of the roots of *R. glutinosa* and *C. spinarum*, with percentages of 72.19% and 74.85%, respectively. The antibacterial activity of crude extracts and isolated compounds was evaluated using disc diffusion and broth micro dilution methods. DCM/MeOH (1:1) extracts of *A. schimperi*, and *R. glutinosa*, exhibited maximum inhibition of *S. aureus* with ZI of 15.6 ± 0.47 , and 17.3 ± 0.81 , respectively, compared to ciprofloxacin (31.0 ± 0.0 mm). MeOH extract of *R. glutinosa* showed the highest antibacterial activity against *S. aureus* with ZI of 18.0 ± 0.0 mm, compared to ciprofloxacin (31.0 ± 0.0 mm, at 0.5mg/mL). DCM/MeOH (1:1) and MeOH extracts of *C. spinarum* also showed promising activity against *S. pyogenes* with ZI of 18.6 ± 0.57 and 18.3 ± 0.81 mm, respectively, compared to ciprofloxacin (30.0 ± 0.0 mm at 0.5mg/mL). DCM/MeOH (1:1) and MeOH extracts of *T. vogelii* demonstrated maximum activity against *P. aeruginosa* with MIC values of 0.5 ± 0.0 and 0.66 ± 0.2 mg/mL, respectively, compared to ciprofloxacin (0.078 ± 0.0 $\mu\text{g/mL}$). Maackian (**103**) showed maximum antibacterial activity against *P. mirabilis* and *P. aeruginosa* with MIC values of 0.5 ± 0.0 and 0.83 ± 0.0 mg/mL, respectively, compared to ciprofloxacin (0.312 and $0.078 \mu\text{g/mL}$). Lupeol (**107**) and methyl gallate (**114**) showed maximum inhibition of *S. aureus* with ZI of 17.3 ± 0.81 and 18.6 ± 0.57 mm, respectively, compared to ciprofloxacin (31.0 ± 0.0 mm). EO extracts from *W. uniflora* and *A. schimperi* exhibited maximum inhibition of *S. aureus* (ZI of 17.6 ± 0.47) and *E. coli* (ZI of 17.3 ± 0.81 mm). DCM/MeOH (1:1) and MeOH extracts of the roots of *W. uniflora*, *T. vogelii*, *A. schimperi*, *R. glutinosa*, and *C. spinarum* demonstrated promising DPPH radical scavenging with IC_{50} values ranging from 12.58 to 44.0 $\mu\text{g/mL}$, compared to ascorbic acid (5.83 $\mu\text{g/mL}$). Compounds **98, 99, 102, 103, 105, 108, 114, and 115** displayed strong DPPH radical scavenging with IC_{50} values of 6.48, 9.48, 10.43, 10.73, 10.97, 10.66, 7.48, and 6.07 $\mu\text{g/mL}$, respectively, compared to ascorbic acid (5.83 $\mu\text{g/mL}$). DCM/MeOH(1:1) and MeOH extracts of *A. schimperi* showed antiviral activity against MDCK cells of anti-influenza A and B with IC_{50} values of 2 and 25.1 $\mu\text{g/mL}$, respectively. The docking analysis results revealed that compounds **101, 103, and 104**, showed strong binding affinity towards PDB ID: 2UV0, PDB ID: 6F86, and PDB ID: 3T07 targets with binding energies of -7.6, -6.6 and -6.8 kcal/mol respectively, compared to ciprofloxacin with respective binding energies of -7.3, -7.4, and -7.2 kcal/mol. Compounds **101, 102, and 115** also showed strong binding interaction towards PDB ID: 4M8I, PDB ID: 1E9Y and PDB ID 6UKD receptors with respective binding energies of -7.9, -7.0 and 5.7 kcal/mol compared to ciprofloxacin (ranging -6.8 to -8.8 kcal/mol). Towards PDB ID: 1DNU receptor, compounds **96, 98, 99, 102, 104, 105, 108, 114, 115, 116, and 117**

*exhibited promising binding free energies from -4.8 to -10.0 kcal/mol, compared to ascorbic acid (-5.7 kcal/mol). Towards topoisomerase II α (PDB ID 4FM9) receptor, compounds **97**, **98** and **103** displayed the lowest minimum binding energies of -9.1, -9.8, -9.2 kcal/mol respectively compared to vosaroxin (-7.8 kcal/mol). Compounds **97**, **101**, **102**, **103**, **104**, **105**, **107**, **108**, **114**, **115**, **116**, and **117** obeyed Lipinski's rule of five with no violations. The proTox II screening analysis results revealed that all compounds under investigation were found to be non hepatotoxicity, carcinogenic, mutagenic, and cytotoxic. Given the important antimicrobial and antioxidant activities demonstrated by plant extracts and isolated compounds, further research should focus on the development of these extracts into therapeutic agents. Future studies should include detailed safety evaluations, in vivo studies, and clinical trials to confirm efficacy and safety for potential therapeutic applications. Additionally, exploring the mechanism of action and optimizing extraction methods could enhance the yield and effectiveness of these plant-based antimicrobial, antioxidant, and antiviral agents.*

Keywords: *Phytochemical, Antibacterial, Inhibition zone, Cytotoxicity, Molecular docking, Binding energy, Isolated compounds*

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the Study

Since ancient times, natural products have played a vital role throughout the world in treating and preventing human and livestock diseases. Natural-based medicines have come from various sources including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates (Hosseinzadeh *et al.*, 2015b). Natural products are important sources of new and good lead compounds for further drug discovery (Lahlou, 2013). Many of the conventional drugs produced by the pharmaceutical industry come from molecules isolated from plants or other living organisms. About 35% of the annual global market of medicine is either from natural products or related drugs; mainly including plants (25%) followed by microorganisms (13%) and animal (3%) sources (Najmi *et al.*, 2022). Among 177 anticancer-approved drugs, about 70% of entities are natural products or their derivatives (Saikat and Chakraborty, 2017). Primarily, plants are the source of medicine and directly contribute to developing new drugs in addition to agrochemicals, cosmetics, and food (Da Silva and Rodrigues, 2014). Throughout the ages, humans have relied on plant products for their basic needs for the production of foodstuffs, shelter, clothing, means of transportation, fertilizers, flavors and fragrances, and, not the least, medicines (Karunamoorthi *et al.*, 2013).

Humans have learned that plants have been a good source of drugs for a long time (Dzobo, 2022). Plants are the basis of abundant chemical constituents that exhibit various therapeutic properties and are continuously explored to develop novel drugs (Nasim *et al.*, 2022). Medicinal plants have long been utilized for the prevention and treatment of various disease conditions such as diabetes, oxidative stress, malaria, typhoid fever, schistosomiasis, onchocerciasis, lymphatic, African dengue, and trypanosomiasis (Ojah, 2020). Numerous scientific investigations have demonstrated the maximum therapeutic values and minimum side effects of herbal remedies (Maridass and De Britto, 2008). According to the World Health Organization (WHO), about 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants (Selvamohan *et al.*, 2012). The

medicinal importance of plant-derived compounds lies not only in their pharmacological or chemotherapeutic effects but also in their role as template molecules for the discovery of new drug substances (Phillipson, 1994). The effectiveness of traditional medicine has been acknowledged because of its better compatibility with the human body, better cultural acceptability all over the world, and lesser side effects (Mustafa *et al.*, 2017). Phytochemicals are secondary metabolites that are organic compounds synthesized by plants which do not directly involved in the fundamental processes of growth, development, or reproduction (Chaachouay and Zidane, 2024). Thousands of structurally differing secondary metabolites have evolved during plant development as a means for plants to defend themselves against herbivores, bacteria, fungi, and viruses. These classes of compounds include alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, and tannins are pharmacologically active compounds attributed to the therapeutic values of the corresponding medicinal plants (Rios and Recio, 2005). Plants produce these chemicals to protect themselves but recent research demonstrated that many of these compounds can protect humans and animals against different diseases (Muthee *et al.*, 2016). Plant secondary metabolites have been reported to possess antispasmodic, antimalarial, anticancer, antibacterial, antiviral, hypoglycemic effects, antioxidants, cardioprotective agents, immune system promoting, and skin protection from UV radiation properties (Carsanba *et al.*, 2021; Shakya, 2016; Tungmunthum *et al.*, 2018).

Centuries ago, the widespread use of plants for medicinal purposes was documented, primarily in the form of crude drugs such as tinctures, powders, and various herbal formulations (Gullo *et al.*, 2006). Plant-derived organic compounds are also still used for treating various diseases either in their natural form (as pure drug or phytomedicine) or serve as lead molecules for the development of synthetic semisynthetic analogs with improved drug efficacy (Najmi *et al.*, 2022). The use of plants as medicines has involved the isolation of active compounds, beginning with the isolation of morphine (**1**) from opium in the early 19th century and latterly discovery of historically important modern drugs such as quinine (**2**), vincristine (**3**), emetine (**4**), artemisinin (**5**), silymarin (**6**), cocaine (**7**), pilocarpine (**8**), codeine (**9**), topotecan (**10**) and digoxin (**11**), (Figure 1) from medicinal plants signify the huge potential that still exists for the production of many more novel pharmaceuticals (Balunas and Kinghorn, 2005; Chaachouay and Zidane, 2024; Geyid *et al.*, 2005; Katiyar *et al.*, 2012; Ulrich-Merzenich *et al.*, 2007). Several mechanisms

underlie the remedial action of plant-derived compounds. Phytochemicals can act by disrupting microbial membranes (Ginovyan *et al.*, 2017) or impairing cellular metabolism, control biofilm formation and by reducing microbial toxin production (Upadhyay *et al.*, 2014).

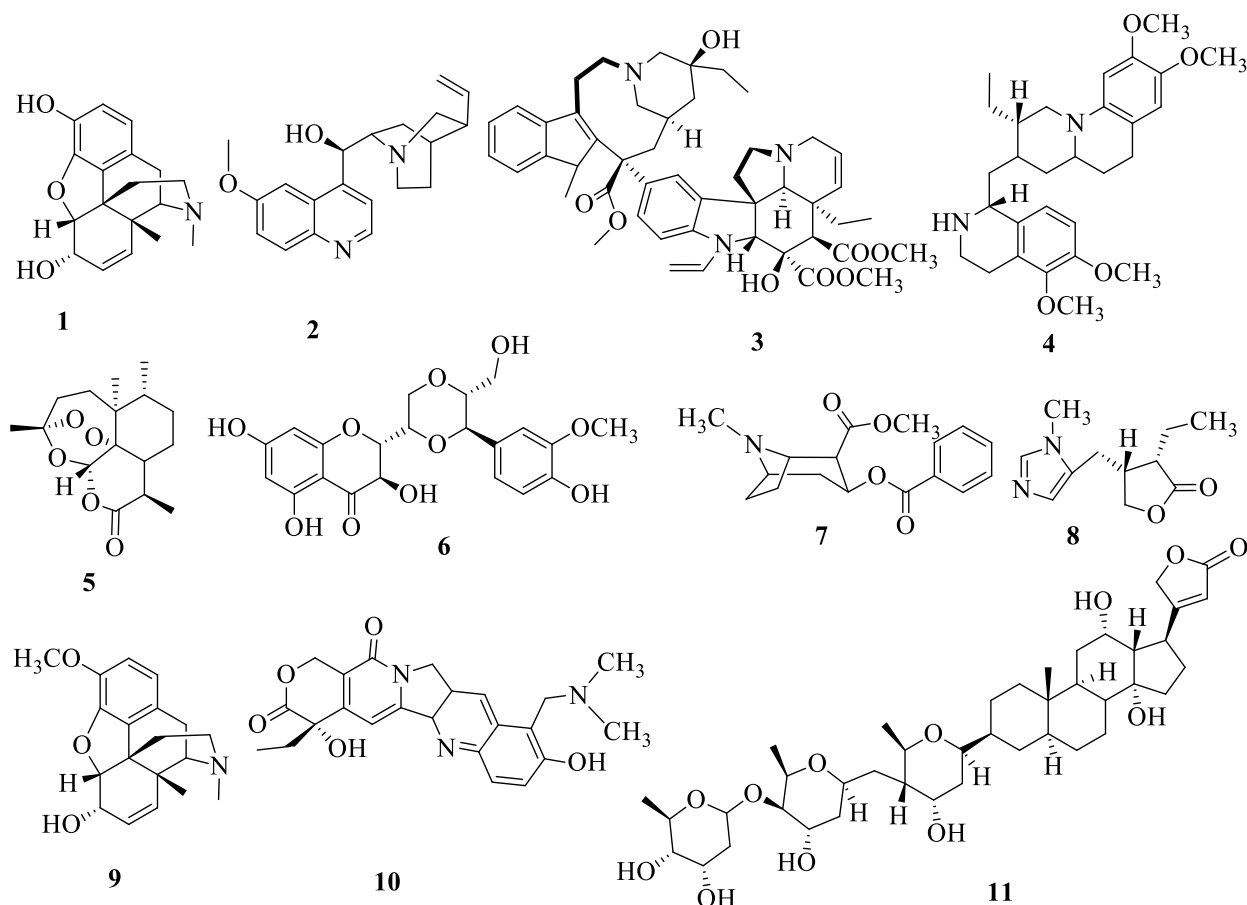


Figure 1: Structure of historically important drugs (**1-11**) isolated from plants.

Conventionally, the process of drug design, development, and commercialization is a tedious, time-consuming, and cost-intensive process (Tripathi *et al.*, 2016). On average, the standard process of discovery and development of drugs to marketing takes from 10 to 15 years. Furthermore, the average cost for research and development of each effective drug is estimated at USD 1.8 billion (Prada-Gracia *et al.*, 2016).

Drug discovery involves a multi-step process that begins with the identification of a suitable drug target, validation of the drug target, hit to lead discovery, optimization of lead molecules, and preclinical and clinical phases (Gurung *et al.*, 2021). Recently, computational approaches have been introduced as a key component in drug design and discovery workflows using a wide range of software packages available for analyzing molecular docking simulations such as AutoDock, GOLD, and FlexX (Alkali, 2018). The application of rational drug design as an integral part of computer-aided drug design provides useful insights into understanding the binding affinity and molecular interaction between target and ligand (Sliwoski *et al.*, 2014). The computer-aided drug design method utilizes the 3D structures of potential drug targets (e.g., enzymes, receptors, or ion channels) that are involved in the process of disease and the assembly of possible candidates to design novel molecules or modify existing agonists/antagonists against the target specifically (Gu *et al.*, 2023). Molecular docking has become an essential aspect of *in-silico* drug development by predicting the interaction between a small molecule and a protein at the atomic level (Agu *et al.*, 2023). Molecular docking works to achieve an optimized conformation for both the protein and ligand, the relative orientation between protein and ligand such that the free energy of the overall system is minimized (Rangaraju and Rao, 2013).

In Ethiopia, traditional medicine is not only part of the struggle of the people to fulfill their primary healthcare but also an integral component of the cultural beliefs and attitudes (Tadeg *et al.*, 2005b). Plants have been used in the prevention and treatment of various human and livestock diseases from time immemorial throughout the country (Hishe and Asfaw, 2014). The extensive use of traditional medicine in Ethiopia could be attributed to efficacy against certain types of ailments, economic affordability, physical accessibility, and cultural acceptability as compared to modern medicine (Maryo *et al.*, 2015). Ethnobotanical studies revealed that a wider range of Ethiopian medicinal plants are being used in the treatment of various diseases in the traditional healthcare system of the country. The areas of cancer and infectious diseases have a leading position in the utilization of medicinal plants as a source of remedy. For instance, *Aerva javanica*, *Asparagus africanus*, *Bersama abyssinica*, *Brucea antidysenterica*, *Cucumis prophetarum*, *Dorstenia barnimiana*, *Kalanchoe pinnatifida*, *Stephania abyssinica*, *Withania somnifera*, and many other species have been reported on their

cytotoxicity and antimicrobial properties (Abate *et al.*, 2022; Mulugeta Alemu Lulekal *et al.*, 2024; Tesfaye *et al.*, 2020a). Similarly, *Eucalyptus globulus*, *Allium sativum*, *Zingiber officinale*, *Ruta chalepensis*, *Ocimum lamiifolium*, and *Catha edulis* have been reported for their potential for the management of respiratory tract disorders (Teka and Maryo, 2023). Identifying new biologically active compounds from the local medicinal plants was vital in modern drug development. However, even though Ethiopia is the home of medicinal plants, different infectious and chronic diseases still threaten public healthcare. Moreover, the lack of experimental validation regarding the efficacy of the therapeutic potential of medicinal plants against specific target diseases demands a scientific investigation to support the traditional values of the plants. For instance, ethnobotanical survey of Ethiopian medicinal plants revealed that *Woodfordia uniflora*, *Tephrosia vogelii*, *Acokanthera schimperi*, *Rhus glutinosa*, and *Carissa spinarum* have been used for the treatment of various human and livestock diseases (Das *et al.*, 2007). However, previous phytochemical investigations done on these plants are very limited compared to their profound traditionally therapeutic uses. Considering this fact, the work carried out in this study involves evaluating antiviral, bacterial, and antioxidant activities, and molecular docking analysis of secondary metabolites isolated from these Ethiopian medicinal plants.

1.2. Statement of the Problem

Microbial diseases continue to threaten the world regardless of efforts and progress in developing modern medicine. The impact of microbial diseases is particularly significant in developing countries like Ethiopia, where access to modern medications is limited and prices are often unaffordable (Lulekal *et al.*, 2014b). Currently, emerging and re-emerging transmissible diseases continue to inflict danger on mankind (Aschale *et al.*, 2021). The extensive, inappropriate, irregular, and indiscriminate uses of antibiotics have resulted in the emergence of antimicrobial resistance, making many currently available medications ineffective (Vaou *et al.*, 2021). The influence of antimicrobial resistance (AMR) is a huge concern, which results in the greatest loss to the individual and social economy. It is estimated that by 2050, the death rate due to AMR will balloon to 10 million lives per year at the expense of one hundred trillion dollars (Anand *et al.*, 2019). This alarming issue making that infectious diseases have become a serious global health problem, with a significant increase in morbidity

and mortality. Recently, more bacteria have become resistant to available antibiotics. In response to the negative effects and growing bacterial resistance, the pharmaceutical industry urgently seeking antimicrobial medications that are less harmful to the host and more effective against microorganisms (Haddou *et al.*, 2023). The spread of multidrug-resistant pathogens and the deteriorating number of available antibiotics forced researchers to seek renewed interest in utilizing plant-based sources to identify potent novel antimicrobial agents (Keita *et al.*, 2022).

In Ethiopia, ethnobotanical surveys conducted in different parts of the country showed that numerous medicinal plants have been used for the treatment of various infectious diseases (Habtamu *et al.*, 2010). Despite the rich heritage of traditional medicine in Ethiopia, many medicinal plants remain underexplored in terms of scientific research. There is a pressing need for systematic investigations aimed at isolating bioactive compounds from these traditional medicinal plants and rigorously evaluating their biological activities. Such studies could unlock the potential of these plants, providing valuable insights into their therapeutic properties and contributing to the development of new pharmacological agents (Tesfaye *et al.*, 2020b). Thus, this study aimed to evaluate cytotoxicity, antibacterial, and antioxidant activities, and molecular docking studies of secondary metabolites derived from *W. uniflora*, *T. vogelii*, *A. schimperi*, *R. glutinosa*, and *C. spinarum*.

1.3. Justification of the Study

Ethiopia is endowed with a huge potential for medicinal plants and their uses that provide a wide contribution to the treatment of human and livestock ailments (Kebede *et al.*, 2016). Various traditional medicinal plants are still used as an alternative solution for different human and livestock health problems (Araya *et al.*, 2015). *W. uniflora* is used for the management of dysentery, toothache, as a sedative, and as medication for skin ailments (Eui Jin Kim *et al.*, 2021; Kroes *et al.*, 1993; Syed *et al.*, 2013). *T. vogelii* have insecticidal, food parasitocidal, antifeedant, antibacterial, and pest crop control properties (Mofor *et al.*, 2013). Different parts of *A. schimperi* are used for the treatment of headaches, eye disease, skin infection, snake bites as anti-venom, and tonsillitis (Abrha *et al.*, 2018). *R. glutinosa* has been used for the management of ectoparasites and scabies (Yemane *et al.*, 2018). *C. spinarum* is traditionally used to treat typhoid fever, chickenpox, fever, skin diseases, gastric ulcers, asthma, infertility,

hypertension, and kidney complications (Berhanu *et al.*, 2020). Regardless of the great role of traditional medicine in primary healthcare, the studies presented so far on medicinal plants were limited to ethnobotanical surveys (Dubale *et al.*, 2023), and their phytochemistry investigation is found to be insignificant compared to the wealth of Ethiopian ethnomedicinal plants (Lulekal *et al.*, 2014a). The lack of experimental justification regarding the efficacy of the therapeutic potential of medicinal plants against specific infectious diseases demands insightful scientific investigations. The process of identifying new biologically active compounds from the local traditional medicinal plants have a vital role in developing modern drugs. Taking this into account, Ethiopian traditional medicinal plants must be investigated for isolation of chemical constituents and examined for antimicrobial activities. Therefore, herein, we investigated the isolation, and evaluation of biological activities of the roots of *W. uniflora*, *A. schimperi*, *T. vogelii*, *R. glutinosa* and *C. spinarum*.

1.4. Objectives

1.4.1. General objective

The overall objective of this study was to evaluate the antibacterial, antioxidant, and antiviral activities of extracts, essential oils, and isolated compounds from the roots of *W. uniflora*, *A. schimperi*, *T. vogelii*, *R. glutinosa*, and *C. spinarum* and conduct molecular modeling studies of these compounds.

1.4.2. Specific objectives

- ✓ To isolate compounds from the roots of *W. uniflora*, *T.vogelii*, *A. schimperi*, *R. glutinosa*, and *C. spinarum* using silica gel column chromatography.
- ✓ To elucidate the structure of the isolated compounds using spectroscopic (IR, ¹H, and ¹³C (1D and 2D NMR)) techniques
- ✓ To extract essential oils from the roots of *W. uniflora*, *T. vogelii*, *A. schimperi*, *R. glutinosa*, and *C. spinarum* using hydrodistillation
- ✓ To analyze the essential oils of the studied plants using GC-MS
- ✓ To evaluate the antibacterial activities of extracts and isolated compounds against Gram-positive bacteria (*S. aureus*, *S. pyogenes*, *E. faecalis*, *S. epidermidis*, and *S. agalactia*)

and Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *S. dysenteriae*, and *P. mirabilis*) using agar disk diffusion and broth dilution method.

- ✓ To evaluate the antioxidant potential of crude extracts and the isolated compounds using DPPH assay.
- ✓ To evaluate the antiviral activity of crude extracts and isolated compounds against influenza A and B using MTT assay
- ✓ To conduct *in silico* molecular docking analysis of compounds towards antibacterial and antioxidant target proteins using the Auto Dock Vina 4.2. program
- ✓ To predict the ADMET analysis of the isolated compounds using the SWISSADME/T online server

1.5. Significance of the Study

Ethiopia is home to above 6500 higher plant species (Bultum *et al.*, 2019). Many medicinal plants play a major role in treating various diseases and protecting human health, especially in rural areas of Ethiopia. Phytochemical investigations on the identification of the chemical makeup and evaluation of the biological activities of medicinal plants are in great demand. To the best of our knowledge, the result of this study may:

- ✓ Provide Justification for the ethnobotanical use of the plants
- ✓ Help to get new antiviral and antimicrobial natural compounds
- ✓ Help to get new antioxidant metabolites
- ✓ Provide lead compounds for the development of modern drugs
- ✓ Fill the gap in the previous studies on selected plant species
- ✓ Used as a reference for further work on the studied medicinal plants.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Traditional Medicinal Plants

World Health Organization (WHO) defines herbal medicine as plant-derived material and products that have therapeutic and other positive effects on human health and contain raw or processed ingredients from one plant or a combination of different plants (Zaidi *et al.*, 2022). The active components of various parts of medicinal plants such as seeds, roots, leaves, fruit, flowers, or even the whole plant have direct or indirect therapeutic effects (Mulugeta Alemu Asfaw *et al.*, 2024). Indigenous communities have developed various traditional healthcare systems using locally available resources for the improvement of the well-being of human beings (Enyew *et al.*, 2014). Historical accounts of traditionally used medicinal plants depict that different medicinal plants were in use as early as 5000 to 4000 BC in China and 1600 BC by Syrians (Yirga, 2010). The aspiration to capture the wisdom of traditional healing systems has led to a resurgence of interest in herbal medicines, particularly in Europe and North America, where herbal products have been incorporated into so-called alternative, “complementary”, “holistic” or “integrative” medical systems (Hosseinzadeh *et al.*, 2015a).

Plant-derived compounds have been separated and analyzed for their medicinal properties and are still acknowledged for their therapeutic uses in the present day. Previous investigation on drug development indicated that, out of 119 drugs, 74 % were discovered due to chemical studies directed at isolating the active substances from plants used in traditional medicine (Cragg and Newman, 2005). In addition to the biologically active plant-derived secondary metabolites that have found direct medicinal application as drug entities, many other bioactive plant compounds have proven useful as "leads" or model compounds (templates) for drug syntheses or semi-syntheses (Balandrin and Klocke, 1988; Balandrin *et al.*, 1993). Natural product chemists continue to explore a variety of lead structures, which may be used as templates for the development of new drugs by the pharmaceutical industry. There is no doubt

that natural products have been, and will be, important sources of new pharmaceutical compounds (Lahlou, 2013).

2.1.1. Ethiopian traditional medicinal plants

Ethiopia is well-known for its rich biodiversity, which includes a vast array of medicinal plants with significant therapeutic potential. Medicinal plants have been practiced since ancient times and become an important source of the healthcare system throughout the country (Temesgen *et al.*, 2020). Most people in Ethiopia still depend on traditional medicine mainly due to the shortage of pharmaceuticals, inadequate coverage of the modern medical system, and unaffordable prices of modern drugs (Teshome *et al.*, 2023). The existence of many languages, cultures, and beliefs has contributed to the high diversity of traditional knowledge and practices on medicinal plants (Abebe and Teferi, 2021). Nearly 90% of the country's livestock and 80% of humans depend on plant-derived traditional medicine to prevent, alleviate, and treat various health problems (Tahir *et al.*, 2021).

The Ethiopian flora is estimated to have between 6500 and 7000 species, of which 10-12% are endemic (Bultum *et al.*, 2019). About 887 plant species have been reported to be utilized as traditional medicine in Ethiopia (Demisse, 2001). Amongst these, 26 species are endemic and they are becoming gradually rare and are on the average of extinction (Gonfa *et al.*, 2020). In Ethiopia, medicinal plants are used in the treatment of arthritis, colds, constipation, diabetes, dysentery, eclampsia, gastritis, gonorrhea, headache, hemorrhoids, hepatitis, herpes simplex, kwashiorkor, leprosy, malaria, measles, rabies, rheumatism, scabies, syphilis, and toothache (Aragaw *et al.*, 2020). The existing literature demonstrated that medicinal plants such as *Achyranthes aspera*, *Acokanthera schimperi*, *Zingiber officinale*, *Ximenia americana*, *Ekbergia capensis*, *Dodonaea angustifolia*, *Coleus barbatus*, and many more plants have been reported to their antiviral activity (Fekadu *et al.*, 2024). A similar study conducted by Tesfaye *et al.*, (2020) revealed that *Crissa spinarum*, *Croton macrostachyus*, *Cucumis prophetarum*, *Withania somnifera*, *Vernonia amygdalina*, *Linum usitatissimu*, *Kniphofia foliosa*, *Euphorbia tirucalli*, *Podocarpus falcatus*, *Plumbago zeylanica* and *Bersama abyssinica* have been reported for their anticancer activity (Tesfaye *et al.*, 2020a). The indigenous knowledge system in Ethiopia is not fully documented when compared to available multiethnic, cultural, and flora diversity (Enyew

et al., 2014). The traditional knowledge in Ethiopia is passed verbally from generation to generation and valuable information can be lost whenever a conventional medical practitioner passes without conveying his traditional medicinal plants knowledge (Birhanu *et al.*, 2015). Even though most of the Ethiopian population profoundly depends on medicinal plants for their primary healthcare needs, minimal work has been done so far to record and analyze the associated knowledge and validate the therapeutic values of the demanded plants (Nuro *et al.*, 2024). Despite the significant role of medicinal plants in supporting national primary healthcare, the efforts on antimicrobial screening of the country's medicinal flora remained minimal and were found to be insignificant compared to the wealth of Ethiopian ethnomedicinal plants and associated knowledge.

2.2. Ethnobotanical Uses, Phytochemistry, and Pharmacological Activities of the Selected Medicinal Plants

2.2.1. Ethnobotanical uses of the selected medicinal plants

2.2.1.1. Ethnobotanical description of *W. uniflora*

The genus *Woodfordia* (Lythraceae) comprises two well-known morphologically similar species namely *W. fruticosa* and *W. uniflora*. *W. fruticosa* is widely found in Asia while *W. uniflora* (Figure 2) is extended in the Middle East and East Africa (Graham, 1995). In southern Asia, the dried flowers of *Woodfordia* species have been used as an astringent for treating ulcers, wounds, and diarrhea, and its leaves have been used for their antibiotic and sedative properties (Yu *et al.*, 2021a). Literature reviews concerning *W. uniflora* highlight its extensive history of medicinal use, particularly in the treatment of various ailments such as rheumatism, bowel complaints, and hematuria (Das *et al.*, 2007). Additionally, the plant has been recognized for its properties as an alexiteric, its efficacy in alleviating dysentery and toothache, and its sedative effects. Furthermore, *W. uniflora* has been utilized in traditional medicine to address skin ailments (Kim *et al.*, 2021; Kroes *et al.*, 1993; Syed *et al.*, 2013). In Ethiopia, the fresh root portion of *W. uniflora* has been also used for the treatment of liver disorders (Garedew and Bizuayehu, 2018).



Figure 2: *Woodfordia uniflora* (Photo taken by Bihon Abera, in 2022, Lalibela, North Wollo, Amhara Regional State, Ethiopia)

2.2.1.2. Ethnobotanical uses of *T. vogelii*

Tephrosia vogelii Hook (Fabaceae) (Figure 3) is a soft woody branching herb widely found in the tropical region of Africa with a wide range of therapeutic applications (Mlozi *et al.*, 2022). Its well-known properties are ichthyotoxic, insecticidal, food parasitocidal, antifeedant, and pest crop control (Mofor *et al.*, 2013). The root part of the plant has been practiced for the treatment of dyspepsia, diarrhea, rheumatism, asthma, hypertension, and urinary disorders (Masete, 2021). *T. vogelii* is used by smallholder farmers in several African nations as an organic pesticide to manage pests on cattle, in cultivated fields, and as a medication for skin disorders (Mpumi *et al.*, 2020). The species is also used ethnomedically in East and West Africa as a purgative, emetic, bactericide, abortifacient, and treatment for schistosomiasis and parasite infections (Hanolo, 2021).



Figure 3: *Tephrosia. vogelii* (Photo taken by Bihon Abera, in 2023, Wolaita Sodo, South Regional, State, Ethiopia)

The leaf extract also exhibited significant activity against lice, mites, fleas, and mosquito larvae (Mlozi *et al.*, 2020). It is also used for the treatment of ringworms, yaws, and constipation (Mlozi *et al.*, 2020; Owor *et al.*, 2020). Literature reports indicated that different parts of the plant have an attractive option for the treatment of cardiovascular problems and also provide a natural source for antihyperlipidemic medications (Bawa *et al.*, 2022). The ethnomedical use and pharmacological investigations established that *T. vogelii* is a promising candidate for bioactive secondary metabolites that could be used to develop pharmaceuticals with a wide range of applications, from promoting agricultural efficiency to maintaining public health (Antonio *et al.*, 2019).

2.2.1.3. Ethnobotanical uses of *A. schimperi*

Acokanthera schimperi (Apocynaceae) (Figure 4) is found in the margins of dry forests, in relict forests, thickets, grasslands, and bushlands, at 1100- 2400m altitude and with 600-1000 mm rainfall (Maundu and Tengnäs, 2005). In Ethiopia, different parts of *A. schimperi* locally called “*Merenz*” (in Amharic) and “*Kararu*” (in Afaan Oromo) are used alone or in combination with other species of plants for the treatment of headache, eye disease, leprosy, rheumatic pain, elephantiasis, curing of skin infection, snake bite as anti-venom, and tonsillitis (Abrha *et al.*, 2018). Its leaves and bark are applied to the skin to treat skin disorders and inflammatory-related diseases in Ethiopia, while in Kenya and Tanzania, the infusion of its pounded root is drunk in small quantities to treat sexually transmitted diseases and common cold (Chaithanya *et al.*, 2018). The leaves and bark are also used to treat different diseases and show antiviral activity in the cases of skin disorders caused by viruses, and antimalarial activity (Gebremariam *et al.*, 2006). In addition, Ethiopian traditional healers used this indigenous plant for the treatment of epilepsy, amnesia, eye disease, syphilis, rheumatic pain, elephantiasis, scabies, leprosy, wounds, eczema, and warts (Mohammed *et al.*, 2014). Dried tender stems, leaves, or roots are crushed with water and squeezed a drops through the nasal region to treat the evil eye (Enyew *et al.*, 2014). The dried bark is ground, powder mixed with water, and taken orally to treat stomach aches while roots are burned on fire and fumigated for curing Hepatitis in Ethiopia (Birhan *et al.*, 2017). The leaves of *A. schimperi* are crushed, powdered, mixed with honey, and then eaten orally for the treatment of malaria (Kassahun *et al.*, 2019). The root of *A. schimperi*

is combined with the root of *C. ficifolius* pounded together and mixed with water and 1 liter is given to cattle once a day for 3 days for the treatment of rabies (Meresa *et al.*, 2017).



Figure 4: *Acokanthera schimperi* (photo taken by Bihon Abera, September 2023, Lalibela, North Wollo, Amhara Regional State, Ethiopia)

2.2.1.4. Ethnobotanical uses of *R. glutinosa*

Rhus glutinosa (“*Xaxeeysa*” in Afaan Oromo and “*Embis*” in Amharic) (Figure 5) is an indigenous tree species in Ethiopia (Enyew *et al.*, 2014). The powdered leaves of *R. glutinosa* are administered for seven days to the affected parts of the body for the treatment of wounds (Fassil and Gashaw, 2019). The dry/fresh stem bark of *R. glutinosa* is orally given for deficiency of vitamin C (Tamene, 2020). In Eritrea, the leaves of *R. glutinosa* have been used for the management of ectoparasites and scabies (Yemane *et al.*, 2018). In Ethiopia, the dried roots and leaves of *R. glutinosa* are used for the treatment of hemorrhoids (Gidey, 2010).



Figure 5: *Rhus glutinosa* (Photo taken by Bihon Abera, August, 2023, Lalibela, North Wollo, Amhara Regional State, Ethiopia).

2.2.1.5. Ethnobotanical uses of *C. spinarum*

Carissa spinarum L., (Apocynaceae) (Figure 6) is found to be widely distributed throughout tropical regions of Africa, Southern Asia, Australia, and various islands of the Indian Ocean (Ansari and Patil, 2018). Different parts of *C. spinarum* (“Hagmsa” in Afaan Oromo) and “Agam” in Amharic) have been used as a traditional remedy for the treatment of headaches, chest complaints, rheumatism, edema, gonorrhea, syphilis, and rabies (Yaya *et al.*, 2022). The dried root bark is grinding and given for the treatment of evil spirits and eye diseases (Assefa *et al.*, 2021). Its root is extensively used in Ethiopian folk medicine to treat various ailments, either alone or in the form of mixtures (Ali and Engidawork, 2022). It has been also reported as useful in the treatment of rheumatism, purgative and snake repellent, and wounds, and has emerged as a good source of traditional medicine for the treatment of, arthritis, microbial infection, epilepsy, viral infection, and cancer diseases (Ayalew *et al.*, 2022). *C. spinarum* has been used in the treatment of microbial infections such as venereal, respiratory, gastrointestinal infections, snake bites, gonorrhea, and sickle cell anemia (Maobe *et al.*, 2022). The dried bark of the root is ground into a fine powder and administered as a remedy for the treatment of evil spirits and various eye ailments (Berhanu *et al.*, 2020). *C. spinarum* produces edible fruits and its roots are traditionally used for their purgative properties as well as to treat worm-infested wounds in animals (Harwansh *et al.*, 2010).



Figure 6: *Carissa spinarum* (Photo taken by Bihon Abera, August 2023, Lalibela, North Wollo, Amhara Region, Ethiopia).

2.2.2. Phytochemistry of the selected plants

2.2.2.1. Phytochemistry of *W. uniflora*

Previous phytochemical investigations have shown that a class of secondary metabolites such as phenolic compounds, tannins, and flavonoids are abundantly present in *Woodfordia* species but most of these studies were based on *W. fruticosa* (Nitha *et al.*, 2012; Parekh and Chanda, 2007). Yu and his colleagues (2021) carried out a phytochemical investigation on methanol extract of leaves of *W. uniflora* leaves using liquid chromatography/mass spectrometry (LC/MS)-based analysis results in the isolation of four flavonoid-type analogs procyanidin B3-3-O-gallate (**12**), rhamnetin 3-O-(6-galloyl)- β -D-glucopyranoside (**13**), quercetin 3-O-(6-galloyl)- β -D-glucopyranoside (**14**), and rhamnetin 3-O- α -L-rhamnopyranoside (**15**) (Yu *et al.*, 2021b) (Figure 7). A similar study conducted by Yu *et al.*, (2020) reports the isolation of polyphenolic compounds (**16-24**) from the leaves of *W. uniflora* (Yu *et al.*, 2020b). Kim and his coworkers (2021) also reported the isolation of a polyphenol, catechin-7,4 -O-digallate (**25**) from the leaves of *W. uniflora* (Kim *et al.*, 2021) (Figure 7).

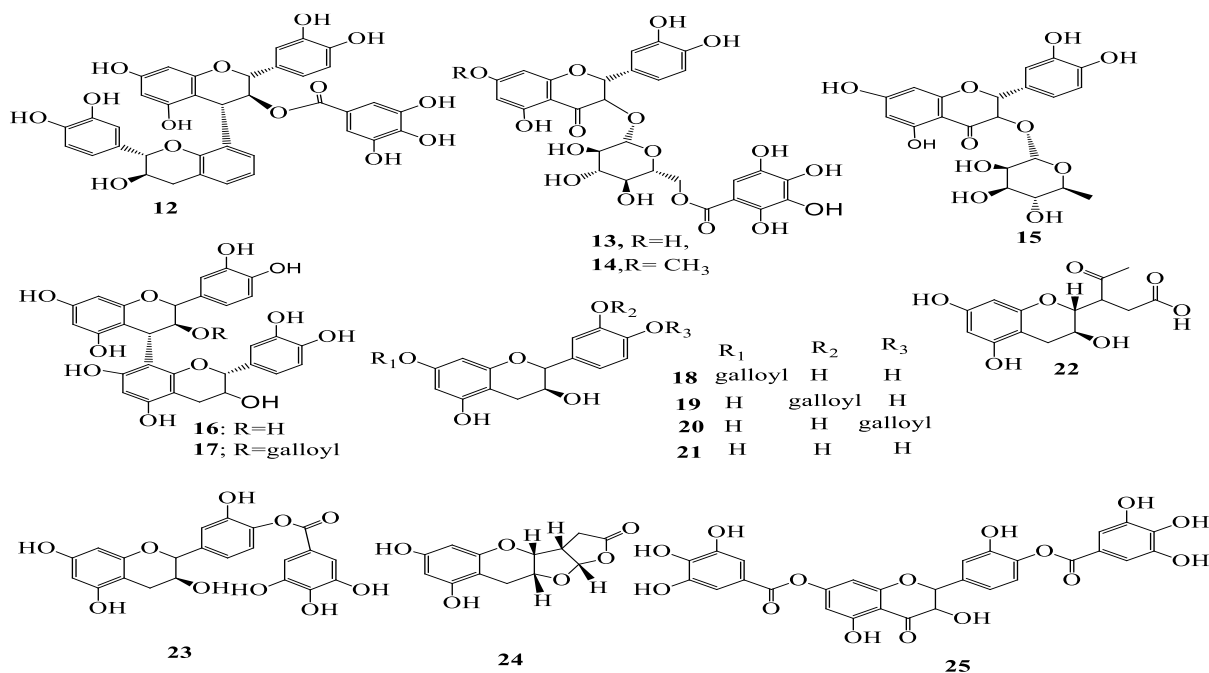


Figure 7: Structure of compounds (**12-25**) reported from leaves *W. uniflora*

2.2.2.2. Phytochemistry of *T. vogelii*

A phytochemical investigation done on the genus *Tephrosia* showed the presence of flavonoids, terpenoids, and sterols. It should be noted that flavonoids are the most abundantly isolated and identified compounds in the genus *Tephrosia* (Touqeer *et al.*, 2013). Yadeta and Tessema (2019) also reported compound **26** and **27** from DCM/MeOH (1:1) extract of the roots of *T. vogelii* (Yadeta and Tessema, 2019) (Figure 8).

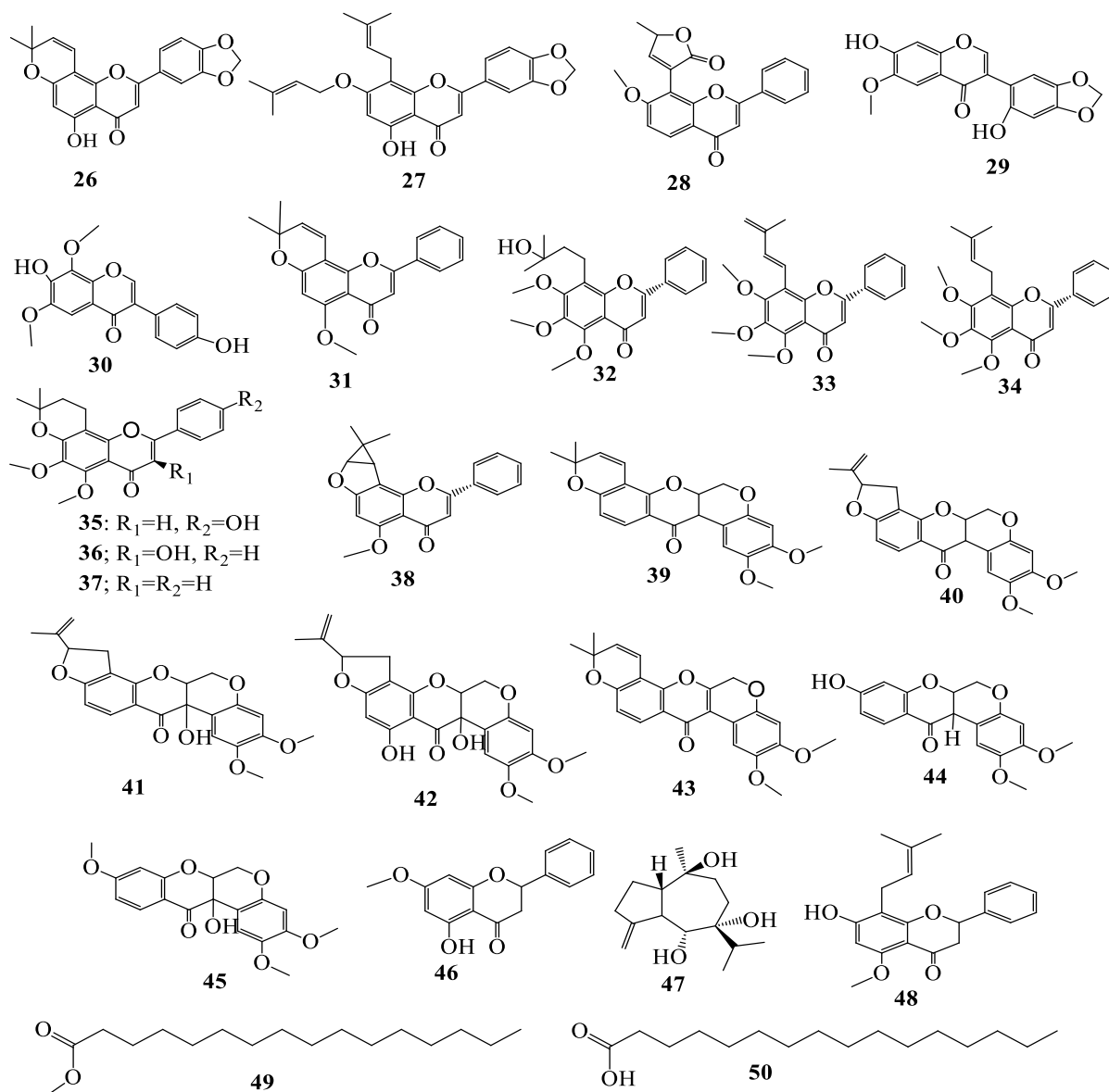


Figure 8: Structure of compounds (**26**-**50**) reported from *T. vogelii*

Tole and Neme (2019) have studied the phytochemistry of *T. vogelii* and reported the isolation of compound **28** from methanol extract of the stem bark of the plant (Tole and Neme, 2019). Vogelisoflavone A (**29**), vogelisoflavone B (**30**) and opongaflavone (**3**) were also reported from dichloromethane/methanol (1:1) extract of seedpods of *T. vogelii* (Owor *et al.*, 2020). A related study conducted by Stevenson *et al.*, (2012) indicated that methanol extracts of the leaves of *T. vogelii* result in the isolations of prenylate flavonoids (**32-38**) (Stevenson *et al.*, 2012). Mikami *et al.*, (2018) also found compounds (**39-43**) from ethanol and methanol extracts of the seed of *T. Vogelii* (Mikami *et al.*, 2018). Mlozi and his colleagues (2022) have reported the identification of compounds (**44-50**) from the GC-MS analysis of the roots and leaves extract of *T. vogelii* (Mlozi *et al.*, 2022) (Figure 8).

2.2.2.3. Phytochemistry of *A. schimperi*

Previous studies on preliminary phytochemical investigation of different parts of *A. schimperi* demonstrated the presence of alkaloids, flavonoids, phenols, terpenoids, and tannins (Yemata *et al.*, 2019). Matebie and his colleagues (2019) identified caryophyllene (**51**), damascenone (**52**), derylacetone (**53**), dihydroactinidiolide (**54**), and (E)-2-tridecenal (**55**) from leaves extract of *A. schimperi* using steam distillation (Matebie *et al.*, 2019). Matebie *et al.*, (2019) also reported lupan-20-ol-3(β)-yl-3-hydroxyoctadecanoate (**56**), along with seven known triterpenoids, 28-nor-urs-12-ene-3,17-diol (**57**), ursolic aldehyde (**58**), alagidiol (**59**), 3-hydroxy-oleana-11,13-dien-28-oic acid (**60**), oleanolic acid (**61**) and ursolic acid lactone (**62**) from ethanol: water (95:5) extract of the leaves of *A. schimperi* (Matebie *et al.*, 2019) (Figure 9). Guliye *et al.*, (2015) examined the phytochemical investigation of *A. schimperi* and reported the isolations of acaricidal coumarins **63** and **64** from methanol extract of the leaves of *A. schimperi* (Guliye, 2015) (Figure 9).

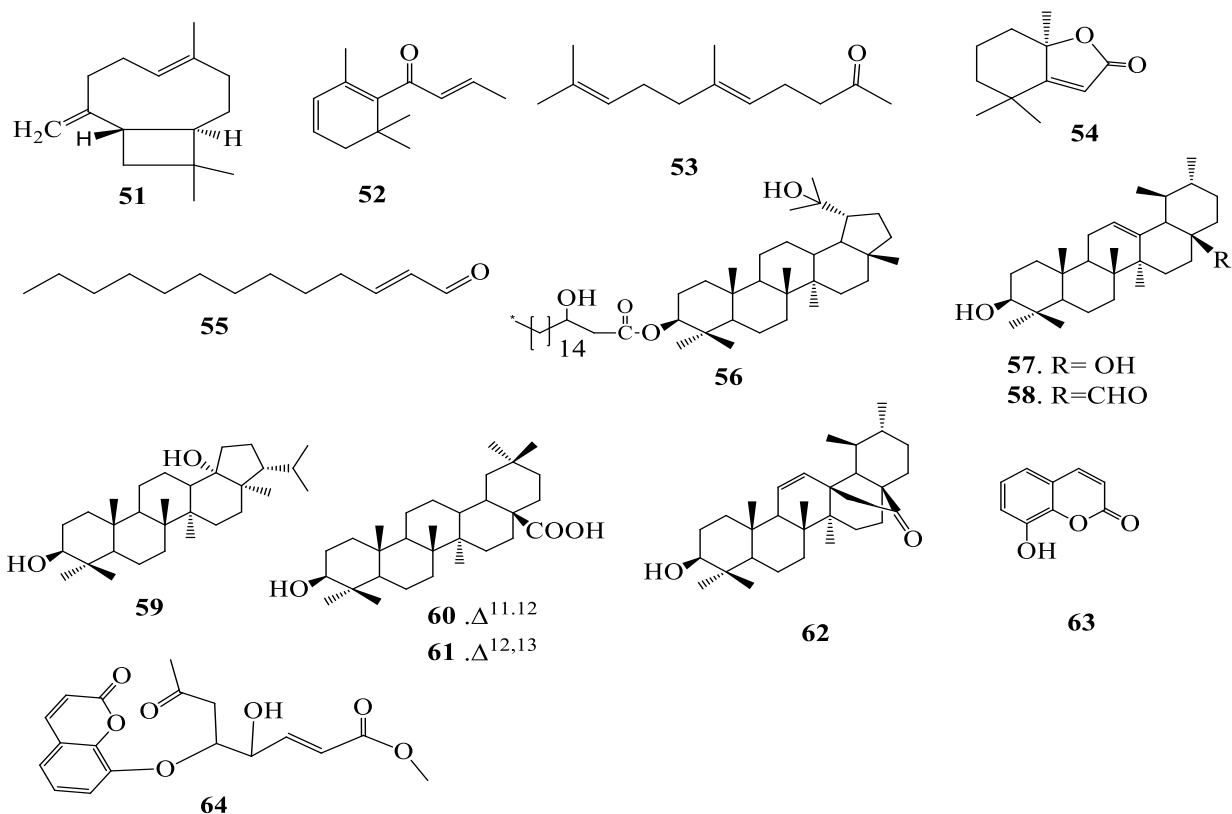


Figure 9: Structure of compounds (51-64) isolated from the leaves of *A. schimperi*.

2.2.2.4. Phytochemistry of *R. glutinosa*

Preliminary phytochemical screening analysis of the genus *Rhus* revealed the presence of flavonoids, terpenoids and glycosides (Opiyo *et al.*, 2021). Sisay and his colleagues (2022) conducted phytochemical investigation on *R. glutinosa* and reported the isolations of acacetin (65), kaempferol (66), rhusflavanone (67), rhusflavone (68), avicularin (69), quercetin (70), myricetin (71), quercitrin (72), hyperoside (73), myricetin-3-O- β -D-galactopyranoside (74) and shikimic acid (75) from DCM-MeOH (1:1) the leaves of *R. glutinosa* (Mammo *et al.*, 2022) (Figure 10).

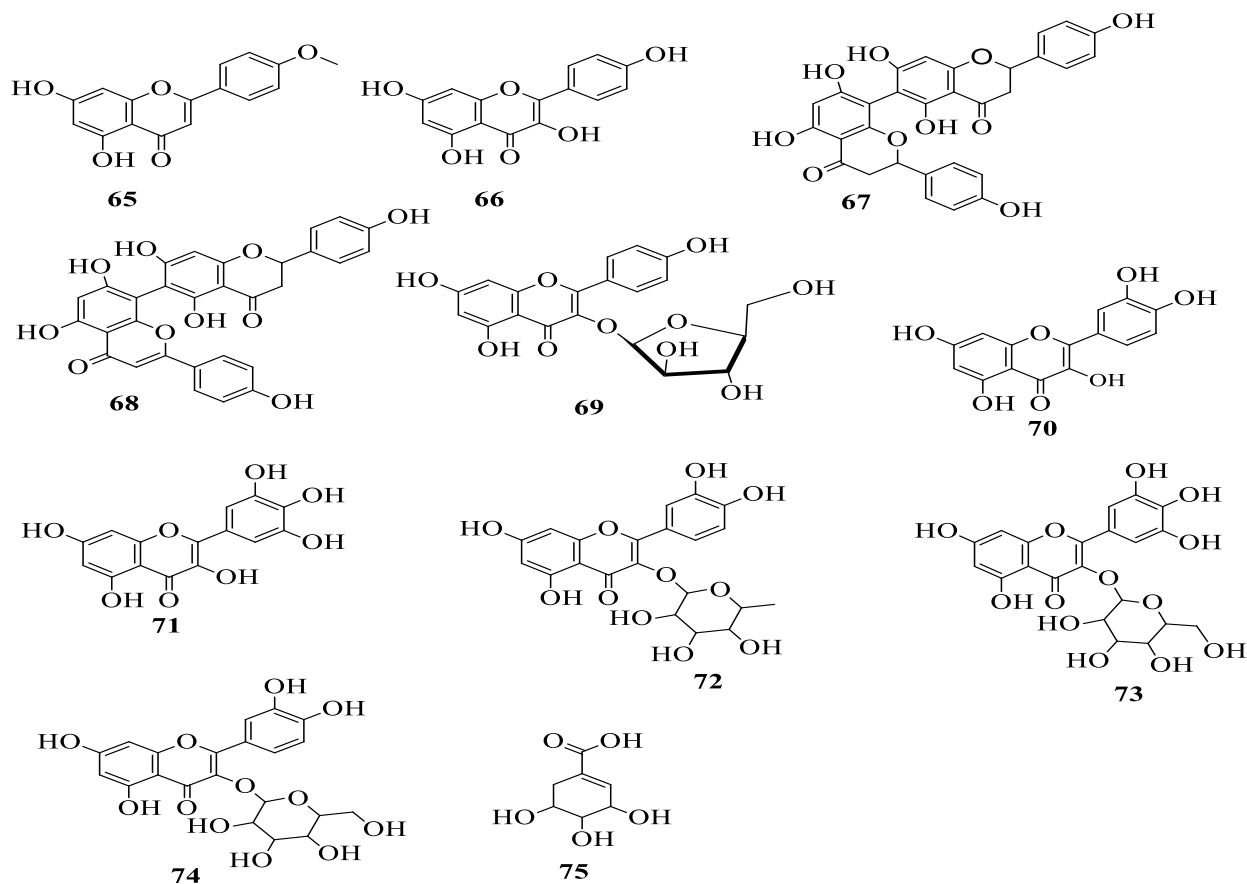


Figure 10: Structure of compounds (65-75) isolated from the leaves of *R. glutinosa*

2.2.2.5. Phytochemistry of *C. spinarum*

Phytochemical analysis of *C. spinarum* reported the presence of tannins, alkaloids, anthraquinones, lignans, anthocyanins, gallic tannins, cardiac glycosides, flavonoids triterpenoids, phenolic compounds, saponins, steroids, and sterols (Berhanu *et al.*, 2020). A study investigated by Jagadeeshwar Rao *et al.*, (2005) reports in the isolation of compound (76-86) from chloroform extract of the stem of *C. spinarum* (Jagadeeshwar Rao *et al.*, 2005) (Figure 11). Liu *et al.*, (2021) have studied the phytochemical investigation on *C. spinarum* and reported the isolations of compounds (87-95) from the methanol extract of root bark of *C. spinarum* (Liu *et al.*, 2021) (Figure 11).

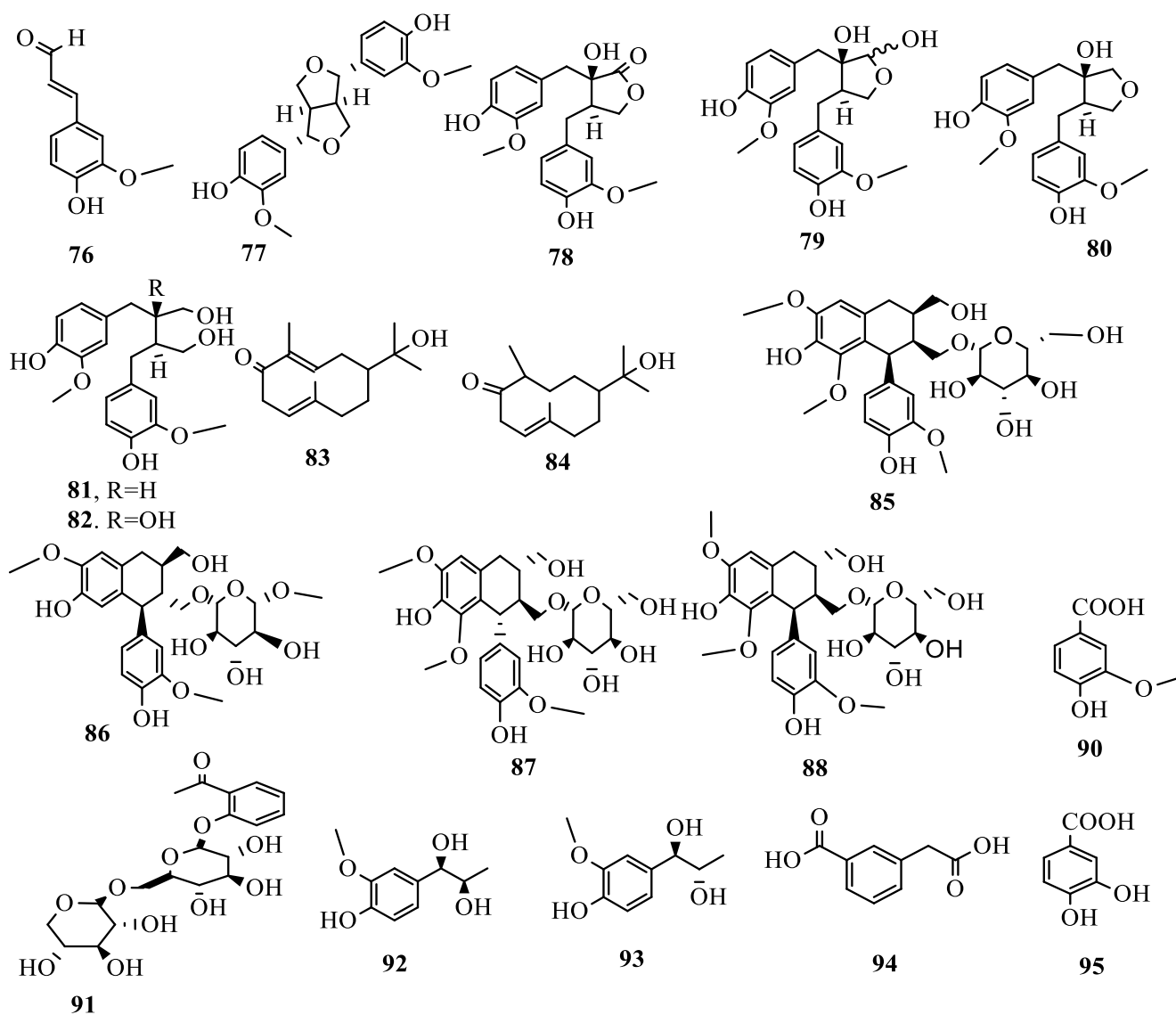


Figure 11: Structure of compounds (76-95) reported from *C. spinarum*

2.2.3. Pharmacological activities of the selected plants

2.2.3.1. Pharmacological activities of *W. uniflora*

A study conducted by Yu *et al.*, (2020a) demonstrated that compound **16**, isolated from the leaves of *W. uniflora* showed antifungal activity against *C. neoformans* H99, *C. neoformans* JEC21, and *C. albicans* SC5314 with IC₅₀ values of 1.9, 3.8 and 14.8 μ M respectively, compared to fluconazole with respective MIC values of 25.5, 6.5, 1.6 μ M (Yu *et al.*, 2020a). According to Kim and his colleague's (2021) investigation, catechin-7,4 -O-Digallate (**24**), isolated from

the leaves of *W. uniflora* exhibited anti-inflammatory activity by inhibiting NO production through the downregulation of inducible nitric oxide synthase expression in lipopolysaccharide (LPS)-stimulated following the treatment of immortalized mouse Kupffer cells (ImKCs) (Kim *et al.*, 2021). Literature reported also indicated that flavonoids bearing galloyl moieties isolated from *W. uniflora* showed antibacterial properties against MRSA, by inhibiting biofilm formation (De Jesus *et al.*, 2023). Methanol extract of flower and leaves of *W. fruticosa*, morphologically a similar species with *W. uniflora* had potent scavenging of DPPH radical with an inhibition percentage of 93.48 and 84.17% respectively at 200 µg/mL, compared to ascorbic acid (percentage of inhibition of $69.28 \pm 0.31\%$) (Grover *et al.*, 2014a). A study investigated by the same author showed that methanol extract of the leaves of *W. fruticosa*, displayed promising antimicrobial activity against *Zymomonas mobilis*, *Staphylococcus epidermis*, *Micromonospora sp.*, *Staphylococcus aureus*, and *Alternaria solani* with MIC values of 13.2, 18.8, 33.86, 20.6, and 19.38 µg/mL, respectively compared to ampicillin with respective MIC values of 15.2, 23.5, 33.2, 20.6, and 19 µg/mL (Grover *et al.*, 2014b). A study carried out by Rahman *et al.*, (2023) revealed that dichloromethane extract of the barks of *W. fruticosa* exhibited antibacterial activity against the growth of *S. boydii*, *S. lutea*, and *S. paratyphi* with inhibition zones of 15, 14 and 12 mm respectively, compared to ciprofloxacin (inhibition ranging from 40-50 mm) (Rahman *et al.*, 2023). As indicated in the literature reports, the phytochemistry and biological activity of *W. uniflora* studied so far mainly focused on the leave part of the plant. Thus, this study was design to explore the chemical constituents and biological activity of the roots of *W. uniflora* aimed to fill the gap of the previous study.

2.2.3.2. Pharmacological activities of *T. vogelii*

Mlozi *et al.*, (2020) have studied on evaluation of the antimicrobial activity of *T. vogelii* using the broth dilution method and found that methanol extract of the roots of *T. vogelii* exhibited moderate activity against *S. aureus*, *K. pneumonia*, *S. typhia*, and *E. coli* with MIC values of 1.25, 2.5, 2.5, and 10 mg/mL respectively compared to ciprofloxacin (MIC values ranging from 0.15625 to 0.625 mg/mL). The same authors also reported that the roots and leaves of *T. vogelii* showed maximum antifungal activity against *C. albicans* (MIC value of 5mg/mL) and *C. neoformans* (MIC value of 1.25 mg/mL), compared to fluconazole (MIC value of 0.625 mg/mL) (Mlozi *et al.*, 2020). A similar study carried out by Mofor *et al.*,(2013) revealed that methanol

extract of the leaves of *T. vogalii* showed antifungal activity against *T. rubrum*, *T. soudanense*, and *T. violaceum* with MIC values of 27.5, 22.5 and 27.5 µg/mL, respectively. The aqueous extract of *T. vogellii* also showed moderate antifungal activity against all tested fungal strains with MIC values of 27.5 µg/mL, compared to amphotericin B (MIC value of 2.4 µg/mL) (Mofor *et al.*, 2013). A study conducted by Masete (2021) indicated that ethanol extract of the leaves of *T. vogellii* had moderate antibacterial activity *S. aureus*, *P. auriginosa*, and *S. typhi* with ZI ranges of 10 to 13 mm, compared to ciprofloxacin (ZI ranging from 38-40 mm) (Masete, 2021). Nenotek and Ludji (2020) evaluated the efficacy of the seed extract of *T. vogellii* and *A. squamosa* on the larvae of *Helicoverpa armigera*. The authors found that the mixture of *A. squamosa* and *T. vogellii* seed extracts killed *H. amigera* larvae with values of LC₅₀ and LC₉₅ of 0.07% and 2.07%, respectively (Nenotek and Ludji, 2020). Wanga and his colleagues (2007) have investigated the antibacterial activity of the leaves of *T. vogellii* and found that DCM extract exhibited significant antibacterial activity against *S. aureus*, *E. coli* and *F. phoseolida* with MIC values ranges between 0.25 to 6.4 µg/mL (Wanga *et al.*, 2006). Similarly, Inalegwu and Sodipo (2015) have studied on evaluation of the antibacterial activity of the leaves of *T. vogellii*. The authors found that aqueous extract of the leaves of *T. vogellii* demonstrated promising antibacterial activity against *S. pneumonia*, *S. epidermidis*, *N. gonorrhoea*, and *E. coli* with IZ of 25, 24, 20, and 18, mm at 300 mg/mL, respectively compared to ciprofloxacin (ZI ranging from 12-28 mm at 10 mg/mL) and ceftaxidine (ZI ranging from 26-28mm). The methanol extracts of the leaves of *T. vogellii* also showed antibacterial activity against *S. pneumonia*, *S. epidermidis*, *N. gonorrhoea*, and *E. coli* with respective ZI of 18, 17, 15, and 12 mm (Inalegwu and Sodipo, 2015). Even though different parts of *T. vogellii* possess a wide range of remedial agents, the previous study conducted on exploring the chemical makeup and pharmacological activity was limited compared to its wealth medicinal value. Therefore, this work aimed to examine on the isolation of secondary metabolites and evaluation of biological activities of the roots of *T. vogellii*.

2.2.3.3. Pharmacological activity of *A. schimperi*

Taye *et al.*, (2011) have studied the antimicrobial activities of water and methanol extracts of the leaves of *A. schimperi* against *S. aureus*, *S. pyogens*, *E. coli*, and *P. aeruginous*, *Proteus vulgaris*, and found that both extracts inhibited the growth of the tested bacteria with inhibition

zones ranging from 7 to 14 mm, compared to amoxicillin (ZI ranging from 14 to 31 mm) (Taye *et al.*, 2011). According to Abrha *et al.*, (2018) study, ethanol extract of the leaves of *A. schimperi* showed considerable inhibition of DPPH, NO, and superoxide radicals with IC₅₀ values of 53.12, 53.48, and 91.08 µg/ml respectively, compared to ascorbic acid with respective IC₅₀ values of 48, 41.23 and 37.77 µg/mL (Abrha *et al.*, 2018). Tadege and his coworkers (2005) conducted a study on the evaluation of the pharmacological potential of *A. schimperi*. According to the author's report methanol extract of the leaves of *A. schimperi* exhibited significant antiviral activity against influenza virus A, coxsackievirus B3, and HSV-1 by inhibiting their replication. The extract also displayed significant antibacterial activity against *S. aureus* and *P. aeruginosa* and antifungal activity against *T. mentagrophytes* (Tadege *et al.*, 2005a).

Getachew and his colleagues (2012) examined the anthelmintic potential of the leaves of *A. schimperi* and the results revealed that the methanol extract showed notable anthelmintic activities with 50% inhibition hatch of an egg of *H. contortus* at 0.85 mg/ml, compared to albendazole (50% inhibition hatch of egg at 0.09 µg/ml) (Getachew *et al.*, 2012). According to Kenubih *et al.*, (2021) study, the ethyl acetate fraction of leaves of *A. schimperi* showed antibacterial activity against *K. pneumonia*, *S. typhi*, *E. coli*, *P. aeruginosa*, and *S. aureus* with ZI of 15.67 ±0.66, 18.25± 0.0, 16.67 ±0.33, 17.67± 0.33, and 16.25 ±0.33 mm respectively at 200 mg/mL, compared to chloramphenicol (ZI ranging from 21.67±0.66 to 23.33±0.33 mm) (Kenubih *et al.*, 2021). A study conducted by Gebre-Mariam and his colleagues (2006) revealed that the methanol extract of the leaves of *A. schimperi*, collected from Ethiopia showed anti-coxsackievirus B3 activity (Gebre-Mariam *et al.*, 2006). As shown in the literature reviews, the previous studies conducted on the phytochemistry analysis and pharmacological activities of *A. schimperi* were only focused on the leaves parts of the plant. Thus, this work aimed to explore on the isolation of secondary metabolites and assessment of biological activity of the roots of *A. schimperi*.

2.2.3.4. Pharmacological activity of *R. glutinosa*

Previous studies conducted on the assessment of the biological activity of the genus *Rhus* demonstrated crude extracts of the *R. glutinosa* possess antimicrobial, cytotoxicity, anticancer,

antioxidant, antiviral, anti-inflammatory, and antimalarial activities (Opiyo *et al.*, 2021). Birhan *et al.*, (2020) have studied the *in vitro* evaluation of the anthelmintic activity of condensed tannins extract of *R. glutinosa* on egg hatchability and larval development inhibition of *Haemonchus contortus* in sheep. The authors found that 70% acetone in aqueous extract of the leaves *R. glutinosa* demonstrated significant inhibition of egg hatchability and larval development with IC₅₀ values of 0.21 mg/mL, compared to albendazole, (IC₅₀ value of 0.08 mg/mL) and 2.27 mg/mL, respectively, compared to ivermectin (IC₅₀ value of 0.66 mg/mL) (Birhan *et al.*, 2020). A related study investigated by Alemu *et al.*, (2014) indicated that condensed tannin extracted from *Rhus glutinosa* had significant egg inhibition egg hatching inhibition of *H. contortus* inhibition (59.67-76%), compared to ivermectin, which inhibited egg hatchability by 50.67-74% (Alemu *et al.*, 2014). Although, different parts of *R. glutinosa* showed numerous therapeutic agents, phytochemical analysis and evaluation of biological activities carried out on plant was very limited. Therefore, this study aimed to examine the chemical constituents and biological activities of the roots of *R. glutinosa*.

2.2.3.5. Pharmacological activity of *C. spinarum*

Sanwal and Chaudhary (2011) studied the antimicrobial activity of the leaves *C. spinarum*. The authors found that methanol extract displayed moderate antimicrobial properties against *E. coli*, *B. subtilis*, *S. aureus*, *Streptococcus sp.*, and *A. niger* with MIC values of 125 ± 10, 512 ± 43, 110 ± 28, 165 ± 20, 256 ± 30 µg/mL respectively, compared to silver sulfadiazine (32-64 µg/mL) (Sanwal and Chaudhary, 2011). A related study investigated by Dhatwalia and his co-workers (2021) demonstrated that the root extract of *C. spinarum* showed antibacterial activity against *P. aeruginosa* and *S. aureus* with MIC values of 8.0 and 312 µg/mL respectively (Dhatwalia *et al.*, 2021). According to Tolo *et al.*, (2006) study, the root extract of *C. spinarum* showed significant *in vitro* antiviral activity ($p < 0.05$) at 1 mg/mL against herpes simplex viruses HSV-I (EC₅₀ of 15.1 ± 0.57 µg/mL), HSV-II (EC₅₀ of 6.9 ± 1.27 µg/mL), HSV-I APr (EC₅₀ of 8.1 ± 1.56 µg/mL), and HSV-I TK- (EC₅₀ of 11.1 ± 5.66 µg/mL), compared to acyclovir against HSV-I (EC₅₀ of 0.91 ± 0.46 µg/mL), HSV-II (EC₅₀ of 0.87 ± 0.44 µg/mL), HSV-I APr (EC₅₀ > 5.0 µg/mL), and HSV-I TK- (EC₅₀ > 5.0 µg/mL) (Tolo *et al.*, 2006). Harwansh *et al.*, (2010) studied the anthelmintic activity of root extracts of *C. spinarum* against an adult Indian earthworm *Pheretima posthuma* and the researchers found that the methanol (100 mg/ml) and

chloroform extract (50 and 100 mg/ml) have equivalent potency compared to piperazine citrate (10 mg/ml) in the time taken for both paralysis and death of *P. posthuma* (Harwansh *et al.*, 2010). According to Liu *et al.*, (2021) study, the dichloromethane fraction of *C. spinarum* showed COX-2 inhibition with IC_{50} value of $0.5 \pm 0.0 \mu\text{g/mL}$ while the ethyl acetate, petroleum, and butanol fractions demonstrated COX-2 inhibition with IC_{50} value $0.2 \pm 0.0 \mu\text{g/mL}$, compared to using indomethacin (IC_{50} of $0.4 \pm 0.1 \mu\text{g/mL}$) (Liu *et al.*, 2021). A study carried out by Fiky *et al.*, (1996) showed that ethanol extract of the leaves of *C. spinarum* (2g/kg) exhibited antidiabetic activity in comparison with the reference drugs (metformin (500 mg/kg) and 3 mg/kg of glibenclamide) in diabetic adult male albino rats (El-Fiky *et al.*, 1996). Motivated by the wide range of biological properties of *C. spinarum* and limited phytochemical analysis, we designed this study to explore the isolation and evaluation of secondary metabolites from the roots of *C. spinarum*.

2.3. Molecular Docking

Drug discovery is a lengthy process that takes around 10-15 years and costs up to 2.558 billion USD for a drug to reach the market (Gurung *et al.*, 2021). It is a multi-step process that begins with the identification of a suitable drug target, validation of the drug target, hit to lead discovery, optimization of lead molecules, and preclinical and clinical studies (Budipramana and Sangande, 2022). Modern pharmaceutical research is progressively shifting toward the exploration of compounds produced by plants due to their medicinal capabilities. The combination of biotechnology and synthetic biology has provided new opportunities for discovering and developing drugs (Chaachouay and Zidane, 2024). Computational methods identifying possible binding modes and binding site residues located in conserved motifs are plausible and scientifically reputable techniques for recognizing lead phytochemicals (Afrose *et al.*, 2022). Computer-aided drug design (CADD) is an area that consists of many computational strategies for the discovery, design, and development of novel therapeutic agents. CADD has a crucial role in improving active ligands, discovering novel drugs, and understanding biological processes at a molecular level (Muhammed and Yalcin, 2024). In this context, molecular docking is among one of the most popular and successful structure-based *in silico* methods, which helps predict the interactions occurring between molecules and biological targets (Kitchen *et al.*, 2004). Since its first appearance in the mid-1970s, docking has proved

to be an important tool to help understand how chemical compounds interact with their molecular targets, and for drug discovery and development (Pinzi and Rastelli, 2019). Molecular docking is a computational method used to predict the interactions between small molecules, such as nutraceuticals, and larger biomolecules, such as enzymes, receptors, RNA, DNA, and other proteins. The technique involves the simulation of the molecular interactions between the small molecule and the target biomolecule, which can provide insights into the binding affinity, binding site, and possible mechanism of action (Agu *et al.*, 2023). It demonstrates how two or more molecular entities communicate with each other, such as medicines and enzyme or protein receptors (Nirmalraj *et al.*, 2021).

Docking experiments have evolved and made a substantial contribution to drug development by replicating biological occurrences and so providing insights into the molecular and geometrical transitions involved with bimolecular systems (Oyedele *et al.*, 2023). The first step in a docking design is to obtain the target structure, which commonly consists of a large biological molecule (protein, DNA, or RNA). The structures of these macromolecules can be readily retrieved from the Protein Data Bank (PDB) which provides access to 3D atomic coordinates obtained by experimental methods (Torres *et al.*, 2019). Protein molecules can be downloaded from the Protein Data Bank website (www.rcsb.org). The downloaded protein structure in their “pdb” format has to be edited to remove the non-amino acid residues, such as water molecules, ions, and ligands that are in the complex (Pawar *et al.*, 2023). Molecular docking programs use scoring functions to estimate the binding energetics of the predicted ligand-receptor complexes. The structure of the ligands is drawn with programs like ChemDraw or downloaded from chemical libraries or databases like PubChem (Kim *et al.*, 2019). After preparing the target and ligand, the grid maps the binding area, which will be the center of docking calculations. Docking simulation is accomplished by first predicting the molecular orientation of a ligand within a receptor, and then estimating their complementarity through a scoring function (Trott and Olson, 2010). The best docking scoring function is selected depending on the stability of the ligand-protein complex. It is difficult to choose a suitable scoring function that gives a correct binding pattern and the possible ligand. Theoretically, the lower the binding free energy (ΔG) of a protein-ligand complex, the more stable the complex is (Muhammed and Yalcin, 2024).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Chemicals and Instruments

HPLC grade solvents such as methanol (Alpha Chemika, India) ethyl acetate ((Alpha Chemika, India), chloroform (Alpha Chemika, India), dichloromethane (LOBA CHEMIE PVT. LTD., India), and petroleum ether (60-80°C) (LOBA CHEMIE PVT. LTD., India) were used for extraction and isolation of compounds. TLC silica gel Merck pre-coated aluminum plate (silica gel GF₂₅₄ German) was used for TLC analysis, and compounds on TLC were identified with a UV lamp (UV4AC6/2, CBIO, Bioscience, and Technologies. Ltd., China) at 254 and 365 nm and spraying with vanillin/H₂SO₄. Silica gel column chromatography was used to isolate compounds (silica gel mesh size of 60-120 mm as stationary phase). FTIR (in KBr) spectra were recorded in Thermo scientific spectrometer SMART iTR basic in NICOLET iS10 model within a 4000-400 cm⁻¹ spectral range. ¹H and ¹³C (1D and 2D) NMR spectral data were analyzed on a JNM-ECZ 400S (400 MHz, JEOL, Ltd. USA) spectrometer. CDCl₃, CD₃OD, and DMSO-*d*₆ were used as solvents for NMR analysis. A digital melting point apparatus was used to determine the melting points of solid compounds. Agar disc diffusion and broth micro dilution methods were to evaluate the antibacterial activity of the extracts and isolated compounds. A double-beam UV-Vis spectrophotometer (P9-UV-Vis, VWR International Ltd, China) was used to measure absorbance at 517 nm for the DPPH assay. Silica gel (60-120 mesh, LOBA CHEMIE PVT. LTD., India) was used for column chromatography. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to evaluate the radical scavenging activity of the extracts and isolated compounds. The antiviral activity of the extracts and isolated compounds was evaluated using MTT assay. Ciprofloxacin and ascorbic acid were used as the standard drug for antibacterial and antioxidant assays respectively.

3.2. Plant Material Collection and Identification

The roots of *W. uniflora* (in April 2022), *A. schimperi* (in April 2023), *R. glutinosa* (in April 2023), and *C. spinarum* (in June 2023) were collected from Lalibela Town, North Wollo Zone, Amhara Regional State, Ethiopia, located about 710 km north of the capital, Addis Ababa. The

roots of *T. vogelii* were collected in February 2023 from Areka Agricultural Research Institute, Wolaita Sodo, Wolaita Zone, South Regional State, Ethiopia, located about 328 km south of the capital, Addis Ababa. The plants were authenticated by Mr. Melaku Wondafrash and a voucher for the specimens BA001, BA002, BA003, BA004, and BA005 were given, respectively, for *A. schimperi*, *W. uniflora*, *T. vogelii*, *C. spinarum* and *R. glutinosa* and deposited at the National Herbarium, Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia.

3.3. Extraction and Isolation of Compounds

3.3.1. Extraction and isolation of compounds from *W. uniflora*

Powdered roots of *W. uniflora* (700 g) were soaked with 4L of DCM/MeOH (1:1) for 72 hr and the extract was filtered and concentrated using a rotary evaporator at 40 °C. The process was repeated twice and obtained 38 g (5.4%) extract. The marc was soaked with methanol (3 L) for 72 hrs, filtered, and concentrated and the process was repeated twice and obtained 26 g (3.7 %) extract. Preliminary TLC profiles of both extracts were analyzed and both DCM/MeOH (1:1) and MeOH extracts exhibited similar spots under a UV lamp. Afterward, both crude extracts (50g) were combined, dissolved with methanol, and then poured into a 1000 mL separatory funnel containing 400 mL of distilled water. Chloroform (400 mL) was added into a separatory funnel containing an aqueous extract solution and shaken continuously at room temperature. An emulsion was formed between the organic phase (chloroform) and the aqueous phase. An emulsion and chloroform soluble fraction were collected and the process was repeated twice. The collected emulsion fractions were combined, concentrated and washed with methanol and gave compound **96** (3.5 g, 0.5% w/w). The chloroform fractions were combined and concentrated using a rotary evaporator and afforded 4 g yield.

Chloroform soluble fraction (4g) was adsorbed with 4 g of silica gel and subjected to chromatographic separation (silica gel 82 g, 60-120 mesh size as stationary phase) and eluted with increasing gradient of EtOAc in *n*-hexane. A total of 86 fractions (each 100 mL) were collected. The TLC profile of each fraction was detected under a UV lamp (254 and 365 nm) and visualized after spraying with vanillin/H₂SO₄. Fractions 18-21 (eluted with 20% EtOAc in *n*-hexane) were combined and washed repeatedly with *n*-hexane and obtained compound **97** (45 mg). Fractions 40 and 41 were combined and purified by silica gel column chromatography

with increasing the gradient of EtOAc in *n*-hexane as eluent to collect a total of 84 fractions (each 10mL). Subfractions 58 and 59 showed a single spot under UV₂₅₄ nm and afforded compound **98** (26 mg). Fractions 57-62 were combined and purified using gradient-increasing of EtOAc in petroleum ether as eluent to collect a total of 52 (each 10 mL) fractions. Subfractions 33-37 (eluted in 50% EtOAc in petroleum ether) showed a single spot under the UV₂₅₄ lamp and obtained compound **99** (13 mg).

3.3.2. Extraction and isolation of compounds from *T. vogelii*

Powdered roots of *T. vogelii* (500 g) were soaked with 3 L DCM/MeOH (1:1) for 72 hr. The extract was filtered and concentrated using a rotary evaporator at 40°C. The marc left was soaked twice with the same solvent, filtered, and concentrated afforded 15 g (3%) extract. The marc left was soaked with methanol (3 L) three times for 72 hr and concentrated and obtained 6 g (1.2%) extract. Preliminary TLC profiles of DCM/MeOH (1:1) and MeOH extracts were analyzed with a UV lamp (254 and 365 nm) and Vanillin/H₂SO₄. The DCM/MeOH (1:1) extract showed good TLC profiles. DCM/MeOH (1:1) extract (13 g) was adsorbed with 13 g of silica gel and subjected to silica gel chromatographic separation (silica gel 120 g, 60-120 mesh size as stationary phase) and column elution began with petroleum ether and gradually increasing gradient of EtOAc. A total of 120 fractions (each 100 mL) were collected. The TLC profile of each fraction was analyzed under a UV lamp (254 and 365 nm) and visualized after spraying with vanillin/H₂SO₄. Fractions 13 and 14 (eluted with 10% EtOAc in petroleum ether) showed a single spot with equal R_f values, combined and washed with methanol and obtained compound (**100**, 45 mg). Fractions 20-26 (eluted with 20% EtOAc in petroleum ether) were combined and re-chromatographed (eluted with increasing the gradient of EtOAc in petroleum ether) to collect 70 fractions (each 10 mL). Subfractions 15-19 showed a single spot under UV₂₅₄ and afforded compound (**101**, 15 mg), and subfractions 41-44 afforded compound (**102**, 16 mg). Fractions 29-35 were combined and purified with PTLC using a mobile phase of 15% EtOAc in petroleum ether and gave compound (**103**, 11 mg). Fractions 40-46 were combined and re-chromatographed using gradient-increasing EtOAc in petroleum ether as eluent. A total of 38 fractions were collected of which sub fractions 23-28 (eluted with 30% EtOAc in petroleum ether) showed a single spot under the UV₂₅₄ lamp and obtained compound (**104**, 18 mg). Fractions 50-54 (eluted with 40% EtOAc in petroleum ether) were combined and re-

chromatographed using gradient increasing of EtOAc in *n*-hexane to collect a total of 42 fractions. Subfractions 31-35 (eluted with 30% EtOAc in *n*-hexane) showed a single spot under UV₂₅₄ lamp afforded compound (**105**, 23 mg).

3.3.3. Extraction and isolation of compounds from the roots of *A. schimperi*

Powdered roots of *A. schimperi* (600 g) were soaked for 72 hrs with 4 L of DCM/MeOH (1:1) and the extract was filtered and concentrated using a rotary evaporator at 40°C. The process was repeated twice and afforded 20 g (4.9%) extract. The marc was soaked with methanol (3 L) for 72 hrs, filtered, and concentrated and the process was repeated twice and obtained 6 g (1%) extract. Preliminary TLC profiles of DCM/MeOH (1:1) and MeOH extract were examined using a UV cabinet (UV₂₅₄ and UV₃₆₅ nm lamps) and vanillin/H₂SO₄ as a spraying agent and DCM/MeOH extract displayed a good TLC profile.

DCM/MeOH (1:1) extract (15 g) was adsorbed with an equal amount of silica gel and subjected to chromatographic separation (silica gel 150 g, 60-120 mesh size as stationary phase). Column elution was done by increasing the gradient of EtOAc in petroleum ether to collect 130 fractions (each 100 mL). The TLC profile of each fraction was detected under a UV lamp (254 and 365 nm) and visualized after spraying with vanillin/H₂SO₄. Fractions 8-13 (eluted with 10% EtOAc in petroleum ether) afforded compound (**106**, 18 mg) as a single spot. Fractions 17-23 (eluted with 10% EtOAc in petroleum ether) showed a similar spot. Combined fractions, and re-chromatographed using an increasing gradient of EtOAc in petroleum ether to collect a total of 36 fractions (10 mL each). Sub-fractions 19-25 (eluted with 10% EtOAc in petroleum ether) showed a single spot after spraying with vanillin/H₂SO₄ to afforded compound **107** (23 mg). Fractions 32-40 (eluted with 30% EtOAc in petroleum ether) were combined and further purified by column chromatography using gradient increasing of EtOAc in petroleum ether. A total of 35 fractions were collected of which sub-fractions 21-25 (30% EtOAc in petroleum ether) showed a single spot under UV₂₅₄ lamp to afforded compound **108** (26 mg). Fractions 76-79 form a white precipitate and showed a single spot after spraying with vanillin/H₂SO₄ and obtained compound **109** (57 mg). Fractions 93-97 (eluted with 10% MeOH in EtOAc) were mixed and purified with silica gel column chromatography with increasing gradient of MeOH

in EtOAc and collected 52 fractions (10 mL each). Subfractions 36-41 showed a single spot to afford compound **110** (40 mg).

3.3.4. Extraction and isolation of compounds from the root of *R. glutinosa*

Powdered roots of *R. glutinosa* (600 g) were soaked with 4 L of DCM/MeOH (1:1) and the extract was filtered and concentrated using a rotary evaporator at 40 °C. The marc was soaked twice with the same solvent, filtered, and concentrated afforded 15 g (2.5%) extract. The marc was soaked with methanol (3 L) for 72 hrs, filtered, and concentrated, and the process was repeated twice with the same solvent system and obtained 7 g (1.16%) extract. Preliminary TLC profiles of both extracts showed similar spots under a UV lamp. The combined extract (16 g) was adsorbed with an equal amount of silica gel and subjected to silica gel column chromatography and eluted with increasing gradient of EtOAc in petroleum ether to afford 150 fractions (100 mL each). The TLC profile of each fraction was analyzed under UV₂₅₄ and UV₃₆₅ lamps. Fractions 11-16 (eluted in 10% EtOAc in Petroleum ether) showed a single spot and afforded compound **97** (23 mg).

Fractions 26-33 (eluted in 20% EtOAc in petroleum ether) were combined and re-chromatographed using an increasing gradient of EtOAc in petroleum ether. A total of 32 fractions were collected, of which sub-fractions 17-22 showed a single spot under the UV₂₅₄ lamp and gave compound **111** (17 mg). Fractions 26-34 were combined and further purified by column chromatography using gradient increasing of EtOAc in petroleum ether as eluent and 52 fractions (each 10mL) were collected. Subfractions 36-41 showed a single spot under the UV₂₄₅ lamp to afford compound **112** (23 mg). Fractions 41-47 were combined, repeatedly washed with n-hexane, and showed a single spot gave compound **113** (28 mg). Fractions 53-62 (eluted with 50% EtOAc in petroleum ether) were combined and purified by silica gel column chromatography using gradient increasing of EtOAc in petroleum ether to collect a total of 62 fractions (10 mL each). Sub-fractions 28-34 (eluted with 50% EtOAc in petroleum ether) and sub-fractions 49-53 (eluted with 60% EtOAc in petroleum ether) showed a single spot under the UV₂₅₄ lamp and obtained compounds **114** (40 mg) and **115** (70 mg) respectively.

3.3.5. Extraction and isolation of compounds from the root of *C. spinarum*

The powdered roots of *C. spinarum* (600 g) were soaked with 4 L of DCM/MeOH (1:1) and the extract was filtered, and concentrated using a rotary evaporator at 40°C. The process was repeated twice and obtained 16 g (2.66%) extract. The marc was soaked with MeOH (3 L) for 72 hrs, filtered, and concentrated and the process was repeated twice with the same solvent system and gave 10 g (1.66 %) crude extract. Preliminary TLC profiles of both extracts were analyzed under a UV₂₅₄ and UV₃₆₅ nm lamp. The DCM/MeOH (1:1) extract showed a better TLC profile. The DCM/MeOH (1:1) extract (12 g) was adsorbed with an equal amount of silica gel and subjected to silica gel column chromatography (120 g, 60-120 mesh size) eluted with increasing gradient of EtOAc in petroleum ether to afford 120 fractions (100 mL each). Fractions 31-37 (eluted with 30% EtOAc in petroleum ether) were combined and purified by column chromatography using gradient increasing of EtOAc in petroleum ether as eluent to give 45 fractions. Subfractions 28-35 (eluted in 30% ethyl acetate in petroleum ether) showed a single spot under the UV₂₅₄ nm lamp and afforded compound **116** (32 mg). Fractions 53-60 (eluted in 50% EtOAc in petroleum ether) were combined and further purified by column chromatography using gradient-increasing EtOAc in petroleum ether to collect a total of 60 fractions. Subfractions 38-44 showed a single spot under the UV₂₅₄ and UV₃₆₅ nm and gave compound **117** (26 mg).

3.4. Essential Oil Extraction and GC-MS Analysis

Hydro distillation extraction and GC-MS analysis of the essential oil constituents of the studied plants were carried out following the standard procedures as described in the literature (Amin *et al.*, 2022). The powdered roots of *W. uniflora* (60 g), *T. vogelii* (70 g), *A. schimperi* (60 g), *R. glutinosa* (70 g), and *C. spinarum* (60 g) were mixed with distilled water (250 mL) in a separate round bottom flask and connected to a Clevenger apparatus then heated for 3 h at 70 °C. The distillate was collected and transferred to a separatory funnel and then extracted with chloroform (150 mL). The organic phase was dried over anhydrous Na₂SO₄ as a water-absorbent agent and chloroform was removed under a vacuum. Finally, the essential oils were kept at -4°C in the refrigerator for further analysis.

GC-MS analysis of the essential oil was established using an Agilent 7890B gas chromatograph with an Agilent 5977B GC/MSB mass spectrometer equipped with an HP-5MS capillary column (30m×0.25 mm, 0.25µm film thickness). The gas chromatography (GC) condition was adjusted as follows: The oven temperature was adjusted at 70°C for the first two minutes and gradually increased by 5 °C per minute until it reached 300 °C. After that, the temperature was adjusted to remain constant for the next two minutes; the injector temperature and volume were kept at 250°C and 1 µL respectively; with a split ratio of 1:20, and the medium of transport was helium. Condition for mass spectrometry (MS): Ion spring temperature was 230 °C, scan range of 30-500 Amu, EI ionization mode, 70 eV. The relative percentages of the identified compounds were calculated based on chromatograms obtained from the GC/FID/MS system (Adinew, 2014).

3.5. Antibacterial Activity

3.5.1. Disc diffusion method

The antibacterial activities of the crude extracts, EO extracts, and isolated compounds of roots *W. uniflora*, *A. schimperi*, *R. glutinosa*, and *C. spinarum* were evaluated using Mueller Hinton agar disc diffusion following the standard procedure reported in the literature (Balouiri *et al.*, 2016) against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and Gram-positive bacteria (*S. aureus* and *S. pyogenes*). In brief, a few colonies (3-5) of bacterial strains with similar morphologies were introduced into a liquid medium (saline solution) using a sterilized inoculating loop. The turbidity of the suspension was adjusted to 0.5 McFarland barium sulfate standards (10^8 CFU/mL). The experiment was carried out at two-fold serial solutions of 200, 100, and 50 mg/mL of DCM/MeOH (1:1) and MeOH crude extracts, and 5, 2.5, and 1.25 mg/mL of the isolated compounds using DMSO as the diluting agent. Bacterial suspensions were applied onto a petri plate containing MHA. Whatman No 1 paper discs (6 mm) were applied on the surface of the inoculated MHA and about 20µl each test sample was applied onto the disks. The samples were tested in triplicates and expressed as mean±SD. Ciprofloxacin (30 µg) was used as the reference drug.

3.5.2. Broth dilution method

Evaluation of the antibacterial properties of crude extracts, EO extracts, and isolated compounds (**101-105**) of *T. vogelii* was carried out against gram-positive bacterial (*S. aureus*, *E. faecalis*, *S. epidermidis*, and *S. agalactiae*), and gram-negative bacteria (*E.coli*, *P. aeruginosa*, *S. dysenteriae*, *P. mirabilis*) following micro dilution method described by literature (Eloff, 1998). All the bacterial strains were obtained from the Armauer Hansen Research Institute (AHRI), Addis Ababa, Ethiopia. Ciprofloxacin and bacteria-free solvent were used as a positive and negative control, respectively.

3.5.2.1. Media and inoculum preparation

Mueller-Hinton agar (19 g) was weighed and then placed into a conical flask with 500 ml of distilled water prepared following the manufacturer's instructions heated to completely dissolve and sterilized by autoclave at 121°C for 20 minutes. Bacterial strains were subcultured onto sterilized and cooled to 50 °C growth media and incubated overnight at 37 °C for 24 h. About 3-5 colonies of the same morphological type were selected from the cultured media and transferred to the sterilized broth (Muller Hinton broth (MHB) using an inoculating loop. The bacterial suspension was adjusted using a UV spectrophotometer by measuring its absorbance with OD values ranging from (0.08 to 0.1) at a wavelength of 625 nm across a 1 cm path length cuvette (Eloff, 1998).

3.5.2.2. Determination of minimum inhibitory concentration

The MIC value of the analyzed samples was evaluated by the 96-microwell plate broth dilution method following the Clinical and Laboratory Standards Institute protocol (Eloff, 1998; Humphries *et al.*, 2018). Two-fold serial dilution concentrations ranging from 8 mg/mL to 0.0625 mg/mL were prepared for essential oil, crude extract, and isolated compounds, and 10 µg/mL to 0.078125 µg/mL for ciprofloxacin were prepared by dissolving in 5% Tween 80 by adding to the respective wells. A freshly cultured bacterial suspension containing about 5×10^5 CFU/mL was prepared from inoculated in sterile broth to a respective organism of (1.0×10^7 CFU/ml) of bacterial suspensions, and 100 µL suspension was added to each well for inoculation, except to the sterility control column wells. The plates were incubated at 37°C for

18-24 h and then 40µl of a 0.4 mg/mL solution of 2,3,5-Triphenyltetrazolium chloride was added to each well for determination of microbial growth and incubated at 37°C for 30 minutes. MIC values were recorded by observing the presence or absence of pink color. The lowest concentration of each analyzed sample showing no visible pink color was noted as the MIC.

3.6. Antioxidant Activity

The antioxidant activities of crude extracts, EO extracts, and isolated compounds of the studied plants were evaluated following the DPPH assay as described by Sasikumar *et al.*, (Sasikumar *et al.*, 2020) using ascorbic acid as standard. Two-fold serial dilution solutions of 1000, 500, 250, 125, and 62.5 µg/mL were prepared from 1 mg/mL stock solutions. Then, 4 mL of DPPH (4mg/100 mL in methanol) was added to 1 mL of each serially prepared sample and kept in the dark for 30 minutes. The absorbance was measured using a UV-Vis spectrophotometer at 517 nm. The percentage of inhibition of DPPH radical was calculated as;

$$\% \text{ inhibition} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

where A_{control} is the absorbance of DPPH, and A_{sample} is the absorbance of the sample with DPPH solution. The inhibitory concentration, the concentration needed to scavenge 50% of DPPH radical (IC_{50}) was calculated from the Prism dose-response curve (GraphPad, Prism version 4.0 for Windows, GraphPad Software, San Diego, CA, USA) obtained by plotting the percentage of inhibition versus the concentrations (Tundis *et al.*, 2012).

3.7. Antiviral Activity

Cytotoxicity assay. The antiviral activity of DCM/MeOH (1:1) and MeOH extracts of the roots of *W. uniflora* and *A. schimperi* and bergenin (**96**) against MDCK cells of influenza A and B were evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Chavan *et al.*, 2016). Briefly, a series of three-fold dilutions of each test sample in MEM (3.7- 300 µg/mL) were prepared. MDCK cells were incubated for 48 h at 36°C in 5% CO₂ in the presence of the dissolved specimens. The cells were washed twice with phosphate-buffered saline (PBS), and a solution of 3-(4,5-dimethylthiazolyl-2) 2,5-diphenyltetrazolium bromide (ICN Biochemicals Inc. Aurora, Ohio) (0.5 µg/ml) in PBS was added to the wells.

After 1h incubation, the wells were washed and the formazan residue was dissolved in DMSO (0.1 ml per well). The optical density in the wells was then measured on a Thermo Multiskan FC plate reader at the wavelength of 540 nm and plotted against the concentration of the tested samples. Each concentration was tested in three parallels. The 50% cytotoxic concentration (CC₅₀) of all the tested samples was calculated from the data obtained.

Cytoprotection assay. DCM/MeOH (1:1) and MeOH extracts of the roots of *W.uniflora* and *A. schimperi* and bergenin (**96**) were dissolved in 0.1 ml DMSO to prepare stock solutions, and final serial solutions (300 - 3.7 µg/ml) were prepared by adding MEM with 1µg/ml trypsin. The tested samples were incubated with MDCK cells for 1 h at 36°C. Each concentration of the samples was tested in triplicate. The cell culture was then infected with influenza virus A and B for 48 h at 36°C in the presence of 5% CO₂. Based on the photometry results, 50% inhibiting concentration (IC₅₀), the concentration that protected 50% of cells compared to the wells without extracts, was calculated for each extract and the selectivity index (SI, the ratio of CC₅₀ to IC₅₀). GraphPad Prism software was used to calculate the CC₅₀ and IC₅₀ values. The specimens demonstrating SI of 10 or higher were considered active and prospective for further development.

3.8. Molecular Docking Analysis

The prediction of the mode of binding orientation and affinity of the isolated compounds towards the antibacterial, antioxidant, and anticancer target proteins was conducted using the Auto Dock Vina 4.2. Program. Based on the *in vitro* analysis, compounds with promising antibacterial activity were chosen for docking analysis towards the antibacterial target proteins such as pyruvate kinase of *S. aureus* (PDB ID:3t07), *S. epidermidis* FtsZ (PDB ID: 4M8I), DNA gyrase B of *E. coli* (PDB ID: 6F86), LasR protein of *P. aeruginosa* (PDB ID: 2UV0), *P. mirabilis* urease (PDB ID: 1E9Y), and *S. pyogenes* 10782 streptopain (PDB ID: 6UKD) receptors and antioxidant target proteins such as human myeloperoxidase (PDB ID: 1DNU), human peroxiredoxin 5 (PDB ID: 1HD2) and anticancer target such as topoisomerase II α (PDB ID: 4FM9), estrogen receptor alpha with Y537S Mutation (PDB ID: 6OWC), androgen receptor (PDB ID:1T7R), estrogen receptor alpha (PDB ID:1G50), and progesterone receptor (PDB ID-3G8O) receptors.

Protein and ligand preparation

The 3D crystal structures of the selected target proteins (PDB ID: 3t07, PDB ID: 4M8I, PDB ID: 6F86, PDB ID: 2UV0, PDB ID: 1E9Y, PDB ID: 6UKD, PDB ID: 1DNU, PDB ID: 4FM9, PDB ID: 1HD2, PDB ID-6OWC, PDB ID: 1T7R, PDB ID: 1G50, and PDB ID-3G8O receptors were downloaded from the RCSB Protein Databank ([RCSB PDB: Homepage](https://www.rcsb.org/)). The protein preparation was done using the Auto Dock 4.2.6 (MGL tools 1.5.6) tools following the standard protocol (Uttu *et al.*, 2023) by removing the co-crystallized ligand, water molecules, and co-factors. Polar hydrogens and Kollman charges were added to the protein and a pdbqt format file was generated using the Auto Dock Tools 1.5.6 software. The chemical structures of the studied compounds were drawn using the ChemOffice tool (Chem Draw 16.0) assigned with proper 2D orientation, and the energy of each molecule was minimized using ChemBio3D. The energy-minimized ligand molecules were saved in pdbqt format using the Auto Dock Vina tool to carry out the docking simulation.

Docking simulations

A grid box size of 54 × 54 × 54 Å pointing in x, y, and z directions with a grid point spacing of 0.375 Å was established using the graphical user interface program Auto Dock 4.2.6. The Auto Dock Vina searched for the best-docked conformation between isolated compounds and target proteins. Nine different conformations were generated for each ligand in the docking process using the Lamarckian genetic algorithm (LGA) program. The conformations with the most favorable (least) binding free energy were selected for analyzing the interactions between the target receptor and ligands by Discovery Studio visualizer and PyMOL. The ligands are represented in different colors, H-bonds, and the interacting residues are represented in stick model representation (Ahmed *et al.*, 2020; Hussein *et al.*, 2021).

3.9. *In-Silico* Pharmacokinetics Analysis

The pharmacokinetic properties of the studied compounds were assessed to estimate their potential as drug candidates. This estimation was carried out based on the standard protocol adopted by the Lipinski rule (Lipinski *et al.*, 1997). Pro Tox II was employed to predict the toxicities, toxicological endpoints, and LD₅₀ of the isolated compounds (Oshevire *et al.*, 2021).

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. Structural Elucidation of the Isolated Compounds

4. 1.1. Compounds isolated from the roots of *W. uniflora*

Compound 96

Compound **96** was obtained as a white powder with a melting point ranging from 238-240°C. Its TLC profile showed a single spot with *R_f* value of 0.51 using *n*-hexane: EtOAc (1:9) as eluent (Appendix 1). Its ¹H NMR (400 MHz, Table 1, Appendix 2A) displayed a signal at δ 7.05 (1H, s) attributed to one aromatic methine proton. The signals observed at δ 4.94 (1H, d, *J*=10.6), 3.68 (1H, m), 3.44 (1H, dd, *J*=9.5, 8.7), 3.81 (1H, dd, *J*=9.5, 8.7) and 4.05 (dd, *J*= 10.4, 9.5) belong to sp³ oxygenated methine protons. The peak at δ 3.89 (3H, s) was attributed to the methoxy proton. The peaks at δ 3.72 (1H, m)) and 4.02 (1H, m) belong to diastereotopic oxygenated methylene protons.

¹³C NMR and DEPT-135 spectra (Appendix 2B & C) of compound **96** display a total of 14 well-resolved carbons of which the peaks at δ 152.2, 149.6, and 142.2 belong to oxygenated quaternary aromatic carbons, peak at δ 165.7 belong to ester carbonyl whereas the peaks at δ 119.3 and 117.2 suggest the presence of two sp² non-oxygenated quaternary carbons. The presence of sp² methine was evident at δ 110.8 along with five sp³ oxygenated methine carbons resonated at δ 71.9, 74.0, 75.5, 81.3, and 82.9. The peaks at δ 62.6 and 60.8 belong to oxygenated methylene and methoxy carbons, respectively. Its HSQC spectrum (Table 1, Appendix 2D) of the compound showed ¹J direct correlations between signals at δ 4.94, 3.68, 3.44, 3.81, 4.05, 3.89, and 7.05 with carbon signals at δ 74.0, 82.9, 71.9, 75.5, 81.3, 60.8, and 110.8, respectively. The two diastereotopic methylene protons at δ 3.72 and 4.02 displayed a ¹J correlation with carbon observed at δ 62.6. The COSY spectrum (Appendix 2E) of the compound disclosed correlations between protons appearing at δ 3.44 (H-3) with protons observed at δ 3.68 (H-2) and 3.81 (H-4) and *vice versa*. The COSY spectrum also showed the correlations of a proton signal displayed at δ 4.05 (H-4a) with proton observed at δ 4.94 (H-

10b) and cross-coupling correlation of the diastereotopic oxygenated methylene protons displayed at δ 3.72 and 4.02 were observed.

Table 1: ^1H and ^{13}C NMR (in CD_3OD) of bergenin (**96**) (δ in ppm).

C/H- No	Bergenin (96) (δ in ppm)				Literature (Nazir <i>et al.</i> , 2011)	
	^1H NMR (400MHz)	^{13}C NMR (125MHz)	^1H - ^1H	HMBC	^1H NMR (500 MHz)	^{13}C NMR (125MHz)
2	3.68 (1H, m)	82.9	H2 $\rightarrow$ H3	H2 $\rightarrow$ C3, 4, 10b, 11	3.81 ((1H, dd, J= 9.0, 5.7)	83.1
3	3.44(dd, J=9.5, 8.7)	71.8	H3 $\rightarrow$ H2, 4	H3 $\rightarrow$ C2, 4, 11	3.42 ((1H, dd, J = 9.2, 9.0 Hz)	71.9
4	3.81(dd,J=9.5, 8.7)	75.6	H4 $\rightarrow$ H3, 4a	H4 $\rightarrow$ C3, 4a	4.02 (dd, 10.0, 9.2 1H)	75.7
4a	4.05 (dd, J= 10.4, 9.5)	81.3	H4a $\rightarrow$ H4, 10b	H4a $\rightarrow$ C4, 10a, 10b	4.10 (1H, dd, J = 10.4, 10.1)	81.5
6	-	165.7	-	-	-	165.8
6a	-	119.3	-	-	-	119.5
7	7.05 (s)	111.0	-	H7 $\rightarrow$ C6,6a, 8. 9, 10a	7.08 (s 1H)	111.1
8	-	152.2	-	-	-	152.4
9	-	142.2	-	-	-	142.3
10	-	149.3	-	-	-	149.5
10a	-	117.2	-	-	-	117.3
10b	4.94 (d, J=10.4)	74.2	H10b $\rightarrow$ H4a	H10b $\rightarrow$ C2, 4, 4a, 9, 10, 10b	4.95(d, J = 10.4 1H)	74.3
11	4.02 (1H, m); 3.72 (1H, m)	62.6	H-11 $\rightarrow$ H2	H11 $\rightarrow$ C3	3.68(2H, m, H-11)	62.7
*OCH ₃	3.89 (s)	60.8	-	-	3.95(s)	60.9

Its HMBC spectrum (Appendix 2F) displayed aromatic methine proton signal at δ 7.05 correlated with carbon signals at δ 119.3 (C-6), 117.2 (C-10a), 142.2 (C-9), 149.6 (C-10), and 152.2 (C-8). Proton signal displayed at δ 4.94 (H-10b) showed HMBC correlation with oxygenated methine carbons at δ 82.9, (C-2), 75.5 (C-3) 72.0 (C-4), 81.3, (C-4a) and an ester

carbonyl at δ 165.7 (C-6a). Proton signal at 4.02 (H-4a) showed HMBC correlation with carbon signals at δ 71.9 (C-4), 75.5 (C-3') and 74.0 (C-10b). The above spectral data are in good agreement with data reported for bergenin (**96**) (Figure 12) which was previously reported from *Peltophorum dubium*, *Flueggea leucopyrus*, and *lueggea virosa* species (Agber *et al.*, 2020; Al De Oliveira *et al.*, 2017; Nazir *et al.*, 2011; Wijayabandara *et al.*, 2015). Bergenin (**96**) is a cytoprotective and antioxidative polyphenol found in many medicinal plants with a wide spectrum of activities such as hepatoprotective (Lim *et al.*, 2000) anti-inflammatory (Gao *et al.*, 2015), immunomodulatory (Mehta *et al.*, 2022), antitumor (Li *et al.*, 2023), antiviral (Patel *et al.*, 2012), antifungal properties (Prithiviraj *et al.*, 1997).

Compound 97

Compound **97** was obtained as a white crystalline solid with a melting point ranging from 136-138°C. Its TLC profile showed *R_f* value of 0.50 using *n*-hexane: EtOAc 9:1 as the mobile phase (Appendix 1). The FT-IR spectrum (Appendix 3A) of compound **97** showed a broad band at 3413 cm⁻¹ suggesting the O-H stretching vibration of an alcohol group. The bands observed at 2935 and 2860 cm⁻¹ showed the asymmetric and symmetric C-H stretching vibration of sp³ hydrocarbons. The bands displayed at 1464 and 1383.16 cm⁻¹ represent the C-H bending vibration of methyl groups. The bands observed at 1650, and 1048 cm⁻¹ were assigned to C=C and C-O stretching vibration respectively. Its ¹H NMR (400 MHz, in CDCl₃, Table 2, Appendix 3B) exhibited two sets of singlet protons integrated for three protons each at δ 0.70 (H-18) and 1.01 (H-19) suggesting the presence of two angular methyl groups, signals displayed at δ 5.36 (1H, m), 3.53 (1H, m), δ 2.25 (1H, dd,) and 2.02 (1H, m) belong to an olefinic proton (H-6), sp³ methine proton bearing hydroxyl group (H-3) and methylene protons of H-4 and H-7, respectively indicating the characteristic signals for β - Sitosterol skeleton (Kamboj and Saluja, 2011). Its ¹³C NMR and DEPT-135 spectra (Table 2, Appendix 3C& D) showed a total of 29 well-resolved peaks of which six methyls, nine methines, eleven methylenes, and three quaternary carbons were identified. The peaks at δ 140.7 and 121.7 belong to sp² quaternary carbon and sp² methine (C-5 and C-6) whereas the peak observed at δ 71.7 belong to sp³ methine-bearing hydroxyl group (C-3). Two angular methyl carbons appeared at δ 11.8 (C-19) and 19.3 (C-18). The above spectral data showed in good agreement with that β -sitosterol (**97**)

(Figure 12) previously reported from *Baccaurea macrocarpa* species (Pusparohmana *et al.*, 2020).

Table 2: ^1H and ^{13}C NMR (in CDCl_3) of β -sitosterol (**97**) (δ in ppm).

Position C/H-No	β -sitosterol (97) (δ in ppm).		Literature (Pusparohmana <i>et al.</i> , 2020)	
	^1H NMR (400 MHz)	^{13}C NMR (125 MHz)	^1H NMR (500 MHz)	^{13}C NMR (125 MHz)
1	1.84 (m)	37.2	1.85 (m)	37.3
2	1.95 (m)	31.5	1.95 (m)	32.0
3	3.53 (m)	71.7	3.52 (m)	71.9
4	2.25 (dd, J= 1.98; 1.04)	42.3	2.24 (dd, J= 1.44; 1.06)	42.4
5	-	140.7	-	140.8
6	5.36 (m)	121.7	5.35(m)	121.8
7	2.02(m)	31.9	1.99 (m)	31.7
8	1.98 (m)	31.9	2.00(m)	32.0
9	0.95 (m)	50.1	0.94 (m)	50.2
10	-	36.4	-	36.6
11	1.02 (m)	21.0	1.02 (m,)	21.2
12	1.14 (m)	39.7	1.16 (m)	39.8
13	-	42.1	-	42.3
14	1.00 (m)	56.7	1.00 (m)	56.9
15	1.52 (m)	24.3	1.06 (m) and 1.58 (m)	24.4
16	1.09 (m)	28.2	1.09 (m)	28.3
17	1.11 (m)	56.0	1.12 (m)	56.0
18	0.70 (3H,s)	11.8	0.85 (s)	12.1
19	1.01 (s)	19.4	0.82 (s)	19.5
20	1.16 (m)	36.1	1.16 (m)	36.3
21	1.02 (d, 3H, J=5.7)	18.7	1.03 (d, J= 7.2 Hz, 3H)	18.9
22	1.33 (m)	33.9	1.33 (m)	34.0
23	1.16 (m)	26.0	1.16 (m)	26.2
24	0.93 (m)	45.7	0.94 (m)	45.9
25	1.66 (m)	29.1	1.66 (m)	29.2
26	0.87 (d, 3H, J=5.7)	19.8	0.83 (d, J=11 Hz)	19.3
27	0.85(d, 3H, J=5)	19.0	0.84 (d)	19.1
28	0.84 (m)	23.0	1.25 (m)	23.2
29	0.82 (t, 3H)	11.9	0.85 (t)	12.0

Compound 98

Compound **98** was isolated as yellowish gum with *R_f* value of 0.56 using 50% n-hexane: EtOAc (1:1) as eluent (Appendix 1). Its ¹H NMR spectrum (400MHz, in DMSO-*d*₆, Table 3, Appendix 4A) showed the presence of diastereotopic methylene protons at δ 2.65 (H4a, 1H, m) and 2.94 (H4b, 1H, m) attached to sp³ oxygenated methine at δ 4.94, (H-3, 1H, m) and 5.35 (1H, H-2). In total six aromatic protons were observed of which two of them appeared with AB spin pattern at δ 5.82 (H, d, J=2) and 5.93 (1H, d, J=2) (ring A) whereas the remaining four appeared as AA'BB' spin system at δ 6.40 (2H, s,) and 6.80 (2H, s) (ring B). The above ¹H NMR spectra data suggest a catechin skeleton (Saijo, 1982). The signals observed at δ 8.38 (brs), 8.80 (brs), 9.17 (brs), and 9.44 (brs) belong to phenolic hydroxyl groups.

The ¹³C NMR and DEPT-135 (Table 3, Appendix 4B & 3C) spectra of compound **98** showed the presence of aliphatic methylene carbon displayed at δ 26.1, sp³ oxymethine at δ 68.5 and 76.9, sp² methine carbons at δ 105.9 and 109.0, and α,β -unsaturated ester group at δ 165.7. Eight sp² quaternary carbons were observed at δ 119.7, 129.0 132.7, 138.9, 145.8, 146.0, 156.0 and 156.9. Two sp² oxygenated symmetry carbons were displayed at δ 146.0 (C-3'/5') and δ 145.8 (C-3''/5''). The presence of two sp² oxygenated aromatic quaternary carbons at δ 132.7 (C-4') and δ 139.0 (C-4'') suggests 1,3-di-oxygenated a pattern at ring B and galloyl moiety respectively. The weak signals displayed at δ 48.9 and 60.3 are impurities and not a part of the compound. Based on the above aforementioned spectral data and comparison with published literature, compound **98** was identified as epigallocatechin 3-gallate (Figure 12), reported from various medicinal plants (Gangadhar *et al.*, 2011; Ismail *et al.*, 2021; Wang *et al.*, 2020). This compound has been reported from green tea with proven biological activity including as discrepant in cancer, bone regeneration, and the nervous system (Chu *et al.*, 2017).

Table 3: ¹H and ¹³C NMR (in DMSO-*d*₆) of epigallocatechin 3-gallate (**98**) (δ in ppm)

C/H-No	Epigallocatechin 3-gallate (98) (δ in ppm).		Literature (Wang <i>et al.</i> , 2020)	
	¹ H NMR (400MHz)	¹³ C NMR(125MHz)	¹ H NMR (500MHz)	¹³ C NMR (125MHz)
2	5.35 (d, J=5.7),	77.9	5.56 (d)	78.6
3	4.94(dd,J=5.6, 6)	68.5	4.92 (s)	69.9
4	2.65-2.92 (2H, m)	26.1	3.03-2.92 (m, H)	26.8
5	-	156.9	-	156.7
6	5.93 (1H,d, J=2),	95.9	5.94 (1H,d, J=2)	96.5
7	-	156.9	-	157.8
8	5.83 (1H, d, J=2))	94.7	5.94 (1H, d, J=2)	95.9
9	-	156	-	157.8
10	-	97.8	-	99.4
1'	-	129.0	-	130.8
2'&6'	6.40 (2H, s)	105.9	6.49 (s)	106.9
3' &5'	-	146.0	-	146.7
4'	-	132.7	-	133.8
1''	-	119.7	-	121.4
2''	6.81(2H, s)	109.0	6.94 (s, 2H)	110.2
&6''				
3''&		145.8	-	146.7
5''				
4''		139.0		139.8
7''		165.7	-	167.7

Compound 99

Compound **99** was obtained as yellowish gum with *R_f* value of 0.60 using (n-hexane: EtOA;1:9 as a mobile phase (Appendix 1). Its ¹H NMR (400MHz, Table 4, Appendix 5A) spectrum showed the presence of six sets of AA'XX' spin-system at δ 7.14 (2H, d, J = 8.8 Hz, H-2a and H-6a), 6.82 (2H, d, J=8, H-3a, and H-5a), 6.68 (2H, J=8, H-3b, and H-5b), 6.97 (2H, d, J=8, H-2c, and H-6c) and 6.75 (2H, d, J = 8.8, H-3c and H-5c) suggesting the presence of three 1,4-disubstituted aromatic rings. The signals shown at δ 6.23 (1H, t) and 6.18 (2H, d, J=2) attributed to the AB₂ spin system of the aromatic group. The presence of AB spin pattern aromatic protons were observed at δ 6.24 (1H d, J=2, H-12b), 6.06 (1H, d, J=2.2, H-14b), 5.99 (1H, d, J=2, H-12c) and 6.66 (1H, d, J=2.1, H-14c) suggesting the presence of two *meta* coupled aromatic groups. The presence of a trans-olefinic coupled doublet proton system (J=16.4 Hz) were displayed at δ 6.86 (H-7c) and 6.59 (H-8c). The peaks observed at δ 5.18, (H-7a), 5.34 (H-7b),

4.88 (H-8a), and 4.40 (H-8b) were attributed to a dihydrobenzofuran moiety protons (Table 4). Its ^{13}C NMR and DEPT-135 (Table 4, Appendix 5B & C) disclosed the presence of sp^2 quaternary carbons displayed at δ 161.2, 159.0, 158.8 157.7, and 157.1, 146.1, 141.4, 135.2, 133.1, 132.5, 132.2, 118.9 and 118.8, sp^2 methine carbons at δ 130.2, 127.7, 127.5, 115.9, 115.6, 115.4, 105.9, 103.5, 101.5, 96.4 and 96.1. The signals displayed at δ 93.1, 91.5, and 55.5, 52.2 were respectively assigned to two oxymethine and methine carbons of dihydrobenzofuran moiety. The signals displayed at δ 122 and 130.6 were assigned for olefinic carbons of C-ring stilbene. The above spectral data suggest that compound **99** has a stilbene trimer skeleton (Papastamoulis *et al.*, 2015), and comprehensive comparison with literature spectral data compound **99** was found to be identical E-miyabenol C (**99**) (Figure 12), reported herein for the first time from this species and previously reported from *Grapevine cane* and *Carex species* (Arraki *et al.*, 2013; González-Sarriás *et al.*, 2011).

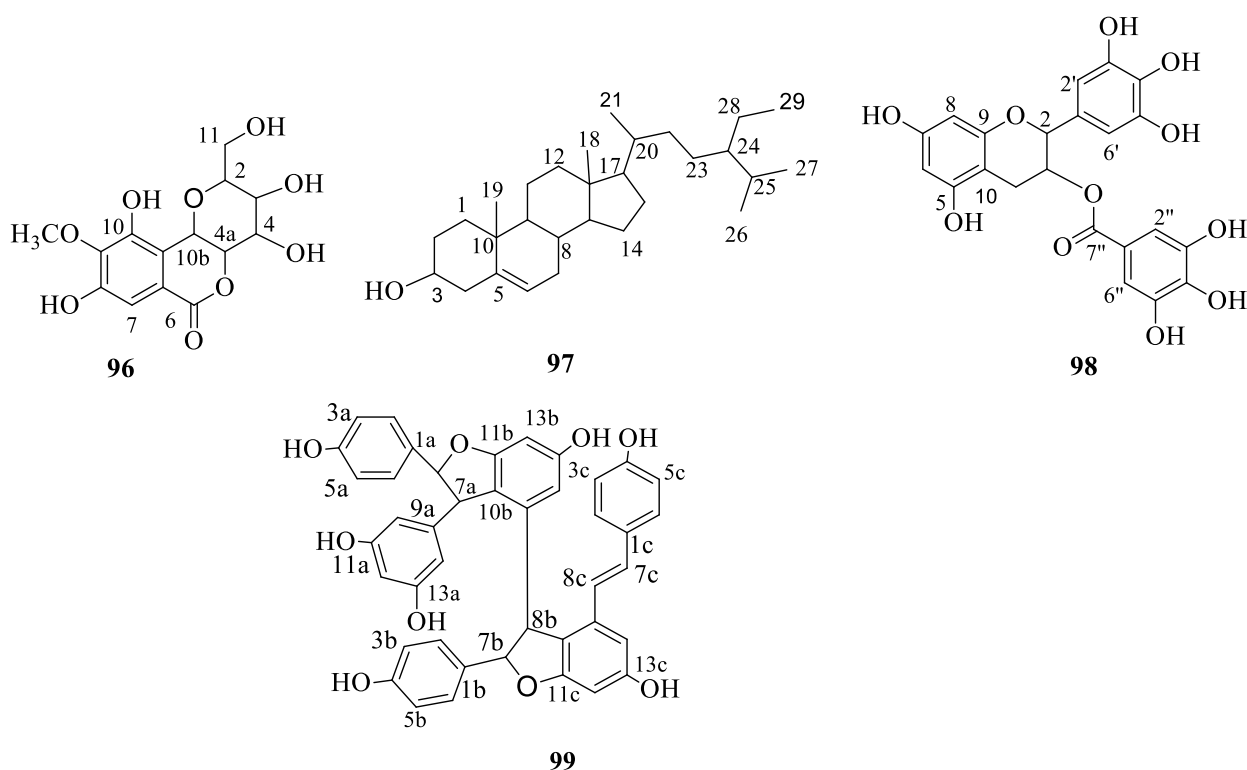


Figure 12: Structure of compounds (**96-99**) isolated from the roots of *W. uniflora*

Table 4: ^1H and ^{13}C NMR (in DMSO- d_6) of E-miyabenol C (**99**) (δ in ppm)

C/H-No	E-miyabenol C (99)		(González-Sarrías <i>et al.</i> , 2011)		C/H-No	E-miyabenol C (99)		(González-Sarrías <i>et al.</i> , 2011)	
	^1H NMR (Jin Hz)	^{13}C NMR	^1H NMR(Jin Hz)	^{13}C NMR		^1H NMR	^{13}C NMR	^1H NMR	^{13}C NMR
1a	-	133.1	-	133.1	12b	6.24,d,J=2	96.4	6.32,d,J=2)	96.3
2a/6a	7.14, d, J = 8.8)	127.5	7.15 d (8.8)	126.9	13b	-	158.8	-	158.8
3a/5a	6.82, d, J=8)	115.6	6.81, d, J =8	115.7	14b	6.06, d, J=2.2	105.9	6.05, d, J=2)	106.7
4a	-	157.7	-	158.1	1c	-	132.2	-	131.5
7a	5.34, d, J = 5.9	93.1	5.38, d, J = 5.9	93.8	2c/6c	6.97, d, J=8	130.2	7.10,d, J= 8.8	127.9
8a	4.88 (1H, d)	55.5	4.62 (1H, s,)	56.4	3c/5c	6.75,d, J = 8.8	115.9	6.74,d,J = 8.8	115.9
9a	-	146.1	-	146.9	7c	6.86,d, J=16.4	130.6	6.87, d,J=16	130.4
10a/14a	6.18, d, J=2.2	105.8	6.15,d, J=2	106.1	8c	6.59,d, J=16.4	122	6.58,d,J=17.6	121.9
11a/13a	-	159.0	-	159.5	9c	-	135.2	-	135.2
12a	6.23,t ,J=1.8	101.5	6.20, t, J=2	101.7	10c	-	118.9	-	120.6
1b	-	132.5	-	132.5	11c	-	159.0	-	161.3
2b/6b	6.40, d,J=8.8)	127.8	6.49,d, J=8	126.6	12c	5.99, d, J=2	96.1	6.32 d, J=2	96.3
3b/5b	6.68, J=8	115.4	6.64, d, J = 8.8	114.8	13c	-	158.8	-	158.8
4b	-	157.1	-	156.8	14c	6.66, d,J=2.1	103.5	6.65, d, J=2.	103.5
7b	5.18, d, J=5	91.5	5.18, brs,	91.4					
8b	4.40, d, J=6.5)	50.2	4.30 d, J=2	50.4					
9b	-	141.4	-	142.6					
10b	-	118.8	-	117.8					
11b	-	161.2	-	161.6					

4.1.2. Compounds isolated from the roots of *T. vogelii*

Compound 100

Fractions 13 and 14 (eluted with 10% EtOAc in petroleum ether) showed a single spot with R_f value of 0.60 using petroleum ether: EtOAc (4:1) as the mobile phase (Appendix 1). Its ¹H NMR spectrum (Table 5, Appendix: 6A) displayed peaks ranging from δ 0.66-5.33. The presence of three sp² methine protons shown at δ 5.02 (1H, m H-23), 5.13 (1H, m H-22), 5.33 (1H, m, H-6) and oxy-methine proton resonated at δ 3.52 (1H, m, H-3) confirms the presence of a steroidal skeleton (Yusuf *et al.*, 2023). The signals displayed at δ 0.66 (s, H-18), 0.79 (s, H-19), 0.99 (d, J=5.6, H-21), 0.80 (d, J=6.2, H-26), 1.23 (d, J=5.8, J=H27), and 0.82 (t, H-29) were attributed to six methyl protons. The peaks displayed at δ 2.21-2.30 (4H, m, H-2, and H-4) attributed to two methylene (CH₂) protons adjacent to carbon (C-3) bearing hydroxyl group and allylic (H-7) proton. Its ¹³C NMR with the aid of the DEPT-135 spectrum (Table 5, Appendix 6B& C) revealed the presence of 46 well-resolved carbons. The peaks that appeared at δ 121.8, 129.3, 138.4, and 140.8 belong to four olefinic carbons. The signals observed at δ 12.0 and 21.2 suggest the presence of two angular methyl carbons C-18 and C-19, respectively. The signal observed at δ 71.9 is a characteristic peak of sp³ oxygenated methine carbon (C-3) of a steroidal skeleton. The ¹³C NMR data supported by the integrals and disparity in single hydrogen peaks indicated that this compound is a mixture of β -sitosterol (**97**) (Figure 12, Table 2,) and stigmasterol (**100**) (Figure 13). In support of this, the ratio of H-6/H-22/H-23/H-3 displayed 0.65/0.35/0.35/0.65 proportions suggesting 2:1 ratio of β -sitosterol (**97**) and stigmasterol (**100**) (Ibrahim *et al.*, 2018; Khan *et al.*, 2020; Pusparohmana *et al.*, 2020)

Table 5: ^1H and ^{13}C (in CDCl_3) of stigmasterol (**100**) (δ in ppm).

C/H- No	Stigmasterol (100)			
	Experimental		(Pusparohmana <i>et al.</i> , 2020)(500Hz)	
	^1H NMR (400MHz)	^{13}C NMR (125MHz)	^1H NMR (500MHz)	^{13}C NMR (125MHz)
1		37.3	1.85 (m)	37.2
2	2.21(2H,m)	31.7	1.95 (m)	31.6
3	3.52(1H, m)	71.3	3.52 (m)	71.7
4	2.23 (2H, m)	42.2	2.23 (m), 2.28 (m)	42.2
5	-	140.8	-	140.7
6	5.33(1H,br)	121.8	5.35 (1H,t)	121.6
7		31.7	1.99b(m)	31.8
8		32.0	2.00 (m)	32.4
9		50.1	0.94 (m)	50.1
10	-	36.5	-	36.4
11		21.1	1.02 (m,)	21.0
12		39.8	1.16 (m)	39.6
13		42.3	-	42.3
14		56.8	1.00 (m)	56.7
15		25.5	1.06(m),	25.5
16		28.3	1.66 (m), 1.25(m)	28.3
17		56.1	1.12 (m)	56.0
18	0.66 (s)	11.8	0.85 (s)	11.9
19	0.79 (3H,s,H-19)	19.1	1.05 (s)	19.1
20	1.98 (m)	40.6	1.16 (m)	40.4
21	0.99 (d, J=5.6)	21.3	1.03(m)	21.5
22	5.13 (1H,m)	138.4	5.00 (dd)	138.3
23	5.02 (1H,m)	129.3	5.15(1H,m)	129.2
24		51.2	1.55 (m)	51.2
25		36.2	1.45 (m)	36.1
26	0.80 (d, J=6.2)	21.03	0.86 (d)	21.2
27	1.23 (d, J=5.8),	23.1	0.84(d)	18.6
28	1.08(m)		1.16 (m)	25.3
29	0.82 (t)	12.0	0.81 (t)	12.1

Compound 101

Compound **101** was isolated as a yellowish gum (*R_f* value 0.56 using petroleum ether: EtOAc (7:3) as a mobile phase (Appendix 1). Its ¹H NMR spectrum (Table 6, Appendix 7A) disclosed the presence of four AB spin multiplicity system of protons observed at δ 6.86 (1H, d, *J*=8.7 Hz, H-10), 8.04 (1H, d, *J*=8.7 Hz, H-11), 5.72 (1H, d, *J*=9.6 Hz, H-3') and 6.77 (1H, d, *J*=9.6 Hz, H-4'). The presence of additional singlet protons resonated at δ 8.44 (1H, s, H-1), 6.55 (1H, s, H-4), and 5.01 (2H, s, H-6) suggest the presence of deguelin skeleton (Vasconcelos *et al.*, 2012). The signals appeared at δ 3.85 (3H, s) and 3.95 (3H, s) attributed to methoxy protons. Methyl protons attached to the pyran moiety were observed at δ 1.48 (6H, s).

The COSY (Appendix: 7B) spectrum of **101** confirmed the ¹H-¹H connectivity of proton displayed at δ 6.86 (H-10) with δ 8.04 (H-11) and at δ 5.72 (H-3') with at δ 6.77 (H-4') and *vice versa*. The ¹³C NMR (Table 6: Appendix 7C) showed the presence of 23 resolved carbons. The signals displayed at δ 157.3, 156.1, 151.2, 148.0, 146.3, 144.1, 110.6, 110.0, 110.6, and 118.7 were assigned to sp² quaternary carbons whereas signals at δ 109.2, 100.6, 114.8, 126.6, 115.4, and 130.6 belong to sp² methine carbons. The presence of two methoxy carbons appeared at δ 56.1 and 56.3. Oxygenated sp³ quaternary and two methyl carbons of the pyran moiety appeared at δ 77.9 and 28.4, respectively. The peaks observed at δ 64.9, corresponding proton appeared δ 5.02 (2H, s) and δ 174.4 belong to oxygenated methylene and α - β unsaturated ketone group respectively. The aforementioned spectral information and supported with literature suggest that compound **101** was identified as 6a, 12a-dehydro-deguelin (Figure 13) previously reported from *Millettia pachycarpa* (Ye *et al.*, 2008).

Table 6: ¹H, ¹³C NMR, and COSY (in CDCl₃) of compound **101**(δ in ppm)

C/H-No	6a,12a-dehydro-deguelin (101)		Literature (Ye <i>et al.</i> , 2008)		
	¹ H NMR (J in Hz)	¹³ C NMR	¹ H- ¹ H COSY	¹ H NMR(Jin Hz)	¹³ C NMR
1	8.44 (1H, s)	109.2		8.45 (1H, s)	109.1
1a		110.6			110.5
2		144.1			144.1
3		148.0			149.0
4	6.55 (1H, s)	100.6		6.56 (1H, s)	100.4
4a		146.3			146.3
6	5.01 (2H, s)	64.9		5.02 (2H, s)	64.8
6a		156.1			156.1
7a		151.2			151.1
8		110.0			110.1
9		157.3			157.2
10	6.86 (1H, d, J=8.7)	114.8	H10 $\longleftrightarrow$ H11	6.86(d d, J=8.7)	114.7
11	8.04 (1H, d, J=8.7)	126.6	H11 $\longleftrightarrow$ H10	8.05(1H,d,J=8.7)	126.5
11a		110.6			111.8
12		174.4			174.3
12a		118.7			118.5
2'		77.9			77.7
3'	5.72 (1H, d, J=9.6)	130.6	H3' $\longleftrightarrow$ H4'	6.76 (1H, d, J=10)	130.5
4'	6.77 (1H, d, J=9.6)	115.4	H-4' $\longleftrightarrow$ H-3'	5.72 (1H, d, J=10)	115.4
2a'	1.48 (6H,s)	28.4		1.49 (6H, s)	28.1
*OCH ₃	3.85 (3H, s)	56.1		3.87 (3H, s)	56.3
*OCH ₃	3.95 (3H, s)	56.3		3.95 (3H, s)	56.9

Compound 102

Compound **102** was obtained as a yellow solid melting point of 198-200°C, with R_f value of 0.54 using petroleum ether: EtOAc (7:3) as a mobile phase (Appendix 1). Its ¹H NMR spectrum (Table 7: Appendix 8A) showed the presence of four AB spin system multiplicity proton displayed at δ 6.44 (H, d, J =8.7, H-10), 7.71 (1H, d, J=8.7, H-11), 5.53 (1H, d, J=10, H-3'), 6.57 (1H, d, J =10, H-4'). Two singlet sp² methine protons were observed at δ 6.55 (1H, s, H-1) and 6.46 (1H, s, H-4). The signals displayed at δ 1.37 (3H, s) and 1.43 (3H, s) were assigned

to the methyl protons attached to the pyran moiety. The presence of two methoxy protons was observed at δ 3.71 (3H, s) and 3.80 (3H, s).

Table 7: ^1H , ^{13}C NMR, and COSY (in CDCl_3) of tephrosin (**102**) (δ in ppm)

C/H-No	Tephrosin (102)		(Ahmad <i>et al.</i> , 1999)		
	^1H NMR (J in Hz) (400MHz)	^{13}C NMR (125MHz)	^1H - ^1H COSY	^1H NMR (J in Hz) (400MHz)	^{13}C NMR (100MHz)
1	6.55 (1H, s)	109.7		6.55 (s)	109.7
1a	-	108.6		-	108.7
2	-	144.0		-	144.0
3	-	151.1		-	151.2
4	6.46 (1H, s)	101.0		6.47 (1H, s)	101.1
4a	-	148.4		-	148.4
6	4.60 (1H, dd, J=10, 2.2)	64.0		4.60 (dd, J= 10, 2.4)	63.9
	4.63(dd,J=10.1,2.2)			4.63 (dd, J =12, 2.4)	
6a	4.55 (dd, J= 10, 2.4)	76.3		4.55(dd,J=10.9, 2.4)	76.3
7a	-	156.7		-	156.6
8	-	109.2		-	109.1
9	-	160.8		-	160.7
10	6.44 (d, J =8.7)	111.9	H-10 $\longleftrightarrow$ H-11	6.44 (d, J 8.7)	111.8
11	7.71 (d, J=8.7)	128.6	H-11 $\longleftrightarrow$ H-10	7.73 (d, J= 8.7)	128.5
11a	-	111.7		-	111.1
12	-	191.4		-	191.0
12a	-	67.5		-	67.4
2'	-	78.1		-	77.9
3'	6.57 (1H, d, J=10)	128.9	H-3' $\longleftrightarrow$ H-4'	6.57 (d, J: 10.1),	128.7
4'	5.53 (1H, d, J=10)	115.4	H-4' $\longleftrightarrow$ H-3'	5.53 (d, J=10.1)	115.4
2a'	1.37 (3H, s)	28.5		3H, s, H-2a	28.0
2b'	1.44 (3H, s)	28.3		1.44 (3H, s)	
*OCH ₃	3.80 (3H,s)	55.9		3.80 (3H, s)	55.8
*OCH ₃	3.71 (3H, s)	56.4		3.71 (3H, s,)	56.4

Two diastereotopic oxymethylene protons were observed at δ 4.63 (1H, dd, J =10, 2.2, H-6 α) and 4.60 (1H, dd, J =10, 2.2, H-6 β). The peak at δ 4.55 (1H, dd, J = 10, 2.4, H-6a) was attributed to oxy-methine proton. The COSY spectrum (Appendix: 8B) showed ^1H - ^1H connectivity observed between protons appeared at δ 6.44 (H-10) with δ 7.71 (H-11) and δ 6.57 (H-3') with δ 5.53 (H-4'). Its ^{13}C NMR supported with its DEPT-135 spectrum (Table 7, Appendix: 8C&

D) of compound **102** showed 23 well-resolved carbons. The signals displayed at δ 160.8, 156.7, 151.1, 148.4, and 144.0 were attributed to sp^2 oxygenated quaternary aromatic carbons. Six sp^2 methine carbons were appeared at δ 111.9, 109.7, 115.4, 128.6, and 128.9. The signals displayed at δ 56.4, 55.9, and 28.6, 28.3 were assigned respectively for two methoxy and methyl carbons. The peaks displayed at δ 76.2 and 64.0 were assigned for oxygenated methine and oxygenated methylene carbons respectively. The peaks displayed at δ 78.1 and 191.4 belong to sp^3 quaternary carbon and α - β -unsaturated ketone respectively. Based on the aforementioned spectrum data and comparison with published literature, compound **102** was identified as tephrosin (Figure 13) previously reported from *Tephrosia purpurea* (Ahmad *et al.*, 1999).

Compound 103

Compound **103** was obtained as a light yellow solid melting point ranging from 177-180 °C. Its TLC profile showed *R_f* value of 0.56 using petroleum ether: EtOAc (3:2) (Appendix 1). The 1H NMR of compound **103** (Table 8, Appendix: 9A) disclosed the presence of ABX spin system of protons displayed at δ 7.24 (1H, d, J = 8.4, H-1) and 6.46 (1H, dd, J = 8.4, 2.2, H-2) and 6.44 (1H, d, J = 2.2, H-4). Two *para*-coupled sp^2 methine protons were observed at δ 6.34 (1H, s, H-10) and 6.77 (1H, s, H-7). The signals resonated at δ 4.21 (1H, dd, J = 9.6, 2.8, H-6 α) and 3.53 (1H, dd J =9.6, 2.8, H-6 β) belong to diastereotopic oxymethylene protons. The peaks observed at δ 5.85 (1H, s, H-12 α) and 5.82 (1H, s, β) were attributed to dioxygenated methylene protons. The signals appeared at δ 5.43 (1H, d, J = 6.9) and 3.46 (1H, t) belonging to sp^3 oxygenated methine H-11a and methine H-6a protons, respectively. The COSY spectrum (Appendix: 9B) established the 1H - 1H cross connectivity of protons appeared at δ 7.24 (H-1) with δ 6.46 (H-2) and δ 3.46 (H-6a) with proton at δ 5.43(H-11a) and *vice versa*. 1H - 1H cross connectivity was also observed for diastereotopic protons displayed at δ 4.21 (H-6 α) with δ 3.53 (H-6 β) (Table 4). The ^{13}C NMR and DEPT-135 spectrum (Table 8, Appendix: 9C&D) of compound **103** displayed a total of 16 well-resolved carbons. The presence of five sp^2 methine carbons was displayed at δ 131.7, 109.3, 102.6, 104.8, and 93.0. The signals that appeared at δ 141.6, 148.2, 154.3, 156.6, and 158.6 belong to sp^2 oxygenated quaternary carbons. The presence of two sp^2 quaternary carbons were observed at δ 111.3 and 118.4. The peaks shown at δ 40.2 and 78.5 suggest sp^3 methine and oxygenated methine carbons, respectively. The

signals resonated at δ 66.0 and 101.2 belong to oxymethylene and methylene dioxy carbons respectively. The combined spectral data and comparison with the literature, compound **103** was identified as maackiain (Figure13) isolated from *Cyclopia genistoides* and *Gueldenstaedtia delavayi* (Peng *et al.*, 2016; Roza *et al.*, 2015).

Table 8: ^1H , ^{13}C NMR, and COSY (in CD_3OD) of maackiain (**103**) (δ in ppm).

C/H- No	Maackiain (103)			Literature (Roza <i>et al.</i> , 2015)	
	^1H NMR (J in Hz) (400MHz)	^{13}C NMR (125MHz)	^1H - ^1H COSY	^1H NMR(J in Hz) (500MHz)	^{13}C NMR (125MHz)
1	7.24 (1H,d, J = 8.4)	131.7	H1 $\longleftrightarrow$ H2	7.23 (1H, d, J = 8.4),	132.0
1a	-	111.3			111.3
2	6.46 (1H,dd,J= 8.4, 2.2)	109.3	H2 $\longleftrightarrow$ H1	6.46 (1H, dd, J = 8.4, 1.9)	109.7
3	-	158.6			158.7
4	6.44 (1H, d, J = 2.2)	102.6		6.53 (1H, d, J = 1.8 Hz)	102.9
4a	-	156.6			156.3
6	4.21 (1H, dd, J= 9.6, 3.2, H-6 α , 3.53 (1H,dd, J=9.6, 2.8 H-6 β))	66.0	H6 α $\longleftrightarrow$ H6 β	4.21 (1H, dd, J = 10.1, 3.8, H-6 β , 3.58 (1H, m, H-6 α))	65.8
6a	3.46 (1H, t)	40.2	H-6a $\longleftrightarrow$ H-11a	3.54 (1H, m)	39.0
7	6.77 (1H, s)	104.8		6.96 (1H, s)	105.3
7a	-	118.4			118.4
8	-	141.6			141.0
9	-	148.2			148.4
10	6.34 (1H, s)	93.0		6.52 (1H, s)	93.2
10a	-	154.3			153.7
11a	5.43 (1H, d, J = 6.9)	78.5	H-11a $\longleftrightarrow$ H-6a	5.50 (1H, d, J = 6.9)	77.9
12	5.85 and 5.82 (2x1H, 2xs)	101.2		5.94 and 5.91 (2x1H, 2xs)	101.0

Compound 104

Compound **104** was obtained as yellowish gum (*R_f* of 0.54 using petroleum ether: EtOAc (3:2) as a mobile phase (Appendix 1). The ^1H NMR spectrum (Table 9, Appendix: 10A) of compound **104** indicated the presence of doublet of doublet methylene protons resonated at δ 2.85 (dd, J=16, 12.5, H-3a) and 3.04 (dd, J= 16, 3.6, H-3b), and oxy-methine proton at δ 5.44 (dd, J=12.5,

3.6). The peaks observed at δ 5.41 (1H, d, J=10.1, H-3'') and 6.53 (1H, d, 10.1, H-4'') were recognized for *ortho*-coupled sp² methine protons. A singlet aromatic methine proton appeared at δ 5.99 (1H, s, H-6). Multiplet peaks integrated with five protons displayed at δ 7.00-7.44 (m) were recognized for unsubstituted B-ring of flavanone skeleton (Amc Arriaga *et al.*, 2009). The signals that appeared at δ 1.41 (3H, s) and 1.43 (3H, s) were assigned for methyl protons attached to pyran ring. The ¹³C NMR and DEPT-135 spectrum (Table 9, Appendix 10 B&C) of compound **104** showed 18 resolved carbons.

Table 9: ¹H and ¹³C NMR (in CDCl₃) of obovatins (**104**) (δ in ppm)

C/H- No.	Obovatins (104)	Literature (Andrei <i>et al.</i> , 2000)		
	¹ H NMR (400MHz)	¹³ C NMR (125Mz)	¹ H NMR (400MHz)	¹³ C NMR (50.3MHz)
2	5.44 (dd, 12.5, 3.6)	79.1	5.45 (dd, 12.5, 3.3)	78.97
3	3.04 (dd, 16, 12.5) 2.85 (dd, 16, 3.6)	43.4	3.10 (dd, 16.7, 12.5) 2.81 (dd, 16.7, 3.3)	43.19
4	-	195.8	-	196.0
5		156.8		157.0
6	5.99 (s)	97.5	5.99 (s)	97.55
7		163.8	-	164.0
8		102.8	-	102.0
9		162.4	-	162.0
10		106.0	-	103.0
1'		138.6	-	139.0
2'/6'	7.40-7.44 (m)	126.1	7.40-7.44 (m)	125.9
3'/5'	7.40-7.44 (m)	128.9	7.40-7.44 (m)	128.7
4'	7.40-7.44 (m)	128.6		128.6
2''		77.9		78.08
4''	5.41(1H, d, 10.1)	115.4	5.40 (d, 10.1)	115.5
3''	6.53 (1H, d, J=10.1)	126.6	6.53(d, 10.1)	126.4
2a''	1.41(3H, s)	28.1	1.41 (s)	28.18
2b''	1.43 (3H,s)	28.4	1.43 (s)	28.42

The signals displayed at δ 28.3, 28.6 and 77.9 were assigned to sp³ quaternary and methyl carbons. The peaks observed at δ 79.1 and 43 recognized the characteristic flavanone skeleton (Amc Arriaga *et al.*, 2009). The signals appeared at δ 156.8, 162.4, and 163.8 were assigned for sp² oxygenated quaternary carbons. The peaks displayed at δ 97.5, 126.1, 128.9, 128.6, 115.4, and 126.6 were recognized to sp² methine carbons. The signals resonated at δ 102.8,

106.0, and 138.6 were recognized for sp^2 quaternary carbons. Based on the aforementioned spectral information and compared with the reported literature, compound **104** was identified as obovatin (Figure 13) isolated from *Tephrosia tunicate* (Andrei *et al.*, 2000).

Compound 105

Compound **105** was obtained as a yellow powder with melting point ranging from 220-222 °C. Its TLC analysis showed *R_f* value of 0.54 using petroleum ether: EtOAc (1;1) as a mobile phase (Appendix 1). The 1H NMR spectrum (Table 10, Appendix 11A) of compound **105** showed the presence of *ortho*-coupled AB pattern signals resonates at δ 6.93 (1H, d, *J*=8.6, H-10), 8.06 (1H, d, *J*=8.6, H-11), 5.78 (1H, d, *J*=9.6, H-3'), and 7.03 (1H, d, *J*=9.6, H-4'). The signals observed at δ 8.97 (1H, s,) and 6.89 (1H, s) were attributed to two *para*-positioned aromatic protons. The signals displayed at δ 3.95 (3H, s) and 4.02 (3H, s) were assignable to methoxy protons. The COSY spectrum (Appendix 11B) showed the 1H - 1H cross connectivity of protons observed at δ 6.93 (H-10) with δ 8.06 (H-11), and δ 5.78 (H-3') with δ 7.03 (H-4'). Its ^{13}C NMR and DEPT-135 (Table 10, Appendix 11 C&D) revealed a total of 23 well-resolved carbons. The peaks observed at δ 99.6, 108.1, 114.9, 116.3, 126.5, and 130.6 were recognized for sp^2 methine carbons. The signals that appeared at δ 108.0, 109, 117.9, and 121.2 were attributed to sp^2 quaternary carbons. The peaks displayed at δ 78.6 and 28.4 were recognized for sp^3 oxygenated quaternary and methyl groups. The presence of six sp^2 oxygenated quaternary carbons was observed at δ 142.2, 146.3, 146.9, 151.2, and 151.5. The signals shown at δ 156.2 and 176.6 were attributed to the α - β -unsaturated ester and ketone groups respectively. The presence of peaks at δ 56.3 and 56.4 were assignable to two methoxy carbons. The above spectra data is identical to the spectra data of 6-oxo-6a, 12a-dehydrodegulin (**105**) (Figure 13) isolated from *Millettia duchesnei* (Ngandeu *et al.*, 2008).

Table 10: ^1H , ^{13}C NMR, and COSY (in CDCl_3) of compound **105** (δ in ppm)

C/H-No	6-oxo-6a, 12a-dehydrodegulin (105)	Lit. (Ngandeu et al., 2008)		
	^1H NMR (J in Hz) (400MHz)	^{13}C NMR (125MH)	^1H - ^1H COSY	^1H NMR (J in Hz) (600MHz) ^{13}C NMR (75MHz)
1	8.97 (1H, s)	108.1		9.02 (1H, s) 108.2
1a		108.0		107.9
2		151.2		151.2
3		146.9		146.9
4	6.89 (1H, s)	99.6		6.92 (1H, s) 99.6
4a		146.3		145.2
6		156.2		156.1
6a		142.1		142.1
7a		151.5		151.5
8		109.6		109.6
9		158.9		158.9
10	6.923(1H, d, J=8.6)	116.3	H10 $\longleftrightarrow$ H11	6.95 (1H, J=8.4) 116.4
11	8.06 (1H, d, J=8.6)	126.5	H11 $\longleftrightarrow$ H10	8.08(1H, d, J=8.4) 126.5
11a		117.9		117.9
12		176.6		176.6
12a		121.2		121.4
2'		78.6		78.6
3'	5.78 (1H, d, J=9.6)	130.6	H3' $\longleftrightarrow$ H4'	6.79(1H, d, J=10.2) 130.8
4'	7.03 (1H, d, J=9.6)	114.9	H-4' $\longleftrightarrow$ H-3'	7.05 (1H, d, J=10) 114.8
2a'	1.50 (6H, s)	28.4		1.52 (6H, s) 28.1
*OCH ₃	4.02(3H, s)	56.4		4.05 (3H, s) 56.3
*OCH ₃	3.95 (3H, s)	56.3		3.98(3H, s) 56.9

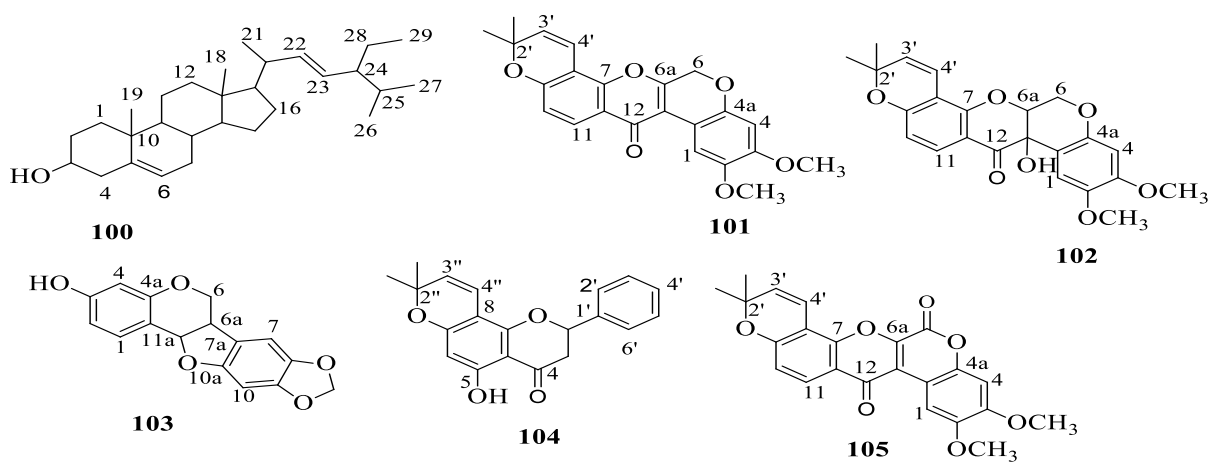


Figure 13: Structure of compound (**100-105**) isolated from the roots of *T. vogelli*

4.1.3. Compounds isolated from the roots of *A. schimperi*

Compound 106

Compound **106** was obtained as a white gum (*R_f* value of 0.54 using petroleum ether: EtOAc (9:1) as a mobile phase (Appendix 1)). The ¹H NMR spectrum (Table 11, Appendix 12A) of compound **106** ranging from δ 0.88 to 5.33 ppm. The signals displayed at δ 5.33 (brs) and 2.34 (2H, t) were attributed respectively to olefinic and methylene protons α - to an acyl group. The signals observed at δ 1.63 (2H, m) and 2.02 (4H, m) were recognized for methylene (H-3) and allylic methylene protons (H-8 and H-9) respectively. The signals displayed at δ 1.26 (18H, 9x CH₂, brs) and δ 0.88 (3H, t) were assigned to the long-chain methylene protons and terminal methyl protons respectively.

Table 11: ¹H and ¹³C NMR (in CDCl₃) of oleic acid (**106**) (δ in ppm)

C/H-No	Oleic acid (106)	Literature (Sintayehu and Adane, 2020)
	¹ H NMR (400MHz,)	¹³ C NMR (125MHz)
1	-	180.1
2	2.34 (2H, t)	34.0
3	1.63(m, 2H)	24.6
4	1.26	29.1
5	1.26	29.3
6	1.26	29.0
7	1.32	29.7
8	2.02	29.3
9	5.33	129.9
10	5.33	129.6
11	2.02 (m, 2H)	29.3
12	1.26	29.6
13	1.26	29.5
14	1.26	29.6
15	1.26	29.3
16	1.26	32.0
17	1.26	22.6
18	0.88 (t, 3H)	14.1

The ^{13}C NMR and DEPT-135 spectra (Table 11, Appendix 12B & C) of compound **106** revealed a total of 18 well-resolved carbons. The signal observed at δ 180.4 was an indicative of the presence of a carboxylic acid. The peaks that appeared at δ 129.9 and 129.6 were recognized for olefinic methine carbons. The presence of 14 methylene carbons ranged from δ 22.7 to 34.0, pointing downward in the DEPT-135 spectrum, and one terminal methyl carbon displayed at δ 14.1 indicated the presence of unsaturated 18-carbon fatty acid. The above spectra data and comparison with the literature report indicated that compound **106** was identified as oleic acid (Figure 14) (Sintayehu and Adane, 2020).

Compound 107

Compound **107** was isolated as a white solid with a melting point ranging from 215-216 °C. Its TLC profile analysis showed R_f value 0.54 using petroleum ether: EtOAc (4:1), as eluent (Appendix 1). ^1H NMR spectrum (Table 12, Appendix 13A) of compound **107** ranges from δ 0.74 to 4.67. The ^1H NMR showed the presence of seven singlet methyl protons resonated at δ 0.74, 0.77, 0.81, 0.92, 0.94, 1.01, and 1.66. Doublet protons observed at δ 4.55 (d, J = 2Hz) and 4.67 (d, J =2Hz) were attributed to diastereotopic protons (2H, H- α -29, and H- β -29) suggesting the presence of a lupine-type triterpenoid skeleton (Akwu *et al.*, 2020). The peaks displayed at δ 3.19 (dd, 1H) and 2.36 (1H) were characteristic peaks for H-3 and H-19 of the lupeol skeleton.

The ^{13}C NMR and DEPT-135 spectra (Table 12, Appendix 13B&C) of compound **107** revealed 30 well-resolved carbons. The ^{13}C NMR spectrum suggests the presence of seven methyls, eleven methylenes, six methines, and six quaternary carbons. The appearance of olefinic peaks displayed at δ 109.4 (C-29) and 151.0 (C-20) and oxy-methine at δ 79.1 (C-3) confirmed that compound **107** was a lupane-type triterpenoid. Based on the above spectral information and comparison with literature reports, compound **107** was recognized as lupeol (**107**) (Figure 14) and has been reported to possess antioxidant, antimicrobial, anti-tumor, and anti-carcinogenic activities (Shwe *et al.*, 2019).

Table 12: ^1H and ^{13}C NMR (in CDCl_3) of lupeol (**107**) (δ in ppm)

C/H-No	Lupeol (107)		Literature (Shwe <i>et al.</i> , 2019)	
	^1H NMR (400MHz)	^{13}C NMR (125MHz)	^1H NMR (600MHz)	^{13}C NMR (150MHz)
1		38.9	0.90, 1.65	38.8
2	1.85 (m)	27.4	1.52, 1.67	27.4
3	3.19 (dd, J=5, 4.8)	79.1	3.2(dd)	78.9
4		38.1		38.7
5		55.3	0.67	55.2
6		18.3	1.37, 1.52	18.3
7		34.3	1.39	34.2
8		40.8		40.8
9		50.5	1.25	50.4
10		37.2		37.1
11		20.9	1.20, 1.40	20.9
12		25.2	1.06, 1.62	25.1
13		38.0	1.66	38.0
14		42.9		42.8
15		27.5	1.05, 1.60	27.4
16		35.6	1.35, 1.45	35.5
17		42.9		42.9
18		48.4	1.36, 1.37	48.2
19	2.36 (m)	48.0	2.40 (m), 1.45 (o)	47.9
20		151.1		150.9
21		29.8	1.3(o), 1.91(m)	29.8
22		40.1	1.18, 1.37 (o)	39.9
23	0.92 (s)	28.0	0.90 (s)	27.9
24	0.74 (s)	15.4	0.76 (s)	15.3
25	0.82 (s)	16.2	0.83 (s)	16.1
26	1.01 (s)	16.0	1.03 (s)	15.9
27	0.94 (s)	14.5	0.94 (s)	14.6
28	0.77 (s)	18.0	0.79 (s)	17.9
29	4.55, 4.67(d, J=2Hz)	109.4	4.57, 4.69 (d, J= 1.9 Hz)	109.3
30	1.66 (s)	19.3	1.67 (s)	19.2

Compound 108

Compound **108** was obtained as a yellow powder with a melting point ranging from 90-94 °C. Its TLC profile displayed *R_f* value of 0.58 using petroleum ether: EtOAc (6:4) as a mobile phase (Appendix 1). ¹H NMR spectrum (Table 13, Appendix 14A) of compound **108** showed the presence of one set of ABX spin system aromatic protons observed at δ 6.82 (1H, d, *J*=2.0, H-2), 6.69 (1H, d, *J*=8.0 Hz, H-5) and 6.67 (1H, dd, *J*=8.0, 2.0 Hz, H-6).

Table 13: ¹H and ¹³C NMR (in CDCl₃) of dihydroferulic acid (**108**)

C/H No	Dihydroferulic acid (108)				Lit. (Cho <i>et al.</i> , 2013)	
	¹ H NMR (400MHz)	¹³ C NMR (125MHz)	¹ H- ¹ H COSY	HSQC	¹ H NMR (400MHz)	¹³ C NMR (125MHz)
1	-	132.2				133.9
2	6.82(1d, <i>J</i> =2.0)	111.0		H-2 → C-2	6.79 (d, <i>J</i> =2)	113.1
3	-	146.6			-	148.9
4	-	144.1			-	146.0
5	6.69(dd, <i>J</i> =8L, <i>J</i> =2.0)	114.4	H-5 ↔ H-6	H-5 → C-5	6.69(d, <i>J</i> =8.0)	115.9
6	6.67(dd, <i>J</i> =8.0, 2.0)	120.9	H-6 ↔ H-5	H-6 → C-6	6.64(1dd, <i>J</i> =8.0,2.0)	121.8
7	2.65 (2H, t)	30.9	H-7 ↔ H-8	H-7 → C-7	2.55 (2H, t)	30.8
8	2.88 (2H, t)	36.1	H-8 ↔ H-7	H-8 → C-8	2.82(2H, t)	37.4
9	-	178.0			-	177.1
*OCH ₃	3.84 (3H, s)	55.9		H-3a → C-3a	3.89 (3H,s)	56.5

Two sets of triplet protons integrated for two protons each of methylene groups were resonated δ 2.63 (2H, t, H-7) and 2.87 (2H, t, H-8). The signal observed at δ 3.84 (3H, s) was recognized as a methoxy proton. The COSY spectrum (Appendix 14B) showed ¹H-¹H cross connectivity of protons of H-5 (δ 6.70) with H-6 (δ 6.67) and proton displayed at δ 2.65 (H-7) with δ 2.88 (H-8). Its ¹³C NMR and DEPT-135 spectra (Table 13, Appendix 14C& D) spectrum recognized a total of ten well-resolved carbons. The signals appeared at δ 30.4 and 35.9 disclosed the presence of two aliphatic methylene carbons. The presence of three sp² methine carbons were observed at δ 111.0, 114.4, and 120.9. The signals displayed at δ 132.2, 144.1, and 146.5 were assignable for sp² quaternary carbons. The peaks at δ 55.9 and 178.1 were attributed respectively to methoxy and a carboxylic group. The HSQC spectrum of compound **108**

(Appendix 14E) demonstrated ^1J C-H bond correlations of protons observed at δ 6.82 (H-2), 6.69 (H-5), 6.67 (H-6), 3.84, 2.65 (H-7), and 2.88 (H-8) with δ 111.0 (C-2), 114.4 (C-5), 120.9 (C-6), 55.9 (C-3a), 30.9 (C-7), 36.1 (C-8) respectively. Based on the above spectroscopic data and compared with the reported literature, compound **108** was identified as dihydroferulic acid (Figure 14), previously reported from *Suaeda japonica* (Cho *et al.*, 2013).

Compound 109

Compound **109** was obtained as a white powder with a melting point ranging from 261-263°C. Its TLC analysis showed *R_f* value 0.45 using 100% EtOAc as a mobile phase (Appendix 1). The FT-IR spectrum (Appendix 15A) of compound **109** showed the presence of O-H stretching vibration at 3368 (br) cm^{-1} . The presence of an intense band at 1745 cm^{-1} was assigned to the C=O stretching of an ester group. The bands shown at 2925 and 1046 cm^{-1} are attributed to the symmetric sp^3 C-H stretching and C-O stretching respectively. The ^1H NMR spectrum (Table 14A, Appendix 15B) of compound **109** ranges from δ 0.74 - 5.88 ppm. The presence of a doublet proton observed δ 4.90 (1 H, d, $J=17.6$, H-21 α), δ 4.98 (1 H, d, $J=17.6$, H-21 β), and an olefinic singlet proton displayed at δ 5.90 (1 H, s, H-22) was an indicative of a characteristic feature of the cardenolide skeleton (Thakur *et al.*, 2021). The signals displayed at δ 0.74 (H-18) and 0.94 (H-19) were recognized for two angular methyl singlet protons of the steroidal skeleton. The peaks resonated at δ 4.77 (s), and 3.29 (3H, s) were recognized for anomeric and methoxy protons respectively.

^{13}C NMR and DEPT-135 spectra (Table 14, Appendix 15C&D) of compound **109** showed a total of 30 well-resolved carbons. The presence of oxy-methylene carbon observed at δ 73.9, olefinic carbons δ 116.5 and δ 177.0, and carbonyl carbon at δ 175.9 confirms the presence of butenolide ring (Pecio *et al.*, 2019). The peaks displayed at δ 15.0, and 17.8 were attributed respectively to C-18 and C-19 peaks for two steroidal angular methyl carbons. The signals displayed at δ 84.8 and 54.4 were assignable to sp^3 oxygenated quaternary and a methoxy carbon respectively. The presence of rhamnose sugar moiety was confirmed due to signals of oxy-methine carbons observed at δ 98.2, 75.0, 69.6, 68.4, 67.5, and methyl carbon at δ 15.5. The abovementioned spectral information and comparison with published data suggested that compound **109** was recognized as acovenosigenin A- 3-O- α -L-hamnopyranoside

(Figure 14), isolated herein for the first time from this species and previously reported from the leaves of *A. oblongifolia* (Abd-Alla *et al.*, 2021).

Table 14: ^1H and ^{13}C NMR (in CD_3OD) of compound **109** (δ in ppm)

Compound 109			Literature (Abd-Alla <i>et al.</i> , 2021)	
C/H-No	^1H NMR (400MHz)	^{13}C NMR (125MHz)	^1H NMR(400MHz)	^{13}C NMR (100MHz)
1	3.80 (1H, s))	72.9	3.71 (1H d)	72.5
2	α 1.89 (m), β 1.97 (m)	31.9	1.89 (m), 1.97 (m)	31.8
3	α , 4.03 (1H, d)	71.2	4.25 (br d)	72.3
4	1.62 (m)	28.3	1.84 (M)	28.3
5	β , 1.89 (m)	30.6	1.89 (m)	30.4
6	α ,1.36 m, β , 1.89, m	26.3	α ,1.36,m, β ,1.84 (m)	26.3
7	α ,1.78m, β 1.26m	21.1	α ,1.76 m, β 1.27m	21..0
8	β 1.62(m)	21.5	1.65 (m)	21.9
9	α 1.49(m)	36.6	1.49 (m)	37.6
10	-	41.3	-	40.3
11	α ,1.36 (m), β , 1.36(m)	21.1	α ,1.35(m), β , 1.35(m)	21.2
12	α , 1.36 (m) β 1.59 (m)	39.2	α , 1.34 (m) β 1.54 (m)	39.9
13	-	49.7	-	49.4
14	-	84.8	-	85.4
15	α , 2.02 (m) β , 1.73	32.5	α , 2.10 (m) β , 1.73 (m)	33.2
16	α , 2.04 (m) β , 1.89 (m)	26.8	α , 2.17 (m) β , 1.89 (m)	26.9
17	α 2.71 (dd)	50.5	2.79 (m)	50.8
18	0.74 (3H, s)	15.0	0.89 (m)	15.8
19.	0.94 (3H,s)	17.8	1.10 (m)	18.8
20	-	177.0	-	174.5
21	4.90 (d, J=17.6), 4.98 (d, J=17.6)	73.7	4.86 (d, J=18), 4.99 (d, J=18)	73.4
22	5.90 (s)	116.5	5.89	117.9
23	-	175.9	-	174.2
1'	4.77 (s)	98.2	5.02 (s)	97.7
2'	3.81 (m,)	68.4	3.88 (m)	68.4
3'	3.51 (m)	75.0	3.36 (m)	75.0
4'	3.80 (m)	69.6	3.88 (m)	69.8
5'	4.03 (m)	67.5	4.01 (m)	66.7
6'	1.32 (d, J=3.7)	15.5	1.34 (d, J=3.8)	16.6
*OCH ₃	3.29 (s)	54.8	3.48(s)	55.7

Compound 110

Compound **110** was isolated as a white solid with a melting point ranging from 182-186°C. Its TLC analysis showed R_f value of 0.60 using DCM: MeOH (7:3) as a mobile phase (Appendix 1). ¹H NMR of spectrum (Table 15, Appendix 16A) of compound **110** ranging from δ 3.30 to 5.24 ppm. The signals observed at δ 5.24 (d, J=5.8), 5.17 (d, 4.08), 4.80 (dd), 4.52 (m), 3.88 (t), 3.78 (d), 3.65 (t), and 3.30 (t) were recognized for sp³ oxy-methine protons. The peaks shown at δ 3.41 (H-10), 4.45 (H-11), and 5.08 (H-12) were assigned to oxy-methylene protons.

Table 15: ¹H and ¹³C NMR (in DMSO-*d*₆) of sucrose (**110**) (δ in ppm).

C/H	Sucrose (110)		Literature (Bhat <i>et al.</i> , 2019)	
	¹ H NMR (400MHz,)	¹³ C NMR (125MHz)	¹ H NMR (400MHz)	¹³ C NMR (100MHz)
1	3.88 (t)	73.2	4.01	72.8
2	3.65 (t)	70.2	3.62	69.2
3	3.78 (t)	72.9	3.98	72.6
4	4.80 (dd, J=5.36, 5.68)	71.9	4.71	71.1
5	5.24 (d, J=5.8),	92.1	5.37	92.2
6	-	104.3	-	103.7
7	5.17 (d, 4.08)	77.4	5.36	76.4
8	3.30 (t)	74.6	3.2	74.07
9	4.52(t)	82.9	4.18	81.4
10	3.41(t)	62.6	3.69	61.4
11	4.45 (m)	61.8	4.16	60.1
12	5.08 (d, J=7.2)	63.5	4.03	62.4

The ¹³C and DEPT-135 spectra (Table 15, Appendix 16B& C) revealed a total of 12 well-resolved carbons. The signal observed at δ 92.1 was attributed to anomeric carbon. Eight sp³ oxy-methine carbons were observed at δ 92.1, 82.7, 77.4, 74.6, 73.2, 72.9, 71.9, and 70.2 ppm. The signals at δ 63.5, 62.6, and 61.8 were recognized as three oxy-methylene carbons. The above spectroscopic data was similar to the spectroscopic data of sucrose (**110**) (Figure 14) reported in the literature (Bhat *et al.*, 2019).

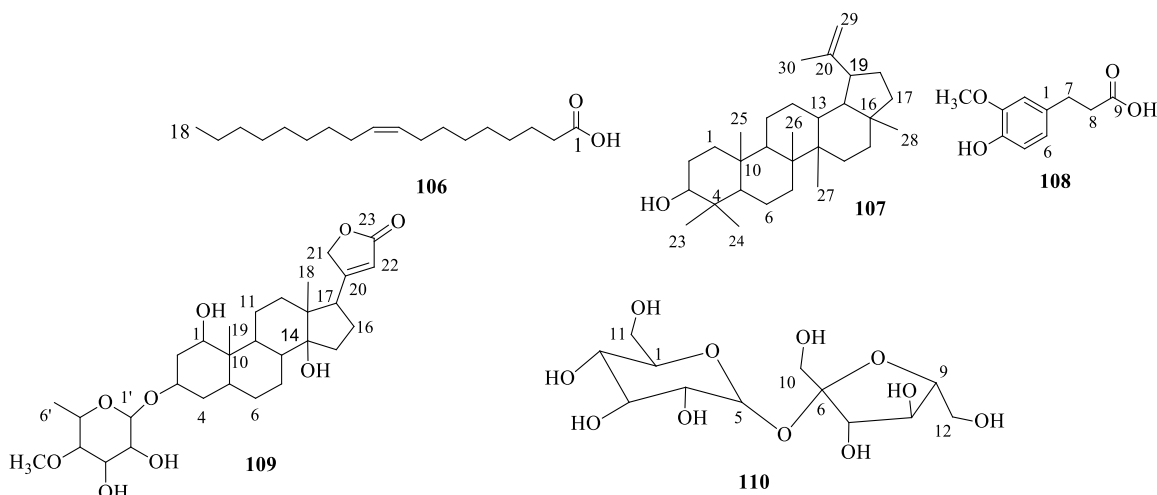


Figure 14: Structure of compounds (**106-110**) isolated from the roots of *A. schimperi*

4.1.4. Compounds isolated from the roots of *R. glutinosa*

Compound 97

β -sitosterol (**97**) was also isolated from the roots of *R. glutinosa*. Its structural elucidation was discussed and presented in Table 2 (Appendix 3A-D).

Compound 111

Compound **111** was obtained as yellowish gum with R_f value of 0.56 using petroleum ether: EtOAc (4:1) as a mobile phase (Appendix 1). The FT-IR spectrum (Appendix 17A) of compound **111** showed the presence of O-H vibration and C=O stretching of an ester group at 3390 (br) and 1733 cm^{-1} respectively. The bands shown at 2923, 1616, and 1024 cm^{-1} were assigned to sp^3 C-H, C=C, and C-O stretching respectively. ^1H NMR spectrum (Table 16, Appendix 17B) of compound **111** demonstrated the presence of olefinic protons resonated at δ 6.11, d ($J=7.9$), δ 6.88 (m) and 5.33 (2H m, H-16' & H-17'). The peaks displayed at δ 2.53 (4H, m) and 2.00 (4H, m) were recognized to be allylic protons for (H-6 & H-4) and (H-15' & H-18') respectively. A multiplet peaks observed at δ 1.22 (H-2' to H-13') were attributed to methylene protons.

Table 16: ^1H and ^{13}C NMR (in CDCl_3) of compound **111** (δ in ppm)

C/H-No	Compound 111			Literature (Saraux <i>et al.</i> , 2023)	
	^1H NMR (400MHz)	^1H - ^1H COSY (400MHz)	^{13}C NMR (125MHz)	^1H NMR (600MHz)	^{13}C NMR (100MHz)
1	-		198.3		198.6
2	6.11, d (J=7.9)	H-2 $\longleftrightarrow$ H-3	129.4	6.11, dt (10.1, 1.8)	129.8
3	6.88 (m)	H-3 $\longleftrightarrow$ H-2 H-3 $\longleftrightarrow$ H-4	146.4	6.88, ddd (10.1, 4.7, 3.4)	146.9
4	2.53(m)	H-4 $\longleftrightarrow$ H-3	38.3	2.54, m	38.6
5	-		74.2	-	74.5
6	2.53, m		50.5	2.54, m	50.8
1'	1.56, dd (16.2, 7.4)		42.2	1.57, dd (16.0, 7.4)	42.4
2'	1.22		23.1	1.31, m	23.5
3'-11'	1.22, m		29.9	1.25, m	30.0
12'	1.22		29.7	1.25, m	30.0
13'	1.22		29.6	1.25, m	30.0
14'	1.22		29.4	1.25, m	30.0
15	1.99, m	H-15' $\longleftrightarrow$ H-16'	32.3	1.99, m	33.0
16'	5.33	H-16' $\longleftrightarrow$ H-15'	129.6	5.40, m	129.7
17'	5.33	H-17' $\longleftrightarrow$ H-18'	131.6	5.40, m	132.2
18'	1.99	H-18' $\longleftrightarrow$ H-17'	27.1	1.99, m	26.0
19'	0.93, t		14.2	0.96, t (7.4)	14.5

The peak observed at δ 0.86 (3H, t) was assigned to terminal methyl protons. The COSY spectrum of compound **111** (Table 16, Appendix 17C) showed ^1H - ^1H connectivity of protons displayed at δ 6.88 (H-3) with δ 6.11 (H-2) and δ 2.53 (H-4) and *vice versa*. COSY spectrum also confirms the ^1H - ^1H cross connectivity of olefinic protons (H-16' and H-17') with their respective neighboring allylic protons of H-15' and H-18' respectively. Its ^{13}C NMR spectrum supported by its corresponding DEPT -135 spectrum (Table 16, Appendix 17D&E) revealed a total of 19 well-resolved carbons. The signal resonated at δ 198.3 was recognized for the presence of a ketone group. The signals observed at δ 146.7, 131.6, 129.6, and 129.4 were assigned to sp^2 olefinic carbons. The presence of sp^3 oxygenated quaternary carbon was displayed at δ 74.2. The signals observed at δ 38.3, and 50.5 were attributed to the α -carbon (C-6) to the carbonyl carbon, and allylic carbons (C-4) respectively. The signals resonated at δ 42.2 and 14.1 were assigned to the carbon attached to sp^3 oxygenated quaternary carbon (C-1') and

terminal methyl (C-19') carbons respectively. Based on the above spectroscopic data and comparison with the literature reports, compound **111** was identified as (14E)-5-(heptadec-14-enyl)-5-hydroxycyclohex-2-en-1-one (Figure 15), previously isolated from the root bark of *Lannea acida* (Saraux *et al.*, 2023).

Compound 112

Compound **112** was isolated as a white gum with *R_f* value of 0.58 using petroleum ether: EtOAc (7:3) as a mobile phase (Appendix 1). Its ¹H NMR spectrum (Table 17, Appendix 18A) showed the presence of olefinic protons observed at δ 5.38 (m), 5.43 (m), 5.69 (m), 5.78 (m), and 5.66 (m). A proton observed at δ 4.48 (1H, m) was assigned to the proton attached to oxy-methine carbon (H-3). The peaks observed at δ 2.12 (m) and 2.19 (m) were recognized for H-2 and H-6 respectively. A multiplet peaks resonated at δ 1.99 (m) were attributed to allylic protons. The peaks shown at δ 0.97 (3H t), 0.96, (3H, t), and 0.87 (3H, t) suggest the presence of three terminal methyl groups.

Its ¹³C NMR and DEPT-135 (Table 18, Appendix 18B and C) of compound **112** showed the presence of olefinic carbons resonated at δ 125.9, 129.3, 129.9, 130.7 and 131.6. The peaks observed at δ 66.0 and 72.5 were attributed to sp³ oxygenated quaternary and oxygenated sp³ quaternary carbon respectively. The signals shown at δ 42.9 and 43.4 were recognized for allylic carbon (C-6) and the carbons next to oxy-methine carbon (C-1') respectively. The peak resonated at δ 37.5 was assigned to the carbon attached to oxygenated quaternary carbon. The signals shown at δ 23.1, 26.9, 27.1, 27.2, 29.7, 29.8 and 32.7 were assigned to methylene carbons. The presence of three methyl protons displayed at δ 0.97 (3H t), 0.96, (3H, t), 0.87 (3H, t) supported with the appearance of three methyl carbons resolved at δ 14.4, 14.2 and 14.1 was indicative of the presence of three fatty acid skeleton. Based on the ¹³C NMR data and comparison with literature reports, compound **112** was found in a mixture of 1-((E)-heptadec-14-enyl)cyclohex-4-ene-1,3-diol (**112a**), 1-((E)-nonadec-16-enyl)cyclohex-4-ene-1,3-diol (**112b**), and 1-((E)-heneicos-18-enyl)cyclohex-4-ene-1,3-diol (**112c**) (Figure 15), isolated from the root bark of *Lannea acida* (Saraux *et al.*, 2023).

Table 17: ¹H NMR (in CDCl₃) of compounds **112a-112c** (δ in ppm).

C/H- No	112a (400MHz)	(Saraux <i>et al.</i> , 2023) (600MHz)	112b (400MHz)	(Saraux <i>et al.</i> , 2023) (600MHz)	112c (400MHz)	(Saraux <i>et al.</i> , 2023) (600MHz)
2	2.00 (m,)	2.00 (m)	2.00 (m)	1.99, (m)	1.95(m)	1.95, (m,)
3	4.48, m	4.50, m	4.48, m	4.50, m	4.48,m	4.43, m
4	5.69, ddt	5.70,ddt (m)	5.69,(m)	5.70,(m)	5.66, (m)	5.65,(m)
5	5.78,d,J=10.8	5.80,d,J=10	5.78, d,	5.78,d,J=10.8	5.76,J=10.8	5.75,d (10.1)
6	2.00, m, 2.19, m	2.00,m,2.19,	1.99, m, 2.19, m	1.99, m, 2.19, dq (18.3, 2.8)	1.95,m, 2.14, m	1.95, m, 2.14, m
1'	1.48, m	1.50, m	1.48, m	1.52, m	1.46, m	1.46, m
2'	1.46,m	1.47, m	1.46, m	1.45, m	1.45, m	1.24, m
3'- 11'	1.23, m	1.29, m	1.23, m	1.27, m	1.23,m	1.24, m
12'	1.25, m	1.29, m	1.25, m	1.27, m	1.23, m	1.24, m
13'	1.99, m	1.99, m	1.25, m	1.27, m	1.23	1.24, m
14'	5.43,m	5.40, m	1.25	1.27, m	1.23	1.24, m
15'	5.43,m	5.40, m	1.99, m	1.99, m	1.23	1.24, m
16'	1.99, m	1.99, m	5.43, m	5.40, m	1.23	1.24, m
17'	0.97 (3H t)	0.96, t (7.4)	5.43, m	5.40, m	1.99, m	1.95, m
18'			1.99, m	1.99, m	5.38, m	5.38, m
19'			0.96, (3H, t)	0.93, t (7.4)	5.38, m	5.38, m
20					1.99	1.95, m
21					0.87 (3H, t)	0.93(t, 7.4)

Table 18: ^{13}C NMR (in CDCl_3) of compound **112a** -**112c** (δ in ppm)

C/H-No	112a (125MHz)	(Saraux <i>et al.</i> , 2023) (150MHz)	112b (150MHz)	(Saraux <i>et al.</i> , 2023) (150MHz)	112c (125MHz)	(Saraux <i>et al.</i> , 2023) (150MHz)
1	72.5	72.5	72.5	72.5	72.5	72.6
2	37.5	37.6	36.5	37.6	37.5	37.3
3	60.0	66.1	60.66.0	66.2	66.0	65.9
4	130.7	130.7	130.7	130.7,	129.9	130.6
5	125.9	126.0	125.9	126.0	125.9	125.8
6	42.9	43.0	42.9	43.1	42.9	42.9
1'	43.3	43.4	43.5	43.5	43.3	43.5
2'	23.1	23.1	23.1	23.2	23.1	23.1,
3'-11'	29.7	29.7	29.8	29.8	29.7	29.7
12'	29.7	29.7	29.8	29.8	29.7	29.7
13'	32.7	32.7	29.8	29.8	29.7	29.7
14'	129.4	129.5,	29.8	29.8	29.7	29.7
15'	131.6	131.9	32.7	32.7	29.7	29.7
16'	27.2	25.7	129.4	129.7,	29.7	29.7
17'	14.4	14.1	131.6	132.0	32.7	32.7
18'			27.1	25.8,	129.4	129.5,
19'			14.2	14.1,	131.6	131.9
20'					26.9	25.7
21'					14.0	14.1

Compound 113

Compound **113** was obtained as a white crystal with *R_f* value of 5.0 using petroleum ether: EtAOc (2:8) as eluent (Appendix 1). The ^1H NMR of compound **113** (Table 19, Appendix 189) exhibited olefinic proton displayed at δ 5.33 (1H, d, H-6), oxy-methine proton δ 3.51 (1H, m, H-3) and angular methyl protons observed at δ 0.66 (s, H-18) and 0.97 (s, H-19) proves the presence of steroidal skeleton (Mailafiya *et al.*, 2020). Besides, the observed signals in line with the β -sitosterol skeleton, the appearance of α -glycosidic moiety was also confirmed by the presence of anomeric proton displayed at δ 4.36 (d, $J=7.8$, H-1', the corresponding carbon resonated at δ 101.2), and oxy-methylene protons resonated at δ 4.26 (2H, dd, $J=12, 2.2$). The presence of olefinic protons appeared at δ 5.15 (m) and 5.02 (m) and the allylic protons were

shown at δ 2.02 and 2.73 indicating the presence of unsaturated moiety. The ^{13}C NMR and DEPT-135 of compound **113** (Table 19, Appendix 19B &C) confirm the presence of β -sitosterol skeleton due to the presence of oxy-methine carbon δ 79.7 (C-3), olefinic carbons δ 122.0 (C-6), δ 140.2 (C-5) and angular methyl carbons observed at δ 11.9 (C-18) and 12.0 (H-19). The signals observed at δ 101.2, 73.4, 76.1, 73.8, 70.4, and 63.6 prove the presence of glucoside moiety. The attachment of sugar moiety to C-3 of β -sitosterol was established by the weak-field shift to δ 79.7 ppm of the C-3 signal in the ^{13}C NMR spectrum compared to the corresponding signal in the spectrum β -sitosterol-glycoside (δ 71.9 ppm). The presence of an ester group observed at δ 174.4, olefinic carbons δ 130.3, 130.1 128.1 127.9, and methyl carbons at δ 14.1 were an indicative of unsaturated fatty ester moiety. Based on the above spectroscopic information and comparison with the literature report, compound **113** was identified as β -sitosterol-3-glyco-6' octadeca-(9Z, 12Z, 15Z)-trienoyl (Figure 15) (Kovganko and Kashkan, 1999; Marina *et al.*, 1990).

Table 19: ^1H and ^{13}C NMR (400Mz, in CDCl_3) of compound **113** (δ in ppm)

C/H-No	Compound 113		Lit. (Marina <i>et al.</i> , 1990)		C/H	Compound 113		Lit. (Marina <i>et al.</i> , 1990)	
	^1H NMR	^{13}C NMR	^1H NMR	^{13}C NMR		^1H NMR	^{13}C NMR	^1H NMR	^{13}C NMR
1	1.84 (m)	37.3	1.85 (m)	37.3	26	0.91(d,J=5)	19.9	0.83(d,J=11)	19.3
2	1.95 (m)	31.6	1.95 (m)	32.0	27	0.81(d,J=7)	19.1	0.84 (d)	19.1
3	3.51 (m)	79.7	3.52 (m)	79.0	28	0.83 (m)	23.1	1.25 (m)	23.2
4	2.26 (dd)	42.2	2.24(dd)	42.4	29	0.79 (t, 3H)	11.9	0.85 (t)	12.0
5	-	140.0	-	140.8	1'	4.36(d,J=7)	101.1	4.39(J=7.7)	101.0
6	5.33 (t, 1H)	122.0	5.35	121.8	2'	3.34 (m)	73.4	3.38(m)	74.0
7	2.02(dd)	31.9	1.99 (m)	31.7	3	3.43 (m)	70.4	3.38 (m)	70.5
8	1.98 (m)	32.0	2.00(m)	32.0	4	3.34(m)	76.1	3.38(m)	76.3
9	0.95 (m)	50.1	0.94 (m)	50.2	5	3.43 (m)	73.4	3.38(m)	76.2
10	-	36.4	-	36.6	6	4.25(d)	63.6	4.25(d)	63.4
11	1.02 (m)	21.1	1.02 (m,)	21.2	1''	-	174.4	-	173.6
12	1.14 (m)	39.7	1.16 (m)	39.8	2''	2.30	34.0	2.30	33.9
13	-	42.1	-	42.3	3''	1.26	24.5	1.26	24.6
14	1.00 (m)	56.7	1.00 (m)	56.9	4''	1.24	29.2	1.26	29.2
15	1.52 (m)	24.3	1.58 (m)	24.4	5''	1.24	26.9	2.08	26.9
16	1.09 (m)	28.2	1.09 (m)	28.3	6''	1.24,m	26.1	1.26, m	26.4
17	1.11 (m)	56.0	1.12 (m)	56.0	7''	1.24,m	29.3	1.26, m	29.4
18	0.66 (3H,s)	12.0	0.85 (s)	12.1	8''	2.79	25.5	2.79	25.7
19	0.99 (s)	19.4	0.82 (s)	19.5	9'',10''	5.15 (m)	128.1	5.36	128.1
20	1.16 (m)	36.1	1.16 (m)	36.3	11''	2.79 (m)	25.6	2.79 (m)	25.7
21	0.91(d,J=6)	18.7	1.03(d,J=7)	18.9	12'',13'	5.15 (m)	129.7	5.36 (m)	127.7
22	1.33 (m)	34.0	1.33 (m)	34.0	14''	2.08 (m)	27.2	2.08 (m)	27.3
23	1.16 (m)	26.2	1.16 (m)	26.2	15'',16'	5.0 (m)	130.0	5.36 (m)	129.6
24	00.84 (m)	45.7	0.94 (m)	45.9	17''	2.02	22.6	2.08	22.6
25	1.66 (m)	29.1	1.66 (m)	29.2	18''	0.88 (3H, t)	14.1	0.98 (t,)	14.0

Compound 114

Compound **114** was obtained as a white solid with a melting point ranging from 198-200°C. Its TLC profile analysis showed *R_f* value 0.55 using petroleum ether: EtOAc (1:1) as a mobile phase (Appendix 1). The FT-IR spectrum (Appendix 20A) of compound **114** showed a broad band observed at 3280, cm⁻¹ representing the O-H stretching vibration, and the intense band that appeared at 1701 cm⁻¹ was assignable to C=O stretching of an ester group. The bands displayed at 2931 and 1450 cm⁻¹ were attributed to the C-H stretching vibration and C-H bending vibration of methyl groups respectively. The presence of the C=C and C-O stretching vibrations were observed at 1608, and 1009 cm⁻¹ respectively. Its ¹H NMR spectrum (400MHz, Appendix 20B) showed the presence of aromatic protons displayed at δ 6.90 (2H, s, H-2/6) and methoxy proton at δ 3.70 (3H, s). The ¹³C NMR and DEPT-135 spectra (125MHz, Appendix 20C & D) of compound **114** revealed a total of six well-resolved carbons. The presence of a symmetry sp² methine carbons (C-2/C-6) was displayed at δ 109.0. The signals observed at δ 119.8 (C-1), 138.9 (C-4), and 146.1 (C-3/C-5) were recognized to be sp² quaternary carbons. The presence of a methoxy carbon resonated at δ 52.0 and α - β unsaturated carbonyl δ 166.9 was indicative of the presence of an ester group. Based on the above combined spectral data and comparing it with the literature report, compound **114** was identified as methyl gallate (Figure 15) (Esmat *et al.*, 2012).

Compound 115

Compound **115** was isolated as a white solid with melting point ranging from 232-236 °C. Its TLC analysis showed *R_f* value of 0.51 using petroleum ether: EtOAc (2:3) as a mobile phase (Appendix 1). The FT-IR spectrum (Appendix 21A) of compound **115** showed the presence of O-H, C=O, C=C, and C-O stretching vibrations at 3345, 1680, 1600, and 1052 cm⁻¹ respectively. The bands shown at 1446, and 1320cm⁻¹ were recognized for C-O-H bending. ¹H NMR spectrum (400MHz, Appendix 21B) of compound **115** showed the presence of singlet aromatic proton integrated for two protons observed at δ 6.87 (2H, s, H-2/6). Its ¹³C NMR spectrum supported its DEPT-135 spectrum (125MHz, Appendix 21C&D) showed a total of five well-resolved carbons. The presence of sp² methine carbon (C-2/C-6) was displayed at δ 109.0. The signals observed at δ 119.8 (C-1), 138.9 (C-4), and 146.1 (C-3/C-5) were recognized

as sp^2 quaternary carbons. The peak observed at δ 168.0 was attributed to the presence α - β unsaturated carboxylic acid. Based on the above spectroscopic information and comparison with the literature reported, compound **115** was identified as gallic acid (Figure 15) (Esmat *et al.*, 2012).

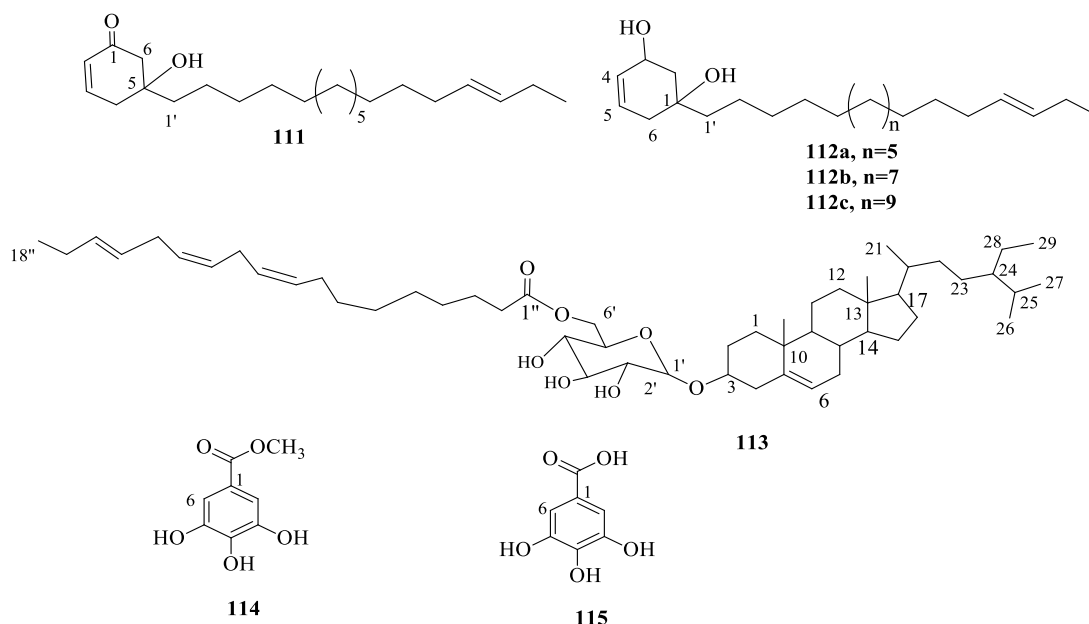


Figure 15: Structure of compounds (**111-115**) isolated from the roots *R. glutinosa*

4.1.5. Compounds isolated from the roots of *C. spinarum*

Compound **116**

Compound **116** was isolated as white gum with *R_f* value of 0.54 using petroleum ether: EtOAc (7:3) as a mobile phase (Appendix 1). The ^1H NMR (Table 20, Appendix 22A) spectrum of compound **116** showed the presence of two *ortho*-coupled olefinic protons appeared at δ 6.20 (1H, d, $J=9.8$ Hz, H-1) and 6.72 (1H, d, $J=9.8$ Hz, H-2). The presence of four singlet methyl protons was evident at δ 1.21 (3H, s, H-15), 1.26 (3H, s, H-13), 1.27 (3H, s, H-14), and 1.91 (3H, s, H-11). The presence of other aliphatic protons were also displayed at δ 2.02 (1H, t, $J=12.6$ Hz, H-6 α), 2.97 (1H, d, $J=12.6$ Hz, H-6 β), 1.40 (1H, m, H-7), 1.59 (1H, m, H-8 α), 1.76 (1H, m, H-8 β), 1.24 (1H, td, H-9 α) and 1.87 (1H, m, H-9 β).

Table 20: ^1H and ^{13}C NMR (in CDCl_3) of dehydrocarissone (**116**) (δ in ppm)

C/H-No	Dehydrocarissone (116)		Literature (Uegaki <i>et al.</i> , 1988)	
	^1H NMR (400MHz)	^{13}C NMR (125MHz)	^1H NMR (500MHz)	^{13}C NMR (125MHz)
1	6.20 (1H, d, J=9.8 Hz)	156.6	6.23 (1H, d, J=9.8 Hz,	156.6
2	6.72 (1H, d, J=9.8).	126.3	6.75 (1H, d, J=9.8 Hz)	126.1
3	-	186.6	-	186.5
4	-	129.7	-	129.2
5	-	160.3	-	160.5
6	2.02 (1H, t, J = 12.6 Hz, H-6 α), 2.97 (1H, d, J = 12.6 Hz, H-6 β)	28.8	2.04 (1H, t, J = 13 Hz, H-6 α), 2.97 (1H, d, J = 13 Hz, H-6 β)	28.7
7	1.40 (1H, m, H-7 α)	50.5	1.37 (1H, tt, J = 13, 3.5 Hz, H-7)	50.5
8	1.59 (1H, m, H-8 α), 1.76 (1H, m, H-8 β)	22.0	1.60 (1H, qd, J = 13, 3.5 Hz, H-8 α), (1H, dm, J = 13 Hz, H-8 β)	22.0
9	1.24 (1H, m, H-9 α), 1.87 (1H, m H-9 β).	38.07	1.30 (1H, td, H-9 α), 1.87 (1H, dt, J = 13, 3 Hz, H-9 β)	38.0
10	-	40.3	-	40.3
11	1.91 (3H, s H-11)	10.5	1.93 (3H, s)	10.4
12	-	72.5	-	72.3
13	1.26 (3H, s,)	27.0	1.28 (3H, s)	26.9
14	1.27 (3H, s,)	27.8	1.29 (3H, s)	27.7
15	1.21 (3H, s)	23.5	1.23 (3H, s)	23.4

Its ^1H - ^1H spectrum (Appendix 22 B) confirmed the ^1H - ^1H connectivity of protons displayed at δ 6.20 (H-1) with δ 6.72 (H-2). Its ^{13}C NMR and with the aid of the DEPT-135 spectrum (Table 20, Appendix 22 C & D) showed the presence of fifteen well-resolved carbons. The singlets displayed at δ 126.3 (C-2), 129.7 (C-4), 156.6 (C-1), and 160.3 (C-5) were attributed to olefinic carbons. The presence of α - β unsaturated carbonyl carbon and sp^3 oxygenated quaternary carbon appeared at δ 186.6 (C-3) and 72.5 (C-12) respectively. The signals observed at δ 10.5 (C-11), 23.5 (C-15), 27.0 (C-13), and 27.8 (C-14) were assigned to methyl carbons. Based on the above spectroscopic information and comparison with the published literature, compound **116** was identified as dehydrocarissone (Figure 16) isolated from *Nicotiana undulata* (Uegaki *et al.*, 1988).

Compound 117

Compound **117** was isolated as a yellow powder with melting point ranging from 200-205 °C. Its TLC profile displayed R_f value of 0.56 using petroleum ether: EtOAc (6:4) as a mobile phase (Appendix 1). The ^1H NMR spectrum (Table 21, Appendix 23A) of compound **117** showed the presence of two *ortho*-coupled aromatic protons resonated at δ 6.25 (1H, d, $J=10$, H-3) and δ 7.92 (1H, d, $J=10$, H-4), and two singlet aromatic protons δ 6.90 (1H, s, H-5) and 6.83 (1H, s, H-8). The peak observed at δ 3.94 was attributed to methoxy protons. The ^1H - ^1H COSY spectrum of compound **117** (Appendix 22B) showed the ^1H - ^1H cross connectivity of protons displayed at δ 6.25 (H-3) and at δ 7.92 (H-4). Its ^{13}C NMR and its DEPT-135 spectrum (Table 21, Appendix 23C&D) revealed a total of 10 well-resolved carbons. The signals appeared δ at 150.3, 149.7, 144.1, and 111.7 were recognized for sp_2 quaternary carbons. The peaks observed at δ 143.4, 113.5, 107.5, and 103.2 were attributed to sp^2 methine carbons. The signals assigned to methoxy and α - β unsaturated ester carbon were resonated at δ 56.5 and 161.5 respectively. The aforementioned NMR data of compound **117** was fully correlated with the NMR data of scopoletin (Figure 16) reported from *Saussurea eopygmaea* (Zhang *et al.*, 2011).

Table 21: ^1H and ^{13}C NMR (in CDCl_3) of scopoletin (**117**) (δ in ppm)

C/H No.	Scopoletin (117)		Literature (Zhang <i>et al.</i> , 2011)	
	^1H NMR (400MHz)	^{13}C NMR (125MHz)	^1H NMR (300MHz)	^{13}C NMR (75MHz)
2		161.5		161.5
3	6.25 (1H, d, $J=10$, H-2)	107.5	6.21(d, 1H, $J = 9.3$ Hz)	107.6
4	7.92 (d, 1H, $J = 9.3$ Hz,	143.4	-	143.4
5	6.90 (1H, s)	113.5	7.21 (s, 1H)	113.4
6		144.1		144.1
7	150.3			150.3
8	6.83 (1H, s)	103.2	6.78 (s, 1H)	103.7
9		149.7		149.8
10		111.7		111.5
*OCH ₃	3.93 (3H, s)	56.3	3.87 (s, 3H)	56.5

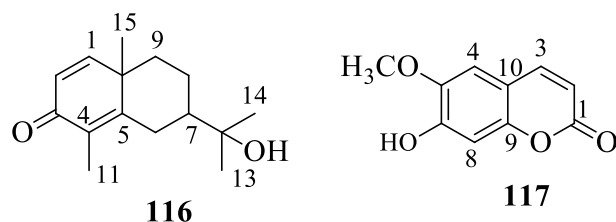


Figure 16: Structure of compounds (**116** and **117**) isolated from the roots of *C. spinarum*

4.2. GC-MS Analysis of Essential Oils

4.2.1. Essential oils profile and GC-MS analysis of *W. uniflora*

Medicinal plants are a rich source of natural oils such as essential and fixed oils which are used traditionally for nutritive as well as medicinal value (Ali *et al.*, 2023). Essential oils (EOs) are concentrated liquids of complex mixtures of volatile organic compounds that can be extracted from leaves, flowers, or seeds of different plants, which give a characteristic odor and flavor to the plants (Barros *et al.*, 2022). EOs are added to many herbal products and they are known to be very effective against different fungi and bacteria (Tandukar *et al.*, 2022). Essential oil chemical constituents have a low molecular weight (below 300 g/mol) and are rich in terpenes, sesquiterpenes esters, aldehydes, and phenols (Ghavam *et al.*, 2020). The breakdown of phospholipids and oily substances during metabolic change also produces non-terpenoid aliphatic hydrocarbon found in essential oil. Fixed oils are the portion of a nonvolatile oil that mostly consists of plant-derived plant materials such as fats, resins, and waxes (Daga *et al.*, 2021).

In the present study, hydrodistillation of air-dried powdered roots of *W. uniflora* (60 g) afforded 8 mg (0.013% w/w yields). The GC-MS analysis results in the identification of seven compounds comprising about 99.99% of the total oil constituents (Appendix 24). The retention time, molecular formula, and percentages of peak area of the identified oils are presented in Table 22. The chemical profiles of the extracted oils confirmed that essential oils, fixed oils, phthalates, and long-chain aliphatic hydrocarbons were the constituents of the oil part of the plant's roots. Cyclopropene, 2, 3- dimethyl-3-phenyl, a phenyl derivative of hemiterpenes, and (1R, 7S, E)-7-isopropyl-4,10- dimethylenecyclodec 5-enol, represent a typical sesquiterpenoid, accounts for about 14.97 % of the total oil components are the representative essential oils

found in the roots of *W. uniflora*. Palmitic acid, tricosane, and eicosane, which account for about 42.81 % of the total oil component were non-terpenoid aliphatic hydrocarbons representative of the fixed oil component of the roots of the plant. The phthalate component represents about 34.93% of the total oil of the roots of *W. uniflora* (Table 22). Among the identified oils, dibutyl phthalate and palmitic acid were the major constituents with percentages of abundance of 28.66 and 22.71% respectively (Appendix 23). A previous study conducted on essential oil analysis indicated that palmitic acid was the major constituents of *Hippophae rhamnoides* L (29.3%) (Cakir, 2004), *Myrtus communis* L (15.7%) (Cakir, 2004), *Coriandrum sativum* L (13.22%) (Mekides Assefa *et al.*, 2024) and *Rheum ribes* (27.0%) (Naemi *et al.*, 2014).

Table 22: Essential oils identified from roots of *W. uniflora*

Compound name		Molecular formula	RT)	RI	Lit.(Babushok <i>et al.</i> , 2011)	%Area
Cyclopropene, 2,3-dimethyl-3-phenyl		C ₁₁ H ₁₂	14.84	1140	-	8.7
Diethyl Phthalate		C ₁₂ H ₁₄ O ₄	20.18	1595	1587	6.27
(1R,7S,E)-7-Isopropyl-4,10dimethylenecyclodec5-enol		C ₁₅ H ₂₄ O	23.84	1695	1694	13.55
Palmitic acid		C ₁₆ H ₃₂ O ₂	24.04	1968	1968	22.71
Dibutyl phthalate		C ₁₆ H ₂₂ O ₄	24.12	1965	1940	28.66
Tricosane		C ₂₃ H ₄₈	27.09	2300	388.8	8.24
Eicosane		C ₂₀ H ₄₂	28.72	2000	331	11.86
Total						99.99

A previous study conducted on essential oil analysis indicated that palmitic acid was the major constituents of *Hippophae rhamnoides* L (29.3%) (Cakir, 2004), *Myrtus communis* L (15.7%) (Cakir, 2004), *Coriandrum sativum* L (13.22%) (Mekides Assefa *et al.*, 2024) and *Rheum ribes* (27.0%) (Naemi *et al.*, 2014).

4.2.2. Essential oil analysis of the roots of *T. vogelii*

Hydro distillation of the roots of *T. vogelii* (70 g) afforded 20 mg (0.028% (w/w)) yields. The retention time, retention index, and percentage abundance of the essential oils of roots of *T. vogelii* are presented in Table 23. GC-MS analysis (Appendix 25) of the EOs results in the identification of a total of 17 compounds, which comprised about 99.99% of the total oil

component. As presented in Table 23, the EOs of the roots of *T. vogelii* have a variety of classes of compounds such as sesquiterpene, oxygenated sesquiterpene, and non-terpenoid aliphatic hydrocarbons. About 95.53% of the total EO constituents were sesquiterpenes and oxygenated sesquiterpenes. Cis-nerolidol (1, 6, 10-dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]) (41.7 %), cadinol (19.7%), 1R,7S, E)-7-Isopropyl-4,10-dimethylene cyclodec-5-enol (3.63%), α -cadinene (3.4%), isoaromadendrene epoxide (3.37%), cyclohexadecane (3.13%) and caryophyllene oxide (3.0%) were the major EO constituents the roots of *T. vogelii*.

Table 23: Essential oils identified of the roots of *T. vogelii*

Compounds		Molecular formula	RT	RI		%Area
				Experi.	Lit.(Babushok <i>et al.</i> , 2011)	
Isocaryophyllene		C ₁₅ H ₂₄	18.126	1411	1404	1.8
5,9-Undecadien-2-one, dimethyl	6,10-	C ₁₃ H ₂₂ O	18.398	1412	1433	1.9
α -Himachalene		C ₁₅ H ₂₄	19.148	1445.1	1444	1.94
γ -cadinene		C ₁₅ H ₂₄	19.299	1513.1	1512	1.88
Copaene		C ₁₅ H ₂₄	19.512	1376.2	1377	1.99
cis-nerolidol		C ₁₅ H ₂₆ O	19.824	1517.0	1517.1	41.7
Caryophyllene oxide		C ₁₅ H ₂₄ O	19.957	1580.6	1581	3.0
Cyclohexadecane		C ₁₆ H ₃₂	20.067	1580.6	1881	3.13
Cadinol		C ₁₅ H ₂₆ O	20.829	1651.9	1660	19.7
α -cadinene		C ₁₅ H ₂₄	20.875	1533.3	1539	3.4
α -cedrene		C ₁₅ H ₂₄	21.256	1412.2	1411	2.9
1R,7S,E)-7-Isopropyl-4,10-dimethylene cyclodec-5-enol		C ₁₅ H ₂₄ O	21.291	1690	1694.5	3.63
Isoaromadendrene epoxide		C ₁₅ H ₂₄ O	21.597	1594	1579	3.37
Humulenol-II		C ₁₅ H ₂₄ O	21.719	2234	2234	2.97
Longifolenaldehyde		C ₁₅ H ₂₄ O	21.944	1668	1631	2.22
Octadecane		C ₁₈ H ₃₈	22.365	296.8	301.7	1.93
Undec-10-ynoic acid, tridec-2-yn-1-yl ester		C ₂₄ H ₄₀ O ₂	23.411	2630	-	2.53
Total						99.99

A study examined by Santos and his coworkers (2020) indicated that α -pinene, limonene, ocimene, nerolidol, spathulenol, and farnesol were identified from the flowers of *T. vogelii* (Dos Santos *et al.*, 2021). According to a study investigated by Bendera (2007) oil nerolidol (7.73%), β -caryophyllene (6.89%), caryophyllene oxide (6.22%), and germacrene D (6.20%) were the

main essential oil components of the leaves of *T. vogelii* (Bendera, 2007). Previously published literature indicated that cis-nerolidol and its trans-isomer had a broad biological spectrum such as antitumor, analgesic, antibacterial, sedative, and fungicide (Abdullah *et al.*, 2022; Chan *et al.*, 2016). An investigation carried out by Chan *et al.*, (2016) demonstrated that nerolidol displayed promising antibacterial activity potent against *S. aureus* FDA 209P, and strains of methicillin-resistant *S. aureus* (MRSA) with MIC values ranging from 512-1024 µg/mL (Chan *et al.*, 2016). Another study investigated by Ugheighele *et al.*, (2020) demonstrated that nerolidol displayed significant anti-urease activity by inhibiting Jack Bean urease with a percentage inhibition of 78.6% with IC₅₀ 34.9 ± 3.57 µM respectively (Ugheighele *et al.*, 2020).

4.2.3. Essential oil analysis of the roots of *A. schimperi*

Hydrodistillation extraction of the roots of *A. schimperi* (60 g) afforded 50 mg (0.08% w/w) yield. GC-MS analysis (Appendix 26) of the essential oil results in the identification of 22 compounds representing 99.83 % of the total essential oil. The retention time, retention index, and percentage of the abundance of the identified essential oils are presented in Table 24. The analyzed essential oils were composed of different classes of compounds such as phenols, aliphatic acids, hydrocarbons, and terpenes. As shown in Table 24, the major constituents of the oils were cedrol (25.85%), squalene (18.77%), 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl (7.82%), cyclopentanone, 2-(1-methylpropyl) (5.76%), hexadecanoic acid (5.99%), 2-Hexenoic acid, (E) (4.545), and 9-Cedranone (3.35%) (Table 24). According to Matebie *et al.*, (2019) study, damascenone (14.83%), dihydroactinidiolide (10.87%), kaur-16-ene (5.74%), and (E)-2-tridecenal (5.51%) were also the major EO components of the leaves of *A. schimperi*. Previous scientific investigations showed that some of the identified compounds have a considerable potential for therapeutic uses. According to Su *et al.*, (2012), cedrol, the major constituent of the root of *A. schimperi* exhibited enormous antimicrobial activity against different pathogenic bacterial and fungal strains (Su *et al.*, 2012). A similar study carried out by Kiran *et al.*, (2009) revealed that cedrol demonstrated antibacterial activity against *P. aeruginosa* with MIC value of 62.5 µg/mL and against *E. coli*, *S. aureus*, *E. aerogenes*, *P. vulgaris*, *S. typhimurium* and *S. epidermidis* with MIC value of 125 µg/mL (Kiran *et al.*, 2010). A related study done by Nazemi *et al.*, (2012) indicated that squalene identified from *Stichopus hermanni* displayed antibacterial activity against *B. subtilis*, *B. cereus*, *S. aureus*, and *N.*

brasiliensis, with MIC values of 500, 50, 100 and 500 µg/mL respectively. The same authors also reported that squalene exhibited cytotoxic activity against the epithelial cancer cell line (KB/C152), and T -lymphocytic leukemia cell line (HUT -78/C185), with IC₅₀ values of 6.21 and 6.29 µg/mL (Nazemi *et al.*, 2022).

Table 24: Essential oils identified from the roots of *A. schimperi*

Compound	Molecular formula	RT	RI		%Area
			Experi.	Lit.(Babushok <i>et al.</i> , 2011)	
2-Hexenoic acid, (E)	C ₆ H ₁₀ O ₂	6.486	981	1933	4.54
Benzylalcohol	C ₇ H ₈ O	7.062	1027.54	-	2.27
Benzeneethanol	C ₈ H ₁₀ O	9.06	1085.5	1088.4	1.11
Benzeneethanol, α-phenyl-	C ₁₄ H ₁₄ O	10.19	1360	1366.3	2.12
Octanoic acid	C ₈ H ₁₆ O ₂	10.41	1175	1165	5.76
Cyclopentanone, 2-(1-methylpropyl)-	C ₉ H ₁₆ O	11.66	1125	1128	1.19
Nonanoic acid	C ₉ H ₁₈ O ₂	12.97	1288	1272	3.13
2(3H)-Furanone, dihydro-5-pentyl-	C ₉ H ₁₈ O ₂	15.53	1312	1320.3	2.93
2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)-	C ₁₃ H ₁₈ O	16.13	1271.8	-	1.02
α-Cedrene	C ₁₅ H ₂₄	16.88	1412.2	1417.7	2.78
Phenol, 2-methoxy-4-propyl-	C ₁₀ H ₁₄ O ₂	17.95	1390	1392	1.81
1,3,5,7-Tetramethyl-adamantane	C ₁₄ H ₂₄	18.63	1162	1169	0.87
2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl	C ₁₁ H ₁₆ O ₂	19.738	1493	-	7.82
Cedrol	C ₁₅ H ₂₆ O	21.45	1589.6	1597.1	25.85
9-Cedranone	C ₁₅ H ₂₄ O	21.927	1630	1638	3.35
Myristic acid	C ₁₄ H ₂₈ O ₂	24.73	1763	1763	0.88
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	C ₁₆ H ₂₂ O ₄	27.05	1847	1847.4	1.63
Germacrene D	C ₁₅ H ₂₄	27.47	1471	1475.9	1.02
Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	28.8	1961	-	5.99
Undec-10-ynoic acid, 3-methylbut-2-en-1-yl ester	C ₁₆ H ₂₆ O ₂	35.92	1820	-	2.3
(3R,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen 3-ol	C ₁₅ H ₂₆ O	40.07	1494	-	3.0
Squalene	C ₃₀ H ₅₀	42.96	2847.1	2831	18.77
Total					99.83

4.2.4. Essential oil constituents of the roots of *R. glutinosa*

Hydrodisillation of extraction of the roots of *R. glutinosa* (70 g) afforded 100 mg (0.14 % w/w) yield. The chemical constituents of the EOs, and their corresponding retention time, retention index, and percentage abundance are presented in Table 25. The GC-MS analysis (Appendix 27) of the EOs of the roots of *R. glutinosa* resulted in the identification of a total of 17 compounds, accounting for 99.92% of the total compositions, of which sesquiterpenes (6.49%), monoterpenes (5.77%), benzenoid compounds (81.71%) and hydrocarbons (4.84) were a class of compounds of EOs of roots of *R. glutinosa*. Among the identified compounds acetophenone, 4'-hydroxy (72.19 %), benzofuran, 2,3-dihydro-2-methyl (6.99%), and 2-oxabicyclo[2.2.2]octane, 1,3,3-trimethyl (3.5%), were the major essential oil component of the root of *R. glutinosa*.

Table 25: Essential oil constituents of the roots of *R. glutinosa*

Compound	Molecular formula	RT	RI		%Area
			Experi.	Lit.(Babushok <i>et al.</i> , 2011)	
(E)-Oct-2-enal	C ₈ H ₁₄ O	4.519	1030	1039	1.18
2-Oxabicyclo[2.2.2]octane, 1,3,3-trimethyl-	C ₁₀ H ₁₈ O	7.805	1027.66	1031.5	3.5
Nonanal	C ₉ H ₁₈ O	10.162	1079.1	1083.8	0.95
Benzofuran, 2,3-dihydro-2-methyl-	C ₉ H ₁₀ O	10.624	130.6	184.6	6.99
4-hydroxy-acetophenone	C ₈ H ₈ O ₂	12.437	1490.7	1442	72.19
dl-Isopulegol	C ₁₀ H ₁₈ O	13.315	1141	1155.6	1.42
Benzene, (2,2-dimethoxyethyl)-	C ₁₀ H ₁₄ O ₂	14.204	2244.1	2240	0.93
Benzofuran, 2,3-dihydro-2-methyl-	C ₉ H ₁₀ O	15.059	1306	1300	0.86
Cyclotetradecane	C ₁₄ H ₂₈	18.23	1673	1680	1.11
1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	C ₁₅ H ₂₆ O	21.302	1517	1523	1.89
Humulene epoxide I	C ₁₅ H ₂₄ O	22.977	1625	1590.7	1.74
Humulenol-II	C ₁₅ H ₂₄ O	23.809	2234	1449	0.87
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	C ₁₆ H ₂₂ O ₄	25.645	1863	-	0.74
Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	26.298	1904.1	-	0.99
Palmitic acid	C ₁₆ H ₃₂ O ₂	26.725	1968	1968.4	0.72
Geranyl benzoate	C ₁₇ H ₂₂ O ₂	26.876	1952		1.99
Carveol	C ₁₀ H ₁₆ O	28.204	1188	1218.0	0.85
Total					99.2

4.2.5. Essential oil constituents of the roots of *C. spinarum*

Hydrodistillation extraction of essential oil (EO) of the roots of *R. glutinosa* (60 g) afforded 55 mg (0.09% w/w) yield. The retention time, retention index, and percentage of abundance of the identified EOs are given in Table 26. The GC-MS analysis (Appendix 28) of the roots of *R. glutinosa* results in the identification of 17 compounds, representing 99.91% of the total oil component. The composition of the EOs were phenolic compounds, monoterpenes, sesquiterpenes, and furancarboxaldehyde. Among the identified compounds, 4-hydroxy-acetophenone (74.7%), 2-methoxy-phenol (7.16%), α -methyl-benzenemethanol (3.97%), and 2-furancarboxaldehyde were the major essential oil components of the *R. glutinosa*. A similar study investigated by Sisay and his coworkers (2022) showed that 2-hydroxy acetophenone (82.97%) was the most prominent essential oil component of the roots of *C. spinarum* (Sisay Yaya *et al.*, 2022).

Table 26: Essential oil constituents of the roots of *C. spinarum*

Compound	Molecular formula	RT	Experi	RI Lit.(Babusho k <i>et al.</i> , 2011)	%Area
2-Furancarboxaldehyde	C ₅ H ₄ O ₂	3.162	835	790	3.02
2-Furancarboxaldehyde, 5-methyl-	C ₆ H ₆ O ₂	5.305	966	966	1.54
Phenol	C ₆ H ₆ O	5.617	950	965	1.56
Phenylacetaldehyde	C ₈ H ₈ O	7.153	1014.1	1014	0.53
α -methyl-benzenemethanol	C ₈ H ₁₀ O	7.569	1038	1035	3.97
2-methoxy-phenol	C ₇ H ₈ O ₂	8.308	1066.8	1070	7.16
4-hydroxy-acetophenone	C ₈ H ₈ O ₂	10.422	1490.7	1475	74.7
Creosol	C ₈ H ₁₀ O ₂	11.034	1162	1163.4	0.8
(1S,4R,5R)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-5-ol	C ₁₀ H ₁₈ O ₂	11.872	1240.7	-	0.42
Guaiacol, 4-ethyl	C ₉ H ₁₂ O ₂	13.333	1282	1286	0.95
2-methoxy-4-vinyl-phenol	C ₉ H ₁₀ O ₂	14.257	1315		1.46
2,6-dimethoxy- phenol	C ₈ H ₁₀ O ₃	15.227	1347	1343	0.85
Phenol, 2-methoxy-4-(1-propenyl)-	C ₁₀ H ₁₂ O ₂	17.728	1436.3	1430	1.12
1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-	C ₁₅ H ₂₆ O	20.448	1510	1517	0.5
2Naphthalenemethanol1,2,3,4,4a,5,6,7-octahydro, 4a,8-tetramethyl-,	C ₁₅ H ₂₆ O	22.111	1630	1641.1	0.84
Geranyl benznate	C ₁₇ H ₂₂ O ₂	28.972	1952		0.49
Total					99.91

4.3. Antibacterial Activity

4.3.1. Antibacterial activity of *W. uniflora*

Evaluation of the antibacterial activity of extracts, EOs, and isolated compounds (**96-99**) of the roots of *W. uniflora* were evaluated against Gram-positive bacteria (*S. aureus* and *S.pyogens*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*) using agar disc diffusion assay (Balouiri *et al.*, 2016). The results of the antibacterial activities of the tested samples are presented in Table 27. All tested samples showed low to moderate activity compared to ciprofloxacin. As shown in Table 27, MeOH extract exhibited antibacterial activity against all tested pathogens, and the maximum activity was recorded against *E. coli* with an inhibition zone (IZ) of 13.6 ± 0.47 mm at 200 mg/mL, compared to ciprofloxacin (IZ of 27 ± 0.0 mm) at 0.5mg/mL. DCM/MeOH (1:1) extract also displayed modest antibacterial activity against *E. coli* and *P. aeruginosa* with maximum IZ of 11.6 ± 0.81 and 10.0 mm respectively but did not show activity against *S. aureus* and *S. pyogenes* at the same concentration (Table 27).

As given in Table 27, bergenin (**96**) and compound **98** exhibited maximum antibacterial activity against *E. coli* with IZ of 11.6 ± 0.47 and 11.3 ± 0.81 mm respectively. The document compiled by Salimo *et al.*, (2023) indicated that bergenin (**96**) isolated from the ethanol extract of *P. dubium* (Fabaceae) had antibacterial activity against *E. coli*, *K. pneumoniae*, and *P. aeruginosa* (IC₅₀ ranging from 0.78 to 1.56 $\mu\text{g/mL}$); however, it had a weak effect against *S. typhi*, *Acenatobacter sp.*, *E. Proteus sp.*, *E. phaeacalis*, and *S. aureus* (IC₅₀ ranging from 3.125 to 6.25 $\mu\text{g/mL}$) (Salimo *et al.*, 2023). A related investigation conducted by Nyemb *et al.*, (2018) revealed that bergenin (**96**) isolated from *C. populnea* roots had an inhibitory effect against *S. typhi* (ATCC6539), *S. typhi* (isolated), *P. aeruginosa* (ATCC9721) and *E. coli* (isolated), IC₅₀ ranging from 8 to 64 $\mu\text{g/mL}$. According to Nyemb and his coworkers (2018) investigation, bergenin (**96**) can easily cross the complex and multilayer lipopolysaccharide cell walls of Gram-negative bacteria so that it weakens them, causing lysis and consequent death (Nyemb *et al.*, 2018). A similar study carried out by Parvez *et al.*, (2019) showed that epigallocatechin-3-gallate (**98**) exhibited antibacterial activity against *S. aureus* and *E. coli* with MIC values of 625 and 1250 $\mu\text{g/mL}$ respectively, compared to gentamicin (MIC value of 32 $\mu\text{g/mL}$) (Parvez *et al.*, 2019). β -sitosterol (**97**) also showed antibacterial action against *E. coli*, *P. aeruginosa*, and

S. aureus with inhibition zone ranging from 7 ± 0.0 - 11 ± 0.0 mm (Table 27). The highest inhibition zone (11.0 ± 0.0 mm) was recorded against *S. aureus*. A related study, investigated by Sen *et al.*, (2012) demonstrated that β -sitosterol (**97**) exhibited antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, and *K. pneumonia* with IZ ranging from 10-14 mm, compared to gentamicin, IZ of ranging from 16-18 mm (Amit Sen *et al.*, 2012). This may be because terpenes exhibit antimicrobial activity by complexing proteins such as adhesion's, substrates, and cell membrane proteins, hence inactivating microbial adhesion which is the first step in the establishment of infections, and also causing membrane disruption (Nweze *et al.*, 2019).

Compound **99** exhibited antibacterial activity against all tested bacterial strains with inhibition zone ranges of 12.0 ± 0.0 to 15.6 ± 0.47 mm at 5 mg/mL, concentration (Table 27). The maximum antibacterial activity was noted against *S. pyogenes* with an inhibition zone of 15.6 ± 0.47 mm. EO extract had antibacterial activity against all tested photogenes with inhibition zones ranging from 14.3 ± 0.81 to 17.6 mm, compared to ciprofloxacin (IZ ranges from 27.0 ± 0 to 31.0 ± 0.0 mm, at 0.5mg/mL) (Table 27). *S. aureus* was the most inhibited bacteria with an IZ of 17.6 mm at 5 mg/mL concentration. The EO extract also displayed antibacterial activity against *P. aeruginosa*, *E. coli*, and *S. pyogenes* with IZ of 14.3 ± 0.81 , 15.0 ± 0.0 , and 15.6 ± 0.47 mm respectively (Table 27). The highest antibacterial potential of the EO extract may be associated with the additive effect of the major and minor oil components. Fajrih *et al.*, (2022) conducted a study on *B. gymnorhiza* and identified 18.52% of palmitic acid which is reported to work synergistically with various active compounds, hence it can increase its antibacterial activity (Fajrih *et al.*, 2022). Another study conducted by Tao *et al.*, (2018) confirmed that tricosane displayed antibacterial activity against *P. fluorescens*, *E. coli*, *B. subtilis*, and *S. cerevisiae* with an inhibition zone ranging from 8.20-15.97 mm at 20 mg/mL (Tao *et al.*, 2018). According to a study conducted by Octarya *et al.*, (2021), eicosane has also been reported to have antibacterial activities (Octarya *et al.*, 2021).

Table 27: Antibacterial activities (mean $\pm$ SD, in mm) of DCM/MeOH (1:1) and MeOH extract of EO extract and compounds (**96-99**) isolated from roots of *W. uniflora*.

Test Sample	Conc. (mg/mL)	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
DCM/MeOH (1:1) extract	200	11.66 $\pm$ 0.81	10.0 $\pm$ 0.0	NI	NI
	100	8.33 $\pm$ 0.81	9.33 $\pm$ 0.81	NI	NI
	50	7.0 $\pm$ 0.0	8.0 $\pm$ 0.0	NI	NI
MeOH extract	200	13.66 $\pm$ 0.47	9.0 $\pm$ 0.0	11.66 $\pm$ 0.47	10.66 $\pm$ 0.47
	100	11.66 $\pm$ 0.81	8.33 $\pm$ 0.81	9.33 $\pm$ 0.81	8.66 $\pm$ 0.47
	50	8.33 $\pm$ 0.57	7.0 $\pm$ 0.0	8.0 $\pm$ 0.0	7.0 $\pm$ 0.0
Bergenin (96)	5	11.66 $\pm$ 0.47	9.33 $\pm$ 0.81	10.66 $\pm$ 0.47	8.0 $\pm$ 0.0
	2.5	10.0 $\pm$ 0.0	8.66 $\pm$ 0.47	9.0 $\pm$ 0.0	7.33 $\pm$ 0.81
	1.25	8.0 $\pm$ 0.0	8.0 $\pm$ 0.0	7.3 $\pm$ 0.81	7.0 $\pm$ 0.0
β -sitosterol (97)	5	10.66 $\pm$ 0.47	9.33 $\pm$ 0.81	11.0 $\pm$ 0.0	NI
	2.5	8.66 $\pm$ 0.0	7.0 $\pm$ 0.0	8.33 $\pm$ 0.81	NI
	1.25	7.33 $\pm$ 0.81	7.0 $\pm$ 0.00	7.66 $\pm$ 0.47	NI
Compound 98	5	11.33 $\pm$ 0.81	8.33 $\pm$ 0.81	NI	7.0 $\pm$ 0.0
	2.5	8.0 $\pm$ 0.0	7.66 $\pm$ 0.47	NI	NI
	1.25	7.0 $\pm$ 0.0	7.33 $\pm$ 0.81	NI	NI
Compound 99	5	12.0 $\pm$ 0.0	14.66 $\pm$ 0.47	15.0 $\pm$ 0.0	15.66 $\pm$ 0.47
	2.5	10.33 $\pm$.81	12.33 $\pm$ 0.81	13.66 $\pm$ 0.57	14.0 $\pm$ 0.0
	1.25	8.33 $\pm$.081	10.0 $\pm$ 0.0	11.33 $\pm$ 0.81	12.33 $\pm$ 0.81
EOs	5	15.0 $\pm$ 0.0	14.33 $\pm$ 0.81	17.66 $\pm$ 0.47	15.66 $\pm$ 0.47
	2.5	11.0 $\pm$ 0.0	12.0 $\pm$ 0.0	14.33 $\pm$ 0.81	13.66 $\pm$ 0.47
	1.25	10.33 $\pm$ 0.81	11.66 $\pm$ 0.47	11.0 $\pm$ 0.0	11.0 $\pm$ 0.0
Ciprofloxacin	0.5	27.0 $\pm$ 0.0	29.0 $\pm$ 0.0	31.0 $\pm$ 0.0	30.0 $\pm$ 0.0

***NI: No Inhibition**

4.3.2. Antibacterial activity of *T. vogelii*

The antibacterial potentials of the EOs, crude extracts, and compounds (**101-105**) of the roots of *T. vogelii* were evaluated following the broth dilution assay (Eloff, 1998). The MIC values of EOs, DCM/MeOH (1:1), MeOH extracts, and compounds (**101-105**) against eight pathogenic bacteria are presented in Table 28. DCM/MeOH (1:1) extract exhibited inhibitory activity against *P. aeruginosa* and *S. aureus* with MIC values of 0.5 and 1.66 mg/mL respectively, compared to ciprofloxacin (0.078 μ g/mL) (Appendix 29). MeOH extract showed maximum inhibitory activity against *p. aeruginosa*, *P. mirabilis*, and *S. aureus* with MIC values of 0.66, 1.66, and 2.0 mg/mL respectively, compared to ciprofloxacin (MIC values ranging from

0.078-0.312 µg/mL) (Appendix 29). The observed antibacterial activity showed good agreement with the reported literature that methanol extract of the roots of *T. vogelii* was capable of suppressing *S. aureus* with MIC value of 1.25 mg/mL followed by *S. typhi* and *K. pneumoniae* at MIC 2.5 mg/mL. The leaves methanol extracts of *T. vogelii* also exhibited high antibacterial activities at MIC 1.25 mg/mL against *S. aureus* and *S. typhi* compared to ciprofloxacin with MIC values ranging from 0.15625 to 0.625 mg/mL (Mlozi *et al.*, 2020). Compound **101** demonstrated promising antibacterial action against *S. epidermidis* and *P. mirabilis* with MIC values of 0.66 and 0.83 mg/mL respectively. Tephrosin (**102**) exhibited moderate inhibitory activity against *S. aureus* with MIC values of 0.83 ± 0.4 mg/mL, compared to ciprofloxacin (MIC value 0.078 ± 0.0 µg/mL) (Appendix 29). Maackiain (**103**) showed significant inhibitory activity against *P. mirabilis* and *P. aeruginosa* at 0.5 and 0.83 ± 0.2 mg/mL respectively. Obovatin (**104**) displayed moderate inhibitory activity towards *P. aeruginosa* with MIC value of 0.83 ± 0.2 mg/mL, compared to ciprofloxacin (0.078 ± 0.0 µg/mL) (Appendix 29).

Compound **105** also displayed antibacterial activity against *S. epidermidis* with MIC value of 0.66 ± 0.4 mg/mL, compared to ciprofloxacin (MIC value of 0.0312 µg/mL) (Table 28) (Appendix 29). Arriaga and his colleagues (2017) reported that tephrosin (**102**) and obovatin (**104**) showed antibacterial activity against *E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. mutans*, and *S. aureus* with MIC values ranging from 64 to 1024 µg/mL (Arriaga *et al.*, 2017). The antibacterial activities of many plant-derived flavonoids use different mechanisms than those of conventional drugs and thus could be of importance in the enhancement of antibacterial therapy (Górniak *et al.*, 2019). Literature reports supported that different flavonoids exhibited antibacterial activity following different mechanism actions such as causing cell-membrane damage, inhibiting various synthase involving the nucleic acid synthesis, the bacterial respiratory chain, or the cell envelope synthesis (Yuan *et al.*, 2021). For instance, according to Wu *et al.* (2013) study, flavonoids exhibited the important structural aspect inhibition of *E. coli* DNA gyrase target protein (Wu *et al.*, 2013). The EO extract also exhibited antibacterial activity against *S. epidermidis* with MIC value of 1.0 ± 0.0 mg/mL, respective to ciprofloxacin (MIC value of 0.312 µg/mL). The antibacterial activities of crude extracts and the studied compounds confirmed that the chemical constituents of the roots of *T. vogelii* have medicinal value for treating pathogenic bacterial-inducing disorders.

Table 28: Minimum inhibited concentration (mean± SD expressed in mg/mL) of EO extract, crude extracts, and isolated compounds (**101-105**)

Extracts/ compound	<i>S. aureus</i>	<i>E. faecalis</i>	<i>S. epidermidis</i>	<i>S. agalactia</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. dysenteriae</i>	<i>P. mirabilis</i>
DCM/MeOH(1:1)	1.66±0.2	2.66±0.9	2.66±0.3	2.0±0.0	2.66±0.4	0.5±0.0	3.33±0.4	4.0±0.0
MeOH.	2±0.0	4.0±0.0	4±0.0	4.0±0.0	2.66±0.9	0.66±0.2	4.0±0.0	1.66±0.4
Compound 101	1.0±0.0	4.0±0.0	0.66±0.4	4.0±0.0	2.0±0.0	1.33±0.9	2.66±0.9	0.83±0.4
Tephrosin (102)	0.83±0.4	2.0±0.0	4.0±0.0	2.0±0.0	2.0±0.0	1.66±0.9	4.0±0.0	1.0±0.0
Maackiain (103)	1.0±0.0	2.66±0.1	2.0±0.0	4.0±0.0	2.0±0.0	0.83±0.2	2.0±0.0	0.5±0.0
Obovatins (104)	4.0±0.0	2.66±0.2	4.0±0.0	4.0±0.0	1.66±0.9	0.83±0.2	8.0±0.0	4.0±0.0
Compound 105	2.0±0.0	2.66±0.4	0.66±0.4	1.33±0.2	2.0±0.0	2.0±0.0	4.0±0.0	1.33±0.2
EOs extract	4.0±0.0	2.66±0.4	1.0±0.0	4.0±0.0	8.0±0.0	4.0±0.0	8.0±0.0	4±0.0
Cipro (µg/mL)	0.078±0.0	0.156±0.0	0.312±0.0	0.156±0.0	0.078±0.0	0.078±0.0	0.312±0.0	0.312±0.0

The observed antibacterial potentials of the oil components may be linked with the synergistic or additive effects of its major and minor constituents (Kuspradini *et al.*, 2016). The chemical diversity of essential oil components may also increase the possibility of existing constituents that can impair essential biochemical reactions needed for the survival of microorganisms (De Sousa *et al.*, 2023). The hydrophobicity of the fixed and essential oils may also significantly contribute to moving across the lipids of the cell membranes of bacteria and disrupt the cell wall structures (Bhavaniramya *et al.*, 2019).

4.3.3. Antibacterial activity of *A. schimperi*

The antibacterial activity of the DCM/MeOH and MeOH extracts, EOs, and isolated compounds (**107**, **108**, and **109**) of the roots of *A. schimperi* were evaluated against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. pyogenes* using disc diffusion assay (Balouiri *et al.*, 2016). The antibacterial activity results of crude extracts, EOs and isolated compounds of *A. schimperi* are

presented in Table 29. The DCM/MeOH (1:1) and MeOH extracts of the root of *A. schimperi* showed maximum antibacterial activity against *E.coli* and *S. aureus* with respective ZI of 16.0 ± 0.0 and 15.66 ± 0.47 mm at 200 mg/mL respectively, compared to ciprofloxacin (27.0 ± 0 to 31 ± 0.0 mm at 0.5mg/mL) (Table 29). MeOH extract also exhibited modest antibacterial activity against *S. pyogenes* and *S. aureus* with ZI of 13.0 ± 0.0 and 14.33 ± 0.81 mm respectively. The observed result showed a good agreement with the literature report (Taye *et al.*, 2011). DCM/MeOH extract also demonstrated modest activity against *S. aureus* and *E. coli* with ZI of 15.66 ± 0.57 and 13.33 ± 0.81 mm respectively.

Table 29: Antibacterial activities (mean $\pm$ SD, in mm) of DCM/MeOH (1:1) and MeOH extract of EO extract and compounds (**96-99**) isolated from roots of *A. schimperi*

Test Sample	Conc. (mg/mL)	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
DCM/MeOH (1:1) extract	200	13.33 ± 0.81	11.0 ± 0.0	15.66 ± 0.57	12.66 ± 0.57
	100	10.33 ± 0.81	9.33 ± 0.81	14.0 ± 0.0	9.33 ± 0.81
	50	7.66 ± 0.47	8.0 ± 0.0	11.66 ± 0.47	7.0 ± 0.0
MeOH extract	200	16.0 ± 0.0	12.0 ± 0.0	13.0 ± 0.0	14.33 ± 0.47
	100	14.66 ± 0.47	11.66 ± 0.47	10.33 ± 0.01	11.66 ± 0.81
	50	11.0 ± 0.0	9.0 ± 0.0	8.0 ± 0.0	10.0 ± 0.0
Lupeol (107)	5	16.66 ± 0.47	12.33 ± 0.81	17.33 ± 0.81	11.66 ± 0.57
	2.5	11.0 ± 0.0	11.0 ± 0.0	13.66 ± 0.57	11.0 ± 0.0
	1.25	9.66 ± 0.47	9.33 ± 0.81	11.0 ± 0.0	9.33 ± 0.81
Dihydroferulic acid (108)	5	12.0 ± 0.0	10.0 ± 0.0	11.0 ± 0.0	14.66 ± 0.57
	2.5	10.66 ± 0.47	7.0 ± 0.0	10.33 ± 0.81	13.0 ± 0.0
	1.25	7.33 ± 0.57	7.0 ± 0.0	8.0 ± 0.0	11.66 ± 0.47
Compound 109	5	12.33 ± 0.81	14.66 ± 0.57	15.33 ± 0.81	13.66 ± 0.57
	2.5	11.33 ± 0.81	9.66 ± 0.57	11.66 ± 0.47	11.33 ± 0.81
	1.25	9.66 ± 0.47	8.0 ± 0.0	10.0 ± 0.0	9.66 ± 0.08
EOs	5	17.33 ± 0.81	11.33 ± 0.81	15.66 ± 0.47	14.0 ± 0.0
	2.5	16.0 ± 0.0	10.0 ± 0.0	13.33 ± 0.81	12.66 ± 0.47
	1.25	14.66 ± 0.47	8.33 ± 0.81	12.0 ± 0.0	10.33 ± 0.81
Ciprofloxacin	0.5	27.0 ± 0.0	29.0 ± 0.0	31.0 ± 0.0	30.0 ± 0.0

A similar investigation done by Kenubih *et al.*, (2021) demonstrated that the DCM extract from the leaves of *A. schimperi* exhibited antibacterial activity against *E. coli* and *S. aureus* with ZI of 16.66 ± 0.47 and 15.0 ± 0.0 mm respectively (Kenubih *et al.*, 2021). As shown in Table 29, Lupeol (**107**) displayed maximum antibacterial activity against *E. coli* and *S. aureus* with ZI of 16.66 ± 0.47 and 17.33 ± 0.81 mm respectively, compared to ciprofloxacin ($27.0-31.0 \pm 0.0$ mm) (Appendix 30). The finding showed good agreement with the literature report demonstrated that lupeol (**107**) exhibited antibacterial activity against *E. coli* and *S. aureus* with ZI of 19 and 20 mm respectively at 3 mg/mL (Ekalu *et al.*, 2019). Lupeol (**107**) also showed modest antibacterial activity against *P. aeruginosa* and *S. pyogenes* with ZI of 12.33 ± 0.81 and 11.66 ± 0.47 mm respectively. A similar study conducted by Musa *et al.*, (2024) showed that lupeol (**107**) exhibited antibacterial activity against *E. coli* and *S. aureus* with MIC values of 2.5 and 5 mg/mL respectively (Musa *et al.*, 2024).

Dihydroferulic acid (**108**) displayed modest antibacterial activity against *S. pyogenes* and *E. coli* with ZI of 14.66 ± 0.47 and 12.0 ± 0.0 mm respectively (Appendix 30). Compound **109** demonstrated antibacterial activity *S. aureus*, *P. aeruginosa* and *S. pyogenes*, with ZI of 13.66 ± 0.47 , 14.66 ± 0.47 and 15.33 ± 0.81 , mm respectively (Table 29). The antibacterial activity outcome of the studied compounds from the root of *A. schimperi* indicated that lupeol (**107**) showed the best antibacterial activity compared to other compounds (**108**, and **109**) which implies that lupeol (**107**) could be the active constituent of the roots of *A. schimperi*. The observed maximum activity of lupeol (**107**) may be associated with its nature of lipophilicity and it readily penetrates the cell membrane and exerts its biological effect (Chaudhari *et al.*, 2012). The EO extract of the roots of *A. schimperi* also exhibited modest antibacterial activity against *P. aeruginosa*, *S. pyogenes*, *S. aureus*, and *E. coli* with respective ZI of 11.33 ± 0.81 , 14.0 ± 0.0 , 14.6 ± 0.46 , 15.6 ± 0.57 and 17.33 ± 0.81 mm, compared to ciprofloxacin (ZI ranging from 27 ± 0.0 to 31.0 ± 0.0 mm). The observed antimicrobial activities of *A. schimperi* essential oil may be thought to arise from the actions of the main compounds or some synergy between the major and minor compounds. A previous study investigated by Su *et al.*, (2012) indicated that cedrol had a promising antibacterial effect against various pathogenic microorganisms (Su *et al.*, 2012).

4.3.4. Antibacterial activity of *R. glutinosa*

The *in vitro* evaluation of the antibacterial activity of the crude extracts, EOs and isolated compounds was assessed against Gram-positive (*S. aureus* and *S. pyogenes*) and Gram-negative (*E. coli* and *P. aeruginosa*) using agar disc diffusion (Balouiri *et al.*, 2016). The antibacterial activity results of the crude extracts and isolated compounds are presented in Table 30. DCM/MeOH (1:1) and MeOH extracts displayed maximum antibacterial activity against *S. aureus* with ZI of 17.3 and 18.0 mg/mL respectively at 200 mg/mL compared to ciprofloxacin (31 ± 0.0 at 0.5mg/mL) (Table 30) (Appendix 30). MeOH extract also exhibited antibacterial activity against *E. coli* and *S. pyogenes* with ZI of 16.33 ± 0.81 and 16.0 ± 0.0 mm respectively. DCM/MeOH (1:1) extract inhibited *S. pyogenes* and *E. coli* with ZI of 15.66 ± 0.47 and 15.0 ± 0.0 mm respectively. The present finding revealed that both plant extracts had promising antibacterial activity against all bacteria tested with varying degrees of activity. The differences in the antibacterial potential of the DCM/MeOH (1:1) and MeOH extracts against the tested bacteria could be attributed to the differences in the chemical constituents and the concentration of the secondary metabolites present in the respective extracts (Kenubih *et al.*, 2021). EO constituents of roots of *R. glutinosa* demonstrated low to promising antibacterial activity with inhibition zones ranging from 9.0 ± 0.0 to 16.0 ± 0.0 mm (Table 30). Maximum antibacterial activity was recorded against *S. aureus* and followed by *S. pyogenes* with ZI of 16.0 ± 0.0 and 13.33 ± 0.81 mm respectively.

Compound **111** exhibited maximum activity against *E. coli* and *S. pyogenes* with ZI of $15.66\pm.47$ and 16.0 ± 0.0 mm respectively. Compound **111**, methyl gallate (**114**), and gallic acid (**115**) also demonstrated antibacterial effects against *P. aeruginosa* with ZI of 14.33 ± 0.81 , 15.66 ± 0.47 and 14.66 ± 0.57 mm respectively compared to ciprofloxacin (ZI of 29.0 ± 0.0 at 0.5mg/mL) (Appendix 30). Methyl gallate (**114**) showed the highest antibacterial action against *S. aureus* and *E. coli* ZI of 18.66 ± 0.57 and 16.33 ± 0.81 mm respectively (Table 30) (Appendix 30). Gallic acid (**115**) displayed maximum antibacterial activity *S. pyogenes* and *S. aureus* with ZI of 16.0 ± 0.0 and 17.66 ± 0.47 mm respectively (Table 30). A related study investigated by Al-Zahrani (2019) revealed that methyl gallate (**114**) and gallic acid (**115**) exhibited inhibitory

activity against methicillin-resistant and susceptible methicillin *S. aureus* strains with MIC values of ranges 3.5 to 25 µg/mL (Al-Zahrani, 2012).

Table 30: Antibacterial activities (mean ± SD, in mm) of DCM/MeOH (1:1) and MeOH extract of EO extract and compounds (**111-115**) isolated from roots of *R. glutinosa*

Test Sample	Conc. (mg/mL)	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
DCM/MeOH (1:1) extract	200	15.0±0.0	12.0±0.0	17.33±0.81	15.33±0.81
	100	13.66 ±0.47	10.33±0.81	15.33±0.04	13.66±0.47
	50	10.0±0.0	9.0 ±0.0	12.0±0.0	11.0±0.0
MeOH extract	200	16.33±0.81	14.33±0.81	18.0±0.0	16.0±0.0
	100	14.0±0.47	13.0±0.0	16.33±0.81	14.33±0.081
	50	11.66±0.47	11.66±0.57	14.0±0.0	12.6±0.57
Compound 111	5	15.66±0.47	14.33±0.81	11.33±0.81	16.0±0.0
	2.5	15.0±0.0	12.66±0.47	10.66±0.47	13.66±0.47
	1.25	13.66±0.57	11.0±0.0	9.3±0.81	12.33±0.81
Methyl gallate (114)	5	16.33 ±0.81	15.66±0.57	18.66±0.57	12.66 ±0.47
	2.5	14.66±0.47	13.33±0.81	17.0±0.0	10.33±0.81
	1.25	13.33±0.81	12.0±0.0	16.33±0.81	8.66±0.57
Gallic acid (115)	5	14.0±0.0	14.66±0.57	16.0±0.0	17.66±0.47
	2.5	12.33±0.81	9.33±0.81	14.33±0.81	15.66±0.47
	1.25	11.0±0.0	8.66±0.47	12.66±0.57	13.33±0.57
EOs	5	12.66±0.47	10.66±0.47	16.0±0.0	13.33±0.81
	2.5	10.0±0.0	9.0±0.0	14.66±0.47	11.6±0.47
	1.25	8.33±0.81	7.0±0.0	12.0±0.0	9.0±0.0
Ciprofloxacin	0.5	27.0±0.0	29.0±0.0	31.0±0.0	30.0±0.0

A study investigated by Pinho and his coworkers (2014) showed that gallic acid displayed antibacterial effects against *S. epidermidis*, *S. aureus*, and *K. pneumonia* with MIC values of 0.0098, 0.0195, 0.00975 mg/mL, and MBC values of 0.0039, 0.039 and 0.0039 mg/mL respectively (Pinho *et al.*, 2014). Phenolic compounds such as methyl gallate (**114**) and gallic acid (**115**) disrupt the bacterial cell wall by proton donation and acidification of the intracellular cytosolic which inhibits the enzyme H⁺-ATPase, needed for the synthesis of ATP (Kwon *et al.*, 2007).

4.3.5. Antibacterial activity of *C. spinarum*

The antibacterial activity of the crude extracts, EOs, and isolated compounds of roots of *C. spinarum* were evaluated against Gram-positive (*S. aureus* and *S. pyogenes*) and Gram-negative

(*E. coli* and *P.aeruginosa*) using agar disc diffusion (Balouiri *et al.*, 2016). The results of the antibacterial activity of crude extracts, EO extract, and isolated compounds (**116** and **117**) are presented in Table 31. DCM/MeOH (1:1) extract displayed inhibition of all tested pathogens with ZI ranging from 13.0 ± 0.0 to 18.66 ± 0.57 mm. The maximum antibacterial activity was noted by *S. pyogenes* with ZI of 18.66 ± 0.57 mm. MeOH extract also demonstrated antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. pyogenes* with respective ZI of 15.66 ± 0.47 , 14.66 ± 0.57 , 17.0 ± 0.0 and 18.33 ± 0.81 mm (Table 31). Both DCM/MeOH (1:1) and MeOH extract showed better inhibition potential against Gram-positive bacteria compared to Gram-negative bacteria. Previous investigation studied by Rubaka *et al.*, (2014) indicated that methanol extract of the leaves and roots of *C. spinarum* displayed antibacterial activity against *S. aureus* and *E. coli* with MIC value of 312 $\mu\text{g/mL}$. The same authors also reported petroleum extract from the leaves of *C. spinarum* showed antibacterial activity against *S. aureus* and *E. coli* with MIC values of 625 and 1250 $\mu\text{g/mL}$ respectively (Rubaka *et al.*, 2014).

Another study carried out by Abdu *et al.*, (2008) indicated that petroleum ether extract of root-bark of *C.spinarum* (syn. *C. edulis*) showed strong antibacterial activity against *E. coli*, *K.pneumonia*, *S. aureus*, and *S. species* with inhibition zones of 19.45 ± 0.14 , 17.36 ± 0.13 , 21.15 ± 0.16 6.54 ± 0.10 respectively, compared to Gentamycin, with respective inhibition zones of 21.65 ± 0.10 , 13.85 ± 0.15 , 14.65 ± 0.15 , 9.50 ± 0.10 (Abdu *et al.*, 2008). A related study investigated by Nguld *et al.*, (2013) indicated that ethanol extract of root bark of *C. spinarum* (syn. *C.edulis*) displayed modest antibacterial activity against *S. typhi*, *S. dysenteriae*, and *S. pyogenes* with inhibition zones of 10, 12 and 11 mm respectively at a concentration of 200 mg/mL (Ngulde *et al.*, 2013).The EO extract of *C. spinarum* demonstrated inhibitory action against *E. coli* and *S. pyogenes* with respective ZI of 16.66 ± 0.47 , compared to ciprofloxacin (27 ± 0.0 mm) and 17.0 ± 0.0 mm (compared to ciprofloxacin 31 ± 0.0 mm (Table 35) (Appendix 30). The EO extract also showed moderate antibacterial activity against *P. aeruginosa* and *S. aureus* with ZI of 14.66 ± 0.57 and 15.66 ± 0.47 mm respectively at 5 mg/mL (Table 31). EOs comprise numerous compounds such as aromatic hydrocarbons, terpene (monoterpenes and sesquiterpenes), terpenoids, esters, alcohols, acids, aldehydes, and ketones, and their antibacterial activity may also be contributed by the minor and major constituents (Angane *et al.*, 2022). The lipophilic character of their hydrocarbon skeleton and the hydrophilic character

of their functional groups may also be of major importance in the antimicrobial action of essential oils components (Bendiabdellah *et al.*, 2013).

Table 31: Antibacterial activities (mean $\pm$ SD, in mm) of DCM/MeOH (1:1) and MeOH extract of EO extract and compounds (**116** and **117**) isolated from roots of *C. spinarum*

Test Sample	Conc. (mg/mL)	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
DCM/MeOH (1:1) extract	200	15.33 $\pm$ 0.81	13.0 $\pm$ 0.0	16.33 $\pm$ 0.81	18.66 $\pm$ 0.57
	100	14.0 $\pm$ 0.0	11.66 $\pm$ 0.57	15.0 $\pm$ 0.0	16.0 $\pm$ 0.0
	50	11.33 $\pm$ 0.81	10.33 $\pm$ 0.81	13.66 $\pm$ 0.57	13.66 $\pm$ 0.47
MeOH extract	200	15.66 $\pm$ 0.47	14.66 $\pm$ 0.57	17.0 $\pm$ 0.0	18.33 $\pm$ 0.81
	100	14.66 $\pm$ 0.47	12.33 $\pm$ 0.81	15.66 $\pm$ 0.57	17.33 $\pm$ 0.81
	50	12.0 $\pm$ 0.0	10.66 $\pm$ 0.47	14.0 $\pm$ 0.0	15.66 $\pm$ 0.47
Compound 116	5	15.0 $\pm$ 0.0	13.66 $\pm$ 0.57	17.33 $\pm$ 0.81	14.66 $\pm$ 0.47
	2.5	13.66 $\pm$ 0.47	11.0 $\pm$ 0.0	13.66 $\pm$ 0.57	12.66 $\pm$ 0.57
	1.25	11.33 $\pm$ 0.81	8.33 $\pm$ 0.81	11.0 $\pm$ 0.0	10.0 $\pm$ 0.0
Scopoletin (117)	5	14.33 $\pm$ 0.81	9.66 $\pm$ 0.57	11.0 $\pm$ 0.0	15.33 $\pm$ 0.81
	2.5	12.66 $\pm$ 0.57	8.33 $\pm$ 0.81	10.33 $\pm$ 0.81	12.66 $\pm$ 0.57
	1.25	9.0 $\pm$ 0.0	7.0 $\pm$ 0.0	8.0 $\pm$ 0.0	11.0 $\pm$ 0.0
EOs	5	16.66 $\pm$ 0.47	14.66 $\pm$ 0.57	15.66 $\pm$ 0.47	17.0 $\pm$ 0.0
	2.5	14.33 $\pm$ 0.81	12.33 $\pm$ 0.81	13.33 $\pm$ 0.81	15.66 $\pm$ 0.47
	1.25	12.0 $\pm$ 0.0	10.0 $\pm$ 0.0	12.0 $\pm$ 0.0	13.33 $\pm$ 0.81
Ciprofloxacin	0.5	27.0 $\pm$ 0.0	29.0 $\pm$ 0.0	31.0 $\pm$ 0.0	30.0 $\pm$ 0.0

Compound **116** displayed antibacterial activity against all tested bacterial strains with ZI ranging from 13.66 $\pm$ 0.57 to 17.33 $\pm$ 0.81mm at 5 mg/mL (Table 31). The maximum antibacterial activity was noted against *S. aureus* (17.3 $\pm$ 0.81 mm) and followed against *E. coli* (15.0 $\pm$ 0.0 mm) (Appendix 30). Scopoletin (**117**) displayed low to moderate antibacterial activity against the tested pathogens with inhibition zones ranging from 9.66 $\pm$ 0.57 to 15.33 $\pm$ 0.81 at 5 mg/mL (Table 31). The maximum inhibition was recorded against *S. pyogenes* (15.33 $\pm$ 0.81) and followed by against *E. coli* (14.33 $\pm$ 0.81) (Appendix 30). Napiroon *et al.*, (2018) explained that scopoletin (**117**) showed to be effective against both strains of *P. aeruginosa* ATCC 27853 (β -lactamase producing strain) and *P. aeruginosa* DMSC 37166 (clinical strain) with MIC values of 0.66 μ M (Napiroon *et al.*, 2018). Yang *et al.*, (2016) reported that the O-CH₃ group and OH group at positions C-6 and C-7 have been shown to significantly affect the antibacterial activity of scopoletin (**117**) (Yang *et al.*, 2016).

4.4. Antioxidant Activity

The evaluation of the radical scavenging potentials of the crude extracts, EOs extracts, and isolated compounds from the studied plants were determined using DPPH assay compared to ascorbic acid (Sasikumar *et al.*, 2020).

4.4.1. Antioxidant activity of *W. uniflora*

The results of the DPPH radical scavenging activity of extracts, EOs, and isolated compounds (**96-99**) of *W. uniflora* are presented in Table 32 and Figure 17. DCM/MeOH(1:1) and MeOH extracts exhibited promising scavenging of DPPH radical with IC₅₀ values of 16.46 and 14.47 µg/mL respectively, compared to ascorbic acid IC₅₀ value of 5.83 µg/mL (Table 32). The maximum scavenging potential of DPPH radicals in the plant extracts may be due to their bioactive molecules, including phenolic compounds which contribute directly to the antioxidative effect of the extracts (Subramanian *et al.*, 2015). The EO of the roots of *W. uniflora* also demonstrated low scavenging of DPPH radical (IC₅₀ value of 99.92µg/mL), compared to ascorbic acid (5.83 µg/mL). Bergenin (**96**), **98**, and **99** also displayed scavenging of DPPH radical with IC₅₀ values of 18.42, 6.48, and 9.48 µg/mL respectively compared to ascorbic acid (IC₅₀ value of 5.83 µg/mL) (Table 32).

Previous studies also indicated that bergenin (**96**) exhibited good scavenging activities toward DPPH, ABTS⁺, and H₂O₂ with IC₅₀ values of 0.043, 0.075, and 0.033 mg/mL, respectively, and antioxidant activity against lipid peroxidation with an IC₅₀ value of 0.365 mg/mL (Sriset *et al.*, 2021). The present study also showed a good agreement reported by Utenova and co-workers (2007) indicating that epigallocatechin-3-gallate (**98**) exhibited scavenging of DPPH radical with percentage inhibition of 99.0±1.2% at 667 µM concentration (Utenova *et al.*, 2007). The lowest inhibition of DPPH radical was noted by β-sitosterol (**97**) (IC₅₀ value of 114.5 µg/mL). Compounds **98** and **99** showed higher inhibition of DPPH radical compared to the crude extracts and other isolated compounds, indicating that these compounds may be the active antioxidant component of the roots of *W. uniflora*. The observed antioxidant potential of the crude extracts and isolated compounds may be due to the presence of hydroxy-aromatic groups that could donate hydrogen-bearing free radicals to stabilize DPPH free radicals.

Table 32: DPPH scavenging potential of DCM/MeOH (1:1) and MeOH extracts, EO extract, and compounds (**96-99**) of the roots of *W. uniflora*

Extract/compound	% DPPH Inhibition each $\mu\text{g/mL}$ concentration					
	62.5	125	250	500	1000	IC ₅₀
DCM/MeOH (1:1) extract	66.3 $\pm$ 0.09	77.1 $\pm$ 0.16	74.9 $\pm$ 0.09	78.7 $\pm$ 0.08	82.1 $\pm$ 0.04	16.46
MeOH extract	73.2 $\pm$ 0.04	77.1 $\pm$ 0.16	81.2 $\pm$ 0.09	85.7 $\pm$ 0.04	89.2 $\pm$ 0.0	14.47
EO extract	26.6 $\pm$ 0.12	34.8 $\pm$ 0.08	42.4 $\pm$ 0.12	51.0 $\pm$ 0.0	59.8 $\pm$ 0.16	99.92
Bergenin (96)	65.6 $\pm$ 0.02	69.3 $\pm$ 0.12	73.8 $\pm$ 0.16	75.7 $\pm$ 0.04	78.6 $\pm$ 0.08	18.42
β -sitosterol (97)	18.4 $\pm$ 0.16	23.6 $\pm$ 0.08	29.7 $\pm$ 0.12	36.5 $\pm$ 0.08	42.3 $\pm$ 0.12	114.5
Compound 98	88.6 $\pm$ 0.08	91.2 $\pm$ 0.12	93.7 $\pm$ 0.02	95.4 $\pm$ 0.04	97.8 $\pm$ 0.04	6.48
Compound 99	82.0 $\pm$ 0.0	85.6 $\pm$ 0.12	88.2 $\pm$ 0.08	91.8 $\pm$ 0.16	94.0 $\pm$ 0.0	9.48
Ascorbic acid	89.8	92.3	94.1	96.4	98.1	5.83

Antioxidants have been investigated to show cell protection from free radicals causing oxidative cleavage which avoids aging and cancer, by interacting with free radicals, and thereby disrupting the oxidation process (Islam *et al.*, 2023). Published literature indicated that phenolic compounds including flavonoids contribute significantly as scavenging free radicals in plants for tolerating oxidative stress by accumulating in various tissues (Kiani *et al.*, 2021). Thus, the chemical constituents of the roots of *W. uniflora* may serve as antioxidants by neutralizing chemicals that cause oxidative damage to biological processes by giving free radicals electrons and passing them off as harmless (Baliyan *et al.*, 2022).

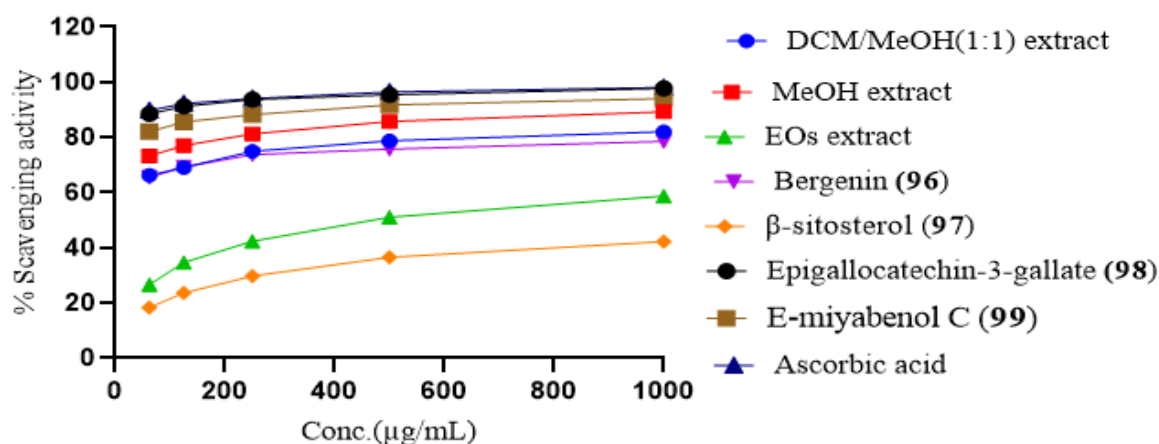


Figure 17: DPPH scavenging potential of DCM/MeOH (1:1) and MeOH extracts, EOs and compounds (**96-99**) of the roots of *W. uniflora*

4.4.2. Antioxidant activity of *T. vogelii*

The radical scavenging properties of EOs, DCM/MeOH(1:1), and MeOH extracts and isolated compounds (**101-105**) of the roots of *T. vogelii* results showed that the extracts and compounds inhibited DPPH radical following a dose-dependent approach, in which the DPPH scavenging activity percentage was directly correlated with concentrations (Table 33 and Figure 18). Tephrosin (**102**), obovatin (**104**), and compound **105** demonstrated powerful scavenging of DPPH radical with IC₅₀ values of 10.97, 10.43, and 10.73 µg/mL, respectively, compared to ascorbic acid with IC₅₀ of 5.83 µg/mL (Table 33, Figure 18). Compound **101** and maackiain (**103**) also displayed promising antioxidant activity with IC₅₀ values of 14.95 and 16.09 µg/mL respectively, compared to ascorbic acid (IC₅₀ value of 5.83 µg/mL). EOs, DCM/MeOH, and MeOH extract also showed notable DPPH scavenging activity with IC₅₀ values of 20.7, 16.90, and 12.58 µg/mL, respectively, compared to ascorbic acid (IC₅₀ value of 5.83 µg/mL) (Table 33). The obtained results indicated that crude extracts and isolated flavonoids displayed a higher scavenging potential of DPPH radical compared to the essential oil extract. The remarkable antioxidant activity of the crude extracts and isolated compounds may be related to the presence of bioactive flavonoids that could able to quench DPPH free radicals.

Table 33: DPPH radical scavenging activity of DCM/MeOH (1:1) and MeOH extracts, EO extract, and isolated compounds (**101-105**) of the roots of *T. vogeli* Literature reports indicated

Extracts/ compounds	% DPPH Inhibition at each µg/mL concentration					
	62.5	125	250	500	1000	IC ₅₀
DCM/MeOH (1:1) extract	60.7±0.01	66.4±0.16	69.3±0.04	72.5 ±0.02	77.1±0.08	16.9
MeOH extract	67.8±0.03	71.3±0.01	75.9±0.04	78.4±0.02	80.1±0.08	12.58
EO extract	50.0 ±0.0	53.4±0.4	57.6± 0.01	62.8±0.4	66 ±0.0	20.7
Compound 101	64.0±0.04	67.7±0.01	71.3±0.03	76.4±0.09	79.0±0.06	14.95
Tephrosin (102)	69.2±0.01	72.8±0.08	75.2±0.01	78.4±0.01	81.6±0.04	10.97
Maackiain (103)	62.8±0.04	67.2±0.016	71.8±0.01	74.4±0.01	78.2±0.06	16.09
Obovatin (104)	74.9±0.02	74.9±0.02	77.5±0.08	80.4±0.01	83.3±0.01	10.43
Compound 105	70.4±0.08	73.8±0.03	76.4±0.02	79.8±0.02	82.0±0.0	10.73
Ascorbic acid	89.8	92.3	94.1	96.4	98.1	5.83

Literature reports indicated that the level of phenolic and flavonoid compounds was correlated with the anti-inflammatory and antioxidant activities of medicinal plants (Sun and Shahrajabian, 2023). Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen and various other free radicals implicated in several diseases (Bravo, 1998). The present study confirmed that phytoconstituents of the roots of *T.vogelii* have a potential for the management of free radical-induced disorders.

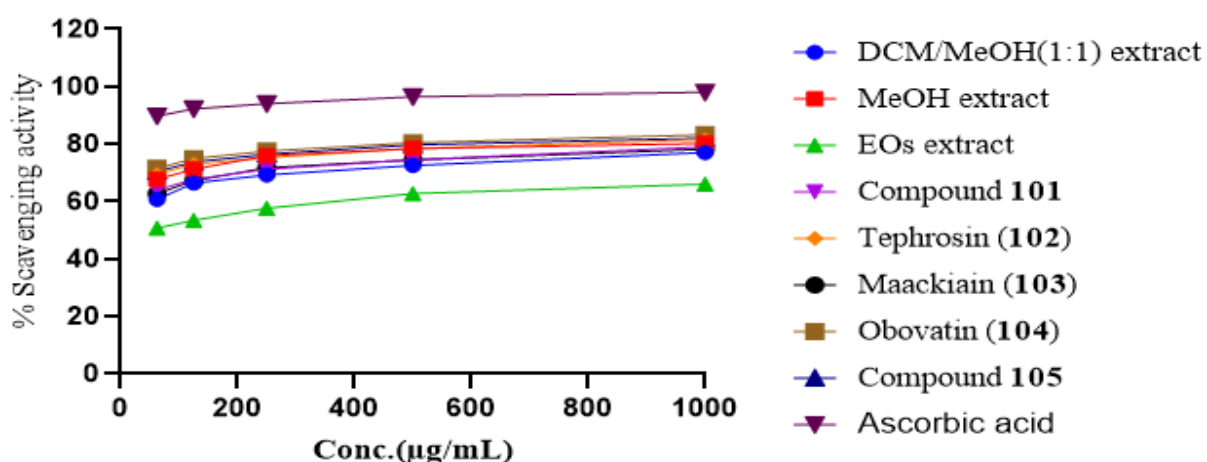


Figure 18: DPPH scavenging potential of DCM/MeOH (1:1) and MeOH extracts, EO extracts, and compounds (101-105) of roots of *T. vogelii*

4.4.3. Antioxidant activity of *A. schimperi*

The crude extracts, EO extract, and isolated compounds of (107, 108, 109) of the roots of *A. schimperi* demonstrated moderate to strong quenching of DPPH free radicals (Table 34, Figure 19). DCM/MeOH (1:1) and MeOH extracts of the roots of *A. schimperi* showed modest inhibition of DPPH radical with IC_{50} values of 43.0 and 32.89 µg/mL respectively, compared with ascorbic acid (IC_{50} value 5.83 µg/mL). A related study investigated by Alemu *et al.*, (2020) showed that 80% methanol extract of the leaves of *A. schimperi* displayed concentration-dependent antioxidant activity with DPPH inhibition percentage of 74.85% at the 200 µg/mL (Belete Kassa Alemu *et al.*, 2020). EOs extract of *A. schimperi* also exhibited scavenging of DPPH radical with percentage inhibition of $68.2 \pm 0.16\%$ (IC_{50} value of 45.84 µg/mL) (Table 34, Figure 19). The radical scavenging activity may be due to the presence of oxygenated

monoterpenes α -cedrene, cedrol, and cedranone (Asres *et al.*, 2012). Dihydroferulic acid (**108**) displayed the highest scavenging of DPPH radical with an IC₅₀ value of 10.66 μ g/mL, compared with ascorbic acid (IC₅₀ value of 5.83 μ g/mL) (Table 34). Lupeol (**107**) and compound **109** also exhibited moderate scavenging of DPPH radical with respective IC₅₀ values of 53.97 and 47.54 μ g/mL respectively, compared to ascorbic acid (IC₅₀ value of 5.83 μ g/mL). The antioxidant potential of lupeol (**107**) showed close agreement in published literature indicated that lupeol established scavenging of DPPH radical with an IC₅₀ value of 44.3 μ g/mL (Gurupriya *et al.*, 2018). Dihydroferulic acid (**108**) displayed higher antioxidant activity compared to the crude extracts and other compounds (**107** and **109**) (Table 34). A similar study investigated by *Deters et al.*, (2008) demonstrated that dihydroferulic acid (**108**) exhibited scavenging of DPPH radical with IC₅₀ value of 19.5 μ M (Deters *et al.*, 2008). The observed promising antioxidant potential of dihydroferulic acid (**108**) could be associated with the nature of the phenolic compound that could easily donate hydrogen to stabilize the DPPH radical (El-Sayed *et al.*, 2015).

Table 34: DPPH radical scavenging potential of DCM/MeOH (1:1), and MeOH extract, EOs extract, and compounds (**107-109**) of the roots of *A. schimperi*

Extract/compound	% DPPH Inhibition at each μ g/mL concentration					
	62.5	125	250	500	1000	IC ₅₀
DCM/MeOH (1:1) extract	43.5 $\pm$ 0.09	51.6 $\pm$ 0.08	58.4 $\pm$ 0.09	65.8 $\pm$ 0.08	71.6 $\pm$ 0.09	43.0
MeOH extract	51.6 $\pm$ 0.08	56.6 $\pm$ 0.16	63.9 $\pm$ 0.08	68.4 $\pm$ 0.08	73.5 $\pm$ 0.04	32.89
EO extract	42.7 $\pm$ 0.16	48.8 $\pm$ 0.04	57.0 $\pm$ 0.0	63.8 $\pm$ 0.08	68.2 $\pm$ 0.16	45.84
Lupeol (107)	37.6 $\pm$ 0.02	45.3 $\pm$ 0.09	51.6 $\pm$ 0.16	58.6 $\pm$ 0.04	65.2 $\pm$ 0.08	53.97
Compound (108)	72.6 $\pm$ 0.04	75.4 $\pm$ 0.08	78.3 $\pm$ 0.12	81.8 $\pm$ 0.08	84.6 $\pm$ 0.12	10.66
Compound 109	41.7 $\pm$ 0.04	49.8 $\pm$ 0.09	57.6 $\pm$ 0.02	63.4 $\pm$ 0.04	69.1 $\pm$ 0.04	47.54
Ascorbic acid	89.8	92.3	94.1	96.4	98.1	5.83

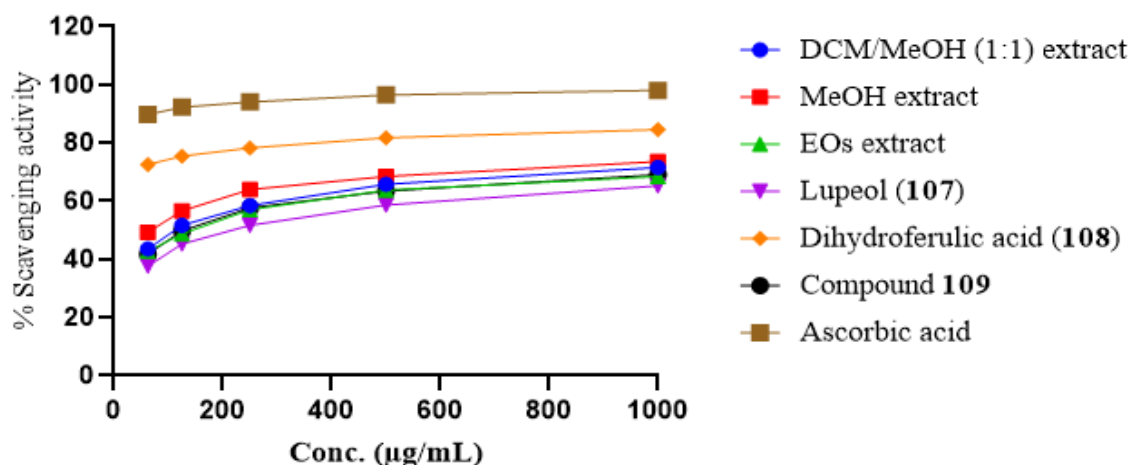


Figure 19: DPPH scavenging potential of DCM/MeOH (1:1) and MeOH extracts, EOs, and compounds (**107-109**) of the roots of *A. Schimperi*

4.4.4. Antioxidant activity of *R. glutinosa*

The DPPH radical scavenging activity of crude extracts, EOs, and isolated compounds (**111**, **114**, and **115**) of the roots *R. glutinosa* demonstrated moderate to promising DPPH radical scavenging activity (Table 35, Figure 20). The DCM/MeOH (1:1) and MeOH extract showed scavenging DPPH radical with respective IC_{50} values of 20.08 and 13.66 µg/mL respectively, compared to ascorbic acid (IC_{50} value of 5.83 µg/mL). EOs extract of *R. glutinosa* also demonstrated promising scavenging of DPPH radical with an inhibition percentage of $66.4 \pm 0.12\%$ (IC_{50} value of 39.6 µg/mL). Compound **111** showed moderate scavenging of DPPH radical with IC_{50} value of 66.86 µg/mL, compared to ascorbic acid (IC_{50} value of 5.83 µg/mL). Methyl gallate (**114**) and gallic acid (**115**) demonstrated powerful quenching of DPPH radical with respective IC_{50} values of 7.48 and 6.08 µg/mL respectively, compared to ascorbic acid (IC_{50} value of 5.83 µg/mL) (Table 35, Figure 20). The obtained inhibition of DPPH radical showed a good agreement with previously reported data (Habtemariam, 2011). The findings indicated that methyl gallate (**114**) and gallic acid (**115**) showed the highest DPPH scavenging activity than the crude extracts and other isolated compounds suggesting that these compounds could be the active antioxidant constituents of the roots of *R. glutinosa*.

Table 35: DPPH scavenging potential of crude extracts, EOs, and compounds (**111**, **114**, and **115**) of the roots of *R. glutinosa*

Extract/compound	% DPPH Inhibition at each $\mu\text{g/mL}$ concentration					
	62.5	125	250	500	1000	IC ₅₀
DCM/MeOH (1:1) extract	58.4 $\pm$ 0.04	63.2 $\pm$ 0.09	67.6 $\pm$ 0.16	72.8 $\pm$ 0.08	76.0 $\pm$ 0.0	20.08
MeOH extract	64.9 $\pm$ 0.08	68.2 $\pm$ 0.04	71.6 $\pm$ 0.08	75.0 $\pm$ 0.01	78.4 $\pm$ 0.08	13.66
EO extract	42.6 $\pm$ 0.04	48.8 $\pm$ 0.12	54.0 $\pm$ 0.0	60.2 $\pm$ 0.08	66.4 $\pm$ 0.12	39.6
Compound 111	32.1 $\pm$ 0.09	39.0 $\pm$ 0.0	46.7 $\pm$ 0.12	53.3 $\pm$ 0.08	59.3 $\pm$ 0.12	66.84
Methyl gallate (114)	83.2 $\pm$ 0.08	85.7 $\pm$ 0.12	89.0 $\pm$ 0.09	92.8 $\pm$ 0.08	95.2 $\pm$ 0.02	7.48
Gallic acid (115)	87.6 $\pm$ 0.04	92.6 $\pm$ 0.08	92.6 $\pm$ 0.08	94.2 $\pm$ 0.01	96.0 $\pm$ 0.08	6.07
Ascorbic acid	89.8	92.3	94.1	96.4	98.1	5.83

The presence of the phenolic hydroxyl group has been comprehensively reported as the main structural descriptor for the antioxidant activity of polyphenols, due to the hydrogen atom ability to scavenge free radicals (Lourençon *et al.*, 2021). Gallic acid (**115**) has been demonstrated as the chief antioxidant component responsible for several plant extracts' efficient antiradical and anticancer properties (Badhani *et al.*, 2015). The present finding confirms that the crude extracts and isolated compounds of the roots of *R. glutinosa* possess antibacterial and antioxidant activity which suggests that the chemical constituents of the studied plant could have a therapeutic value for pathogenic microorganisms and free radical-inducing disorders.

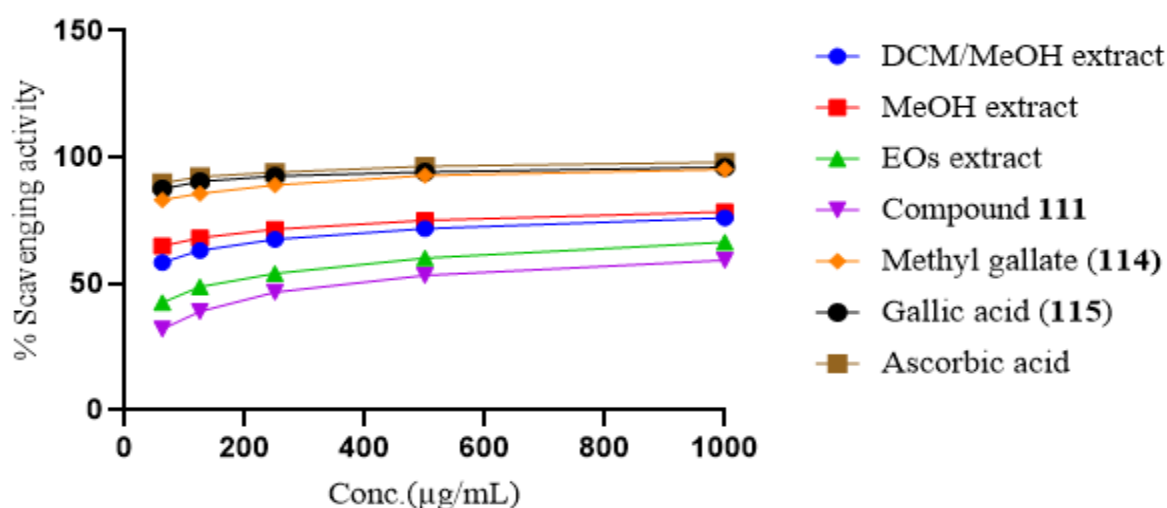


Figure 20: DPPH scavenging potential of DCM/MeOH (1:1) and MeOH extracts, EOs, and compounds (**111**, **114**, and **115**) of the roots of *R. glutinosa*

4.4.5. Antioxidant activity of *C. spinarum*

The radical scavenging activity of crude extracts, EOs, and isolated compounds (**116** and **117**) of the roots of *C. spinarum* displayed dose-dependent inhibition of the DPPH radical (Table 36, Figure 21). DCM/MeOH (1:1) and MeOH extract showed scavenging of DPPH radical with IC₅₀ values of 44.0 and 30.0 µg/mL respectively, compared to ascorbic acid (IC₅₀ value of 5.83 µg/mL) (Table 36, Figure 21). According to Nazareth *et al.*, (2021) investigation, MeOH extract of ripe and unripe fruit of *C. spinarum* showed effective scavenging of DPPH radical with IC₅₀ values of 4.69 and 8.61 mg/mL, respectively (Nazareth *et al.*, 2021). A similar study investigated by Chanda *et al.*, (2014) showed that chloroform extract of the stem of *C. spinarum* displayed scavenging of DPPH radical with IC₅₀ values of 47.03 µM), compared to that of Trolox (IC₅₀ value of 32.19 µM) (Chanda *et al.*, 2014). Ayalew and his coworkers (2022) reported that the aqueous, EtOAc, and DCM extracts of the leaves of *C. spinarum* (syn *C. edulis*) exhibited low scavenging of DPPH radical with IC₅₀ values of 430 ± 0.16, 220 ± 0.06, and 230 ± 0.20 µg/mL, respectively compared to ascorbic acid (IC₅₀ value of 200 ± 0.01 µg/mL) (Ayalew Tiruneh *et al.*, 2022).

Table 36: DPPH radical scavenging potential of DCM/MeOH (1:1), and MeOH extract, EO extract, and compounds (**116** and **117**) of the roots of *C. spinarum*

Extract/compound	% DPPH Inhibition at each µg/mL concentration					
	62.5	125	250	500	1000	IC ₅₀
DCM/MeOH(1:1) extract	46.2±0.12	54.8±0.08	60.2±0.09	67.4±0.04	74.8±0.08	44.0
MeOH extract	53.7±0.04	58.9±0.16	65.6±0.08	71.2±0.12	76.4±0.04	30.0
EO extract	35.4±0.16	41.0±0.0	47.8±0.46	54.4±0.08	60.6±0.12	31.18
Compound 116	48.6±0.12	54.2±0.09	59.6±0.16	65.0±0.0	70.4±0.08	50.41
Scopoletin (117)	71.0±0.0	75.0±0.0	79.2±0.08	83.0±0.0	86.0±0.0	14.33
Ascorbic acid	89.8	92.3	94.1	96.4	98.1	5.83

EOs extract of *C. spinarum* displayed moderate scavenging of DPPH radical with IC₅₀ value of 50.41 µg/mL at a concentration of 1000 µg/mL, compared to ascorbic acid (IC₅₀ 5.83 µg/mL). Compounds **116** and **117** showed scavenging of DPPH radical with IC₅₀ values of 31.18 and 14.33 µg/mL, respectively, compared to ascorbic acid (IC₅₀ 5.83 µg/mL) (Figure 21). A previous study conducted by Gwak *et al.*, (2011) showed that scopoletin (**117**) displayed promising scavenging of DPPH radical with IC₅₀ value of 17.4 µg/mL (Gwak *et al.*, 2011). This finding

confirms that the chemical constituents of the roots of *C. spinarum* have the potential to be natural antioxidants and may be used to manage free radical-induced diseases.

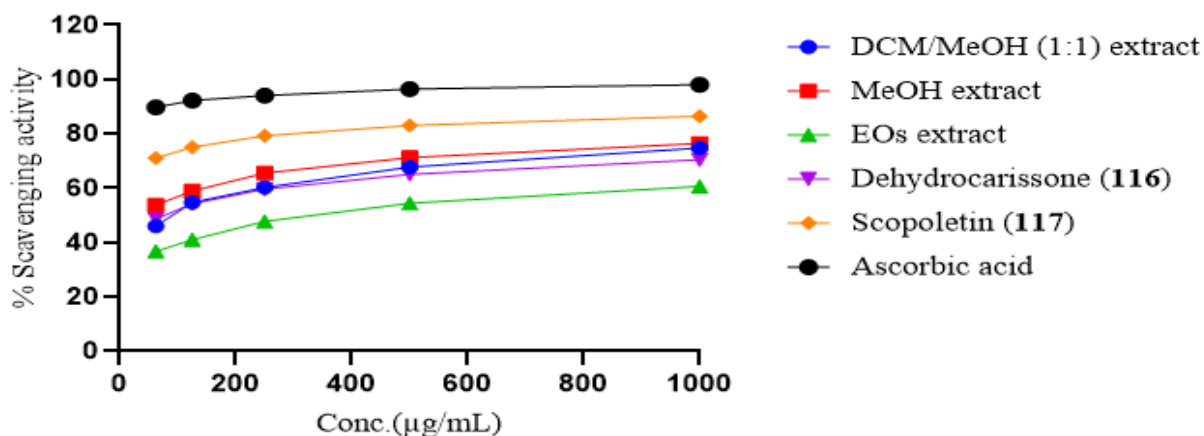


Figure 21: DPPH scavenging potential of DCM/MeOH (1:1) and MeOH extracts, EO extract, and compounds (111, 114 and 115) of the roots of *C. spinarum*

4.5. Antiviral Activity

The *in vitro* anti-influenza activity of DCM/MeOH (1:1) and MeOH extracts of *W. uniflora* and *A. schimperi* and bergenin (96) were evaluated against the MDCK cell line of influenza A and B using MTT assay (Chavan *et al.*, 2016). An antiviral drug should be active against the virus without inducing significant toxicity on the host cell. Therefore, the concentration range in which the extracts will not cause significant toxicity to the host cells, cytoprotection concentration (IC₅₀), and the 50% cytotoxic concentration (CC₅₀) were determined for each test sample. The present finding demonstrated that the cytotoxicity 50% concentration (CC₅₀) of the MeOH extracts of *W. uniflora* and *A. schimperi*, and bergenin (96) were >300 µg/mL indicating that, MeOH extracts and bergenin (96) were not much toxic to the host cells and well tolerated by MDCK cells at the maximum tested concentration (CC₅₀ >300 µg/mL). However, the DCM/MeOH (1:1) extracts of *W. uniflora* and *A. schimperi* exhibited cytotoxicity activity with respective CC₅₀ concentrations of 8.7 and 63.1 µg/mL respectively (Table 37). DCM/MeOH (1:1) and MeOH extracts of the roots of *W. uniflora* and bergenin (96) exhibited antiviral activity against MDCK cells with IC₅₀ values of 3.7, 162, and 33.7 µg/mL respectively (Table 37). A related investigation studied by Rajbhandari and his colleagues (2011) revealed that

bergenin (**96**) isolated from *Astilbe rivularis* displayed important antiviral activity against herpes simplex virus type-1 with an IC₅₀ value of < 6.25 µg/mL (Rajbhandari *et al.*, 2011). According to Hegde *et al.*, (2003) study, bergenin (**96**) had antiviral activity against the NS3 serine protease of the hepatitis C virus (HCV) IC₅₀ of 27.7 µg/mL (Hegde *et al.*, 2003).

DCM/MeOH (1:1) and MeOH extracts of the roots of *A. schimperi* exhibited antiviral activity against MDCK cells with IC₅₀ values of 2 and 25.1 µg/mL respectively (Table 37). DCM/MeOH(1:1) and MeOH extracts of the roots of *A. schimperi* showed antiviral activity against MDCK cells of influenza A and B with respective SI values of 31.5 and 11.9, indicating that the roots of *A. schimperi* have a therapeutic potential for the management of anti-influenza induced disorders. A related study conducted by Gebre-Mariam *et al.*, (2006) showed that the MeOH extract of the leaves of *A. schimperi* displayed antiviral activity against CVB3, with IC₅₀ value of 1.34 µg/mL (Gebre-Mariam *et al.*, 2006). The present study supported by the literature reports confirmed that the DCM/MeOH and MeOH the roots extracts of *A. schimperi* had minimal cytotoxicity and effective antiviral activities indicating their potential as candidates for the development of efficacious and nontoxic drugs against influenza A and B viruses.

Table 37: Antiviral activity of DCM/MeOH (1:1) and MeOH extracts of *W. uniflora* and *A. schimperi* and bergenin (**96**)

Plants	Extracts/ compound	Influenza A			Influenza B		
		CC ₅₀ , µg/mL	IC ₅₀ , µg/mL	SI	CC ₅₀ , µg/mL	IC ₅₀ , µg/mL	SI
<i>W. uniflora</i>	DCM/MeOH (1:1)	8.7	3.7	2.35	8.7	2.35	3.35
	MeOH	>300	162	1.85	>300	162	1.85
	Bergenin (96)	>300	33.3	9	>300	33.3	9
<i>A. schimperi</i>	DCM/MeOH(1:1)	63.1	2	31.5	65.1	2	31.5
	MeOH	>300	25.1	11.9	>300	32.1	11.9

4.6. Molecular Docking Analysis

In *silico* protein target identification and molecular docking analysis have been used for screening the potential drug-likeness potential of natural products for chemical lead discovery against many disorders (Ajayi *et al.*, 2022). The majority of antibiotics work by specifically targeting one or a few protein units that are necessary for bacterial life and the survival of

organisms (Zang *et al.*, 2016). Antibacterial agents prevent bacterial actions by targeting the essential elements of bacterial metabolism such as cell walls, DNA-directed RNA polymerase, protein synthesis, and DNA replication (Aliye *et al.*, 2021). The bacterial pyruvate kinase of *S. aureus* is one of the most important cytoplasmic enzymes involved in carbohydrate metabolism by catalyzing the final rate-limiting step in glycolysis (Akunuri *et al.*, 2022). Pharmacological inhibition of bacterial pyruvate kinase may result in hampered metabolism in MRS, so it has been identified as a promising target for treating infectious diseases associated with *S. aureus*-inducing disorder (Vasu *et al.*, 2015). *S. epidermidis* FtsZ is also another important protein for bacterial viability and is considered to be the major protein of bacterial cell division (Stokes *et al.*, 2013). DNA gyrase is an essential enzyme involved in DNA replication, elongation, and transcription (Alt *et al.*, 2011). LasR protein of *P. aeruginosa* is one of the major actors of bacterium cell-to-cell communication in regulating gene expression and targeting LasR protein offers a promising strategy to interfere with *P. aeruginosa*'s ability to cause infections. (Vetrivel *et al.*, 2021), while *Proteus mirabilis* urease is responsible for nitrogen supplies which is essential for bacterial survival (Grahl *et al.*, 2021). In the ligand-receptor complex formation of a biochemical reaction, an enzyme whose elevated activity may be associated with disease could be the target of interest; a drug that competitively inhibits the enzyme to lower its activity would be an interesting pharmacological precursor (Grinter and Zou, 2014).

Human peroxiredoxin 5 (Prdx5), (PDB ID: 1HD2), is a thioredoxin reductase that reduces H₂O₂, alkyl hydroperoxides, and peroxynitrite. It is implicated in both target receptors for antioxidant compounds and signal transduction pathways (Eze *et al.*, 2021; Salaria *et al.*, 2022). By upregulating Snail, Prdx5 overexpression increases carcinogenicity by encouraging the proliferation and invasiveness of gastric cancer cells (Tayaba Ismail *et al.*, 2019). On the other hand, myeloperoxidase (MPO), secreted by activated neutrophils and macrophages at the site of inflammation involved in the oxidation of protein/lipoprotein during the development of cardiovascular diseases (Shiba *et al.*, 2008). Natural product-derived compounds implicated with MPO inhibitors demonstrated anti-oxidative stress and anti-inflammation to reduce cerebral ischemia-reperfusion disorders (Chen *et al.*, 2020). Topoisomerases are essential enzymes that regulate the conformational changes in DNA topology by catalyzing the concerted breakage and rejoining of DNA strands during normal cellular growth (Topcu, 2001). Inhibition

of human topoisomerase II (topo II) is an effective target in the treatment of a wide spectrum of cancers (Skok *et al.*, 2019). Estrogen receptors (ER α) are another well-known mediator of cell proliferation, especially in breast cancer cells, driving cell proliferation in the presence of estrogen (Shanle and Xu, 2010). Inhibition of ER α can potentially modulate estrogen signaling pathways and affect tumor growth (Ali and Coombes, 2002). Progesterone receptors (PR) are important in breast cancer, particularly in hormone receptor-positive subtypes. PR is involved in mediating the effects of progesterone, which can influence breast cancer growth and response to therapy. PR's role in breast cancer makes it a relevant target for studying compound interactions and potential therapeutic effects (Giulianelli *et al.*, 2013). Molecular docking assessment of natural products towards a particular protein target can provide a hopeful opportunity for the development of effective inhibitors with better binding affinity and reduced toxicity (Magri *et al.*, 2023).

In the present study, the predication of the most favorable binding pattern and affinity of the isolated compounds towards the corresponding binding proteins were carried out against antibacterial target proteins: pyruvate kinase of *S. aureus* (PDB ID: 3T07), LasR protein of *P. aeruginosa* (PDB ID: 2UV0), DNA gyrase B of *E. coli* (PDB ID: 6F86), *P. mirabilis* urease (PDB ID: 1E9Y), *S. epidermidis* FtsZ (PDB ID: 4M8I) and antioxidant target proteins such as human myeloperoxidase (PDB ID: 1DNU), human peroxiredoxin 5 (Prdx5) (PDB ID: 1HD2), myeloperoxidase (PDB ID: 1DNU), and anticancer target such as topoisomerase II α (PDB ID: 4FM9), estrogen receptor alpha with Y537S Mutation (PDB ID: 6OWC), androgen receptor (PDB ID: 1T7R), estrogen receptor alpha (PDB ID: 1G50), and progesterone receptor (PDB ID: 3G8O) receptors using AutoDock Vina 4.2 program. The protein targets for the respective docked compounds were chosen based on the findings obtained *in vitro* results. In the docking analysis, an increase in negative binding energy values reflects a better affinity of the bioactive compounds for binding to receptors. Therefore, compounds that have higher negative binding energies are more efficient (Etminani *et al.*, 2023).

4.6.1. Molecular docking analysis of compounds (104, 107, 114, 116, and 107) against DNA gyrase B of *E. coli* (PDB ID: 6F86) receptor

Based on the *in vitro* antibacterial results, the binding orientation and affinity of compounds (104, 107, 114, 116, and 107) were predicted towards DNA gyrase B of *E. coli* (PDB ID: 6F86) target compared to ciprofloxacin. As shown in Table 38 and Figure 22, the analyzed compounds exhibited minimum binding affinity in their corresponding ligand-protein stable complex with binding free energies ranging from -5.7 to -6.6 kcal/mol, compared to ciprofloxacin (binding energy of -7.4 kcal/mol). The lowest minimum binding free energy was noted by obovatin (104) (-6.6 kcal/mol), followed by lupeol (107) (-6.5 kcal/mol), and methyl gallate (114) (-6.0 kcal/mol) compared to ciprofloxacin (-7.4 kcal/mol).

Obovatin (104) forms two hydrogen bond interactions through the active sites of GLY-A77 and GLU-A50 amino acids (Figure 22). More importantly, Obovatin (104) exhibited a similar pattern of hydrogen bond interaction with ciprofloxacin by interacting through the pocket of GLU-A50 amino acid. Moreover, obovatin (104) also showed hydrophobic/pi-alkyl (through amino acids ASN-A46, ALA-A47, THR-A165) and Vander Waals interactions (through amino acids VAL-A43, VAL120, VALA-167, ILE78, ILE-A94, ASN-A46, and ASP-A73) in its ligand-protein complex. The complex formed between lupeol (107) and PDB ID: 6F86 receptor was stabilized mainly by hydrophobic/pi-alkyl and Vander Waals interactions (Figure 22). Residual amino acids ILE-A186, PHE-A136, THRA-133, GLY-A104, PRO-A135, GLY-A107, GLY-A108, GLY-A71, ALA-A71, ASPA-46, and GLY-A70 were also involved *via* hydrophobic/pi-alkyl and Vander Waals interactions (Figure 22). Obovatin (104), 116, and scopoletin (117) each form three (via GLY-I117, GLY-I118, HIS -I56), three (via GLY-A77, THR-A165, and GLU-A50), and one (via ASP-A73) hydrogen bonds respectively towards the PDB ID: 4F86 receptor in their corresponding stable ligand-protein complex. Moreover, compound 116 showed a similar pattern of hydrogen interaction with ciprofloxacin through the active site of GLU-A50 amino acid (Table 42). The observed strong binding affinity of the studied compounds towards the PDB ID: 6F86 receptor confirms that the investigated compounds have antibacterial properties by disorder of the DNA replication, elongation, and transcription process of *E. coli* microorganisms.

Table 38: Molecular docking values of compounds (**104**, **107**, **114**, **116**, and **117**) against DNA gyrase of *E.coil* (PDB ID: 6F86) receptor

Compounds	Energy (kcal/mol)	H-bond	Residual interactions	
			Hydrophobic/pi-alkyl,pi-sigma	Vander Waal
Obovatin (104)	-6.6	GLY77, GLU50	ASN46, ALA47, THR-165	VAL-A43, VAL120, VALA-167, ILE78, ILE-A94, ASN-A46, ASPA-73
Lupeol (107)	-6.5	-	ILE-A186	ASP-A32, GLY-A33, THR-A34, GLU-A193, ARG-190, LYS-A189, PHE-A41
Methyl gallate(114)	-6.0	GLY-I117, GLY-I118, HIS -I56	-	MET-I120, SER-I120, HIS-I58, ASN-I180, GLU-I181, SER-I182, GLN-I140 GLY-I1115, GLY-I113, HIS-I57, CYS-I114, ARG-I116, TYR-I55
Compound 116	-6.1	GLY-A77, THR-A165, GLU-A50	ILE-A78	ASP-A73, ALA-A47, ILE-A94, ASN-A46, ALA-A53, PRO-A79, ASP-A49, ARG-A76,
Scopoletin (117)	-5.7	ASP-A73	GLU-A50, ILE-78,	PRO-A79, ARG-A75, GLY-77, GLY-A75, THR-A165, ASN-A46, ALA-A46
Ciprofloxacin	-7.4	ARG -A70, GLU -A50,	GLY-A77, ILE-A78, ILE -A94,ASP-A73	PRO-A79, THR-A165, ALA -A47,VAL -A43, VAL- A167, ASN -A46, VAL -A120

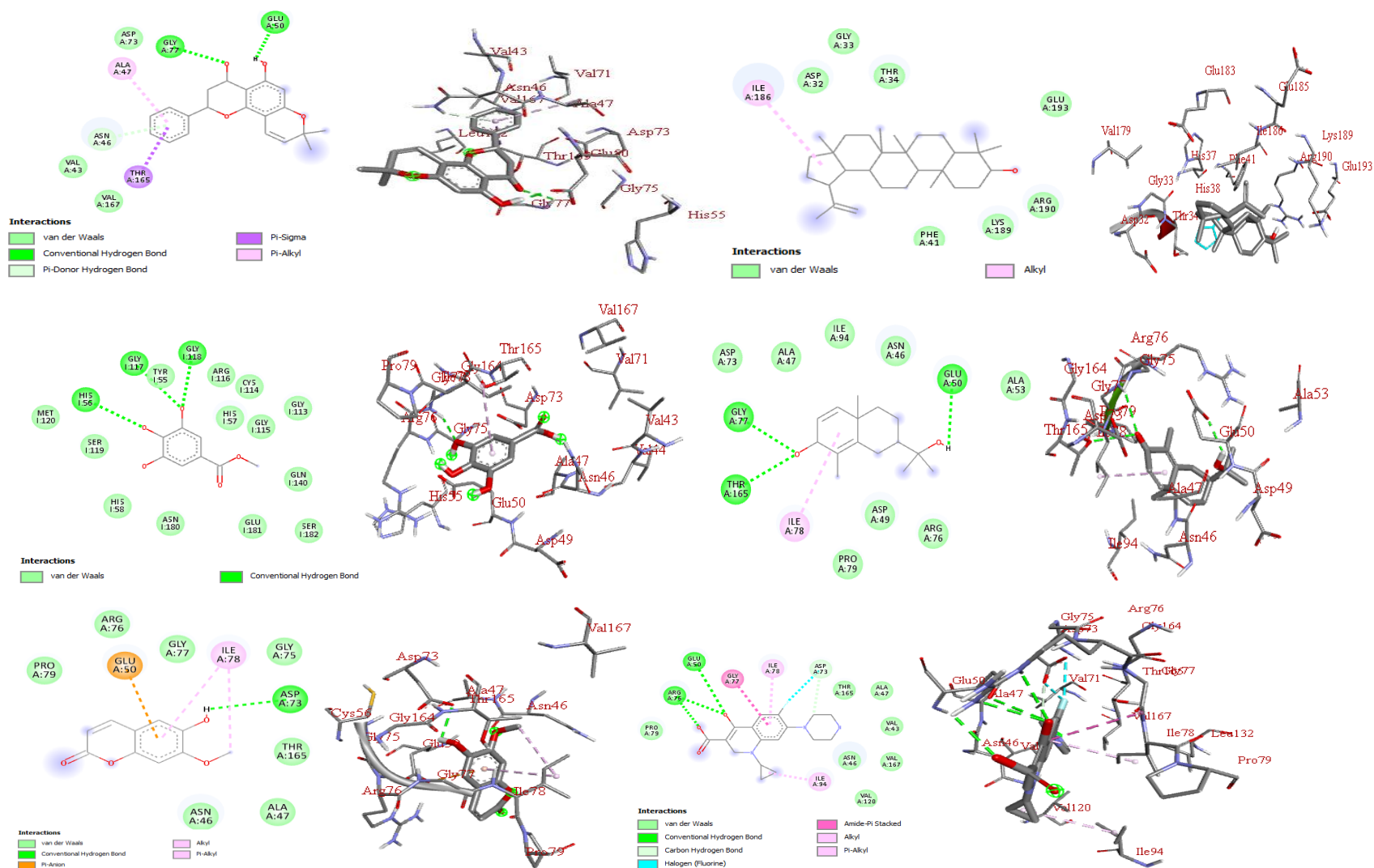


Figure 22: 2D (left) and 3D (right) representation of compounds **104**, **107**, **114**, **116**, and **117** against DNA gyrase of *E. coli* (PDB ID: 6F86) receptor

4.6.2. Molecular docking analysis of compounds (101, 102, 103, 107, 114, and 116) against pyruvate kinase of *S. aureus* (PDB ID: 3T07) receptor

The molecular docking prediction of compounds (101, 102, 103, 107, 114, and 116) towards pyruvate kinase of *S. aureus* (PDB ID: 3T07) receptor are presented in Table 39 and Figure 23. The docking analysis outcomes indicated that all the tested compounds displayed strong binding free energies ranging from -5.9 to -6.8 kcal/mol, compared to ciprofloxacin (-7.2 kcal/mol) (Table 39). Compound 101, tephrosin (102), and maackiain (103) displayed a promising binding affinity with scoring energy of -6.7, -6.5 -6.8 kcal/mol respectively, compared to ciprofloxacin (binding free energy of -7.2 kcal mol). Lupeol (107) and 116 also exhibited minimum binding free energy of -6.5 and -6.8 kcal/mol respectively, whereas, methyl gallate (114) and gallic acid (115) showed similar binding free energy of -5.9 kcal/mol. As depicted in Figure 23, compound 101 showed two hydrogen bond interactions through THR-D366 (2H-bonds) in its ligand-protein complex. Compound 101 also interacts through hydrophobic/pi-alkyl (via THR-C348, ILE-C361, HIS-C365, IEU-D370, ALA-C358) and Vander Waals (via LYS-C349, HIS-365, SER-D362, SER-C361, ASN-D369, THR-C353) interactions.

Tephrosin (102) also interacts with the PDB ID: 3T07 receptor through hydrogen bonds (via HIS-A365, ASN-A369), hydrophobic/pi-alkyl (via HIS-B365, LEU-A368) and Vander Waals (via ILE-B361, SER-A362, ILE-A362, THR-A353, LYS-A341, ASN-B369, THR-A348, THR-B348, SER-A345) interactions (Figure 23). The complex formed between maackiain (103) and PDB ID: 3T07 receptor was stabilized by hydrogen bond (SER-A345), hydrophobic/pi-alkyl (ILE-D343, LYS-D342), and Vander Waals (TYR-B302, GLN-D338, SER-D345, LYS-D345, LYS-D349, ASP-A346) interactions. Lupeol (107) showed hydrogen bond (ASP-D346) and Vander Waals (ASP-A261, ALA-D337, ARG-A264, ASP-D338, GLN-D338, LYS-D342, LEU-D343, and ARG-A347) in its ligand-protein complex. Methyl gallate (114) and gallic acid (115) form five (via ASP-A303, LYS-A260, ASN-A299, ASP-D303, TYR-D302) and three (via THR-C353, ASN-D369, and THR-D366) hydrogen bond interactions (Figure 23). The complex formed between compound 116 and PDB ID: 3T07 receptor was stabilized mainly by hydrophobic/pi-alkyl (LYS-A260, ARG-A264) and Vander Waals (GLY-D338, TYR-D302, ALA-D337, ASP-D399, ASP-A261, ASP-A303) interactions (Table 39, Figure 23).

Table 39: Molecular docking values of isolated compounds (**101**, **102**, **103**, **107**, **114**, **115**, and **116**) towards pyruvate kinase of *S. aureus* (PDB ID: 3T07) receptor

Compounds	Energy (kcal/mo)	H-bond	Residual interactions	
			Hydrophobic, pi-alkyl, pi-sigma	Vander Waals
101	-6.7	THR-D366 (2H-bonds)	THRC348, ILE-C361, HIS-C365, , ALA-C358	LYS-C349, HIS-365, SER-D362, SER-C361, ASN- D369, THR-C353
Tephrosin (102)	-6.5	HIS-A365 ASN-A369	HIS-B365, LEU-A368	ILE-B361,SER-A362,ILE-A362, THR-A353,LYS-A341, ASN- B369, THR-A348, THR-B348, SER-A345
Maackiain (103)	-6.8	SER-A345	ILE-D343, LYS-D342	TYR-B302,GLN-D338,SER- D345, LYS-D345, LYS-D349, ASP-A346
Lupeol (107)	-6.3	ASP-D346	-	ASP-A261, ALA-D337, ARG- A264, ASP-D338, GLN-D338, LYS-D342, LEU-D343,
Methyl gallate (114)	-5.9	ASP-A303, LYS-A260, ASN-A299, TYR-D302	TYR-D302	TYR-A302, ASN-D-299, LYS-D260
Gallic acid (115)	-5.9	THRC353, ASND369, THR-D366	ILE-C361	THR-C348, HIS-C365, HIS- D365, SER-C365, SER-C362, SER-D362, MET-D347, ALA- C358, LEU-D370
116	-6.3	-	LYS-A260, ARG-A264	GLY-D338, TYR-D302, ALA- D337, ASP-D399, ASP-A261, ASP-A303,
Ciprofloxacin	-7.2	ARG-A 264	ASP-D346	LYS -D260, TYRD- 302, TYR- A302, ASN- D299, ASNA- 299, LYS-A260ASN -D267, ARG- D 347, ALA -D337, GLN- D337 ASP- D339, LEU- D343

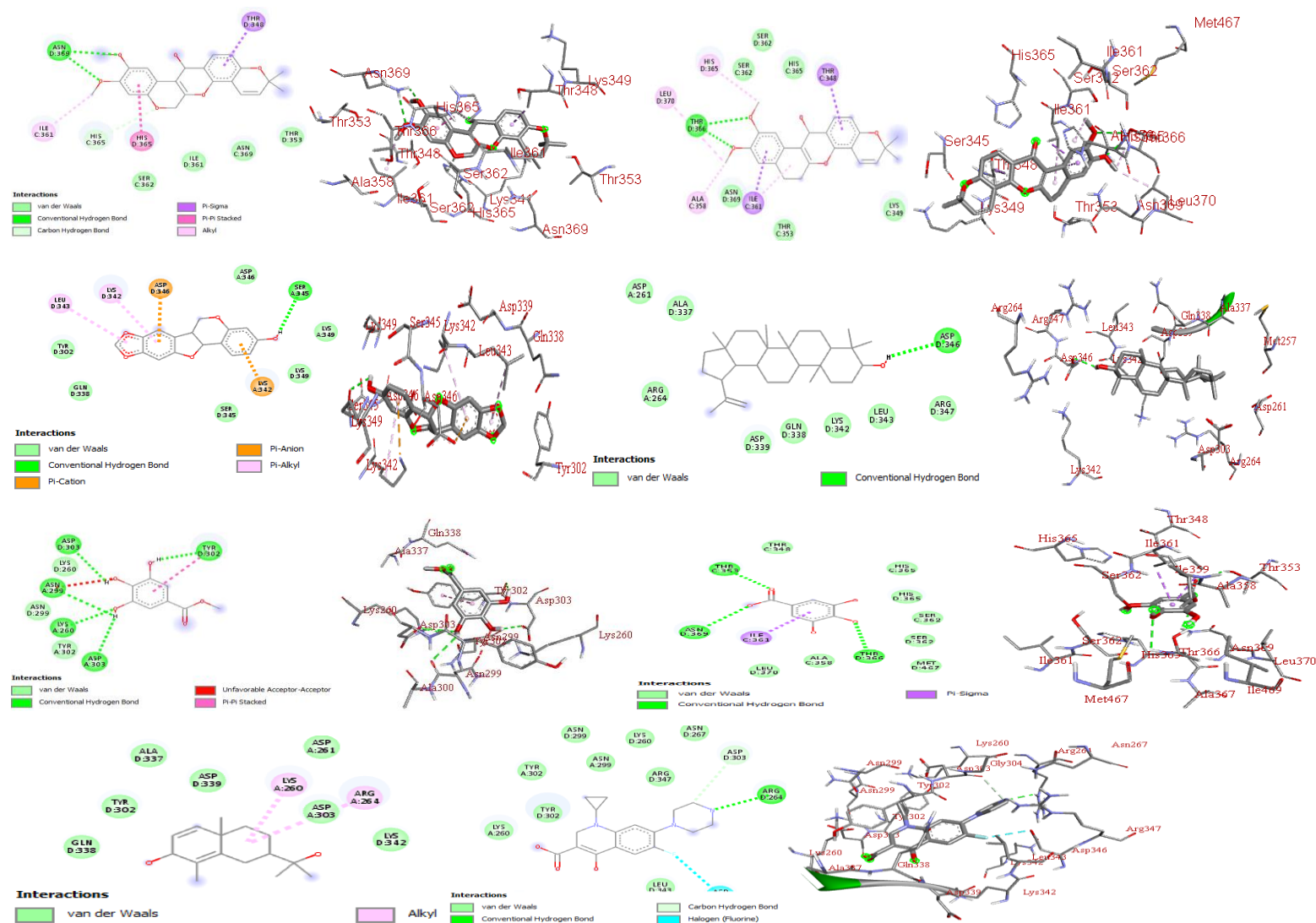


Figure 23: 2D (left) and 3D (right) representation of compounds 101, 102, 103, 107, 114, 115, and 116 against pyruvate kinase of *S. aureus* (PDB ID: 3T07) receptor

4.6.3. Molecular docking analysis of compounds (**101**, **103**, and **104**) against LasR protein of *P. aeruginosa* (PDB ID: 2UV0) receptor

The molecular docking prediction results of compounds (**101**, **103**, and **104**) towards PDB ID: 2UV0 receptor are presented in Table 40 and Figure 24. As illustrated in Table 40, the studied compounds showed minimum binding free energy ranging from -7.1 to -7.6 kcal/mol compared to ciprofloxacin (binding free energy of -7.3 kcal/mol) (Table 40). The lowest minimum binding free energy was noted by compound **101** (binding energy of -7.6 kcal/mol).

Table 40: Molecular docking values of isolated compounds (**101**, **103**, and **104**) against LasR protein of *P. aeruginosa* (PDB ID: 2UV0) receptor

Compounds	Energy (kcal/mol)	H-bond	Residual interactions		Electrostatic interaction
			Hydrophobic, pi-alkyl, pi-sigma	Vander Waal	
101	-7.6	ARGE122 (2H-bonds) TYR-47, THR-G80	LYS-E42, LYS-G42, GLY-G123, LEU-G125	ASP-G43	
Maackiain (103)	-7.4	LYS-E42, HISG119, GLN-G45	GLY-G120	ASPG43,SERG44, ALA-E121, GLU-G124, LEU-G125, SER-G44, HIS - E119, GLY-G123	ASP-G43
Obovatin (104).	-7.1	TYR-F157	THR-H152, PRO-H149, ASP-F5	GLY-F6,-LEU-H148, LEU-F154, TRP F152, TYR-F149,PHE-F7,	ASP-F5,
Ciprofloxacin	-7.3	HIS- G119	GLY-E123, GLY-G123, ARG-122, LYS-E42,	ASP5, SER-A144, TYR-A47, ASP43, LEU-A125, ALA-A121,	

Compound **101** forms four hydrogen bond interactions *via* the active site of ARG -E122 (2H-bonds), TYR-G47, and THR-G80 amino acids. Residual amino acids LYS-E42, LYS-E42, GLY-G123, LEU-G125 GLY-G120 (hydrophobic/pi-alkyl interactions) and ASP-G439 (Vander Waals interaction) were also involved in its ligand-protein complex (Figure 24). Maackiain (**103**) showed three hydrogen bond interactions (*via* LYS-E42, HIS-G119, and GLN-G45) and electrostatic interaction (*via* ASP-G43) in its ligand-protein complex towards PDB ID: 2UV0

receptor. Notably, maackiain (**103**) exhibited a similar pattern of hydrogen bond interaction with ciprofloxacin through HIS- G119 amino acids. Residual amino acids GLY-G120, ASP-G43, SER-G44, ALA-E121, GLU-G124, LEU-G125, SER-G44, HIS-E119, and GLY-G123 were also contributed for the stabilization of the complex formed between Maackiain (**103**) and PDB ID: 2UV0 receptor (Table 40). Obovatin (**104**) displayed one hydrogen bond and electrostatic interactions through TYR-F157 and ASP-F5 amino acids respectively. THR-H152, PRO-H149, ASP-F5 and GLY-F6, LEU-H148, LEU-F154, TRP-F152, GLY-F6, LEU-H148, TYR-F149, PHE-F7, PRO-F149 amino acids also interacted *via* hydrophobic/pi-alkyl and Vander Waals interaction in its ligand-protein complex (Figure 24).

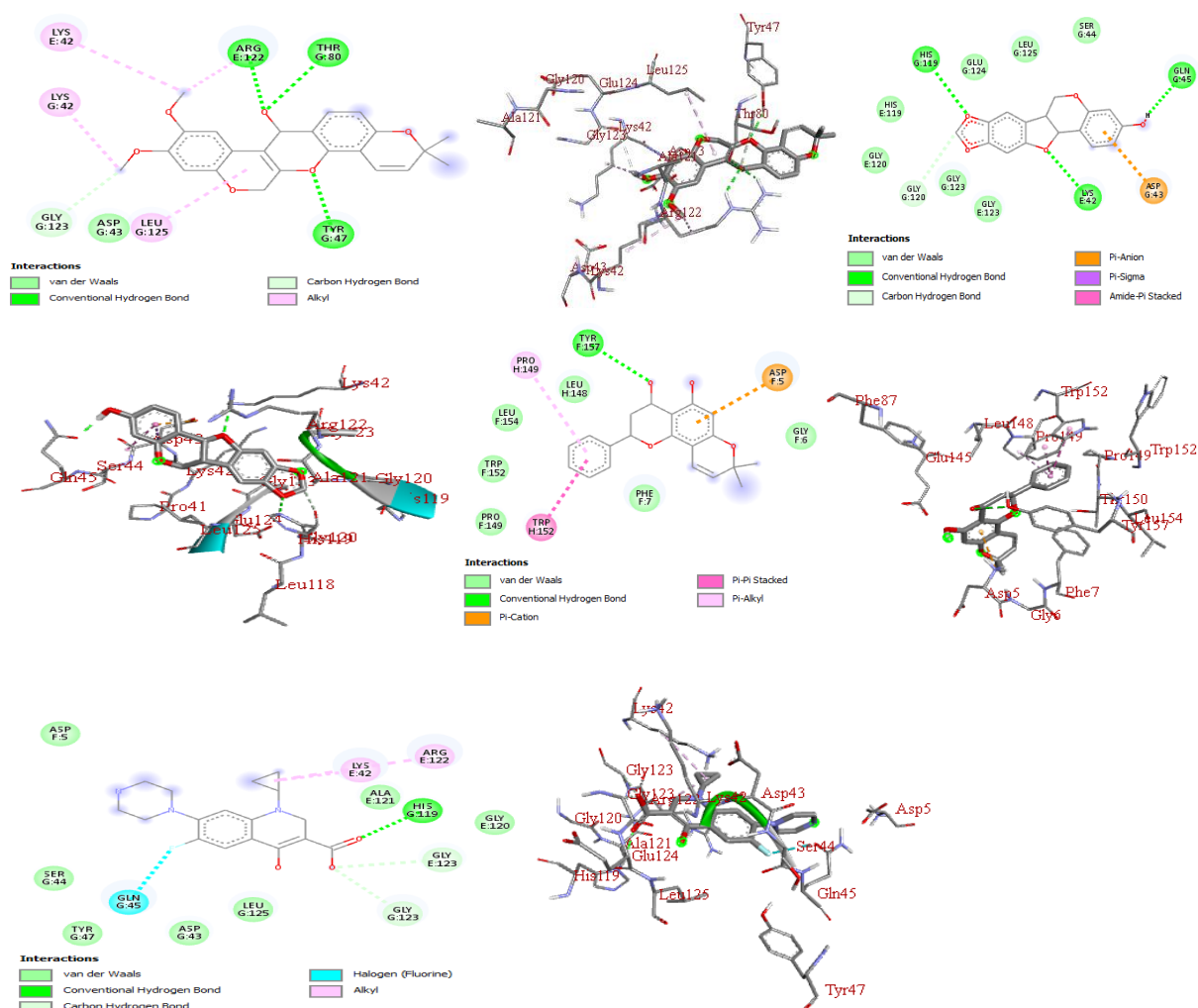


Figure 24: 2D and 3D (left to right) binding interactions of compound (101, 103, 104) and ciprofloxacin against LasR protein of *P. aeruginosa* (PDB ID: 2UV0) receptor

4.6.4. Molecular docking analysis of compounds (**101** and **105**) against *S. epidermidis* FtsZ (PDB ID: 4M8I) receptor

The molecular docking analysis of compounds **101** and **105** towards PDB ID: 4M8I receptor results are presented in Table 41 and Figure 25. Compounds **101** and **105** exhibited comparable binding affinity towards PDB ID: 4M8I receptor with respective binding free energy of -7.9 and -7.7 kcal/mol respectively compared to ciprofloxacin (binding free energy of -8.0 kcal/mol). As mapped in Figure 25, compound **101** forms four hydrogen bonds through the active sites of ASN-3166 (3H- bonds) and ASN-A25 amino acids. Hydrophobic/pi-alkyl (*via* MET-A105, PHE-A123, PRO-A155, and Vander Waals (*via* ASN-A29 and THR-A133) interactions also contributed to its ligand-protein complex stabilization (Figure 25).

Table 41: Molecular docking values of compounds **101** and **105** towards *S. epidermidis* FtsZ (PDB ID: 4M8I) receptor

Compounds	Energy (kcal/mol)	H-bond	Residual interactions	
			Hydrophobic, pi-alkyl, pi-sigma	Vander Waal
101	-7.9	ASN-3166 (3H-bonds), ASN-A25	MET-A105, PHE-A123, PRO A155	ANN-A29, THR-A133
105	-7.7	ASN-A166 (2H-bonds), ASN-A25, GLY-A21, GLYA-22	PHE-183, MET-A105	PHE-A136, THRA-133, GLY-A104,PRO-A135, GLY-A107, GLY-A108, GLYA71, ALA-A71, ASPA-46,
Ciprofloxacin	-8.0	ARG-A70, GLU -A50,	GLY- A 77, ILEA 78, ILE-A94, ASP-A73	PROA -79, THR A165, ALA -A47, VAL- A A43, VAL- A167, ASN -A46,

Compound **105** showed five hydrogen bond interactions towards PDB ID: 6F86 receptor through the active site of amino acids ASN-A166 (2H-bonds), ASN-A25, GLY-A21, and GLYA-22 (Figure 25). Residual amino acids PHE-183, MET-A105, PHE-A136, THRA-133, GLY-A104, PRO-A135, GLY-A107, GLY-A108, GLY-A71, ALA-A71, ASPA-46 and GLY-A70

were contributed for the stabilization of the complex formed between compound **105** and PDB ID: 4M8I receptor (Table 41).

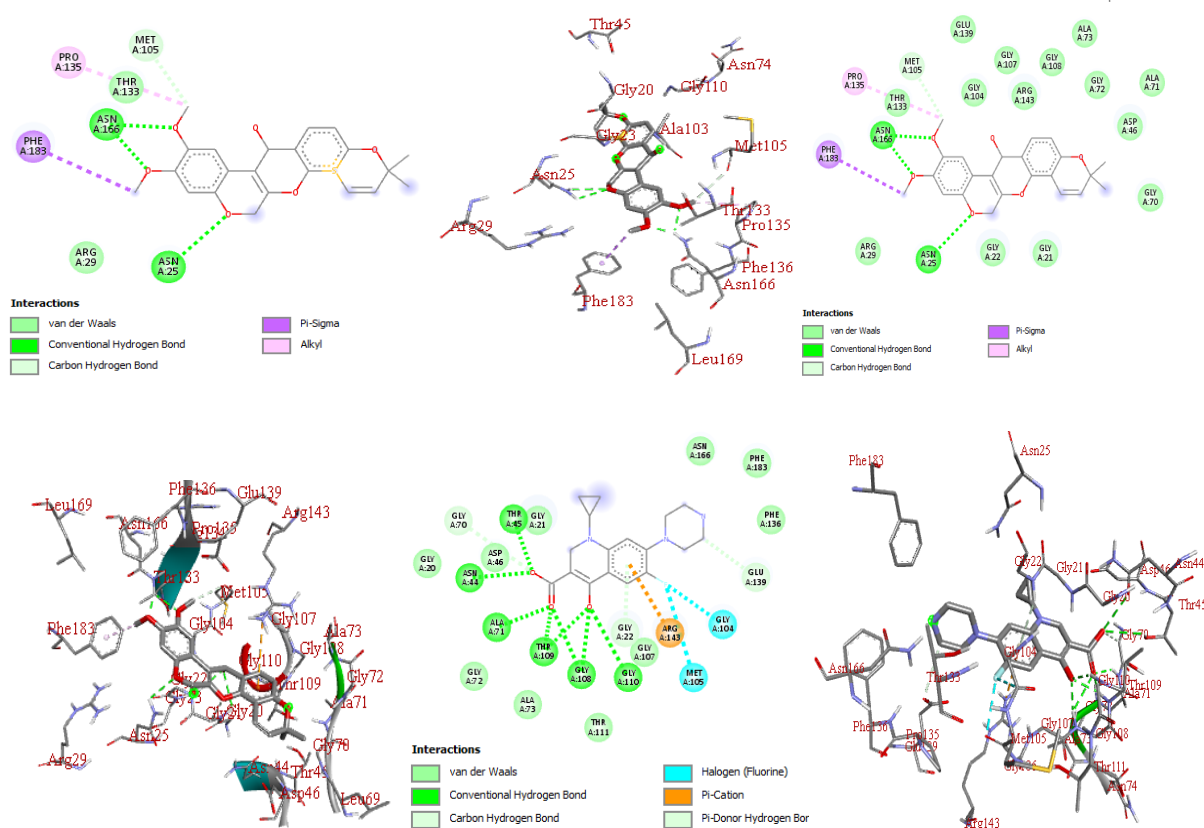


Figure 25: 2D (left) and 3D (right) representation of compounds **101** and **105** against *S. epidermidis* FtsZ (PDB ID: 4M8I) receptor

4.6.5. Molecular docking analysis of compounds (**101-103**) against *P. mirabilis* urease (PDB ID: 1E9Y) receptor

The molecular docking prediction of compounds (**101-103**) were evaluated against *p. mirabilis* urease (PDB ID: 1E9Y) receptor. The docking results are presented in Table 42 and Figure 26. Compound **101**, tephrosin (**102**) and maackiain (**103**) exhibited comparable binding affinity with ciprofloxacin towards PDB ID: 1E9Y receptor with the respective binding energy of -6.7, -7.0 and -62 kcal/mol respectively, compared to ciprofloxacin (binding energy of -7.2 kcal/mol) (Table 46). The complex formed between compound **101** and PDB ID: 1E9Y receptor was stabilized by hydrophobic/pi-alkyl interactions through residual amino acids ASP-B362, GLU-

B222, ALA- B169, ASP-B223, CYS-B321, ALA-B169, HIS-322, ALA-B365, B138, HIS B221, HIS-B 248 and Vander Waals interactions through the active site of GLY-B279, MET B336, ASN- B168, GLN-B364, ARG-B333 and ASP-B165 amino acids (Table 42). Tephrosin (**102**) forms two hydrogen bonds through the active site of AR-B368, and GLY-B367 towards of PDB ID: 1E9Y receptor. Hydrophobic/pi-alkyl interactions (*via* PRO-B302, MET-BB317, ALA-B365) and Vander Waals interactions (*via* CYS-B321, MET-B366, GLU-B364, ASB-168, ILE-B140) were also involved in its ligand-protein complex (Figure 26). The complex formed between maackiain (**103**) and PDB ID: 1E9Y receptor was stabilized mainly by Residual interactions such as Vander Waals (*via* GLY-B280, GLU-B222, ALA-B365, ASN-B168, VAL-B320, ASP-B223, GLY-B279, and ARG-B338) and Hydrophobic, pi-alkyl, pi-sigma (*via* ARG-B338, HIS-B221, ALA-169, CYS-B321, and HIS-B322) interactions (Figure 26). The *in vitro* experimental results supported by the docking analysis confirm that compounds (**101-103**) may have therapeutic potential to address *P. mirabilis*-induced disorders.

Table 42: Molecular docking values of compounds (**101-103**) against *P. mirabilis* urease (PDB ID: 1E9Y) receptor

Compounds	Energy (kcal/mol)	H-bond	Residual interactions	
			Hydrophobic, pi-alkyl, pi-sigma	Vander Waal
101	-6.7	-	ASP-B362, GLU-B222, ALA-B169, ASP-B223, CYS-B321, ALA-B169, HIS-322, ALA-B365, B138,	GLY-B279, MET B336, ASN- B168, GLN-B364, ARG-B333
Tephrosin (102)	-7.0	AR-B368, GLY-B367	PRO-B302, MET-B317, ALA-B365	CYS-B321, MET-B366, GLU-B364, ASB-168, ILE-B140
Maackiain (103)	-6.2	-	ARG-B338, HIS-B221, ALA-169, CYS-B321, HIS-B322	GLY-B280, GLU-B222, ALA, B365, ASN-B168, VAL B320, ASP, B223, GLY-B279
Ciprofloxacin	-7.2	-	ALA-B169, HIS B322, ALA-B365, CYS B321	ASN-B165, ASP-B165, MET -B366, GLY B279, ARG-B338, HIS-B221, HIS-B322

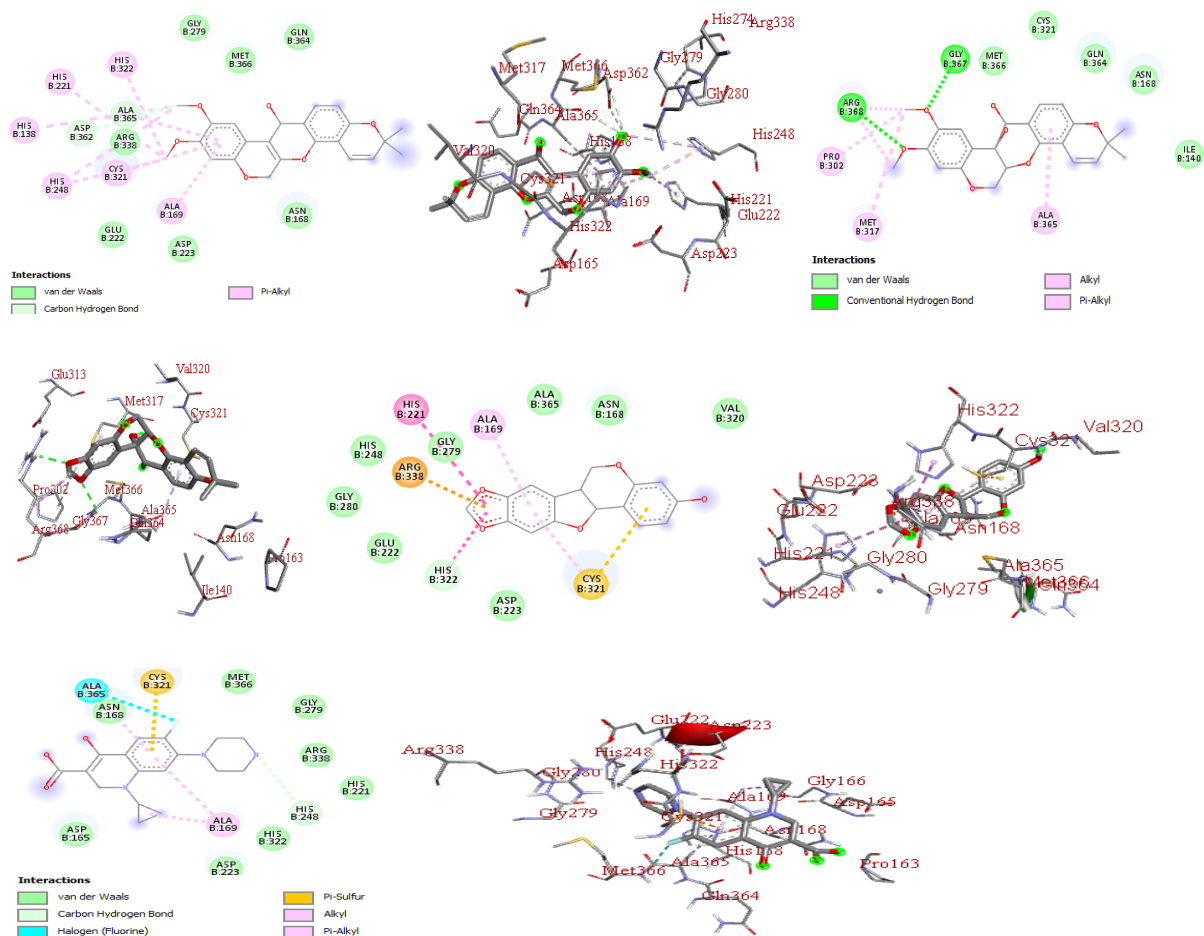


Figure 26: 2D (left) and 3D (right) representation of compounds (**101- 103**) compared to ciprofloxacin against *P. mirabilis* urease (PDB ID: 1E9Y) receptor

4.6.6. Molecular docking analysis of compounds (**115** and **117**) against *S. pyogenes* 10782 streptopain (PDB ID: 6KUD) receptor

The molecular docking analysis of gallic acid (**115**) and scopoletin (**117**) towards PDB ID: 6KUD receptor are given in Table 43 and Figure 27. Gallic acid (**115**) forms four hydrogen bonds through the active sites of SER-A308 (2H-bonds), GLN-A332, and GLN-A390 amino acids (Figure 27). Pi-alkyl (via TYR-A389) and Vander Waals (via SER-A390, SER-A285, ARG-A307, ASN-A306) interactions were contributed in its ligand-protein complex towards PDB ID: 6KUD) receptor (Table 43). Scopoletin (**117**) interacts towards PDB ID: 6KUD

receptor through the active site of GLN-A304 (hydrogen bond), TYR-A389 (pi-alkyl interaction) and SER-A308, GLN-A390, SER-A391, GLN-A332, SER-A285, ASN-306, SER-A286 (Vander Waals interactions) (Figure 27). The molecular docking analysis results revealed that the studied compounds showed strong binding affinity indicating that the compounds may be promising antibacterial agents.

Table 43: Molecular docking values of compounds **115** and **117**, and ciprofloxacin against *S. pyogenes* 10782 streptopain (PDB ID: 6KUD) receptor

Compounds	Energy (kcal/mol)	H-bond	Residual interactions	
			Hydrophobic, pi-alkyl, pi-sigma	Vander Waal
Gallic acid (115)	-5.7	SER-A308(2H-bonds),GLN-A332, GLN-A390	TYR-A389	SER-A390, SER-A285, ARG-A307, ASN-A306
Scopoletin (117)	-5.8	GLN-A304	TYR-A389	SER-A308, GLN-A390, SER-A391, GLN-A332, SER-A285, ASN-306,
Ciprofloxacin	-6.8	ASN-A161, SER-A279	TRP-A359	GLY-A163, ALA-A189, SER-A280, HIS-A340,

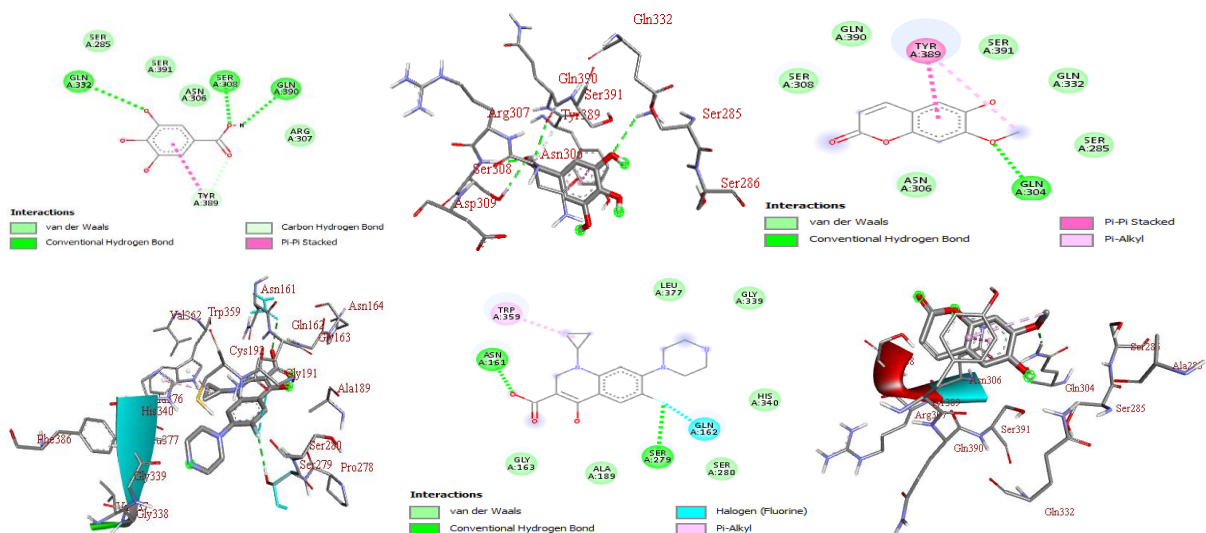


Figure 27: 2D (left) and 3D (right) representation of compounds **115** and **117** towards *S. pyogenes* 10782 streptopain (PDB ID: 6KUD) receptor

4.6.7. Molecular docking analysis of compounds (96 98, 99, 101, 104, 105, 108, 114, 115, and 117) against human myeloperoxidase (PDB ID: 1DNU) Receptor

The molecular docking analysis of results of compounds (96 98, 99, 101, 104, 105, 108, 114, 115, and 117) towards PDB ID: 1DNU receptor are presented in Tables 44, Figures 28 and 29. The analyzed compounds exhibited promising binding affinity towards PDB ID: 1DNU receptor with scoring energy values ranging from -4.8 to -10.7 kcal/mol compared to ascorbic acid (-5.7 Kcal/mol) (Table 44). Compounds 96, 98, 99, 102, 104, 105, and 117 exhibited better binding free energy of -8.0, -10.7, -8.8, -8.7, -9.4, -9.2 and -7.4 kcal/mol respectively compare to ascorbic acid (-5.7 kcal/mol). As shown in Figures 28 and 29, hydrogen bonds, electrostatic, hydrophobic, pi-alkyl/ pi-sigma, and Vander Waals interactions were contributed to the corresponding ligand-protein complex (Table 44). As depicted in Figure 28, Compound 98 forms five hydrogen bonds through the active site of ASP-A98, THR-A-329, GLU-C242, ARG-C333, and MET-A87 amino acids (Figure 28). Similarly, E-miyabenol C (99) and form three (via GLU102, THR238, and ASP214), and two (via THR-A100 (2H-bonds) hydrogen bond interactions towards PDB:1D 1DNU receptors respectively(96) Compounds 102, and 104 each form one hydrogen bond interaction towards PDB ID: 1DNU receptor through the active sites of ARG-C424 and ARG- D323 amino acids respectively. Electrostatic interactions also contributed to the stability of the complex formed between bergenin (96) and the PDB ID: 1DNU receptor via ASP-A94, ARG-C339, and ARG-A239 amino acids. As depicted in Figure 28, various residual amino acids were also contributed for the formation of stable complex formed between analyzed compounds (96, 98, 99, 102, and 104) and PDB ID: 1DNU receptor.

As shown in Figure 29, compound 105 (via ARG-B-31(2H-bonds), ARG-D323) and gallic acid (115) (via TYR-A47, CYS-C119, ALA-C41) each form three hydrogen bond inetractions towards PDB ID: 1DMU receptor. Dihydroferulic acid (108) also interacts with PDB ID: 1DNU target via GLN-C121 and amino acids. Similarly, methyl gallate (14) and scopoletin (117) exhibited one (via ALA-C141), and two (via GLU-A91 and HIS-A95) amino acids respectively in their corresponding ligand-protein complex towards 1DNU receptor. Numerous residual amino acids were contributed to the stabilization of the corresponding complex formed between the analyzed compounds (105, 108, 114, 115, and 117) and 1DNU receptor (Figure 29).

Table 44: Molecular docking values of compounds **96**, **98**, **99**, **103**, **105**, **108**, **114**, **115**, and **117** against human myeloperoxidase (PDB ID: 1DNU) receptor

Compound	Energy (kcal/mol)	H-bond	Residual interactions	
			Hydrophobic, pi-alkyl/ pi-sigma	Vander Waals
96	-8.0	THR-A100 (2H-bonds)	-	GLU-A102, LEU-A420, PHE -A99, ASP-A98, THR-A329, HIS-A339, ARG-A239, ARG-A333
98	-10.7	ASP-A98,THR-A-329,GLU,C242,ARG,C333,	HIS-C336, GLY-A90,ASP-A94,	GLU-A91
99	-8.8	GLU-C102,THR-C238, ASP-C214	LEUC216,ARG-C239, CYS-232,	GLU-C242, PHE-C99, PHE-C366, PHE-C407, HIS-C217, ASP-C218, PRO-C220, PRO-C101, CYS221,
102	-8.7	ARG-C424	HIS-C336, ARG C333,	ASP -A98, ASP -A94, ARG- C239, THR -A100, PHE -A99, GLU- C116, PHE-C336, LEU-415, PHE -C4007, LEU-C420,LEU-C417,ASN-C421,
104	-9.4	ARG- D323	ARG-C323, ILE-D160,ALA-B28,	THR -D159, THR -C159, ALA -D152, ILE C160, ASN-C162, AGR- A31, ALA-A35, PRO- A34
105	-9.2	ARG-B-31(2H-bonds), ARG-D323	ALA-C152,ALA-A28,	THR-D159, ASN-C162, ILE-C160, ALA-C35, PRO-A34, VAL-B30,ASP-D321, IEU-A33, TRP-A32,
108	-5.1	GLN-C121, ASPC-142,	PRO-C127, LYS-C129,ALA-C141	TRP-A47, PRO-A49, PHE-C126, CYS-119, CYS-C143, ILE-C130
114	-4.8	ALA-C141	LYS-C129, PRO-C127	VAL-C120, GLN-C121, ILE-C130, 1EU-C128, CYS-134, ASP-C142
115	-4.9	TYR-A47,CYS-C119,ALA-C41	LYS-C129	PRO-A49, GLY-46, PRO-C127, CYS-143, ASP-C142
117	-7.4	GLU-A91, HIS-A95	HHS-C336, LEU-C417	GLY-A90, PHE-C332, ASP-A94, ARG-C339, ARG-C329, LEU-339, MET-A87, GLY-C335, GLU-A242
Ascorbic acid	-5.7	MET- A87	HIS-C336	TYR-C334, ARG -C333, PHE -C332, GLY-C 335, GLY -A90, GLN -A91, LEU -C338, ILE -C339

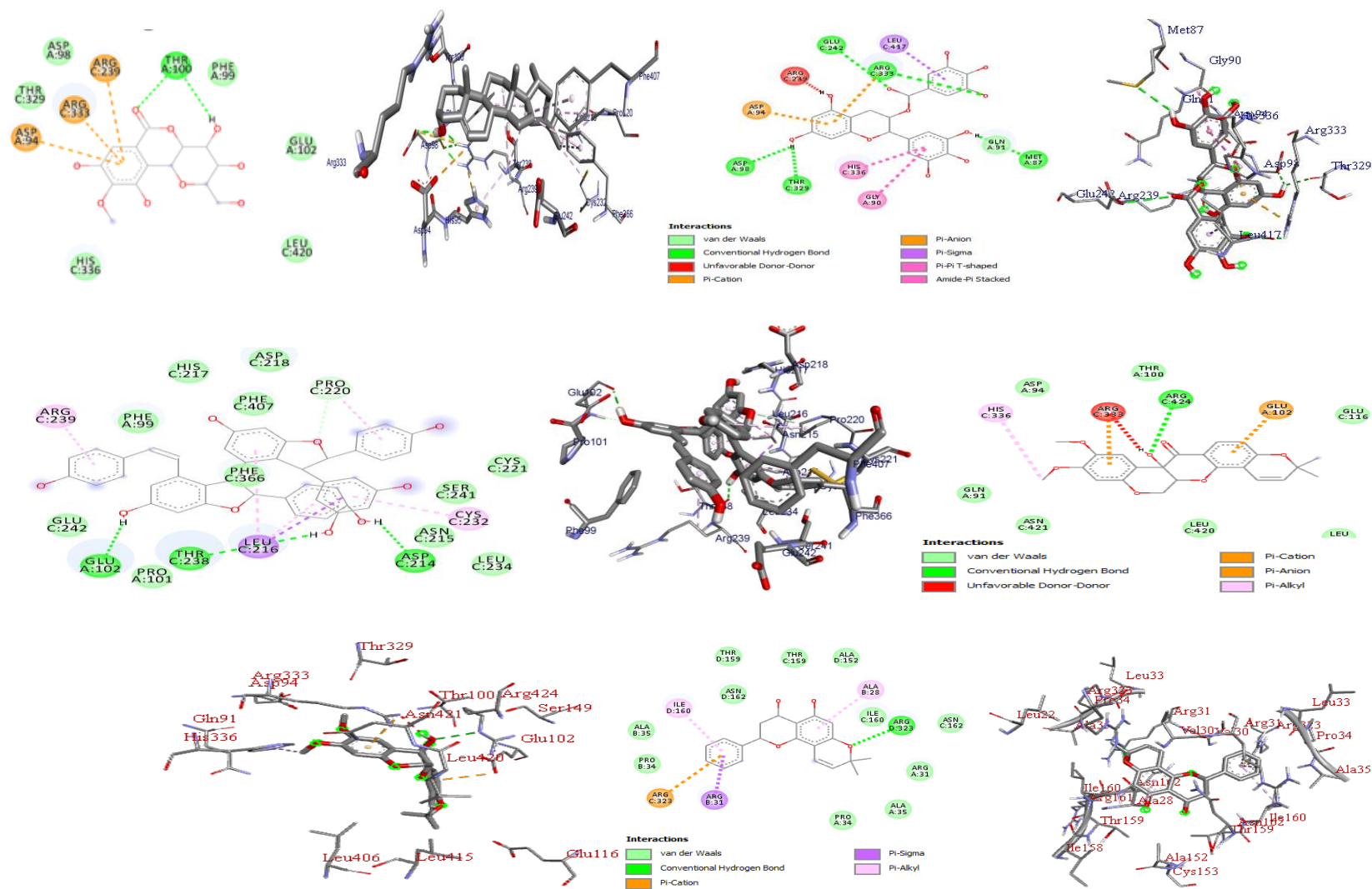


Figure 28: 2D (left) and 3D (right) representation of isolated compounds (**96**, **98**, **99**, **102**, and **104**) against human myeloperoxidase (PDB ID: 1DNU) receptor

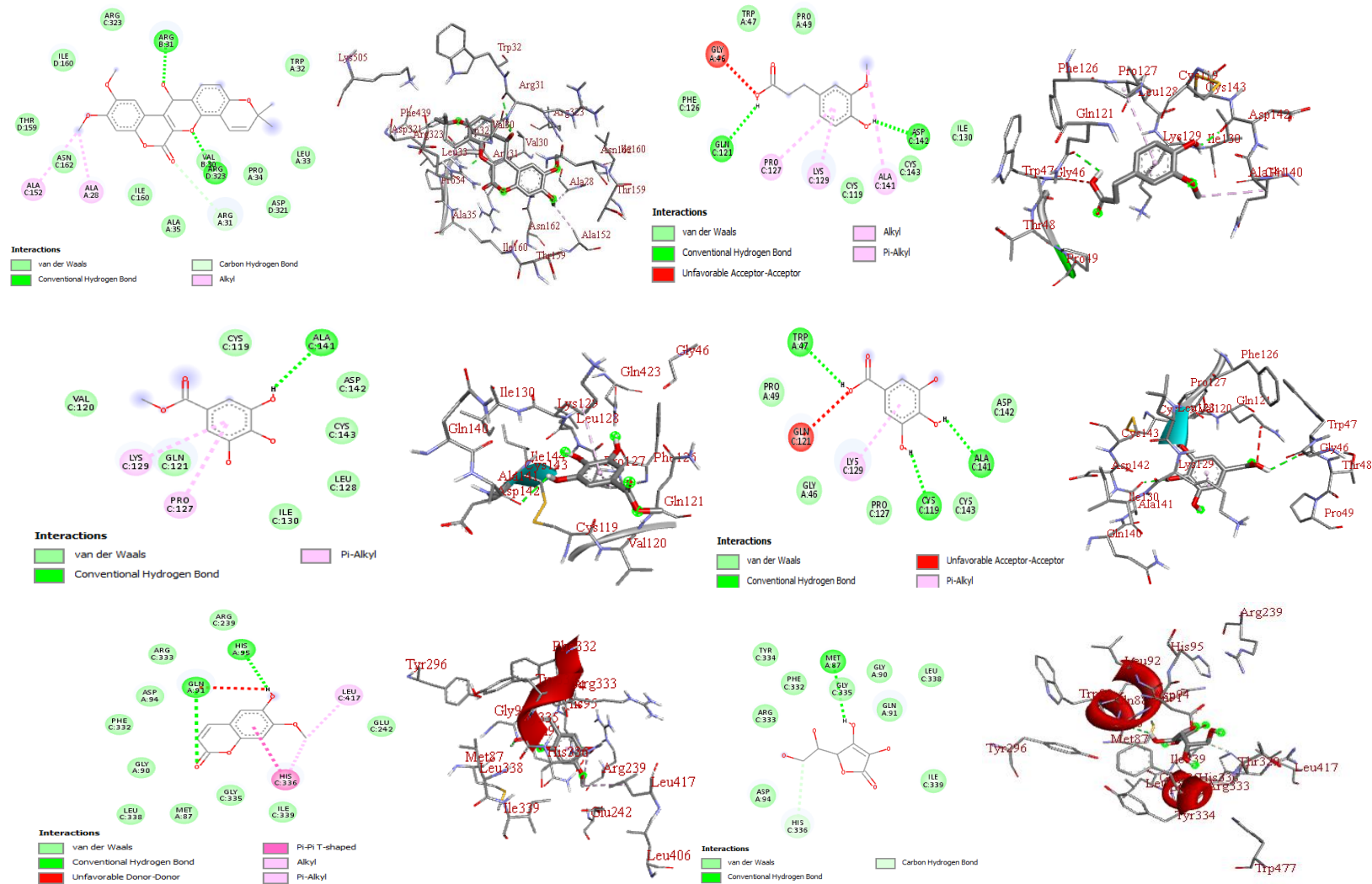


Figure 29: 2D (left) and 3D (right) representation of isolated compounds (105, 108, 114, 115 and 117) and ascorbic acid against human myeloperoxidase (PDB ID: 1DNU) receptor

4.6.8. Molecular docking analysis of compounds (96-99) against human peroxiredoxin 5 (PDB ID: 1HD2) receptor

The molecular docking prediction of compounds (**96**, **98**, and **99**) towards PDB ID: 1HD2 receptor are presented in Table 45 and Figure 30. All the analyzed compounds showed strong binding affinity with scoring energy ranging from -5.2 to -6.3 kcal/mol compared to ascorbic acid (-5.7 kcal/mol) (Table 45). The highest binding free energy was noted by E-miyabenol (**98**) (-6.3 kcal/mol) followed by bergenin (**96**) (-6.2 kcal/mol). Compound **98** forms five hydrogen bond interactions through the active site of THR-A147 (2H-bonds), ARG-A127 (2H-bonds), and CYS-A47 amino acids. Moreover, residual amino acids PRO-A40, PRO-A45, ILE-A119 and GLY-A148, LEU-149, GLY-A46, LEU-A116, PHE-A120, THR-A44 were involved through pi-alkyl and Vander Waals force interactions respectively (Table 45).

Table 45: Molecular docking values of compounds (**96**, **98**, and **99**) towards human peroxiredoxin 5 (PDB ID: 1HD2) receptor

Compounds	Affinity (kcal/mol)	H-bond	Residual Interactions	
			Hydrophobic/ Pi-Alkyl	Van der Waals force
96	-6.2	ILE-A119, ARG A124, ASP-A122, PHE-A120, ASP-A76	-	GLY-A121, THR-A44, ALA-A42, PHE-43, VAL-80, ASP-A77, VAL-A75
98	-6.3	THR-A147(2H-bonds), ARG-A127(2H-bonds), CYS-A47	PRO-A40, PRO-A45, ILE-A119	GLY-A148, LEU-149, GLY-A46, LEU-A116, PHE-A120, THR-A44
99	-5.6	-	LEU-A116, PHE-A120, PRO-A40, PRO-A45, ILE-A119	THR-A44, THR-A47, ARG-129, GLY-A46, ASP-145, LYS-A49
Ascorbic acid	-5.6	LEU-A96, GLT-A92, VAL-A94, GLY-A82,	LYS-93, ALA-A90, GLY-A85, ARG-A80, THR-A81, GLY-A17, GLU-A16	GLU-A91

The complex formed between bergenin (**96**) and PDB ID: 1HD2 receptor was stabilized mainly by hydrogen bond interactions through the active sites of ILE-A119, ARG -A124, ASP-A122, PHE-A120, ASP-A76 and Vander Waals force interactions through GLY-A121, THR-A44, ALA-A42, PHE-43, VAL-80, ASP-A77, and VAL-A75 amino acids (Figure 29). Similarly, the complex formed between E-miyabenol C (**99**) and PDB ID: 1DH2 receptor was stabilized by Vander Waals (*via* THR-A44, THR-A47, ARG-129, GLY-A46, ASP-145, LYS-A49) and Pi-alkyl/pi-pi stacked (*via* LEU-A116, PHE-A120, PRO-A40, PRO-A45, ILE-A119) interactions (Figure 30).

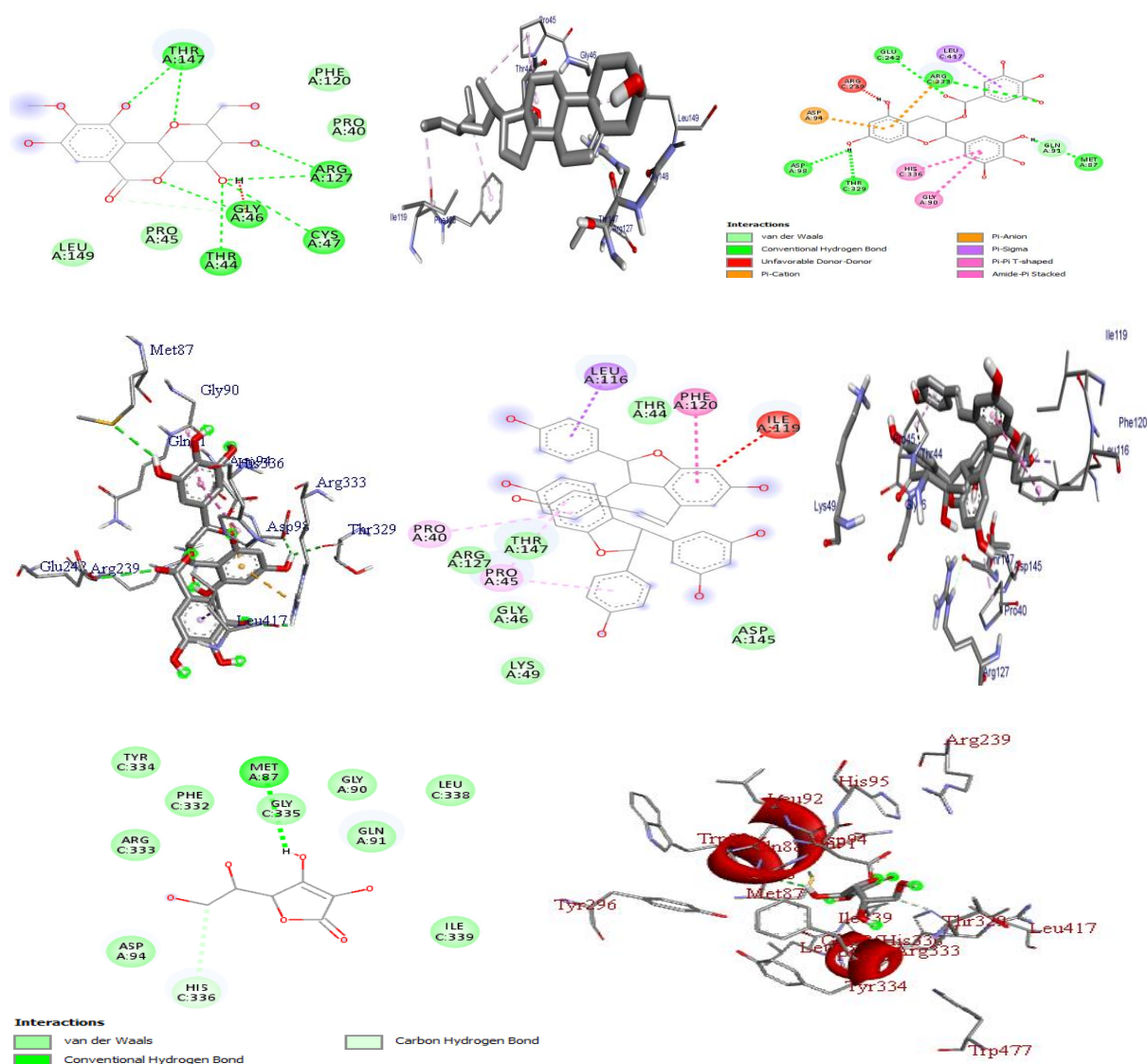


Figure 30: The 2D (left) and 3D (right) representation binding profile of compounds (**96-99**) and ascorbic acid against human peroxiredoxin 5 (PDB ID: 1DH2) receptor

4.6.9. Molecular docking analysis of compounds (96, 98, 99, 102, 103, 105, 108, 114, 115, and 117) against topoisomerase II α (PDB ID: 4FM9) receptor

Molecular docking analysis of compounds (96, 98, 102, 103, 108, 115 and 117) were also screened towards topoisomerase II α (PDB: ID 4FM9) receptor. The results of the docking analysis results are given in Table 46, Figure 31 and F2. As presented in Table 46, compounds (98, 102, and 103) showed a better binding affinity with the respective binding free energy of -9.1, -9.8, -7.9, and -9.2 kcal/mol compared to vosaroxin (binding free energy of -7.8 kcal/mol) (Table 43). Compounds (96, 108, 114, 115, and 117) also exhibited minimum binding-free energies ranging from -6.3 to -7.4 kcal/mol compared to vosaroxin (binding energy of -7.8 kcal/mol). The complex formed between bergenin (96) and PDB ID: 4FM9 receptor was stabilized by hydrogen bonds (*via* GLU-A712, ARG-A727(2H-bonds), VAL-A1006), hydrophobic/pi-alkyl (*via* GLU-A839, GLY-A1007) and Vander Waals interactions (*via* LYS-A670, GLY-A1007, HIS-A1005, TRP-A840, PHE -A1003, ASP-A1004, ILE-A715, LYS-A723 LEU-A722, PRO-A716, PRO-A724) (Table 4, Figure 31). Compound 98 forms four hydrogen bond interactions through the active sites of GLU-A837, GLU-A839, ARG-A727(2H-bonds), and GLU-A712 amino acids (Figure 31). Hydrogen bond interaction was also contributed to the complex stabilization formed between PDB ID: 4FM9 receptor and tephrosin 102 (*via* LYS-A614, (2H-bonds), LYS-A728), maackiain (103) (*via* GLN-AS42, LEU-A685) (Figure 31).

Dihydroferulic acid (108) (*via* GLN-A542, LYS-A550, SER-A547), methy gallate (114) (*via* TYR-A591, LEU-A685, SER-AA591) gallic acid (115) (*via* SER-A591, GLU-A702) and 117 (*via* LEU-A685) form hydrogen bond interactions towards PDB ID: 4FM9 target in their corresponding ligand protein complexes (Figure 32). Residual interactions such as hydrophobic /pi-alkyl and Vander Waals interactions also contributed to the stabilization of the respective ligand-protein complex through the active site of residual amino acids (, Figure 32, Table 46). The outcome of the docking prediction revealed that all the studied compounds exhibited promising binding affinity towards the PDB ID: 4FM9 receptor and are expected to have anticancer potential by inhibiting topoisomerase II α enzymes.

Table 46: Binding profile of compounds (**96, 98, 102, 103, 108, 114, 115, and 117**) against topoisomerase II α (PDB ID: 4FM9) receptor

Compound	Energy (kcal/mol)	H-bond	Residual Interactions	
			Hydrophobic/Pi-cation, Pi/alkyl, Pi-anion	Van der Waals
96	-7.4	GLU-A712, ARG-A727 (2H-bonds), VAL-A1006	GLU-A839, GLY-A1007	LYS-A670, GLY-A1007, HIS-A1005, TRP-A840, PHE – A1003, ASP-A1004, ILE-A715, LYS-A723 LEU-A722,
98	-9.8	GLU-A837, GLU-A839, ARG-A727(2H-bonds), GLU-A712	ARG-A673, ARG-A672, PHE-A1003,	LYS-A728, ILE-A715, SER-A717, TRP-A840, GLY-A1007, MET-A669, CYS-1008, PRO-A838, LEU-A829
102	-7.9	LYS-A614, (2H-bonds), LYS-A728	HIS-758, PRO-A724	ASP-A832, ASP-A831, GLU-A837, LEUA-829, ARG-A727, GLUA-712, HIS-A759
103	-9.2	GLN-AS42, LEU-A685	GLU-A702, LEU-705, PRO-A593	LYS-A550, ASP-A541, SER-A547, ASP-A543, GLN-544, LEU-A592, SER-A591, TYR-A686, TYR-A684, ASP-A683, PHE-A668, GLU-A682
108	-6.3	GLN-A542, LYS-A550, SER-A547	PRO-A593, LEU-A705	THR-540, GLY-546, ASP-A541, ASP-A543, GLY-A702, TYR-A686, TYR-A684, SER-A591, LEU-A685, LEU-592
114	-6.5	TYR-A591, LEU-A685, SER-AA591	ASP-A683	GL-A702, LEU-705, PRO-A593, TYR-A684, LEU-592, ILE-A577, GLU-A542, ASP-A543, TYR-A886, SER-A547, GLU-547, GLN-A544
115	-6.3	SER-A591, GLU-A702,	LEU-AA702	TYR-A684, PRO-A593, LEU-686, TYR-A686, LYS-A550, SER-A547, GLN-A542, ASP-543, GLN-A544, ILE-A577
117	-7.0	LEU-A685	GLN-A542, GLU-A702	TYR-A686, GLU-A542, SER-547, ASP-541, ASP-A543, GLN-A544, LEU-A577, LEU-705, TYR-A684, SER-592
Vosaroxin	-7.8	ARG-A727, ILE-715	PRO-A724, SER-A717,	ARG-A673, LEU-A829, LYS-A676, ARG-A672, ASP-A831, GLU-839, GLY-A1007, TRP-A716, LYS-A723, LEU-A722

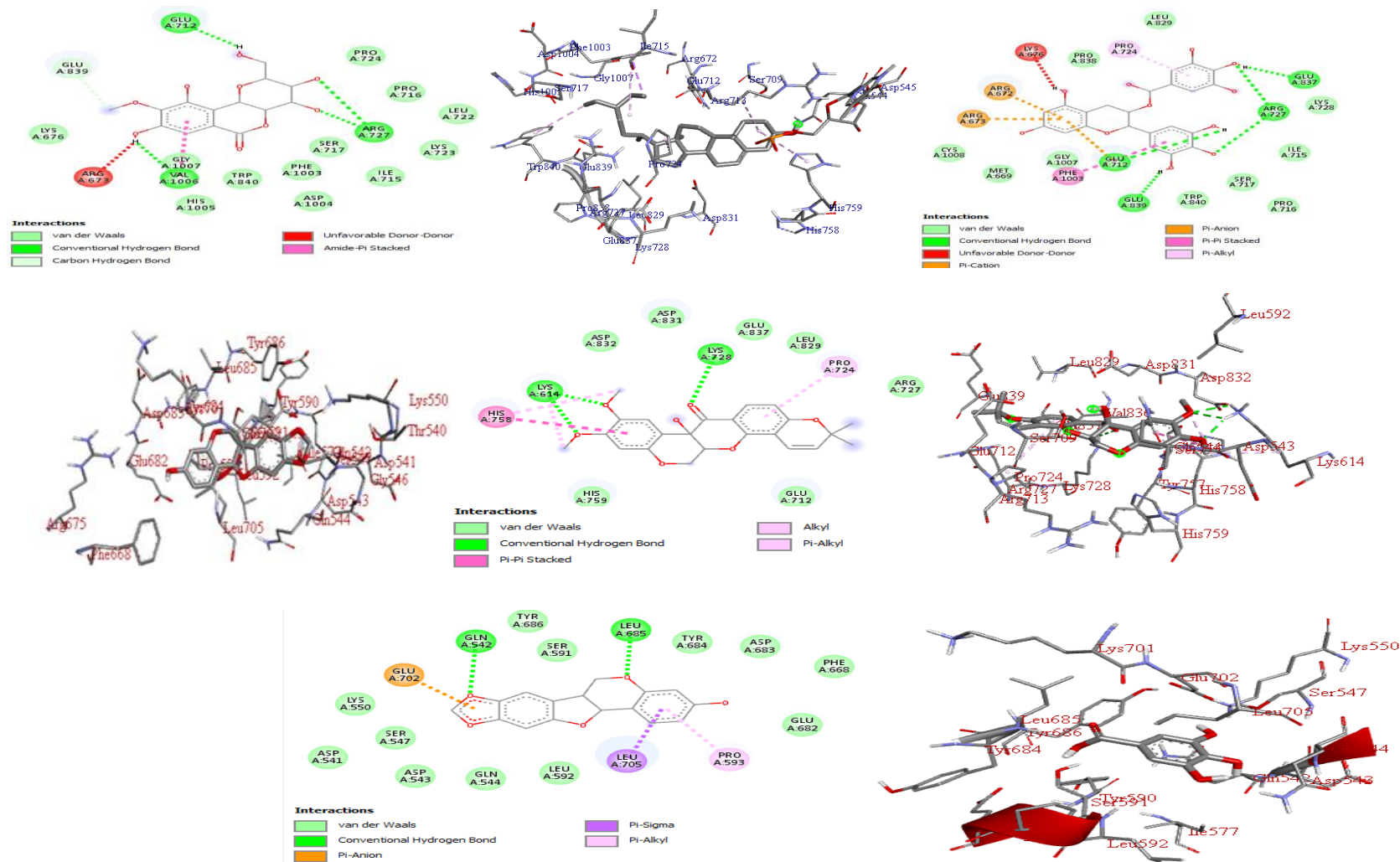


Figure 31: The 2D (left) and 3D (right) representation binding profile of compounds (**96**, **98**, **102**, and **103**) against topoisomerase II α (PDB ID: 4FM9) receptor

4.6.10. Molecular docking analysis of compounds **96**, **98**, **99**, **102**, **103**, **105**, **108**, **114**, **115**, and **117** towards estrogen receptor alpha (PDB ID: 6OWC) receptor

The molecular docking prediction of compounds (**96**, **98**, **102**, **103**, **108**, **114**, **115**, and **117**) were studied towards estrogen receptor alpha with Y537S mutation (ER α -Y537S) (PDB ID: 6OWC) target. The results of docking analysis are presented in Table 47 and Figure 33. As shown in Table 47, all the compounds under investigation demonstrated promising binding affinity towards the PDB ID: 6OWC target with binding energies ranging from -5.8 to -8.8 kcal/mol, compared to vosaroxin (binding energy -7.3 kcal/mol) (Table 47). The strongest binding affinity was noted by maaackiain (**103**) (binding energy of -8.8 kcal/mol). Bergenin (**96**) (-7.0 kcal/mol), **98** (-7.2 kcal/mol), and scopoletin (**117**) (-7.0 kcal/mol) exhibited comparable binding affinity with the standard drug, vosaroxin (-7.3 kcal/mol). As illustrated in Table 47, hydrogen bond, hydrophobic/pi-alkyl/pi-anion, and residual interactions were established the corresponding ligand-protein complex. Bergenin (**96**) showed three hydrogen bond interactions through the active site of THR-AA347, LEU-A346, and GLU-A353 amino acids (Figure 33). Compound **98** showed five hydrogen bond interactions through the active site of GLU-A523 (2H-bonds, TRP-B383 (2H-bonds), and GLY-B580 amino acids towards the PDB: ID 6OWC target (Figure 33). Tephrosin (**102**) and maaackiain (**103**) form two (*via* the active site of TYR-A520, LYS-B520, and GLU-A523) and one (*via* the active site of GLY-A521) hydrogen bond interactions in their corresponding ligand-protein complex (Figure 33).

Gallic acid (**115**) forms three hydrogen bonds *via* TYR-A520, LYS-B520, GLU-A523, and LEU-A346, ARG-A394, and LEU-A387 respectively (Figure 34). Dihydroferulic acid (**108**) also interacts with the PDB ID: 6OWC receptor through the pocket of GLU-A353 and ARG-A394 amino acids. Methyl gallate (**114**), and **117** forms one hydrogen bond each *via* the active sites of GLY-A521, GLY-B346, and ARG-B346 respectively (Figure 34). Various residual amino acids contributed to the stabilization of the corresponding ligand-protein complex (Table 47). The molecular docking prediction results revealed that the studied compounds displayed significant binding affinity towards the PDB ID: 6OWC receptor, suggesting that the compounds under investigation may be important cancer therapies.

Table 47: Binding profile of compounds (**96**, **98**, **102**, **103**, **108**, **114**, **115** and **117**) against estrogen receptor

Alpha with Y537S Mutation (ER α -Y537S) (PDB ID: 6OWC) receptor

Compound	Energy (kcal/mol)	H-bond	Residual Interactions	
			Hydrophobic/Pi-cation, Pi/alkyl, Pi-anion	Van der Waals
96	-7.0	THR-AA347, LEU-A346, GLU-A353	ILE-A424, GLY-A420, MET-A421, LEU-525, GLY-A521, MET-A43	LEU-A384, MET-A388, LEU-391, LEU-A387, ARG-A394, ALA-350, MET-A328, VAL-A418, HIS-A524
98	-7.2	GLU-A523(2H-bonds, TRPB383(2H-bonds), GLY-B580	ARG-A673, LYS-B531, ME-B522, GLU-B523	CYS-B530, LYS-B529, LEU-B525, TYR-B526, LYS-B520
102	-6.3	TYR-A520, LYS-B520, GLU-A523	TRY-A526, LYS-B520	HIS-B524, SER-B527, TYR-B526
103	-8.8	GLY-A521	LEU-A387, LEU-A391, LEU-A346, PHE-A404, LEU-325, MET-A328, MET A343,	MET-A388, LEU-A384, LEU-428, MET-421, ILE-A424, HIS-A524, ALA-A340, GLU-A325, ARG-A394
108	-6.2	GLU-A353, ARG-A394	PHE-B404, MET-A387, LEU-A424	LEU-A-387, LEU-A-428, ILE-A424, MET-A421, GLY-A521, LEU-391
114	-5.8	GLY-B346	ALA-B350, PHE-B404, LEU-B387	ILE-B426, MET-B421, PHE-B425, LEU-B391, MET-B388, ARG-A394, , GLU-B353
115	-6.0	LEU-A346, ARG-A394, LEU-A387	PHE-A404, ALA-A350	LEU-A525, LEU-A384, MET-A388, LEU-A428, GLU-A353, LEU-A391
117	-7.0	ARG-B346	PHE-B404, ALA-B350, LEU-391, LEU-B387	MET-B421, ILE-B424, LEU-B328, MET-B388, LEU-B384, ARG-B394, GLU-B353
Vosaroxin	-7.3	ASN-B519	MET-B522, LYS-B520, CYS-B381, HIS-A519	ASN-B519, GLU-525, TYR-B524, VAL-B548

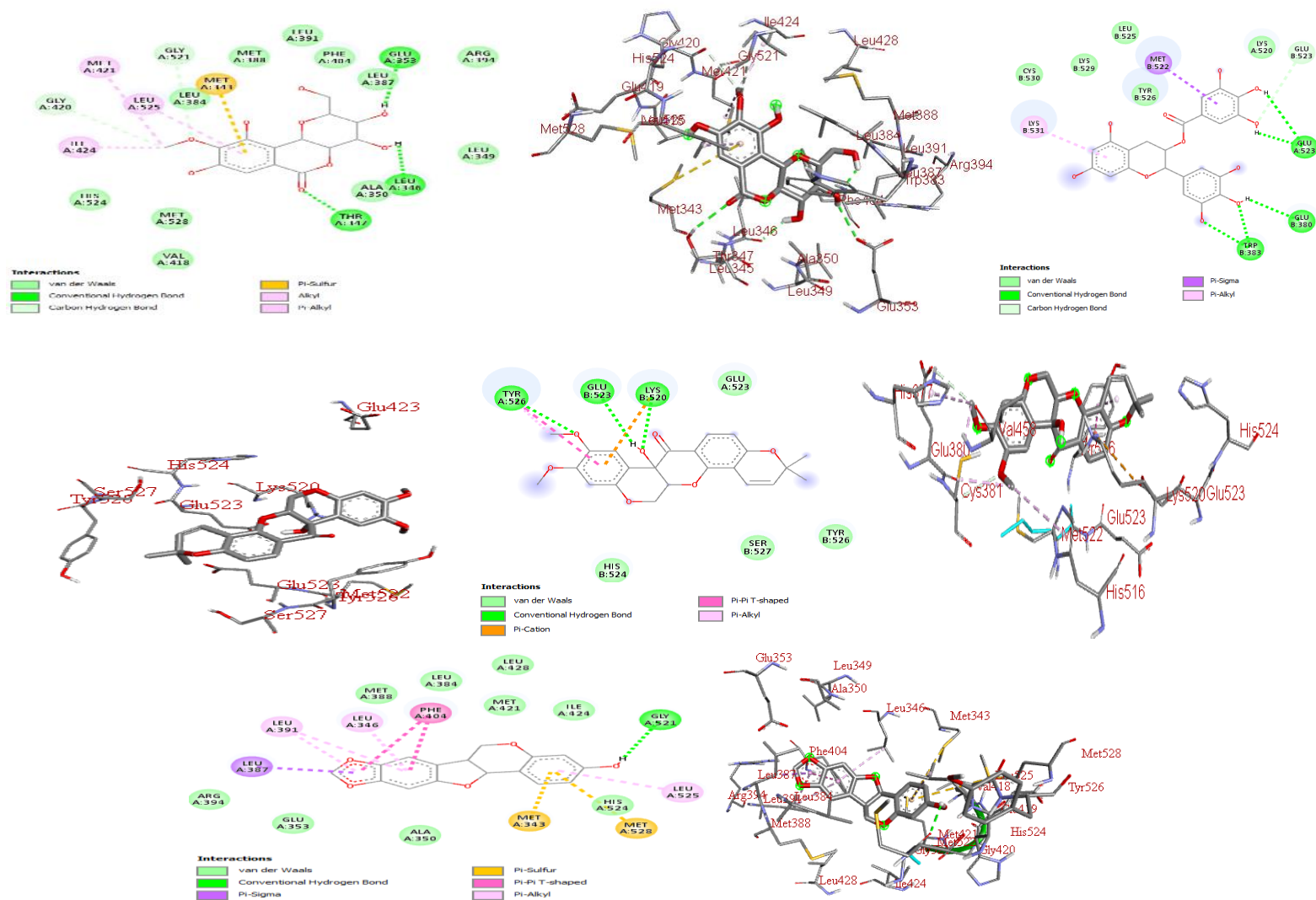


Figure 33: The 2D (left) and 3D (right) representation binding profile of compounds (96, 98, 102, and 103) against PDB ID: 6OWC receptor.

4.6.11. Molecular docking analysis of compounds (96, 98, 102, 103, 108, 114, 115, and 117) against androgen receptor (PDB: ID 1T7R) receptor

The molecular docking analysis of compounds (96, 98, 102, 103, 108, 114, 115, and 117) were screened towards the androgen receptor (PDB: ID 1T7R) receptor. The results of the docking analysis are presented in Table 48 and Figure 35. As illustrated in Table 48, the studied compounds demonstrated significant binding affinity towards the PDB ID: 1T7R receptor with binding energies ranging from -5.0 kcal/mole to -6.9kcal/mol, compared to vosaroxin (binding energy of -5.9 kcal/mol). The best binding energy was noted by tephrosin (-6.9 kcal/mol), followed by dihydroferulic acid (**108**) (-6.2 kcal/mol), and then maackiain (-6.1 kcal/mol), compared to vosaroxin (binding energy of -5.9 kcal/mol) (Table 48). Compound **98** exhibited a similar binding affinity with vosaroxin (binding energy of -5.9 kcal/mol). Compounds (96, 114, 115, and 117) demonstrated promising binding energies of -5.2, -4.8, -5.6, and -5.0 kcal/mol respectively (Table 48). As shown in Table Figures 35 and 36, hydrogen bond interaction, hydrophobic interaction, and residual interactions stabilized the corresponding ligand-protein complex.

Bergenin (**96**) (*via* ASP-A31, LYS-A822, LYS-820), and **98** (*via* Glu-A897 (2H-bonds), MET-A734 amino acids) form three hydrogen bond interactions in their corresponding ligand-protein complex towards PDB: ID 1T7R receptor (Figure35). Tephrosin (**102**) and Maackiain (**103**) exhibited hydrogen bond interactions through the active sites LYS-A905 (2H-bonds) and GLY-A738 amino acids respectively (Figure 35). Dihydroferulic acid (**108**) (GLN-A711 (2H-bonds), THR-A877), methyl gallate (**114**) (*via* LYS-AA822, LYS-A905, and GLN-A738), and 117 (*via* GLN-A902 (2H-bonds), LYS-A822) each for three hydrogen bond interactions in their corresponding ligand-protein complex towards PDB ID: 1T7R receptor (Figure 36). Gallic acid (**115**) also form two hydrogen bonds via the active sites of LEU-A735, GLN-A705 amino acids. Various residual amino acids were involved in the ligand-protein complex formation of the studied compounds and PDB ID: 1T7R receptor (Table 48 and Figure 36).

Table 48: Binding profile of compounds (**96**, **98**, **102**, **103**, **108**, **114**, **115**, and **117**) against androgen receptor PDB ID: 1T7R receptor

Compound	Energy (kcal/mol)	H-bond	Residual Interactions	
			Hydrophobic/Pi-cation, Pi/alkyl, Pi-anion	Van der Waals
96	-5.2	ASP-A31, LYS-A822, LYS-820	LYS-A720, MET-A894, VAL-A730, ILE-737, VAL-A716	LEU-A712, GLN-A738, GLN-A902, ILE-A896,
98	-5.9	Glu-A897(2H-bonds), MET-A734	-	CYS-B530, LYS-B529, LEU-B525, TYR-B526, LYS-B520
102	-6.9	LYS-A905 (2H-bonds)	GLU-A897, GLN-A738, MET-A734, ASP-A731,	VAL-901, ALA-A735, LEU-821, PRO-A819, GLY-A870, ASP-A819, TYR-A739, GLU-A897
103	-6.1	GLY-A738	VAL-A716, LYS-A720, MET-A734, MET-A894	VAL-A730, GLN-A733, PHE-A725, ILE-A737, GLN-A897
108	-6.2	GLN-A711(2H-bonds), THR-A877	LEU-A704, PHE-A876, MET-A780	LEU-A707, GLY-708, LEU-A873, LEU-701, MET-A742, ILE-AA899, PHE-A891, ASN-A705, TRP-741, MET-A895, PHE-A764, PHE-A764, ARG-A752
114	-4.8	LYS-AA822, LYS-A905, GLN-A738,	ALA-A735, GLN-A820,	PRO-A817, LEU-A821, VAL-A901, GLN-A902, MET-A734, ASP-735, TYR-A739
115	-5.6	LEU-A735, GLN-A705	LUE-A705	LEU-707, GLN-A708, MET-A745, TRP-A741, MET-A895, ILE-UA889, PHE—A891, THR-A819, MET-A742, MET-A780, LEU-A873
117	-5.0	GLN-A902, (2H-bonds), LYS-A822	LEU-A821, ASP-A732, LYS-A822, ALA-A735	GLN-A738, MT-A734, ASP-A371, PRO-817, LYS-A905
Vosaroxin	-5.9	-	LYS-A720, MET-A734, ILE-A732, VAL-A716, MET-A894, LEU-A712	VAL-A730, GLN-A733, PHE-725, GLN-A738, ILE-A898

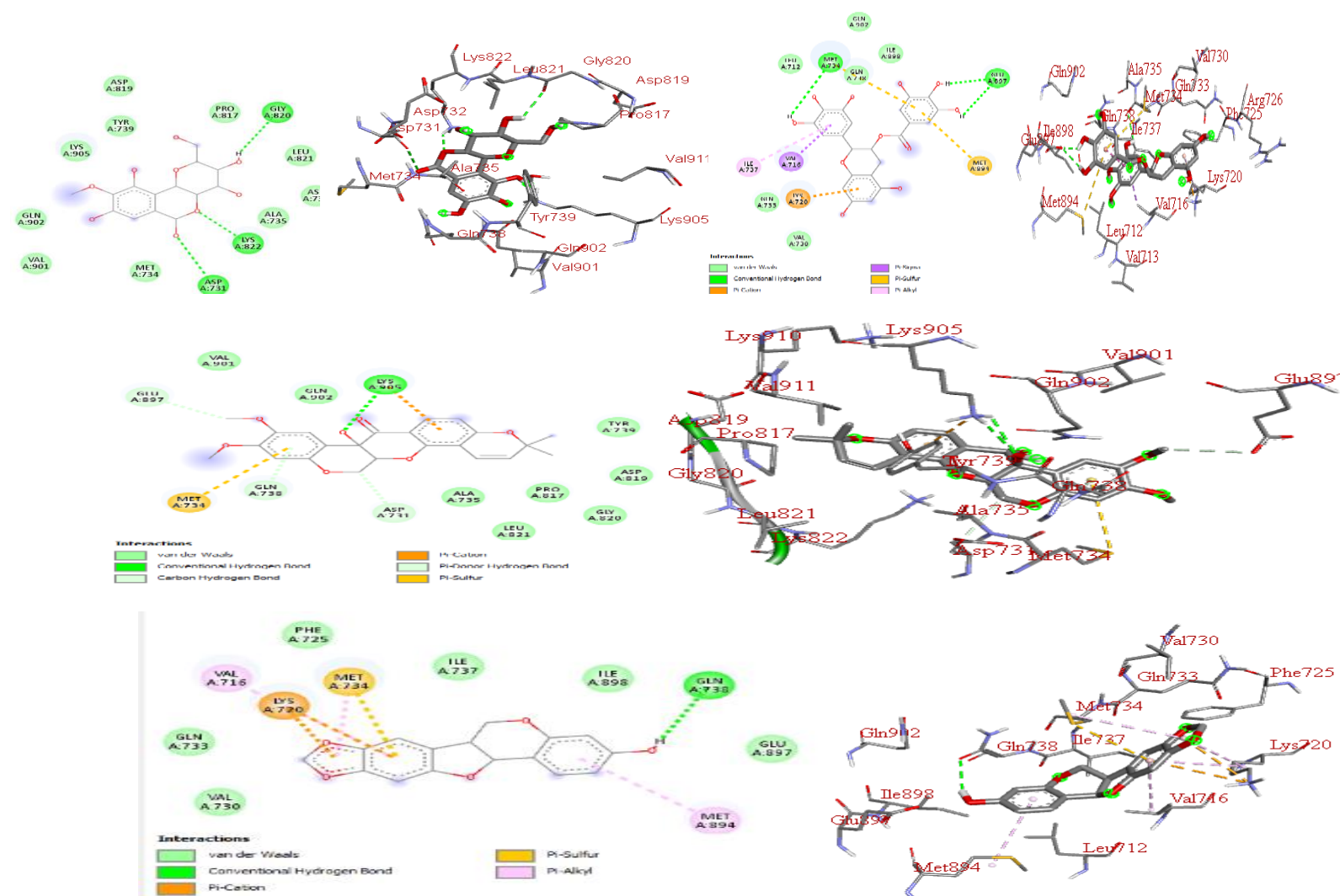


Figure 35: The 2D (left) and 3D (right) representation binding profile of compounds (96, 98, 102, and 103) against androgen receptor (PDB ID: 1T7R) receptor

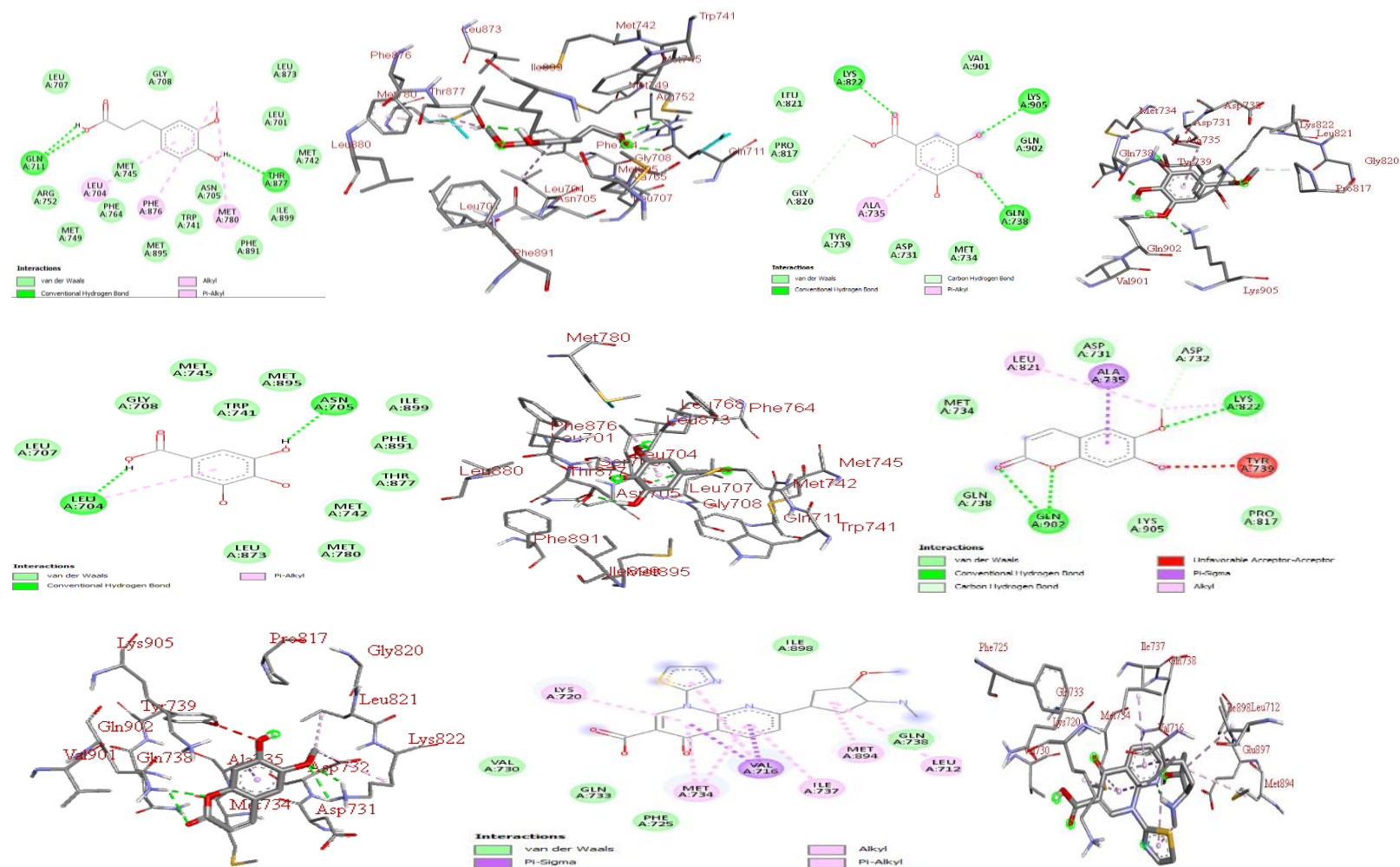


Figure 36: The 2D (left) and 3D (right) representation binding profile of compounds (**108**, **114**, **115**, **117** and vosaroxin against androgen receptor (PDB ID: 1T7R) receptor

4.6.12. Molecular docking analysis of compounds **96**, **98**, **99**, **102**, **103**, **105**, **108**, **114**, **115**, and **117**) towards estrogen receptor (PDB: ID 1G50) receptor

The molecular docking analysis of compounds **96**, **98**, **102**, **103**, **114**, **115**, and **117** were evaluated towards the estrogen receptor (PDB ID: 1G50). The results of the docking analysis are presented in Table 49, Figures 37 and 38. As shown in Table 49, the compounds under investigation demonstrated promising binding affinity with scoring energies ranging from -6.2 to -7.9 kcal/mol, compared to vosaroxin (binding energy -8.0 kcal/mol). The best binding energy was noted by compound **98** (-7.9 kcal/mol), followed by tephrosin (**102**) (-7.8 kcal/mol), and then by maackiain (**103**) (-7.2kcal/mol) (Table 53). Compounds **96**, **114**, and **117** also demonstrated minimum binding energies of -6.5, -6.3, and -6.8 kcal /mol, respectively. As represented in Figure 33, hydrogen bonds, hydrophobic/pi-alky, and Vander Waals interactions contributed to the stabilization of the complex formed between the studied compounds and the PDB ID: 1G50 receptor (Table 49).

Bergenin (**96**) exhibited four hydrogen bond interactions towards PDB ID: 1G50 through the active sites of PHE-C2337, GLN-A314, GLU-C2339, and CYS-C2417 amino acids (Figure 37). Compounds **98** and **102** each form one hydrogen bond interaction through the active sites of ASN-C2413 and PHE-C2337 amino acids. Maackiain (**103**) did not form a hydrogen bond interaction in its ligand-protein complex, rather the complex was stabilized by hydrophobic/pi-alkyl (Figure 37). Dihydroferulic acid (**108**) and gallic acid (**115**) form hydrogen bonds towards the PDB ID: 1G50 receptor through the active sites of GLU-C2353, ARG-C2394 and LEU-C2346, GLN-2353 amino acids respectively (Figure 38). Compounds **114**, and **117**) each form one hydrogen bond interaction towards the PDB ID: 1G50 receptor *via* amino acids ASN-C2413, PHE-C2337, GLU-C2353, and ARG-C2391 respectively (Figure 38). Residual interactions such as hydrophobic/pi-alkyl and Vander Waals interactions also engaged in their corresponding ligand-protein stable complex (Table 49). The results of the molecular docking prediction of the studied compounds revealed the compounds under investigation demonstrated promising binding affinity respectively compared to the reference drug, vosaroxin suggesting that the compounds may potentially be anticancer drugs.

Table 49: Binding profile of compounds (**96**, **98**, **102**, **103**, **108**, **114**, **115**, and **117**) against estrogen receptor alpha (ER α) PDB ID: 1G50 receptor

Compound	Energy(kcal/mol)	H-bond	Residual Interactions	
			Hydrophobic/Pi-cation, Pi/alkyl, Pi-anion	Van der Waals
96	-6.5	PHE-C2337, GLN-A314, GLU-C2339, CYS-C2417	LYS-C2416, ASN-C2413, SER-C2339	SER-A317, VAL-C-2418, GLU-C2419
98	-7.9	ASN-C2413		CYS-B530, LYS-B529, LEU-B525, TYR-B526,LYS-B520
102	-7.8	PHE-C2337	SER-C317, PHE-C2337	ASP-A313, CYS-C2417, PRO-C2330, , SER-A317, PROC-2333, TYR-C2331, ASP-A321, GLN-2414
103	-7.2	-	HIS-C2524, LYS-C2520, GLU-C2523	LYS-C2531, SER-C2527, GLY-C2420, ILE-C2420, GLU-C2427, MET-C2427, ARG-B-1548, LEU-B1543
108	-6.2	GLU-C2353, ARG-C2394	LEU-C384, ALA-C2350, LEU-C2421, LEU-C2424, MET-C2388	TRP-C2383,LEU-C2525, GLY-C2512, HIS-C2425, MET-C2421, PHE-C2404, LEU-C2391
114	-6.3	GLU-C2353	PHE-C2404, LEU-C2387, ALA-C2350	LEU-C22525, LEU-2543, THR-C2347, LEU-C2349, MET-2343, LLEU-2346, LEU-2328, MET-C2380, LEU-2391, ARG-C2394
115	-6.2	LEU-C2346, GLN-2353	LUE-C2383,ALA-C2350, LEU-C2391, PHE-C2404	LEU-C2349, QRG-C2394, LEU-C2304, MET-2388, LEU-C2428, THR-2347
117	-6.8	ARG-C2391,	LEU-C2525,MET, C2343,LEU-C2346, ALA-C2350	LEU-2391,, GLU-C2353, LEU-C2349, PHE-C2404, THR-2347, MET-2388, LEU-C2384, LEU-C2387
Vosaroxin	-8.0	ARG-C2436	ALA-C2340, LEU-C2429, GLY-C2400, PHE-C1461	HIS-C2398, LYS-C2401, ARG-C2412, SER-C1464, LEU-B1469, LYS-B1472, TYR-B1459, ASP-C2426

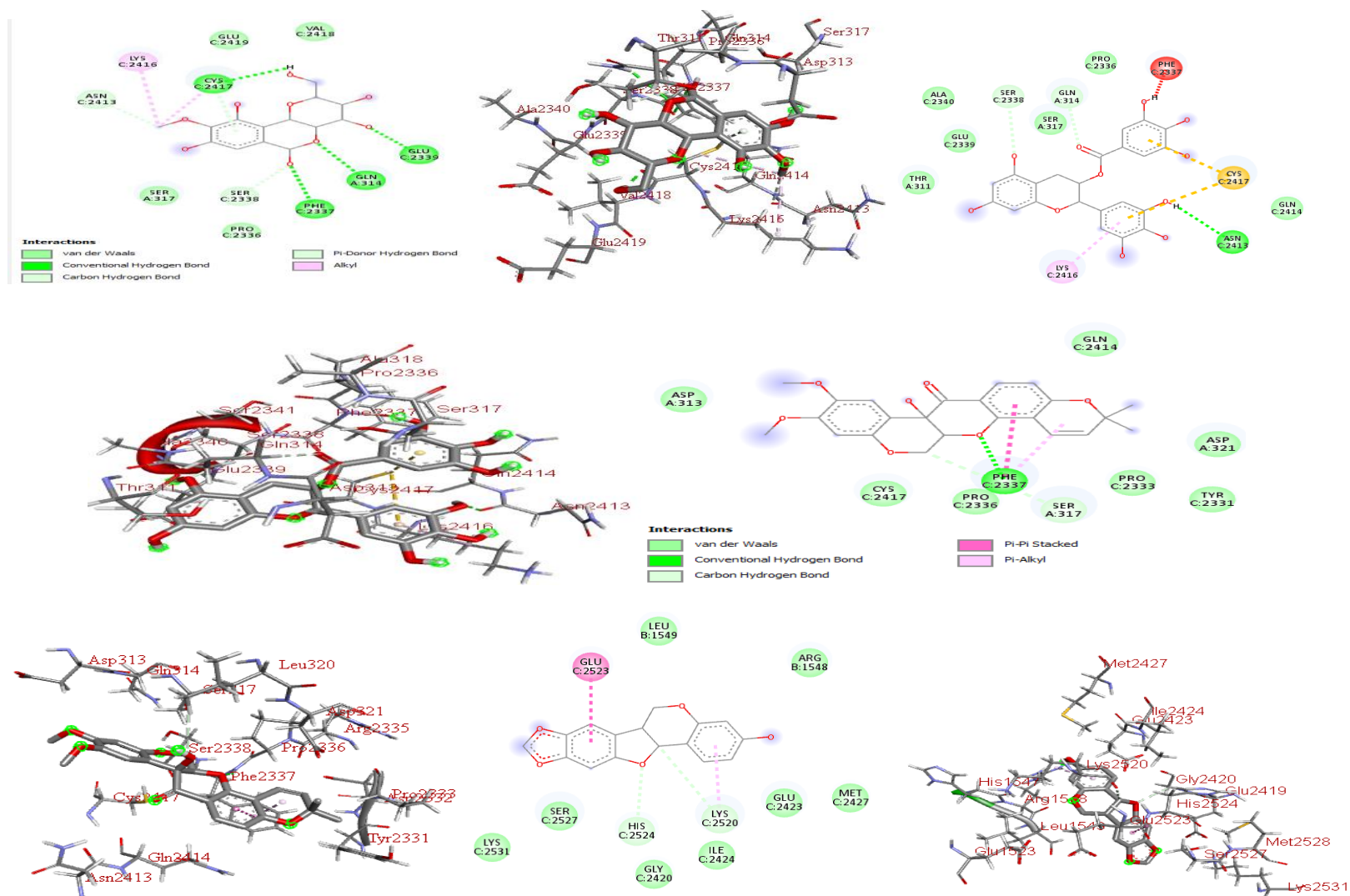


Figure 37: The 2D (left) and 3D (right) representation binding profile of compounds (96, 98, 102, and 103) against PDB ID: 1G50 receptor

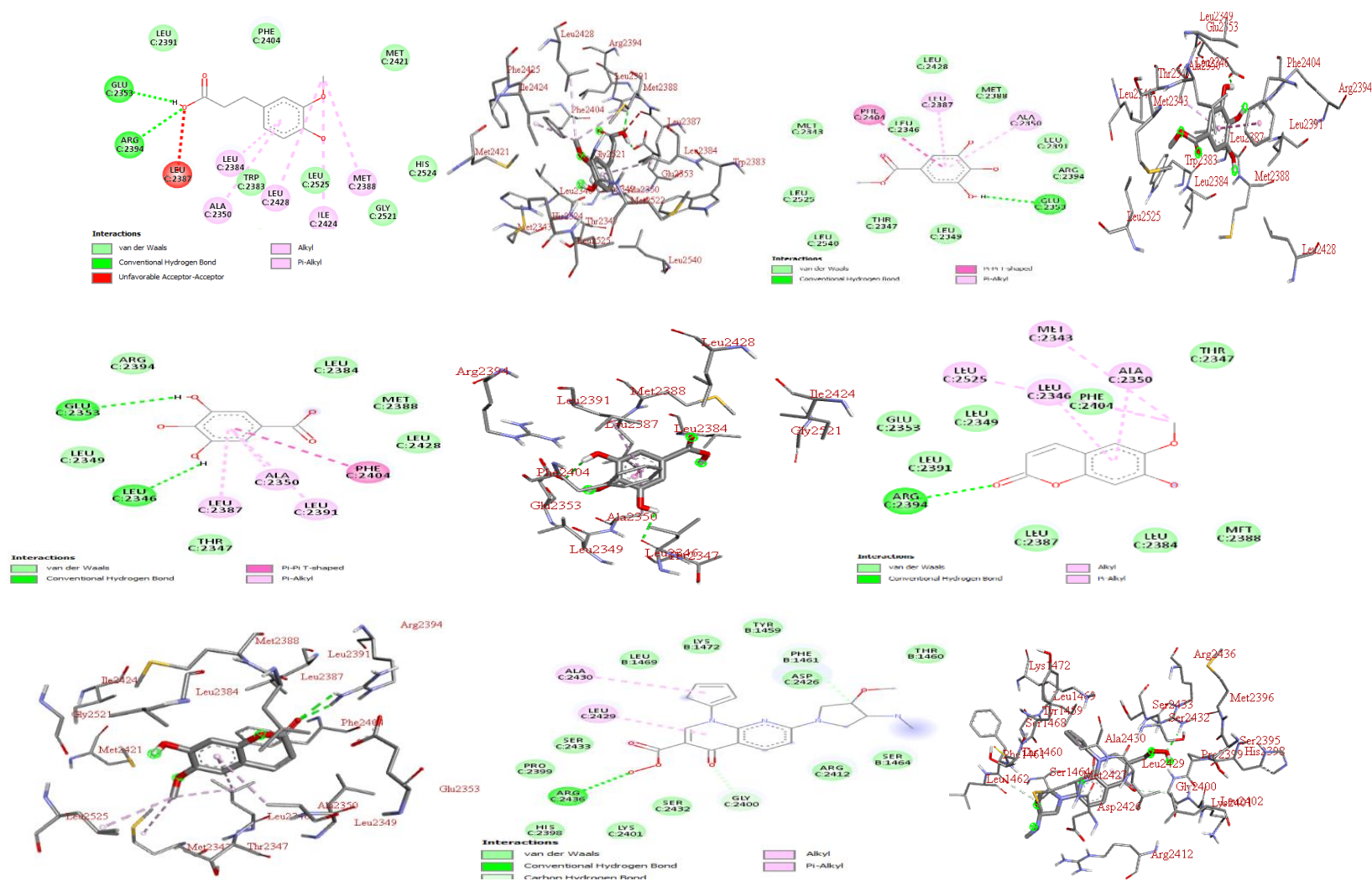


Figure 38: The 2D (left) and 3D (right) representation binding profile of compounds (108, 114, 115, and 117) and vosaroxin against PDB ID: 1G50 receptor

4.6.13. Molecular docking analysis of compounds **96, 98, 99, 102, 103, 105, 108, 114, 115, and 117** against progesterone receptor (PDB ID: 3G8O) receptor

The molecular docking prediction of compounds (**96, 98, 102, 103, 108, 114, 115, and 117**) was evaluated towards the progesterone receptor (PDB ID: 3G8O) target. The results of the docking analysis are presented in Table 50, Figures 39 and 40. Tephrosin (**102**) and maackiain (**103**) exhibited better binding energy of -8.0 kcal/mol, compared to vosaroxin (binding energy of -7.1 kcal/mol). Compounds **96, 98, 108, 114, 115, and 117** also demonstrated promising binding energies of -7.1, 7.9, -6.4, -6.7, and -6.5 kcal/mol respectively, compared to vosaroxin (binding energy of -7.1 kcal/mol). As shown in Table 50, hydrophobic/pi-alkyl, Vander Waals, and hydrogen bond interactions were engaged for the stabilization of the corresponding ligand-protein complex (Table 50). Compound **98** forms three hydrogen bonds through the active sites of ARG-A899, SER-A896, and PHE-B895 amino acids. Bergenin (**96**), (SER-B890 and PHE-A895), and tephrosin (**102**) (SER-A898, and SER-B898), form hydrogen bond interaction in their corresponding ligand-protein complex towards PDB ID: 3G8O receptor. The complex formed between maackiain (**103**) and PDB ID: 3G8O receptor was stabilized by hydrophobic/alkyl (*via* VAL-A884, ILE-A920, PRO-A927, HIS-A88) and Vander Waals (*via* SER-A754, SER-A757, TYR-A753, THR-A829, VAL-A925, GLY-A923, HIS-A888, ASP-A882, LYS-885) interactions (Figure 39).

Dihydroferulic acid (**108**) (SER-A754, and HIS-A882), and methyl gallate (**114**) (HIS-A888, and SER-A754) showed hydrogen bond interactions in their corresponding ligand-protein complex towards PDB ID 1G8O receptor (Figure 40). Gallic acid exhibited three hydrogen bond interactions through the active sites of HIS-888, TYR-A753, and THR-A829 amino acids (Figure 40). Scopoletin (**117**) exhibited one hydrogen bond interaction through the active site of ARG-A766 amino acid. In addition to hydrogen bonds, hydrophobic/pi-alkyl/an-ion, and Vander Waals interactions through various residual amino acids contributed to the stabilization of their corresponding ligand-protein complex (Table 50 and Figure 40). The molecular docking analysis results indicated that the studied compounds showed significant binding affinity towards the PDB ID: 3G8O receptor suggesting that the compounds under investigation may be good therapies for breast cancer.

Table 50: Binding profile of compounds (**96**, **98**, **102**, **103**, **108**, **114**, **115**, and **117**) towards the Estrogen receptor Alpha (ER α)
PDB ID: 3G8O receptor

Compound	Energy(kcal/mol)	H-bond	Residual Interactions	
			Hydrophobic/Pi-cation, Pi/alkyl, Pi-anion	Van der Waals
96	-7.1	SER-B890, PHE-A895	PHE-A896,	ALA-A900, GLN-A897, ARG-A8899, SER-A898, ILE-B896, ARG-B899, PHE-B905, SER-B910,
98	-7.9	ARG-A899, SER-A896, PHE-B895	ALA-B914 ARG-B899, PHE-A896, ILE-B895	GLU-B907, GLU-B911, SER-B910, SER-B896, PHE-B905, GLN-B897, ALA-A900
102	-8.0	SER-A898, SER-B898	PHE-B896, ARG-B899, ILE-B896, PHE-A896	GLU-B907, SER-B910, PHE-B905, PHE-A905, SER-A910, ARG-A899, ILE-A896
103	-8.0	-	VAL-A884, ILE-A920, PRO-A927, HIS-A881	SER-A754, SER-A757, TYR-A753, THR-A829, VAL-A925, GLY-A923, HIS-A888, ASP-A882, LYS-885
108	-6.4	SER-A754, HIS-A882	VAL-A882, HIS-A881, TYR A754, VAL A925,	LEU-A921, LYS-A885, PRO-927, THR-A829, MET-A756, SER-SER-A754
114	-6.7	HIS-A888, SER-A754	HIS-A881, VAL-A884	LEU-A921, LYS-A885, LEU-A880, LEU-A825, ASN-A828, SER-A754, THR-829, VAL-925
115	-6.5	HIS-888, TYR-A753, THR-A829	HIS-888, VAL-A884, HIS-A881	LEU-A921, LYS-A885, ILE-A920, SER-A754, ASN-828, SER-A757, LEU-A880, LEU-A825
117	-6.6	ARG-A766,	MET-759, CYS-A891, MET-756	LEU-A763, MET-A909, GLY-A722, ASB-A719, PHE-A778, LEU-A721, MET-A726
Vosaroxin	-7.1	-	ALAL-B914, ALAL-A90, PHE-B895, ARG-B895,	SER-B-910, GLU-B-907, PRO-B906, SER-B898, ILE-B898, SER-A898, ARG-A899, GLN-A897

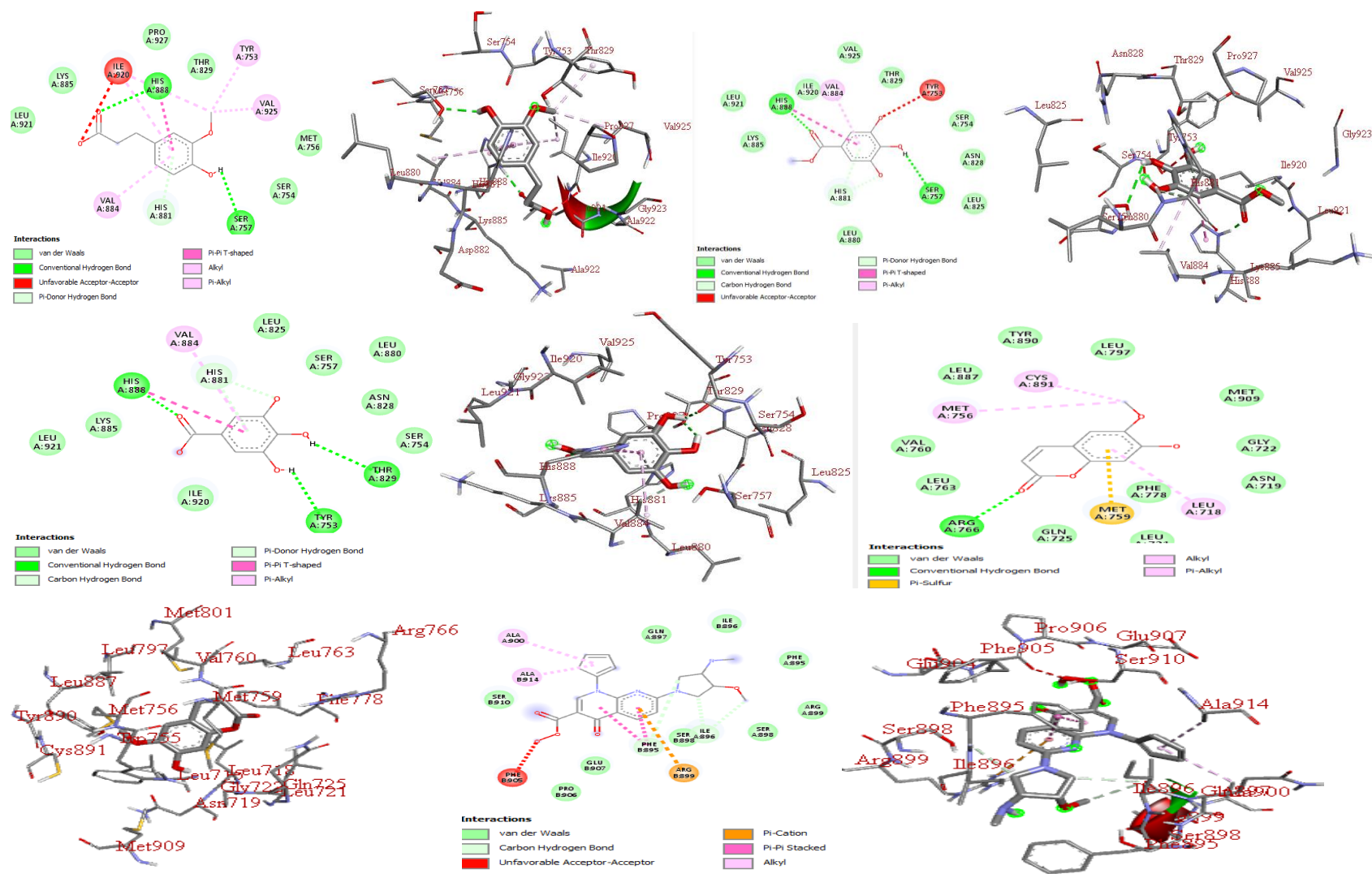


Figure 40: The 2D (left) and 3D (right) representation binding profile of compounds (108, 114, 115, 117 and vosaroxin against PDB ID: 3G8O receptor

4.7. Predication of the Physicochemical and ADMET Parameters of the Isolated Compounds

The physicochemical and ADMET properties of the isolated compounds were evaluated using the Swiss ADME and PreADMET online tools following the standard guidelines described by Maliehe *et al.*, (Maliehe *et al.*, 2020). *In silico* ADMET analysis is a quick approach to find if a compound has acceptable pharmacokinetics and pharmacodynamic properties (Arif Malik *et al.*, 2017). The pharmacokinetic parameters of the isolated compounds were projected on the principle of 'Lipinski's rule of five (Anza *et al.*, 2021; Tegegn *et al.*, 2022). The drug-likeness properties of the studied compounds were evaluated based on the principle of Lipinski's rule of five (RO5'). According to RO5's principles orally active drugs meet the single criteria of hydrogen-bond donors (HBDs) should be less than 5, hydrogen-bond acceptors (HBAs) should be less than 10, the molecular mass should be less than 500 Da, log P should be less than 5 and the total polar surface area (TPSA) should be less than 140 Å² (Olaokun and Zubair, 2023). According to Lipinski's rule, an orally active drug should meet at least four criteria of Lipinski's rule (Abdelrheem *et al.*, 2020).

The results of the physicochemical and ADMET analysis of the analyzed compounds are presented in Tables 51 and 52. Out of the studied compounds, compounds (**97**, **101**, **102**, **103**, **104**, **105**, **107**, **108**, **114**, **115**, **116**, and **117**) obey Lipinski's rule five with zero violations (Tables 51 and 52). The molecular weight of a drug candidate is an important parameter in the drug's mechanism of action. The smaller the molecular weight (MW ≤ 500 g/mole), the lighter molecules can easily be absorbed and diffused in the body, and the poorer drug uptake and bioavailability are associated with greater molecular weights (MW > 500 g/mole (Maliehe *et al.*, 2021). The Swiss ADME estimation results showed that all compounds under investigation have acceptable molecular weights (MW less than 500g/mole), which indicated their capacity to be swiftly absorbed and transported due to their compliance with Lipinski's rule of five (Ammar, 2017). Except for bergenin (**96**) and **98**, the computed TPSA values of the studied compounds were below the limit of 140 Å, indicating that the compounds are expected to be readily permeable from the gastrointestinal tract and favorable oral bioavailability for drug-like candidates (Mahmud Paul *et al.*, 2022).

Table 51: Pharmacokinetics, ADMET, and Toxicity profile of isolated compounds (**96**, **97**, **98**, **101**, **102**, **103** and **104**)

Parameter		Compounds						
		96	97	98	101	102	103	104
Formula		C ₁₄ H ₁₆ O ₉	C ₂₉ H ₅₀ O	C ₂₃ H ₁₇ O ₁₀	C ₂₃ H ₂₂ O ₆	C ₂₃ H ₂₂ O ₇	C ₁₇ H ₁₄ O ₆	C ₂₀ H ₁₈ O ₄
Molecular Weight (g/mole)		328.27	414.71	441.40	394.42	410.42	284.26	322.35
NHD		5	1	7	0	1	1	1
NHA		9	1	11	6	7	5	4
NRB		2	6	5	2	2	0	1
TPSA (Å ²)		145.91	20.23	186.37	63.22	83.45	57.15	55.76
LogP (iLogP)		1.32	4.79	1.53	3.83	3.79	2.77	3.22
RO5' Violation		1	0	3	0	0	0	0
LogKp (cm/s)		-8.33	-2.20	-7.36	-6.06	-6.69	-6.18	-5.41
GIA		High	Low	High	High	High	High	High
BBB		No	No	No	Yes	No	Yes	Yes
Inhibitor Interaction	P-gp Substrate	No	No	No	Yes	Yes	Yes	Yes
	CYP1A2	No	No	No	No	Yes	Yes	Yes
	CYP2C19	No	No	Yes	Yes	Yes	Yes	Yes
	CYP2C9	No	No	Yes	Yes	No	No	Yes
Toxicity	CYP2D6	No	No	Yes	Yes	Yes	Yes	Yes
	CYP2D6	No	No	Yes	Yes	Yes	Yes	Yes
	Hepato	No	No	No	No	No	No	No
	Carcino	No	No	No	No	No	No	No
	Immuno	No	Yes	Yes	Yes	Yes	Yes	No
	Mutagen	No	No	No	No	No	No	No
	Cyto	No	No	No	No	No	No	No
	LD ₅₀ (mg/kg)	10000	5000	3850	4	2500	2500	1060
Toxicity Class		6	5	5	1	5	5	5

Table 52: Pharmacokinetics, ADMET, and Toxicity profile of isolated compounds (**105**, **107**, **108**, **114**, **115**, **116**, and **117**).

Parameter		Compounds						
		105	107	108	114	115	116	117
Formula		C ₂₃ H ₁₈ O ₇	C ₃₀ H ₅₀ O	C ₁₁ H ₁₄ O ₃	C ₈ H ₈ O ₅	C ₇ H ₆ O ₅	C ₁₅ H ₂₂ O ₂	C ₁₀ H ₈ O ₄
Molecular weight(g/mole)		406.38	426.72	194.23	184.15	170.12	234.33	192.17
nHBD		0	1	1	3	4	1	1
nHBA		7	1	3	5	5	2	4
NRB		2	1	4	2	1	1	1
TPSA (Å ²)		88.11	20.23	46.53	86.99	97.99	37.30	59.67
LogP (iLogP)		3.68	4.68	2.01	0.97	0.21	2.74	1.52
RO5' Violation		0	0	0	0	0	0	0
LogKp (cm/s)		-6.12	-1.90	-6.05	-6.81	-6.84	-5.96	-6.39
GIA		High	Low	High	High	High	High	High
BBB		No	No	Yes	No	No	Yes	Yes
Inhibitor Interaction	P-gp substrate	No	No	No	No	No	No	No
	CYP1A2	No	No	No	No	No	No	Yes
	CYP2C19	No	No	No	No	No	No	No
	CYP2C9	No	No	No	No	No	No	No
	CYP2D6	No	No	No	No	No	No	No
	CYP23A4	No	No	No	No	Yes	No	No
	Hepato	No	No	No	No	No	No	No
	Carcino	No	No	No	No	Yes	No	Yes
	Immuno	No	No	No	No	No	No	No
	Mutagen	No	No	No	No	No	No	No
	Cyto	No	No	No	No	No	No	No
LD ₅₀ (mg/kg)		2000	2000	1700	1700	2000	1000	3800
Toxicity Class		4	4	4	4	4	4	5

Cytochrome P450 monooxygenase plays a fundamental part in drug mobilization and elimination in biological systems. Inhibition of isoforms of this enzyme system by drugs could result in poor elimination, resulting in drug-induced toxicity. Cytochrome P450 enzymes exist in isoforms namely CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 are in charge of the metabolism and clearance of drugs and xenobiotics from biological systems (Iheagwam *et al.*, 2019). Blocking the function of CYP 450 isoenzymes may result in undesired drug interactions that may have side effects associated with reduced removal or buildup of drug metabolites (Kirchmair *et al.*, 2015). Therefore it is important to note that a drug candidate should have minimal inhibitory effect against various enzyme isoforms (Lynch and Price, 2007). The present finding indicated that compounds **96**, **97**, **98**, **105**, **107**, **108**, **114**, **115** and **117** were found to be non-inhibitors of CYP1A2, CYP2C19, CYP2C9, CYP2C9, and CYP2D6 isozymes, suggesting that the compounds would be well metabolized in the liver and readily eliminated from the body (Ononamadu and Ibrahim, 2021).

The proTox II screening analysis results indicated all the studied compounds were found to be hepatotoxicity, carcinogenic, mutagenic, and cytotoxic (Tables 51 and 52). The proTox II screening analysis results also demonstrated that all studied compounds exhibited median fatal dose (LD₅₀) values ranging from 547-10000 mg/Kg (Tables 51 and 52). The acute oral toxicity (LD₅₀) predictions showed that compound **96** was found to be non-toxic class 6 (LD₅₀ 10,000), whereas, compounds **97**, **98**, **102**, **103**, **104**, and **117** were classified as class 5 (2000 < LD₅₀ ≤ 5000), highlighting their low toxicity. Compounds **105**, **107**, **108**, **114**, **115**, and **116** were categorized into class 4 (300 < LD₅₀ ≤ 2000), indicating slight toxicity levels. Compound **101** exhibited higher toxic values, class 1 (LD₅₀: 4 mg/kg/day) suggesting potential long-term health risks associated with their usage (Tables 51 and 52).

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

In the present study, silica gel chromatography separation of the roots of *W. uniflora*, *T. vogelii*, *A. schimperi*, *R. glutinosa*, and *C. spinarum* results in the identification of 24 (**96-117**) compounds. Dibutyl phthalate (28.66%), cis nerolidol (41.7%), and cedrol (25.85%), were the major EO constituents of the roots of *W. uniflora*, *T. vogelii*, and *A. schimperi* respectively, whereas, 4-hydroxy acetophenone was the major EO component of the roots of *R. glutinosa* and *C. spinarum* with the percentage abundance of 72.19% and 74.85% respectively. The EO extracts of the roots of *W. uniflora*, and *R. glutinosa* displayed maximum antibacterial activity against *S. aureus* with ZI of 17.6 ± 0.47 mm and 16.0 ± 0.0 mm respectively, whereas, EO extracts of *A. schimperi* and *C. spinarum* exhibited promising antibacterial activity against *E. coil* (ZI of 17.3 ± 0.81 mm) and *S. pyogenes* (17.0 ± 0.0 mm) respectively. The maximum antibacterial potential of the EO components may be linked with the synergistic or additive effects of its major and minor constituents. Tephrosin (**102**) and maackiain (**103**) displayed promising antibacterial activity against *S. aureus* and *P. mirabilis* respectively with MIC value of 0.5 ± 0.0 mg/mL, signifying that flavonoids may be the active antibacterial compounds of *T. vogelii*. Lupeol (**107**), and **116** demonstrated maximum antibacterial activity against *S. aureus* with ZI of 17.3 ± 0.81 mm, their hydrophobicity may also significantly contribute to moving across the lipids of the cell membranes of bacteria and disrupt the cell wall structure. Methyl gallate (**114**) and gallic acid (**115**) also displayed maximum antibacterial activity against *S. aureus* (17.6 ± 0.46 mm) and *S. pyogenes* (ZI of 18.6 ± 0.57 mm) respectively, confirming that phenolic compounds significantly inhibited the growth of Gram-positive bacterial. Compounds **96, 98, 99, 102, 103, 105, 108, 114**, and **115** displayed promising scavenging of DPPH radicals with IC_{50} values of 18.42, 9.48, 10.43, 10.73, 10.97, 10.66, 7.48 and 6.07 μ g/mL respectively compared to ascorbic acid with IC_{50} values of 5.83 μ g/mL, evidencing that polyhydroxy-aromatic groups could donate hydrogen-bearing free radicals to stabilize DPPH free radicals which plays an important role in the prevention of oxidative stress and related free radical-induced disorders. DCM/MeOH (1:1) and MeOH extracts of the roots of *A. schimperi* displayed promising activity against influenza A and B with selectivity index values of 32 and 12 μ g/mL respectively, indicating that the

chemical constituents of the roots of *A. schimperi* may be potentially a candidate for the development of efficacious and nontoxic drugs against influenza A and B viruses. Molecular docking analysis results revealed that compounds **104**, **107**, and **114** displayed strong binding affinity towards DNA gyrase of *E. coil* (PDB ID: 4F86) target with respective binding free energies of -6.6, -6.5, and -6.1 kcal/mol) compared to ciprofloxacin (binding free energy of -7.4 kcal/mol). Towards pyruvate kinase of *S. aureus* (PDB ID: 3T07) receptor, compounds **101** and **103** showed a promising binding affinity with the respective binding free energy of -6.7 and -6.8 kcal/mol compared to ciprofloxacin (binding free energy of -7.3 kcal/mol). Compounds **101**, and **103** displayed better binding affinity towards *P. aeruginosa* (PDB ID: 2UV0) receptor with the respective binding free energy of -7.6 and -7.4 kcal/mol compared to ciprofloxacin (binding free energy of -7.3 kcal/mol). Compounds **96**, **97**, **98**, **99**, **102**, **104**, **105**, **108**, **114**, **115**, **116**, and **117** exhibited promising binding free energies ranging from -4.8 to -10.0 kcal/mol, compared to ascorbic acid (binding free energy of -5.7 kcal/mol). Compounds **101**, **102** and **115** displayed the best binding affinity towards *S. epidermidis* FtsZ (PDB ID: 4M8I) (binding free energy of -7.0 kcal/mol), *P. mirabilis* urease (PDB ID: 1E9Y) (binding energy of -7.9 kcal/mol), and *S. pyogenes* 1078 streptotain (PDB ID: 6KUD) (binding energy of -5.7 kcal/mol) receptors respectively. Compound **98** showed strong binding affinity towards human peroxiredoxin 5 (PDB ID: 1HD2), topoisomerase II α (PDB ID: 4FM9), estrogen receptor (PDB ID: 1G50), with respective binding energies of -6.3, -9.8, and -7.9 kcal/mol respectively. Compound **102** and **103**, exhibited promising binding affinity towards progesterone receptor (PDB ID: 3G8O) with a binding energy of -8.0 kcal/mol. The overall molecular docking study results indicated that the isolated compounds displayed strong binding affinity towards one or more protein targets suggesting that they have the potential to be drug candidates by targeting the essential elements of bacterial biochemical processes, such as DNA replication, carbohydrate metabolism, gene expression, and protein synthesis. The results of the physicochemical and ADMET analysis of the analyzed compounds revealed that compounds **97**, **101**, **102**, **103**, **104**, **105**, **107**, **108**, **114**, **115**, **116**, and **117** obey Lipinski's rule five with zero violations, suggesting their potential to be orally administered drugs. The *in vitro* antibacterial and antioxidant activities of the crude extracts and isolated compounds of the studied plants results supported by the minimum binding energies of the compounds towards the respective

protein targets prove that the chemical constituents of the studied plants could serve as antimicrobial agents by blocking the essential cellular reactions required for the survival of the bacterial and the treatment of free radical-induced diseases.

5.2. Recommendations

- The DCM/MeOH (1:1) and MeOH extracts of the roots of *A. schimperii* showed promising antiviral activity against influenza A and B viruses. Thus, further study on the isolation of the active constituents and *in vivo* analysis is needed to develop lead antiviral drugs.
- In this study, the antibacterial and antioxidant activity of the studied plants' crude extracts and isolated compounds were assessed. Further evaluation of their anticancer, antiviral, and anti-inflammatory activity must be explored to fill the gap.
- Compounds **96, 97, 98, 99, 102, 104, 105, 108, 114, 115, 116** and **117** displayed promising DPPH radical scavenging activity compared to ascorbic acid. Further *in vivo* analysis is needed to validate their therapeutic and cytotoxicity potentials.
- Bergenin (**96**) was obtained in a profound amount (5% of the plant material) from the roots of *W. uniflora*. Using this golden opportunity further work *in vivo* analysis and synthetic modifications is recommended to discover lead drug candidates.
- In the present study, most of the identified compounds are the medium polar constituents of the plants. Advanced hyphenated techniques and reverse phase HPLC are highly recommended to isolate the polar chemical constituent of the plants.

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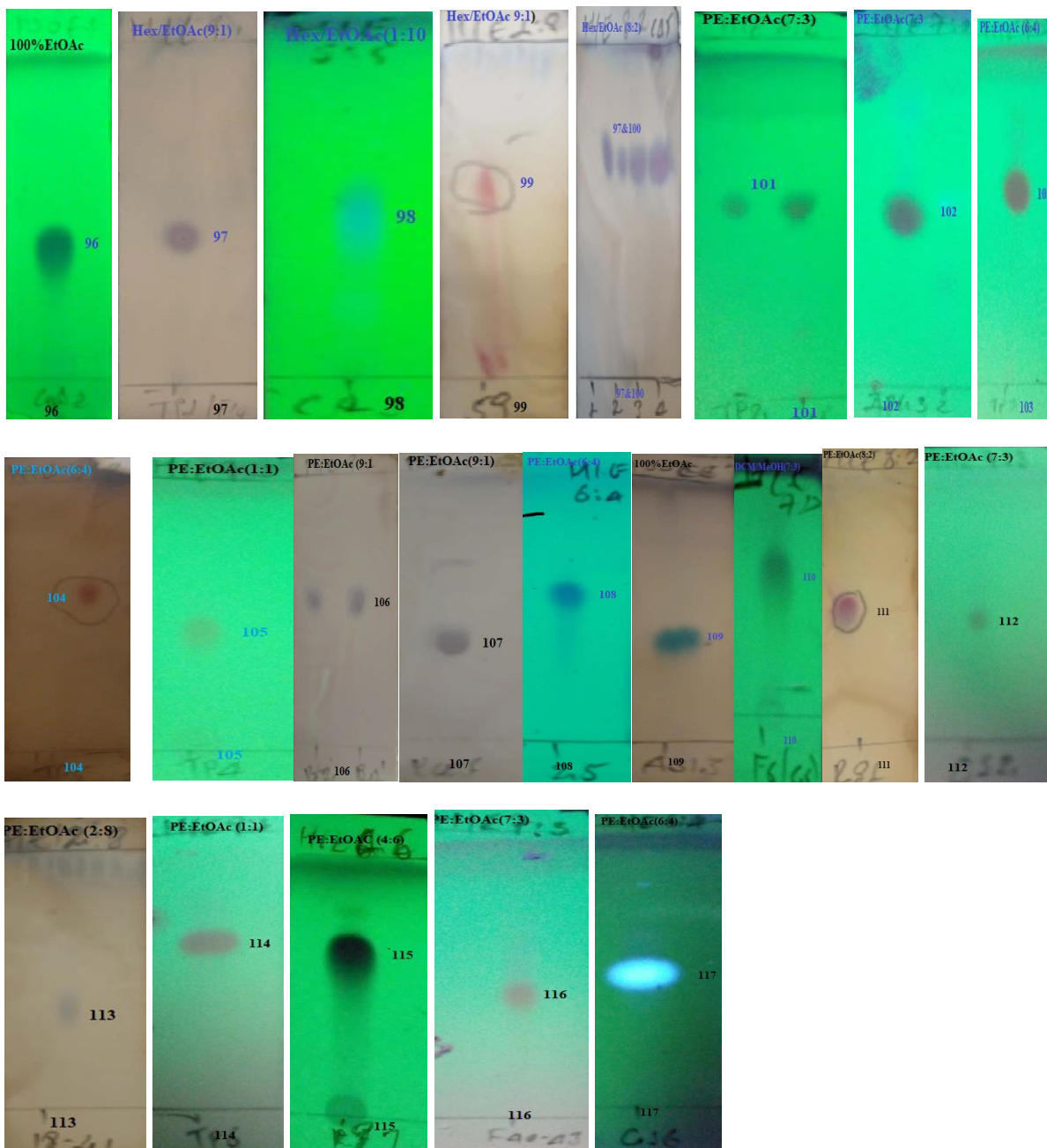
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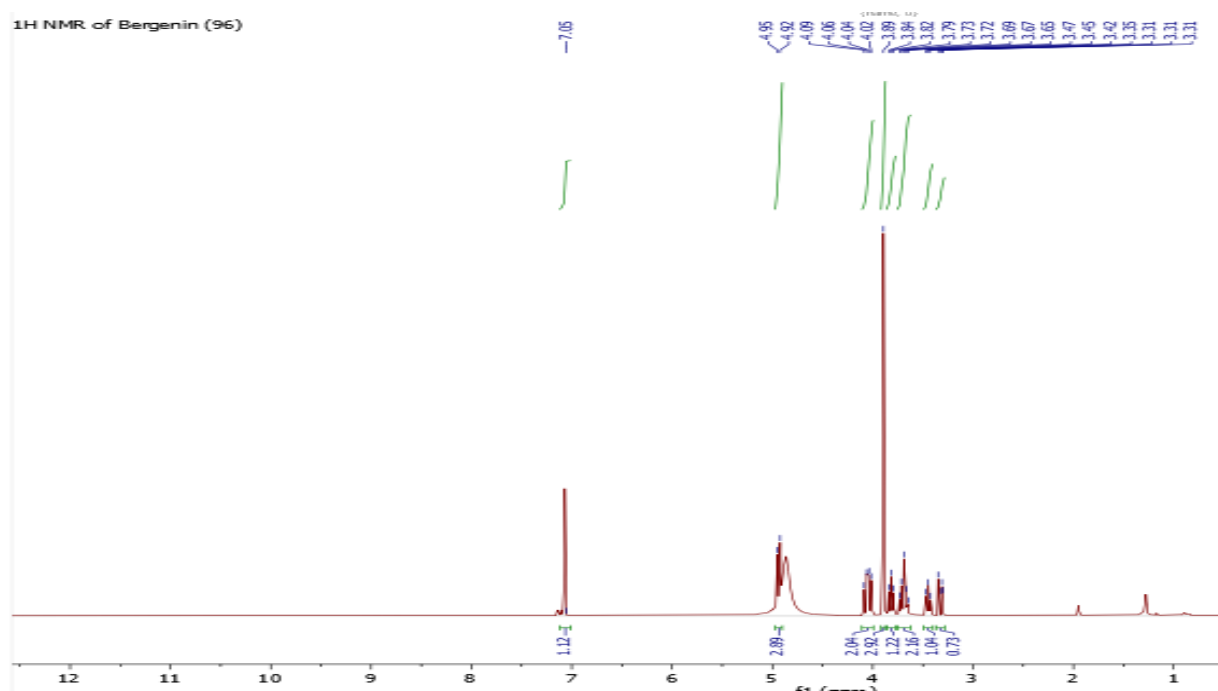
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APPENDICES

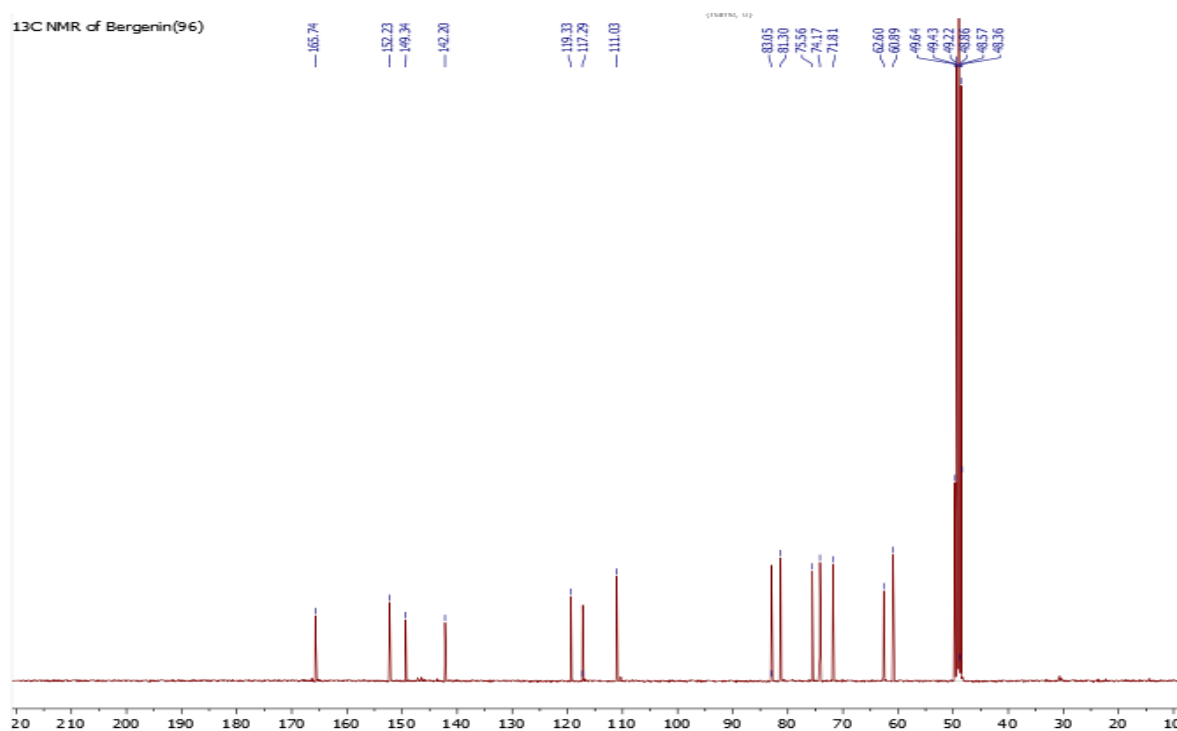
Appendix 1: TLC profile of isolated compounds



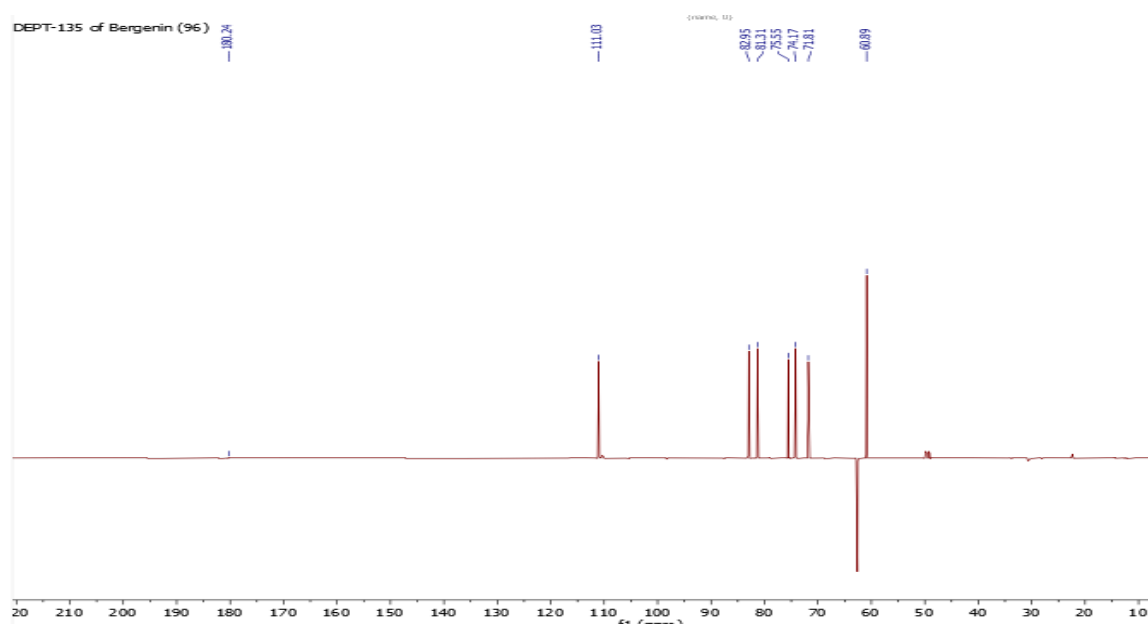
Appendix 1A: ^1H NMR (in CD_3OD) of spectrum bergenin (**96**)



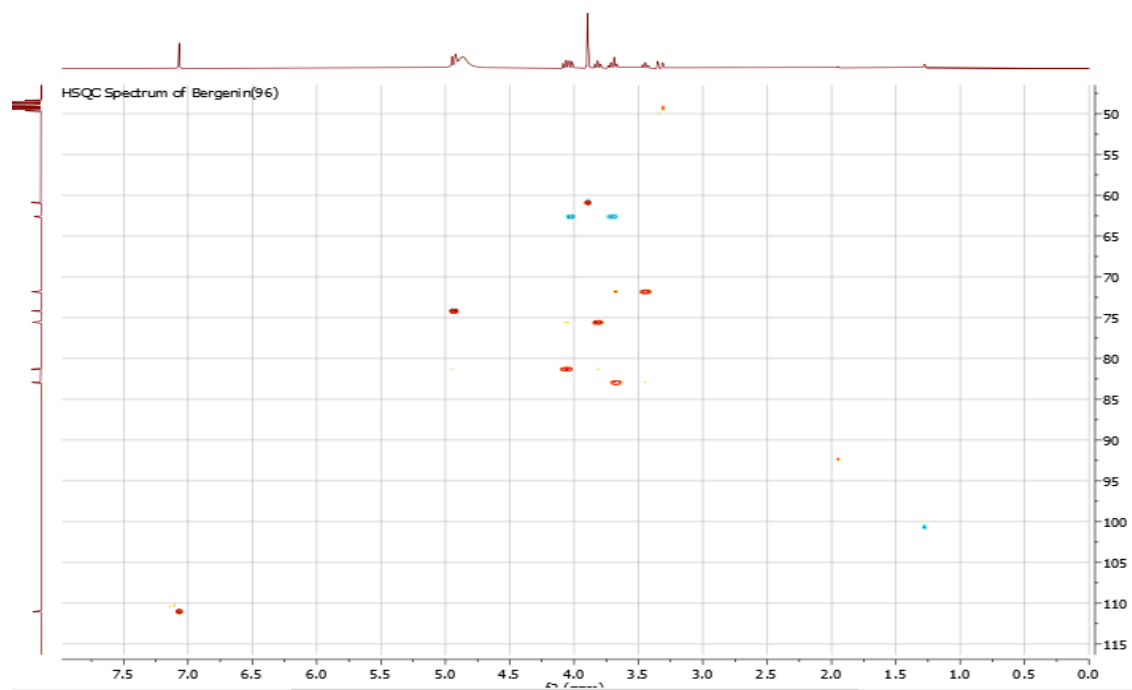
Appendix 2B: ^{13}C NMR (in CD_3OD) spectrum of of bergenin (**96**)



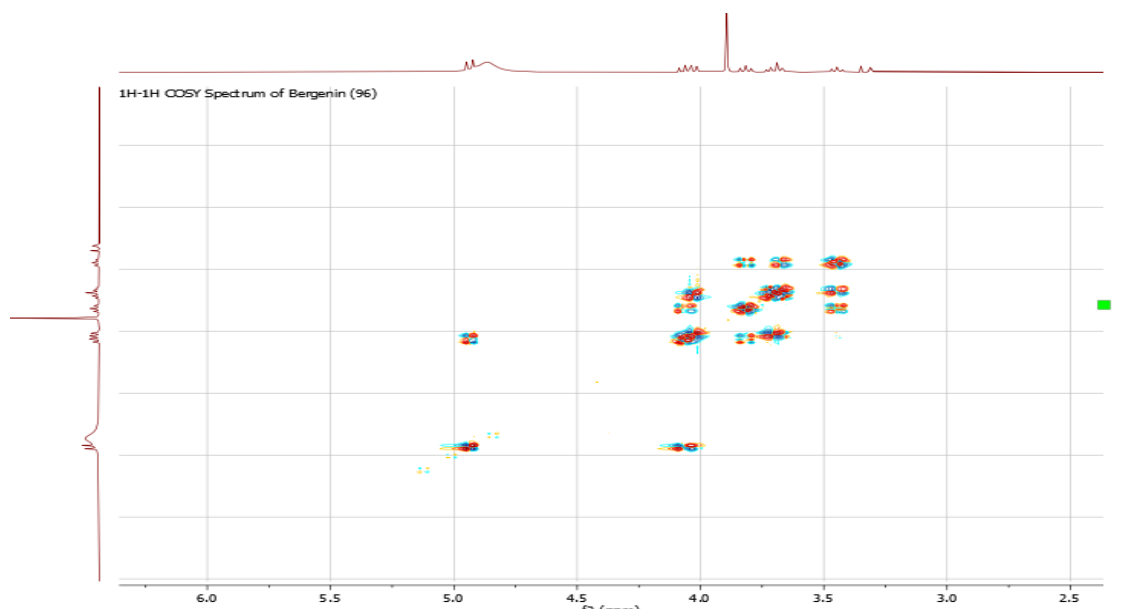
Appendix 2C: DEPT (in CD₃OD) spectrum of bergenin (96)



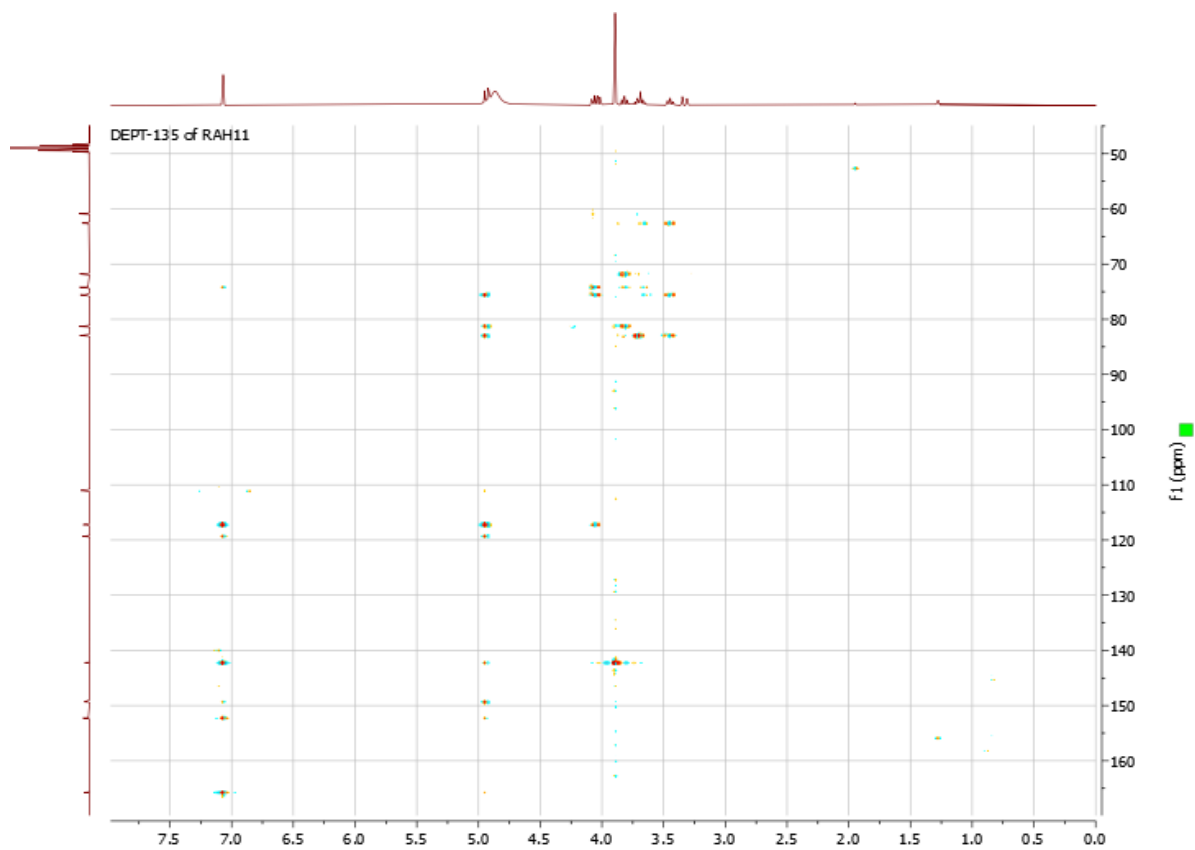
Appendix 2E: HSQC (in CD₃OD) Spectrum of bergenin (96)



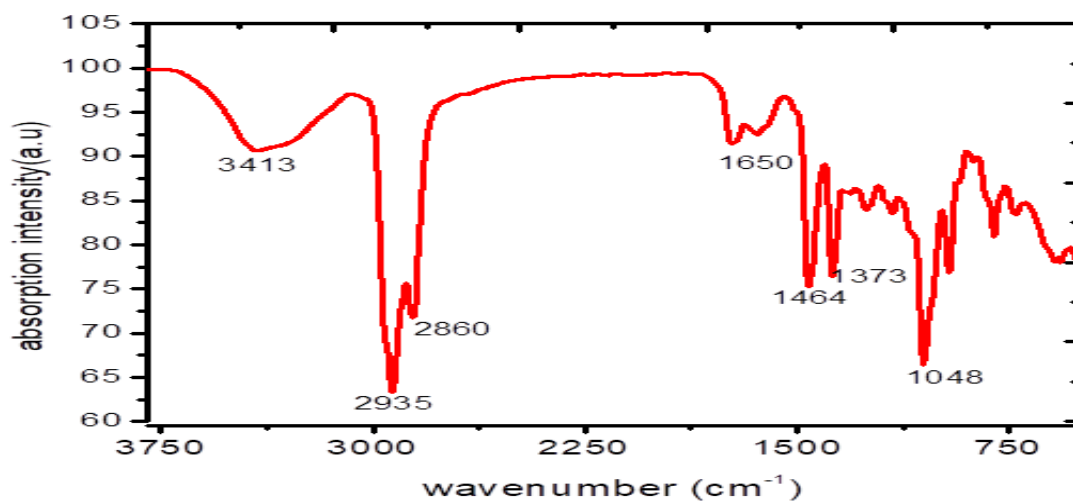
Appendix 2E: ^1H - ^1H COSY (in CD_3OD) spectrum of bergenin (**96**)



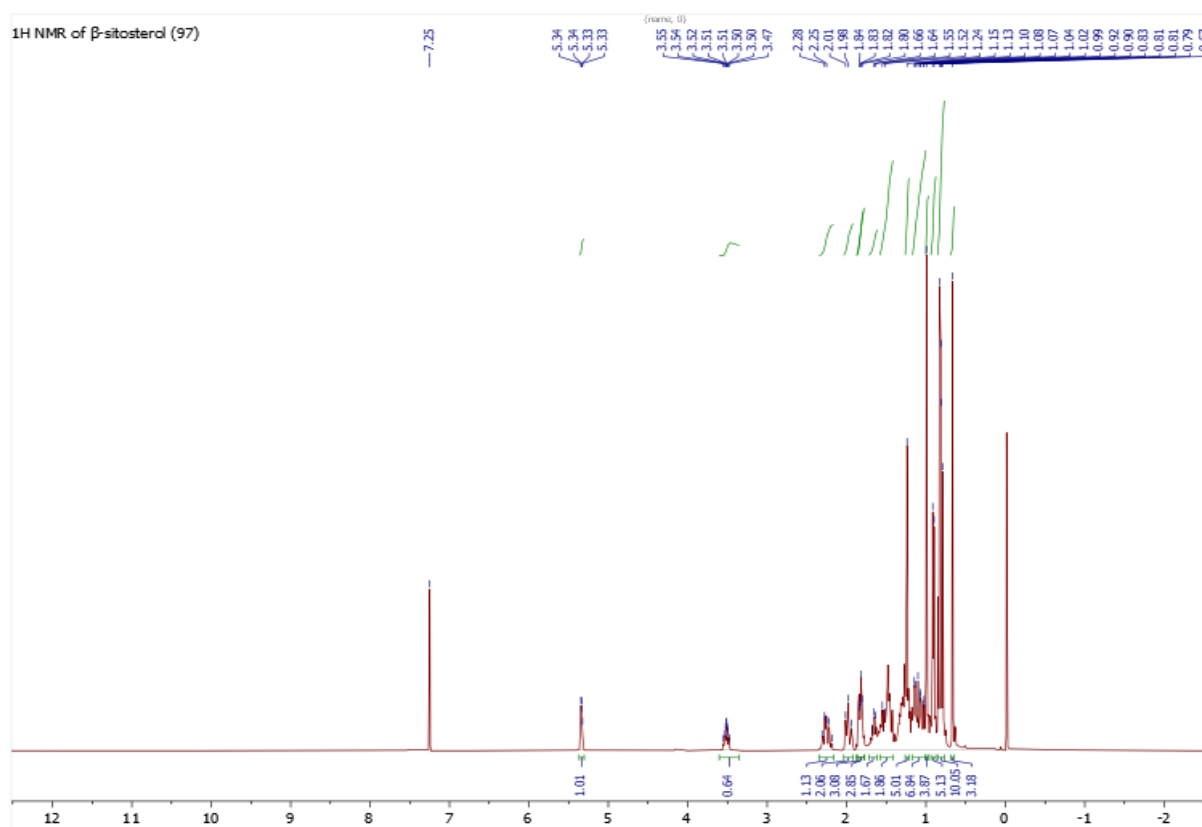
Appendix 2F: HMBC (in CD_3OD) spectrum of bergenin (**96**)



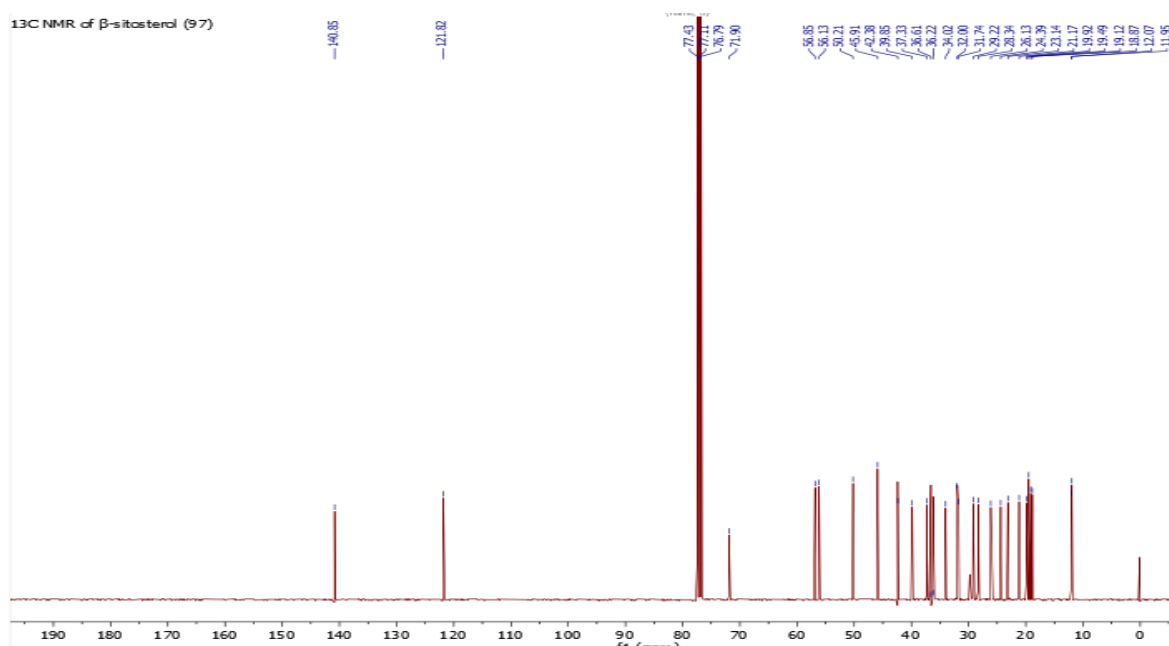
Appendix 3A: FT-IR spectrum of β -sitosterol (97)



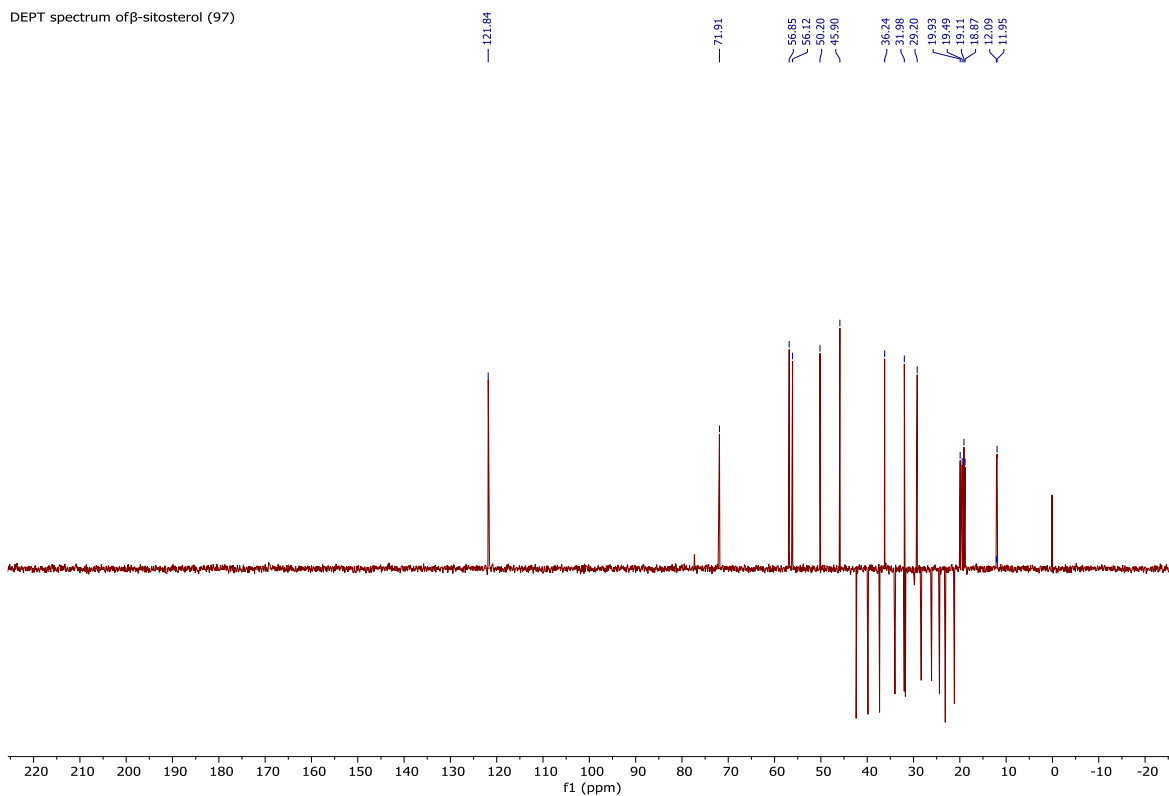
Appendix 3B: ^1H NMR (in CDCl_3) spectrum of β -sitosterol (97)



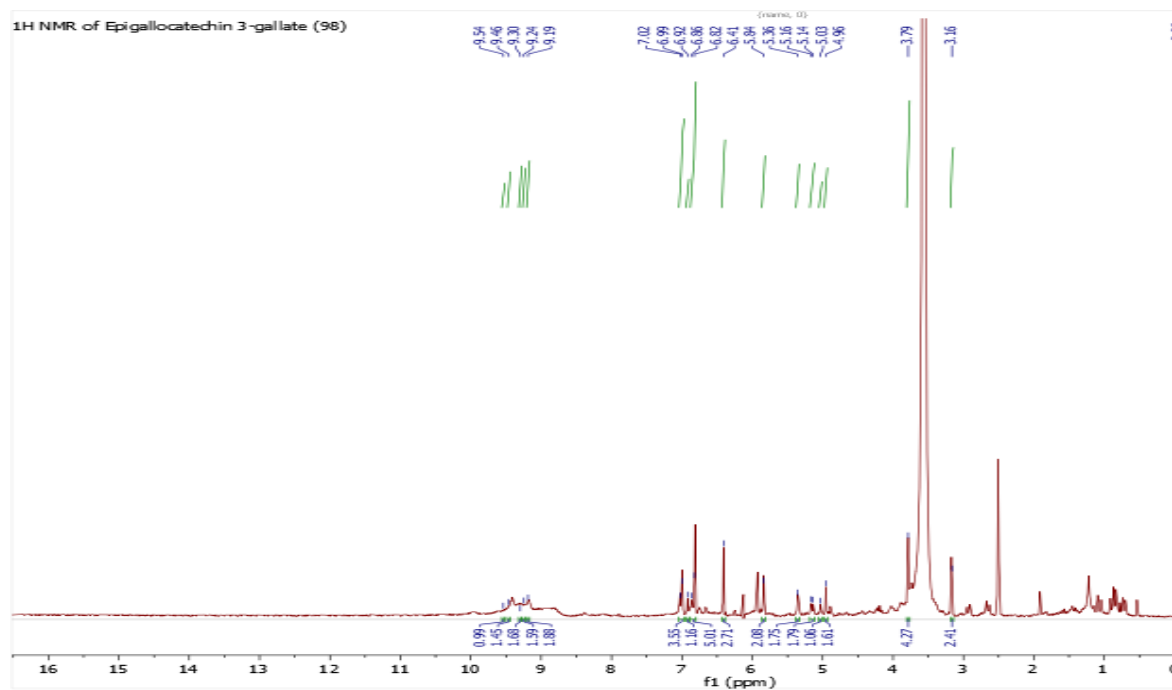
Appendix 3C: ^{13}C NMR (in DCCl_3) spectrum of β -sitosterol (**97**)



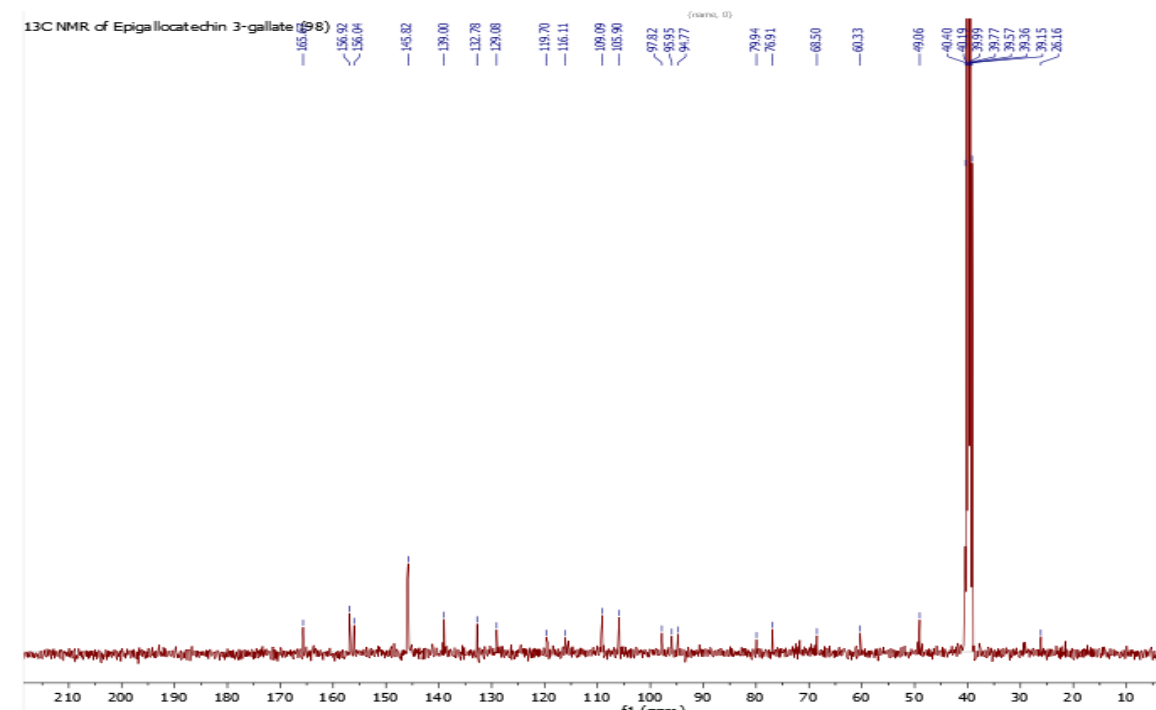
Appendix 3D: DEPT-135 (in CDCl_3) spectrum of β -sitosterol (**97**)



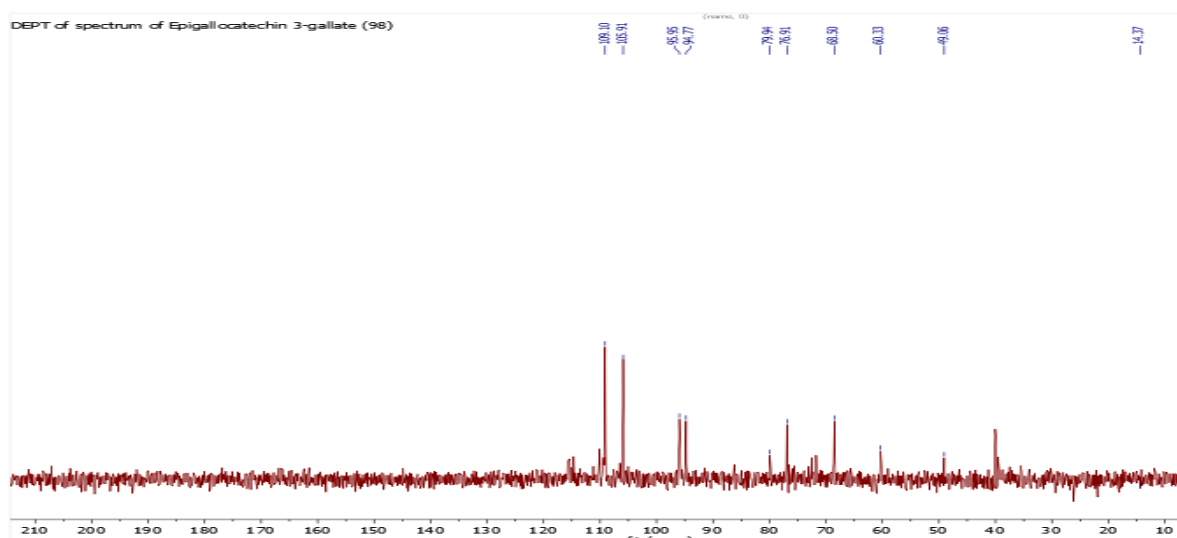
Appendix 4A: ^1H NMR (in $\text{DMSO-}d_6$) spectrum of epigallocatechin 3-gallate (**98**)



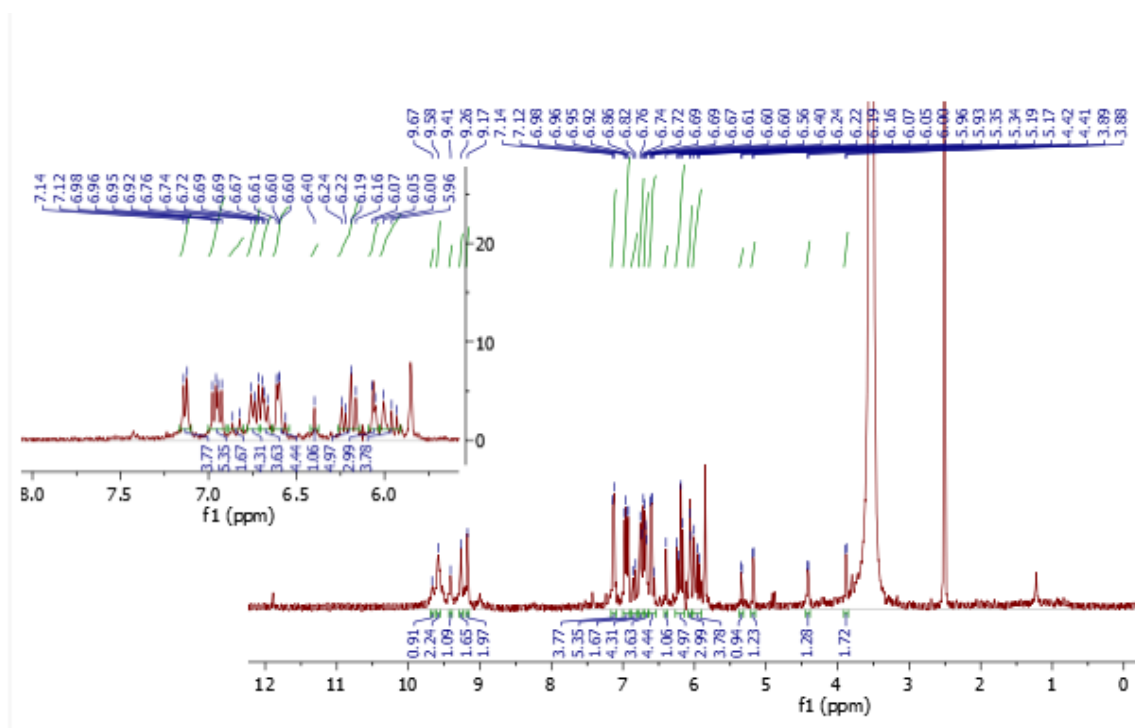
Appendix 4B: ^{13}C NMR (in $\text{DMSO-}d_6$) spectrum of epigallocatechin 3-gallate (**98**)



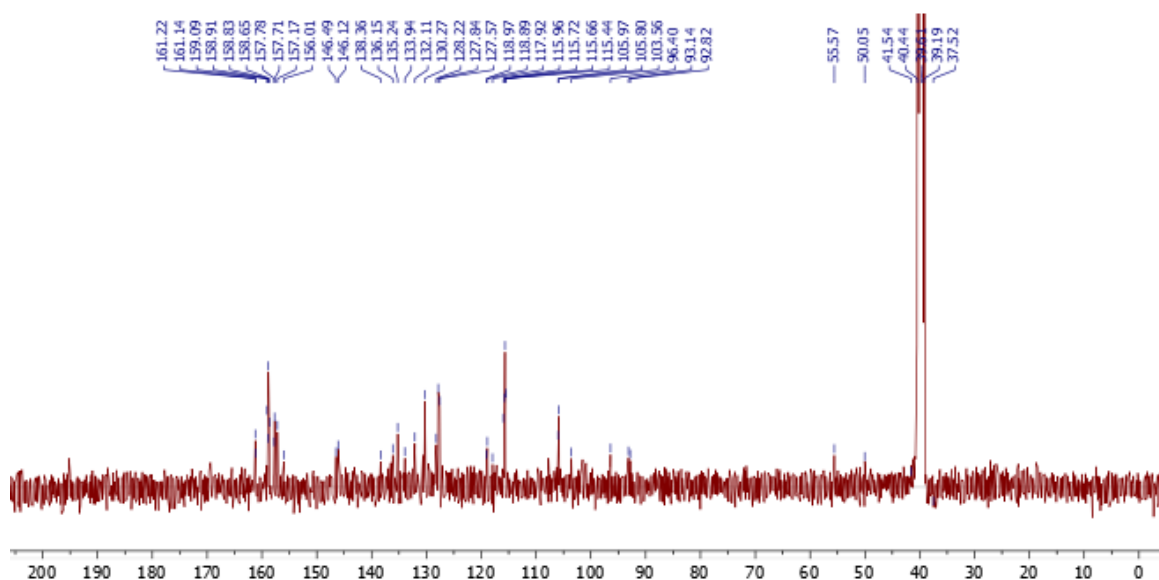
Appendix 4C: DEPT-135 (in DMSO-*d*₆) spectrum of epigallocatechin 3-gallate (**98**)



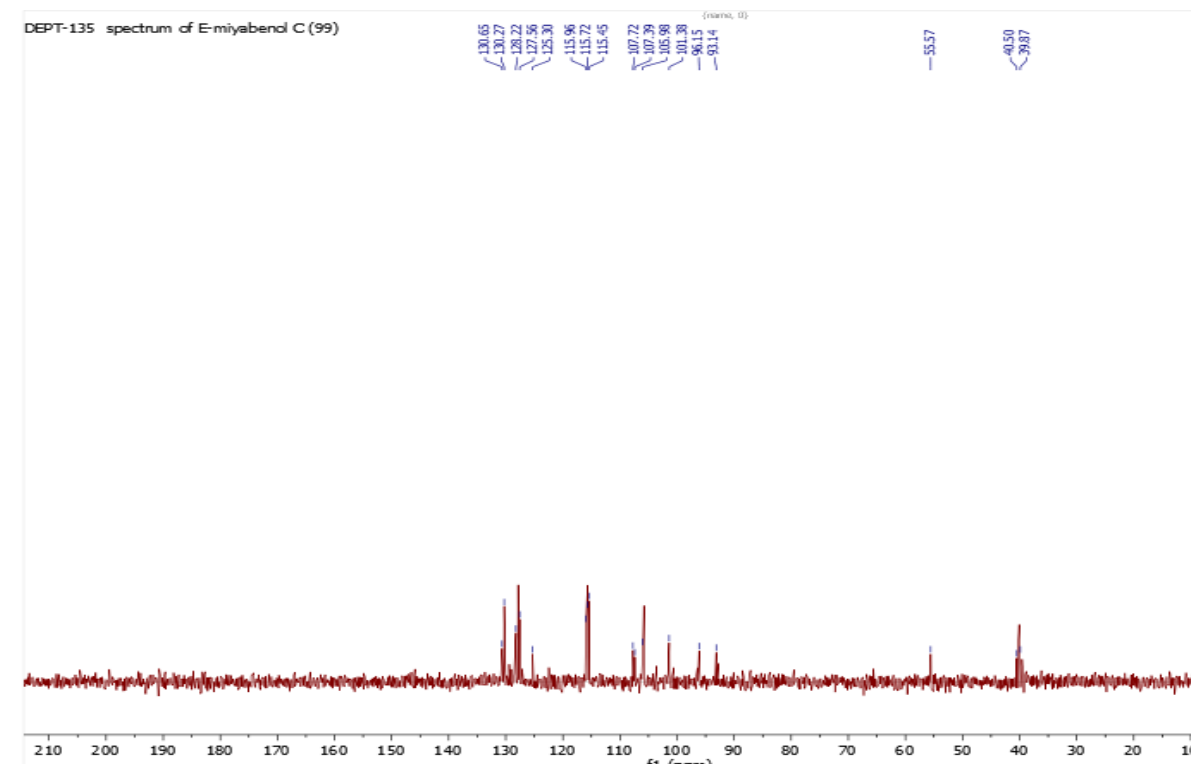
Appendix 5A: ¹H NMR (in DMSO-*d*₆) spectrum of E-miyabenol C (**99**)



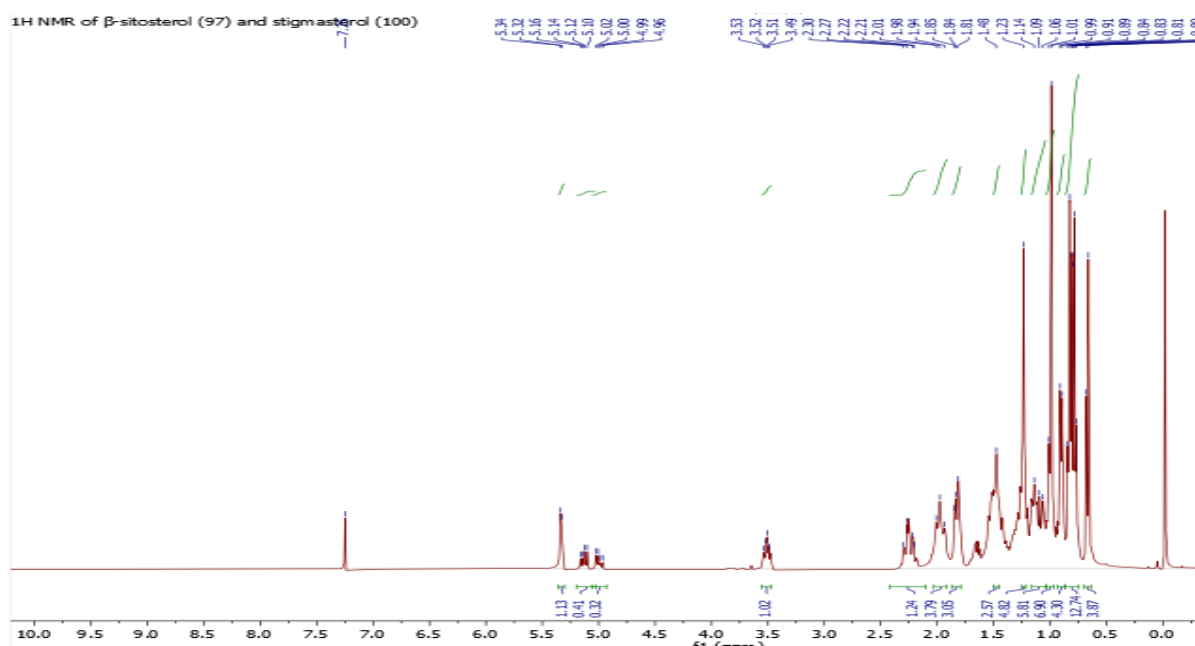
Appendix 5B: ^{13}C NMR (in $\text{DMSO-}d_6$) spectrum of E-miyabenol C (**99**)



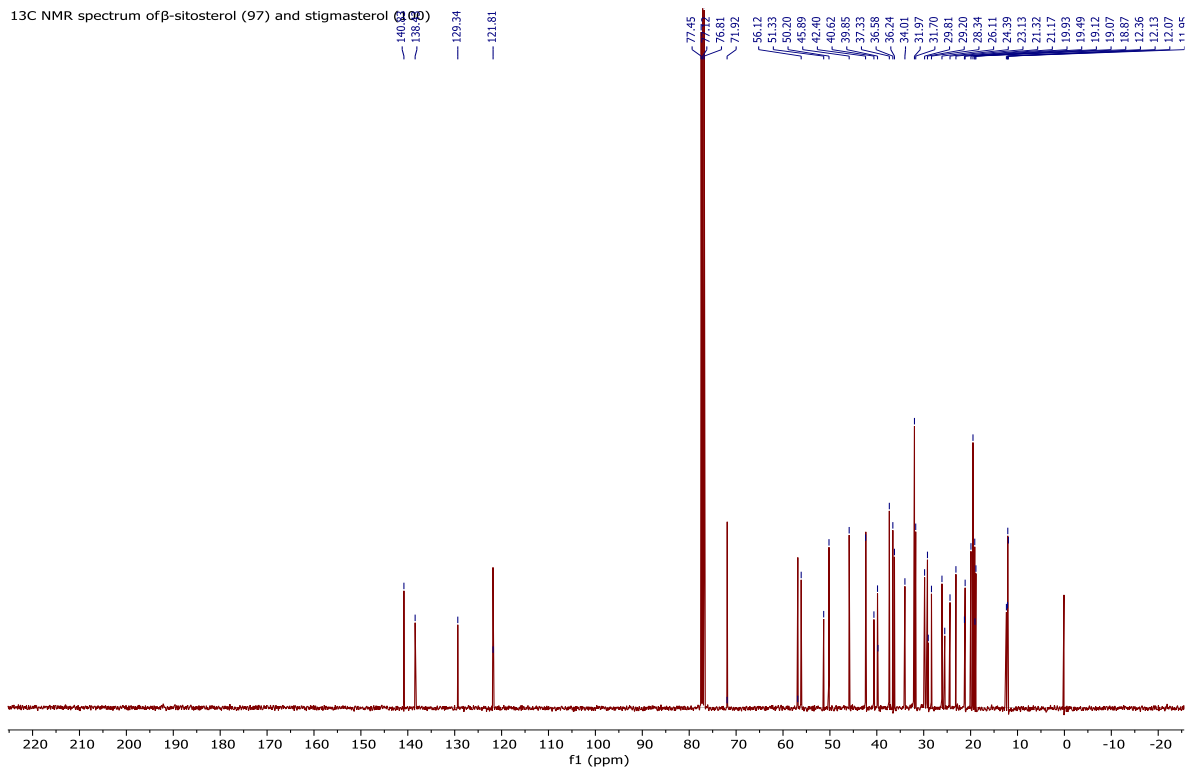
Appendix 5C: DPET-135 (in $\text{DMSO-}d_6$) spectrum of E-miyabenol C (**99**)



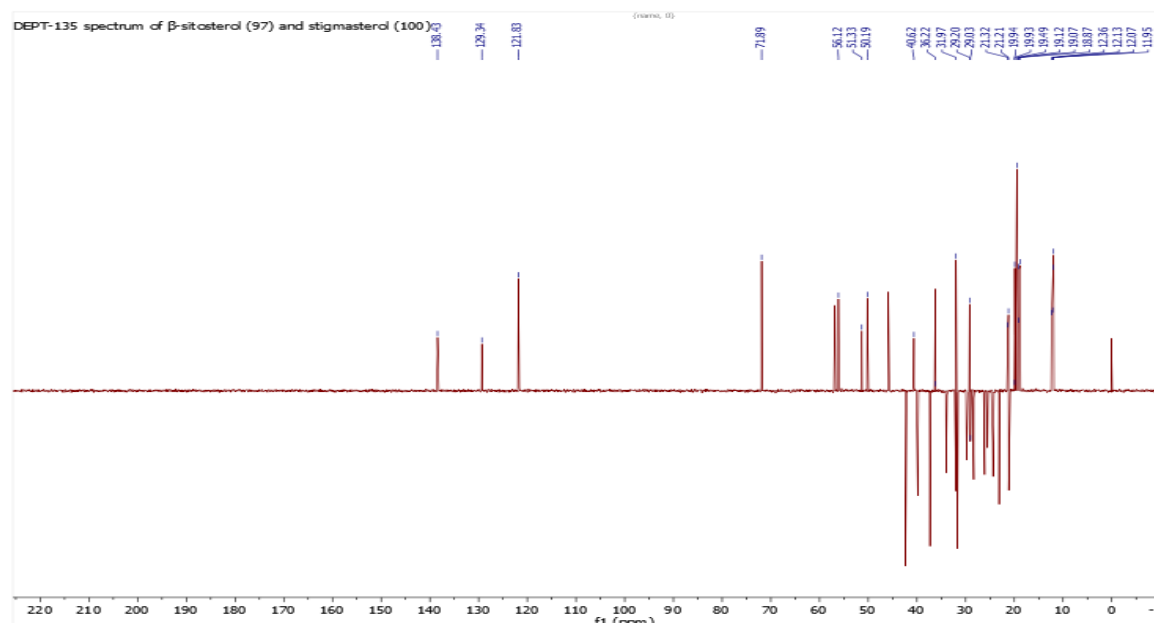
Appendix 6A: ^1H NMR (in CDCl_3) spectrum of β -sitosterol (**97**) and stigmasterol (**100**)



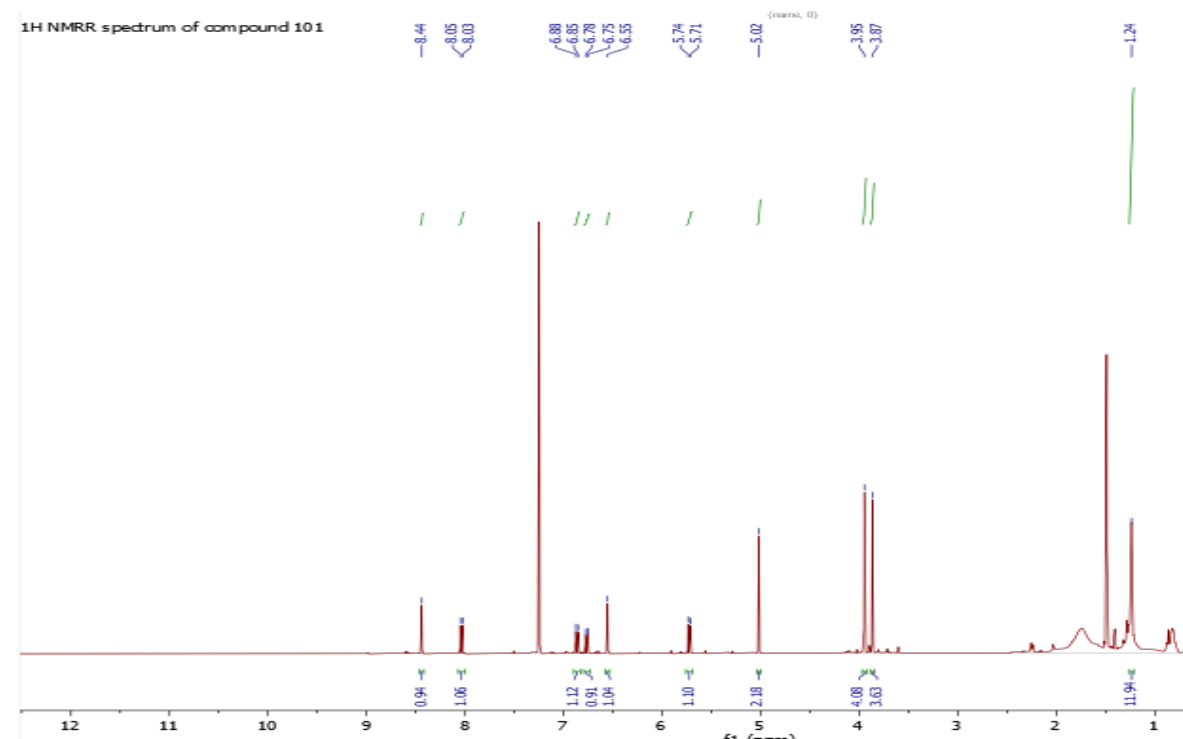
Appendix 6B: ^{13}C NMR (in CDCl_3) spectrum of β -sitosterol (**97**) and stigmasterol (**100**)



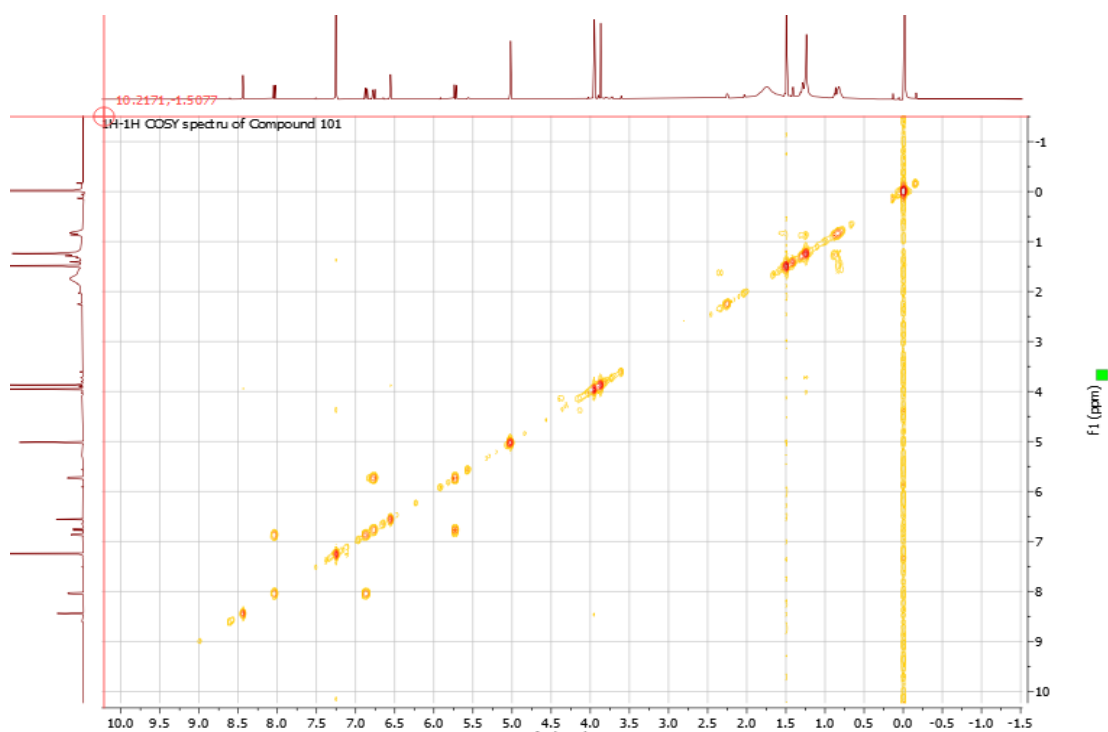
Appendix 6C: DEPT-135 (in CDCl₃) spectrum of β -sitosterol (**97**) and stigmasterol (**100**)



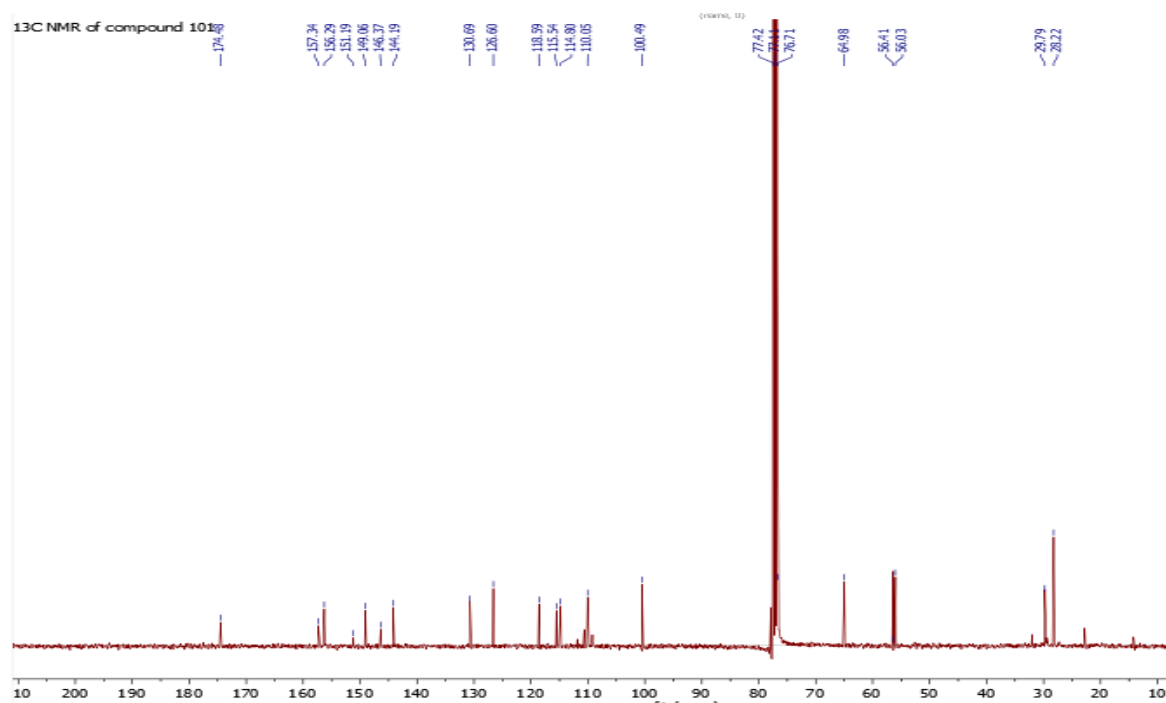
Appendix 7A: ¹H NMR (in CDCl₃) spectrum of compound **101**



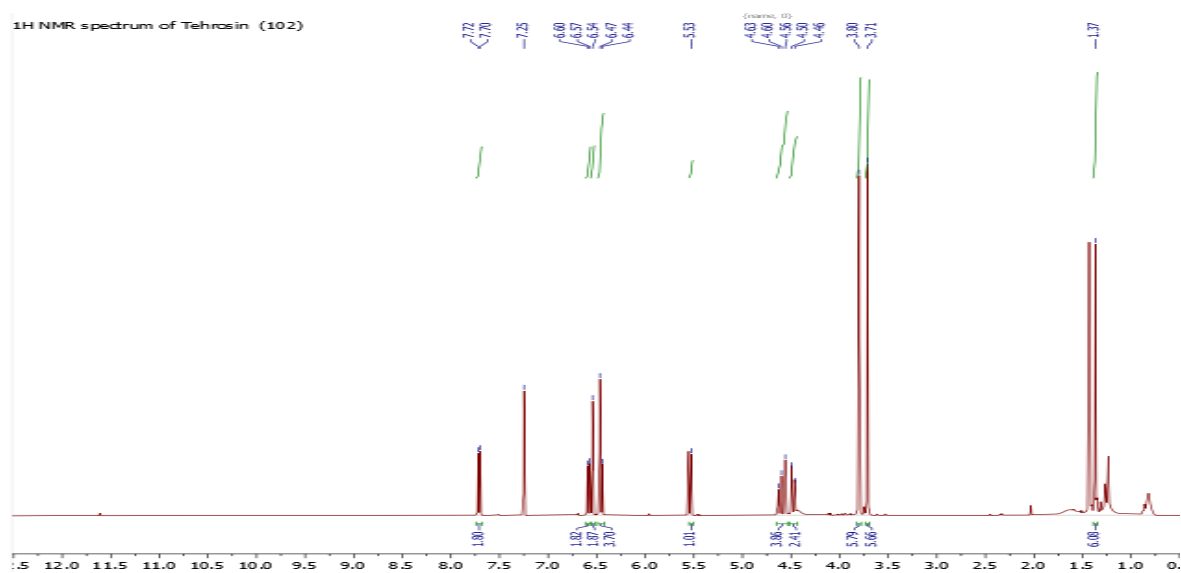
Appendix 7B: ^1H - ^1H COSY (in CDCl_3) spectrum of compound **101**



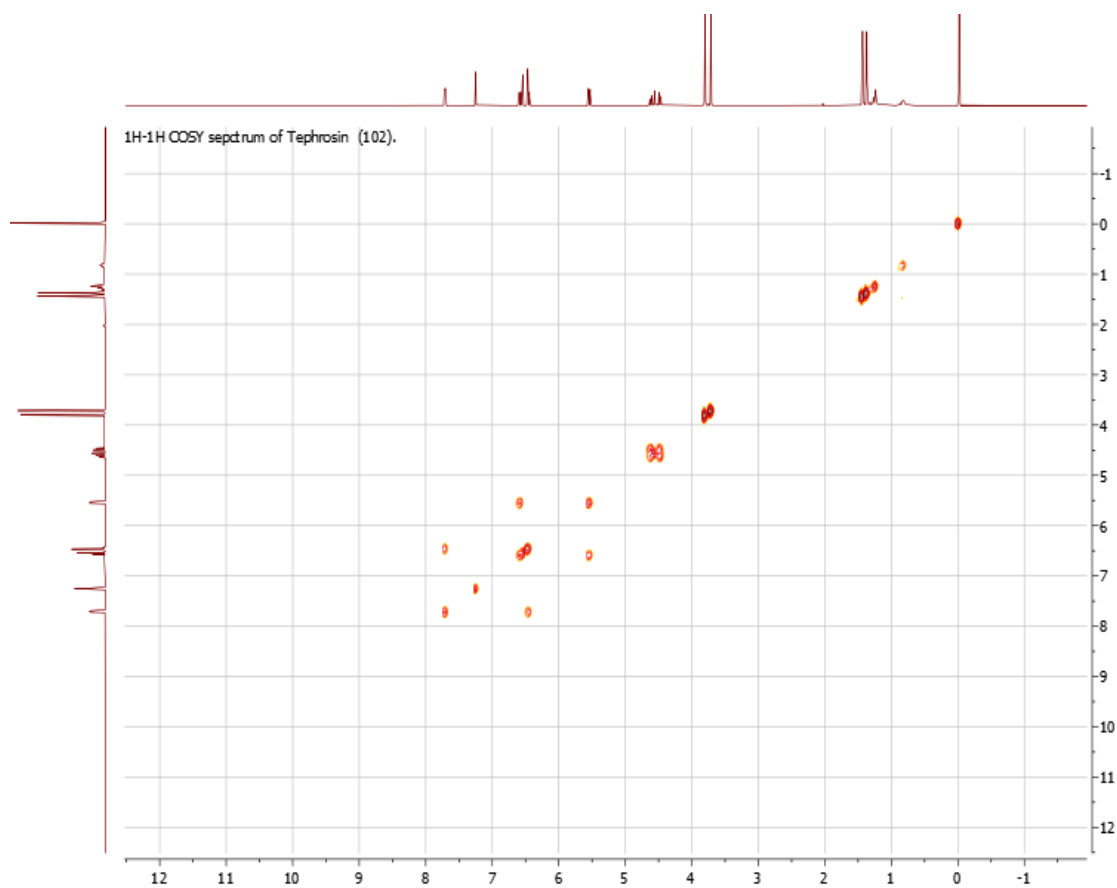
Appendix 7C: ^{13}C NMR (in CDCl_3) spectrum of compound **101**



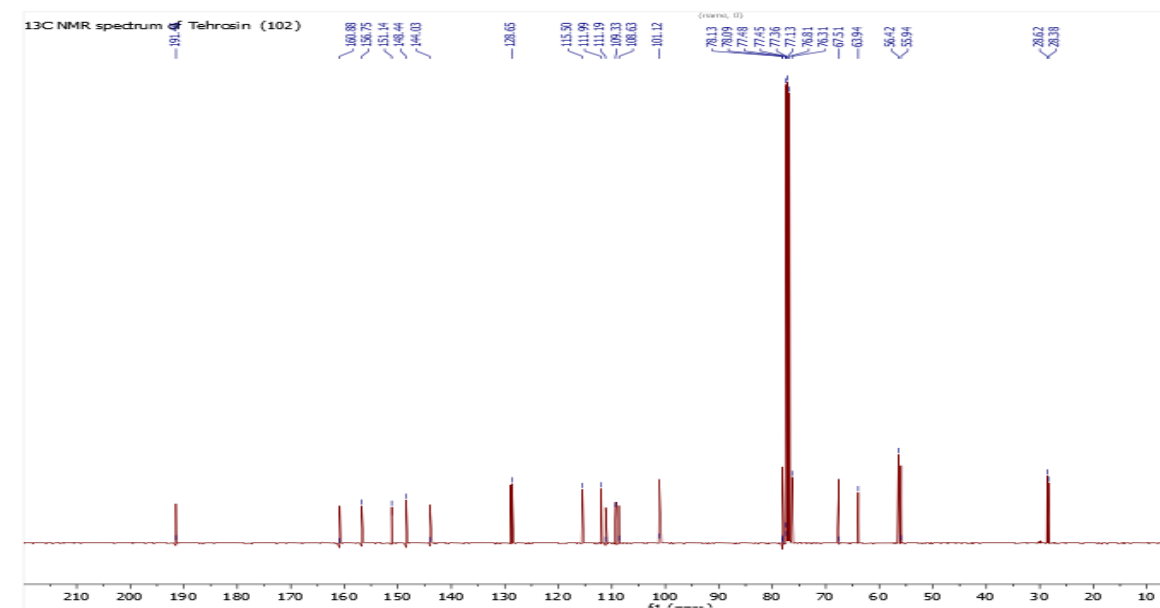
Appendix 8A: ^1H NMR (in CDCl_3) spectrum of tephrosin (**102**)



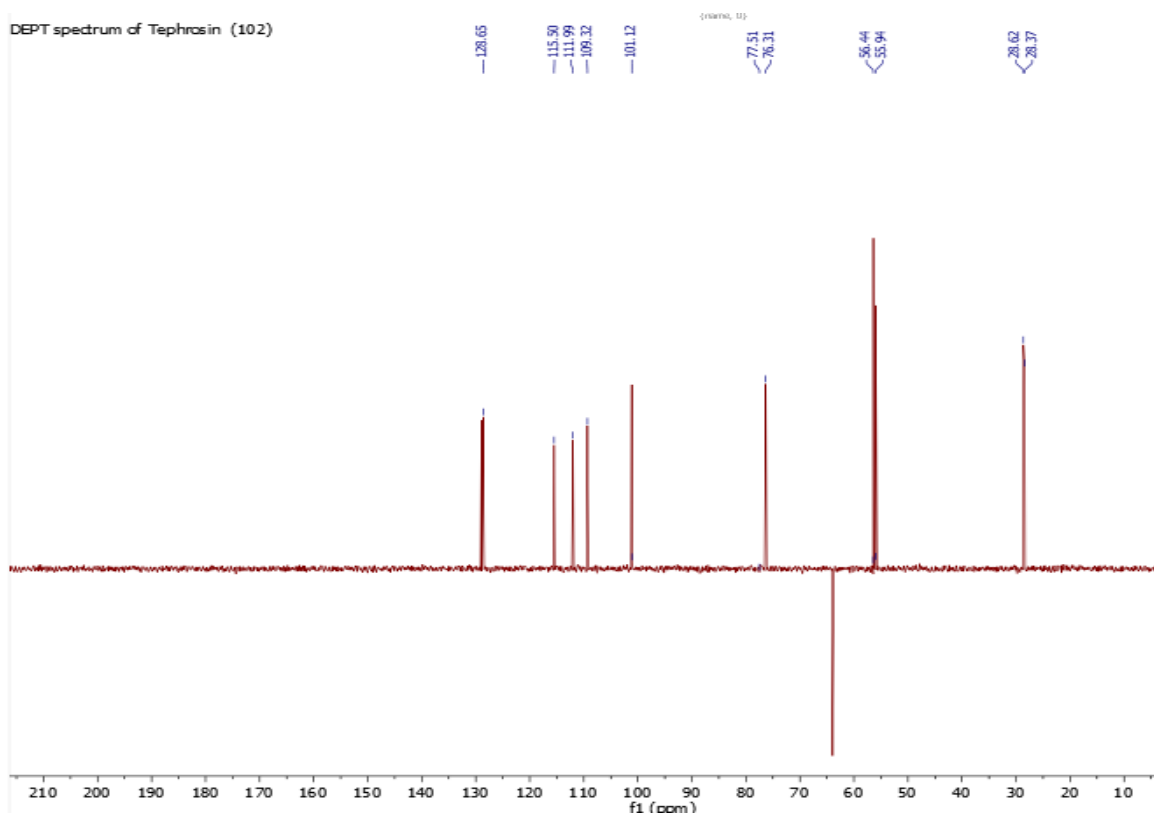
Appendix 8B: ^1H - ^1H COSY (in CDCl_3) spectrum of tephrosin (**102**)



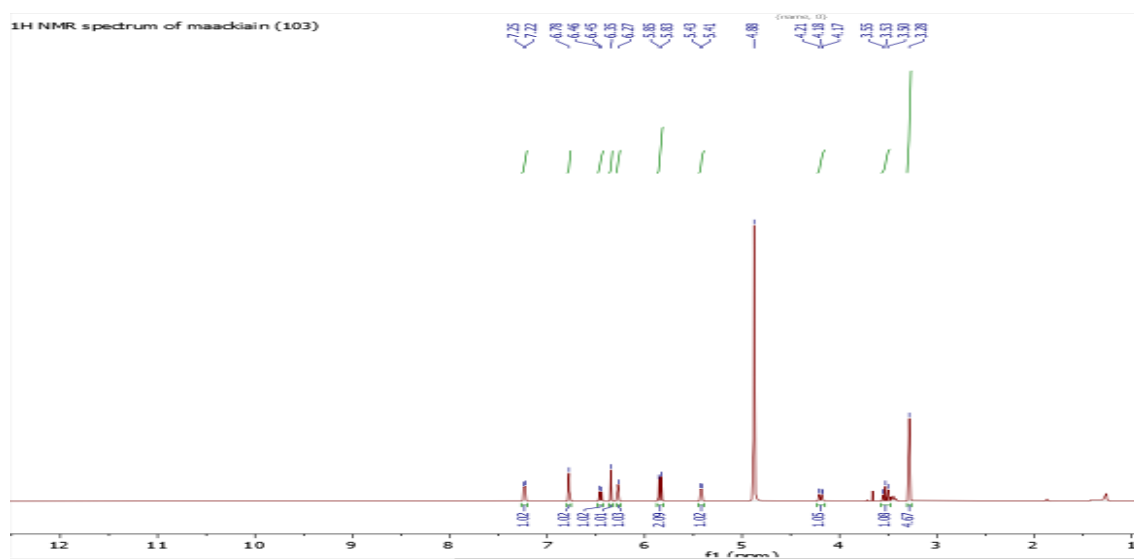
Appendix 8C: ^{13}C NMR (in CDCl_3) spectrum of tephrosin (**102**)



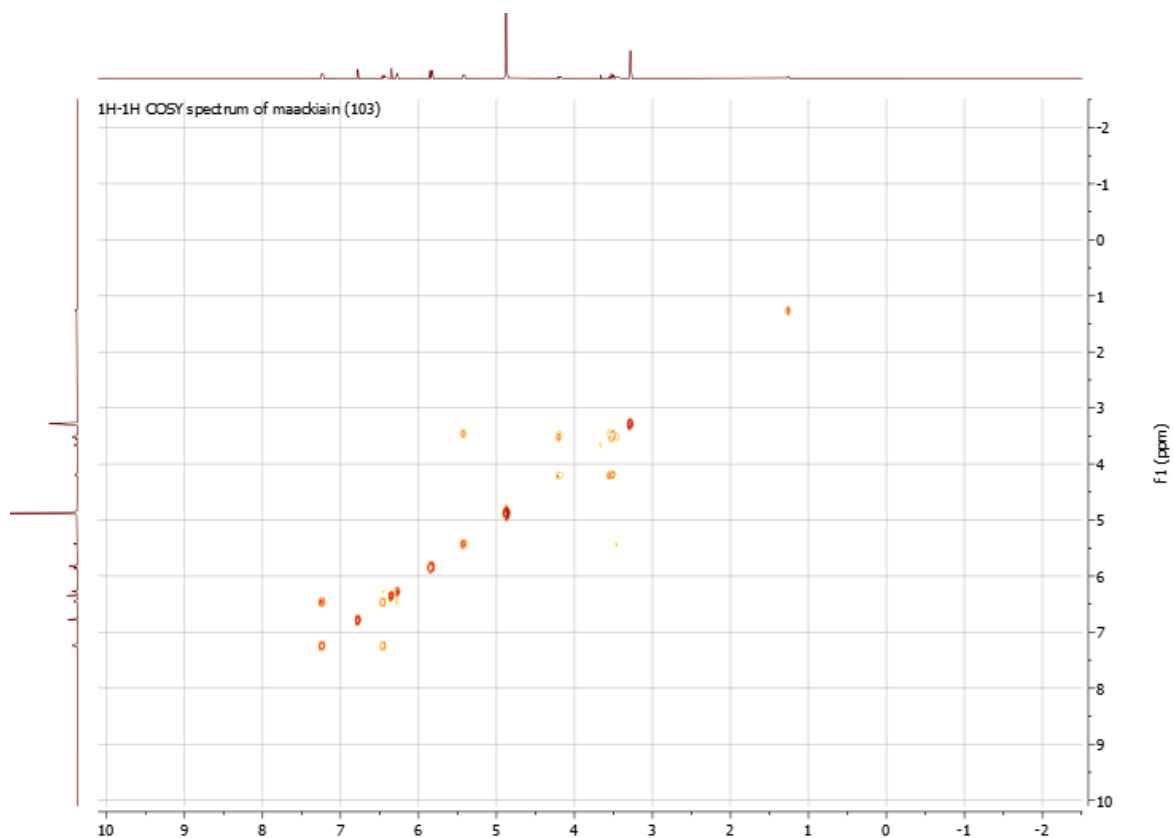
Appendix 8D: DEPT-135 (in CDCl_3) spectrum of tephrosin (**102**)



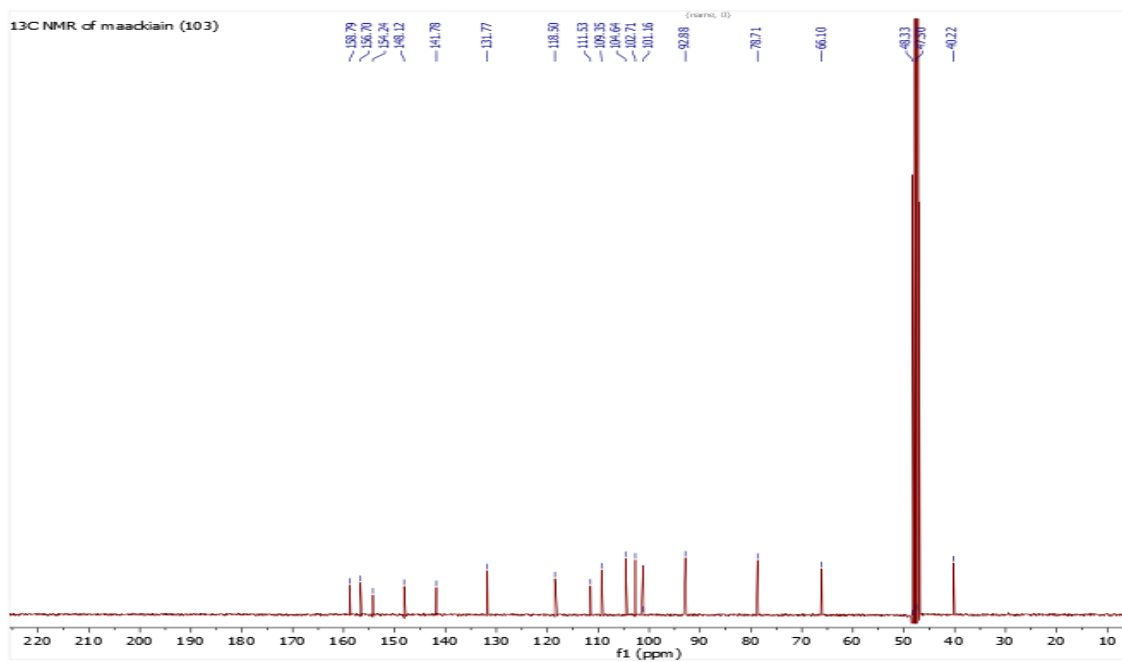
Appendix 9A: ^1H NMR (in CD_3OD) spectrum of maackiain (**103**).



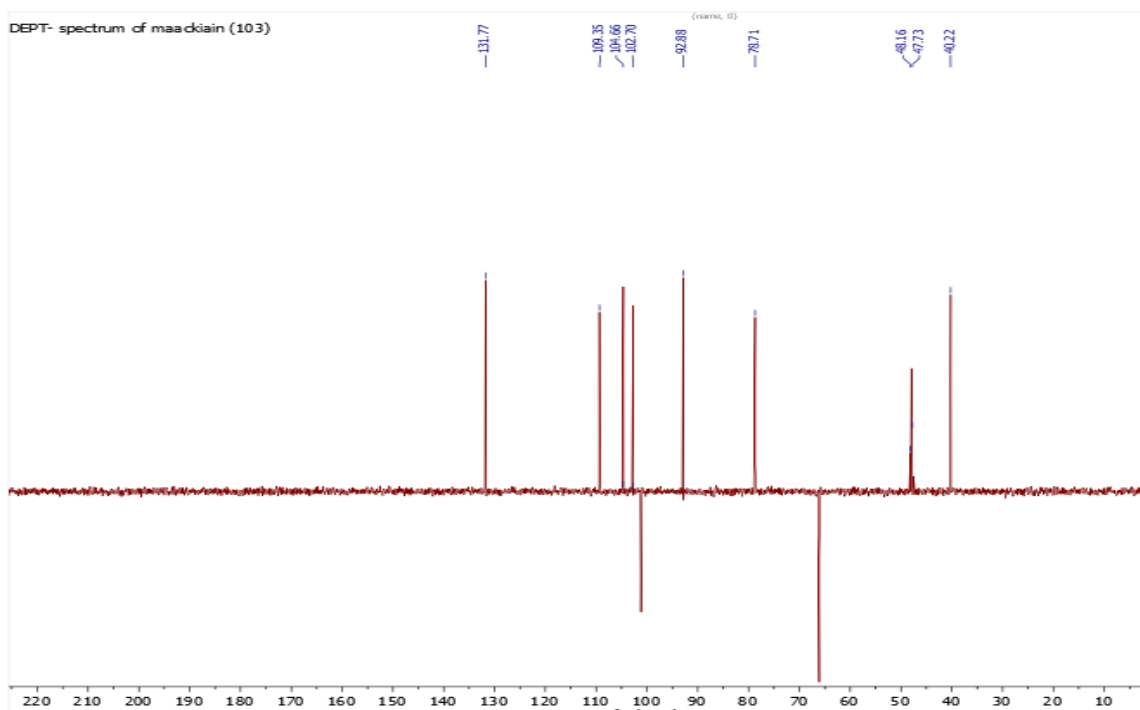
Appendix 9B: COSY (in CD_3OD) spectrum of maackiain (**103**)



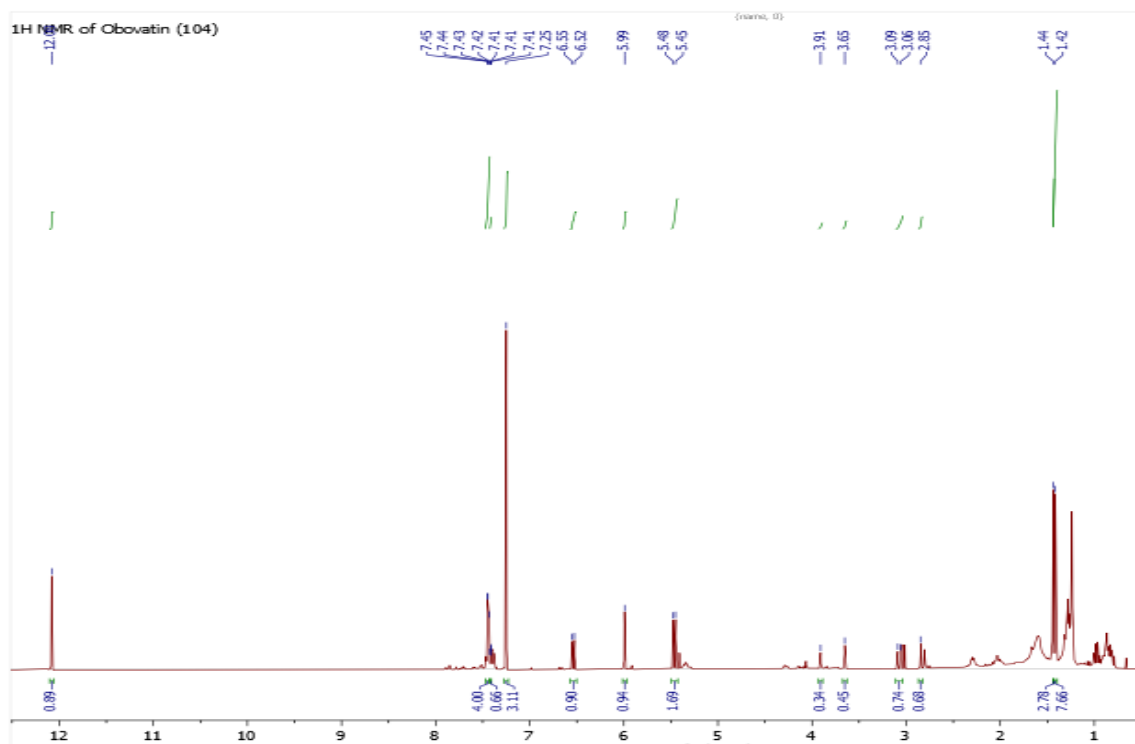
Appendix 9C: ^{13}C NMR (in CD_3OD) spectrum of maackiain (**103**)



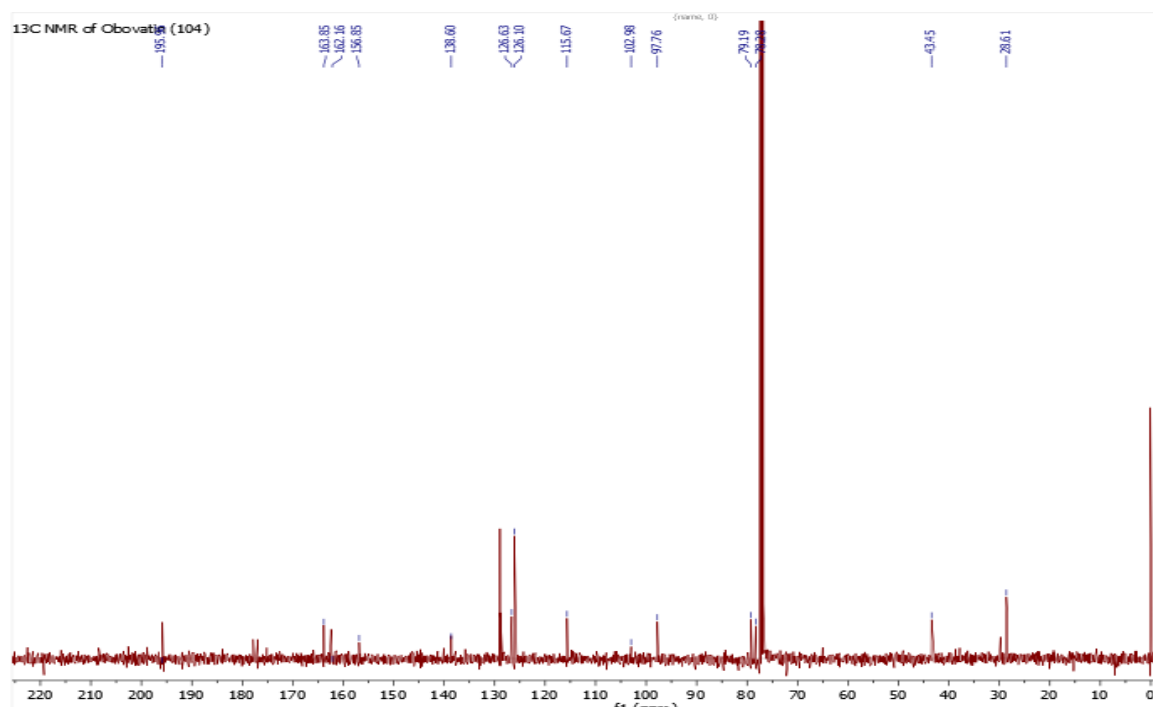
Appendix 9D: DEPT-135 (in CD_3OD) spectrum of maackiain (**103**)



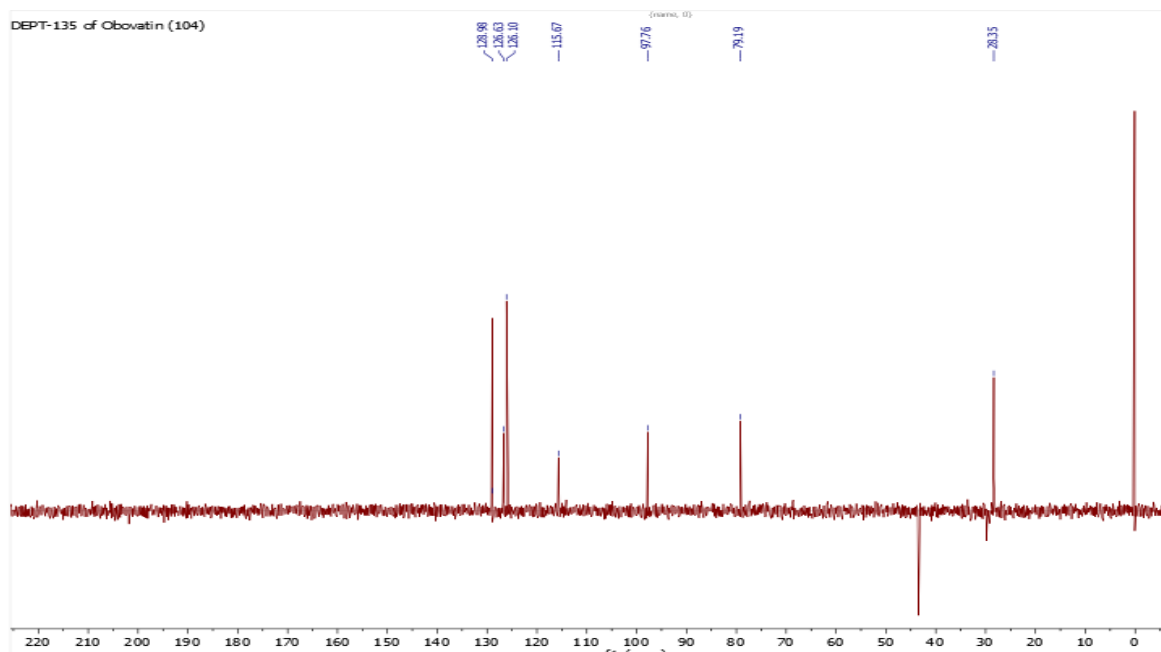
Appendix 10A: ^1H NMR (in CDCl_3) spectrum of obovatin (**104**)



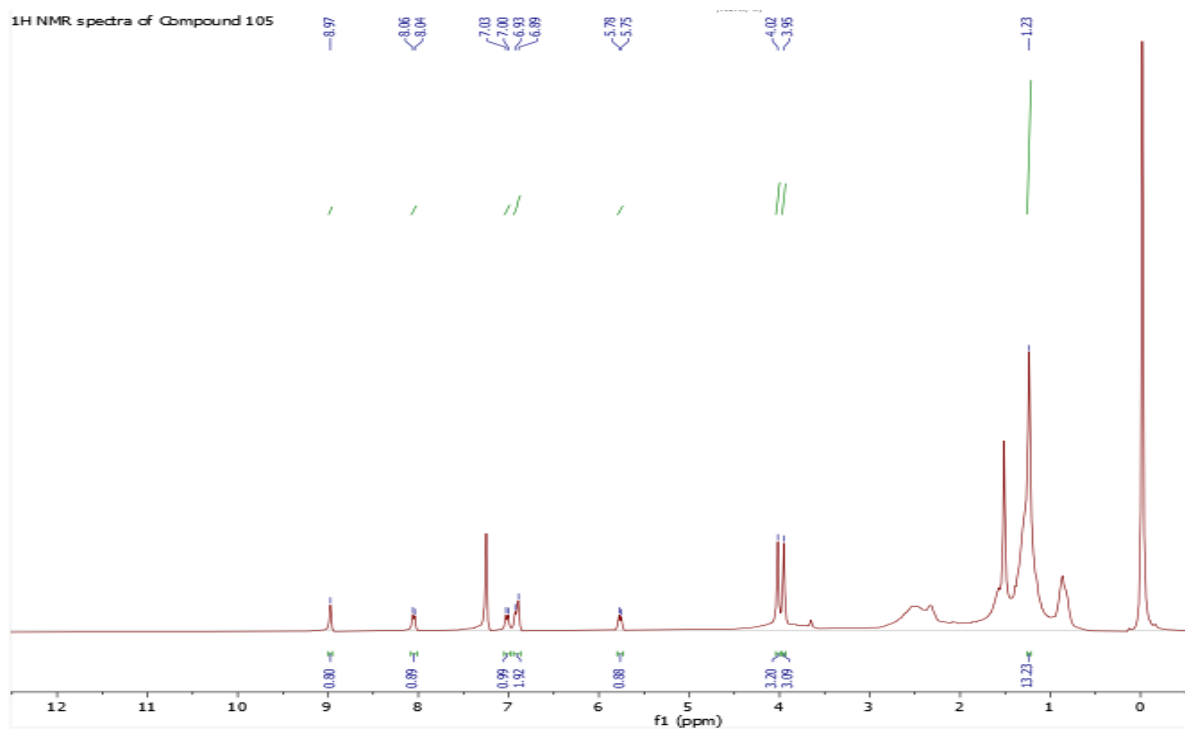
Appendix 10B: ^{13}C NMR (in CDCl_3) spectrum of obovatin (**104**)



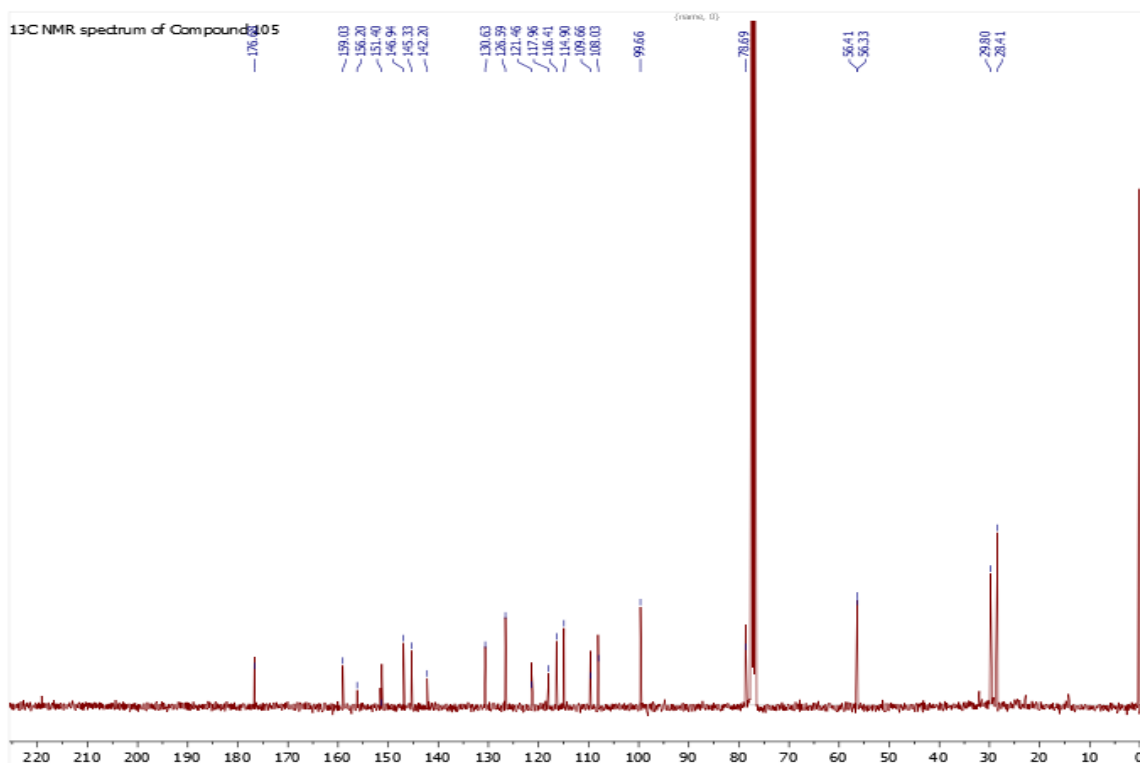
Appendix 10C: EPT-135 (in CDCl₃) spectrum of obovatin (**104**)



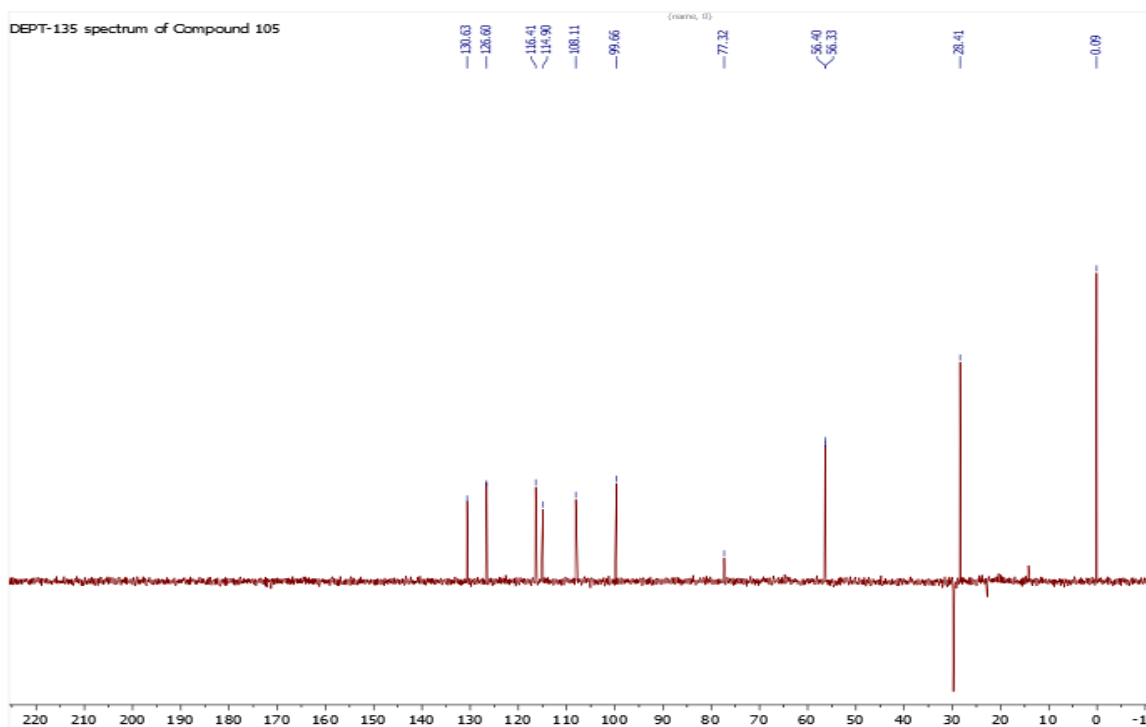
Appendix 11A: ¹H NMR (in CDCl₃) spectrum of compound **105**



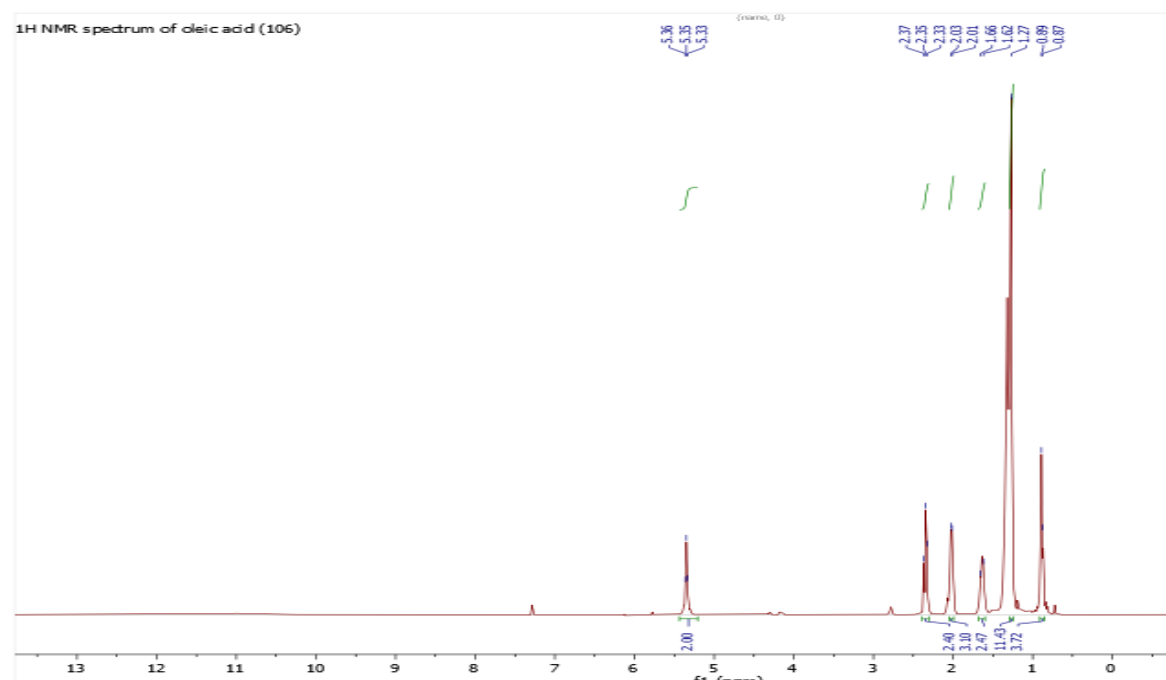
Appendix 11B: ^{13}C NMR (in CDCl_3) spectrum of compound **105**



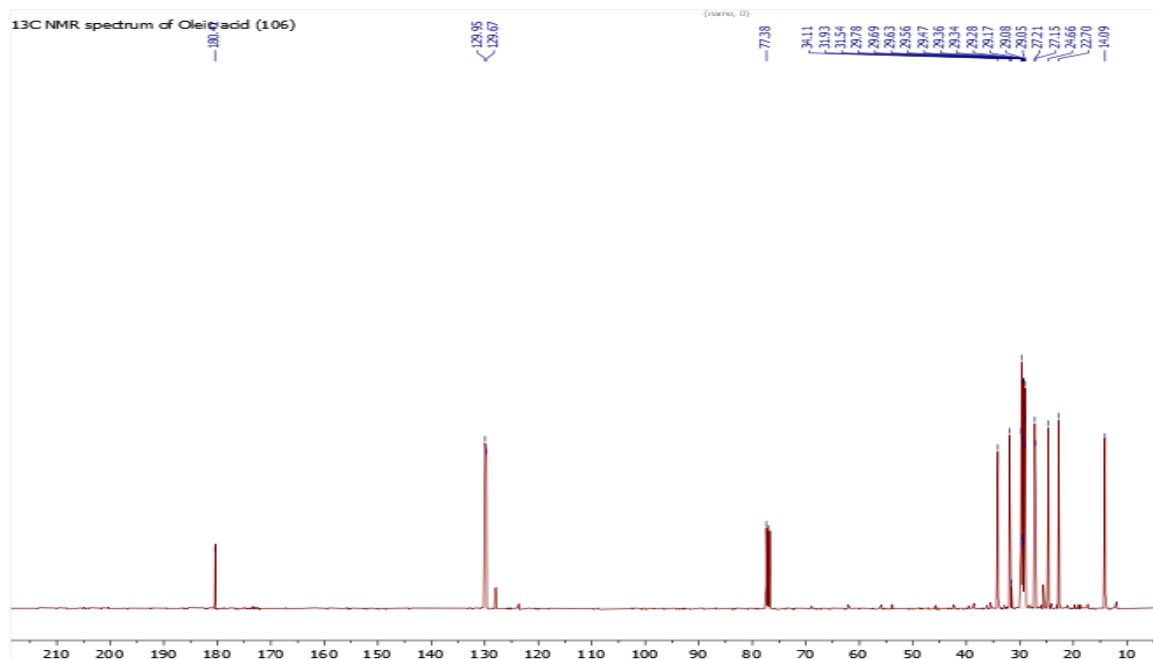
Appendix 11C: DEPT-135 (in CDCl_3) spectrum of compound **105**



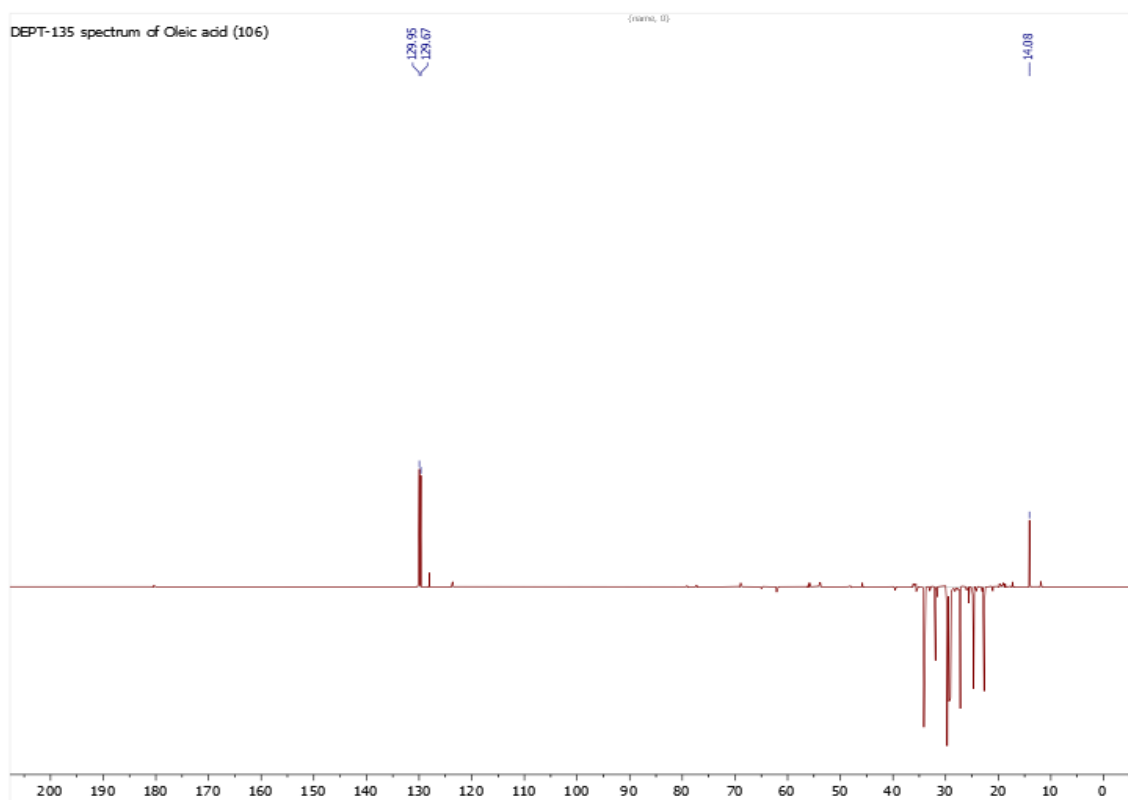
Appendix 12A: ^1H NMR (in CDCl_3) spectrum of oleic acid (**106**)



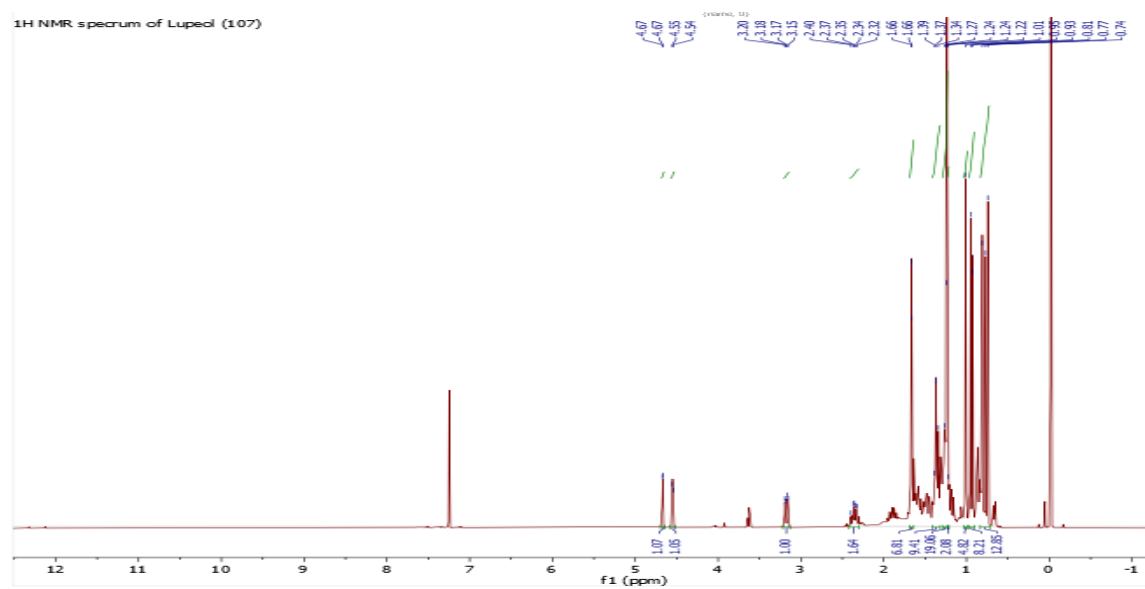
Appendix 12B: ^{13}C NMR (in CDCl_3) spectrum of oleic acid (**106**)



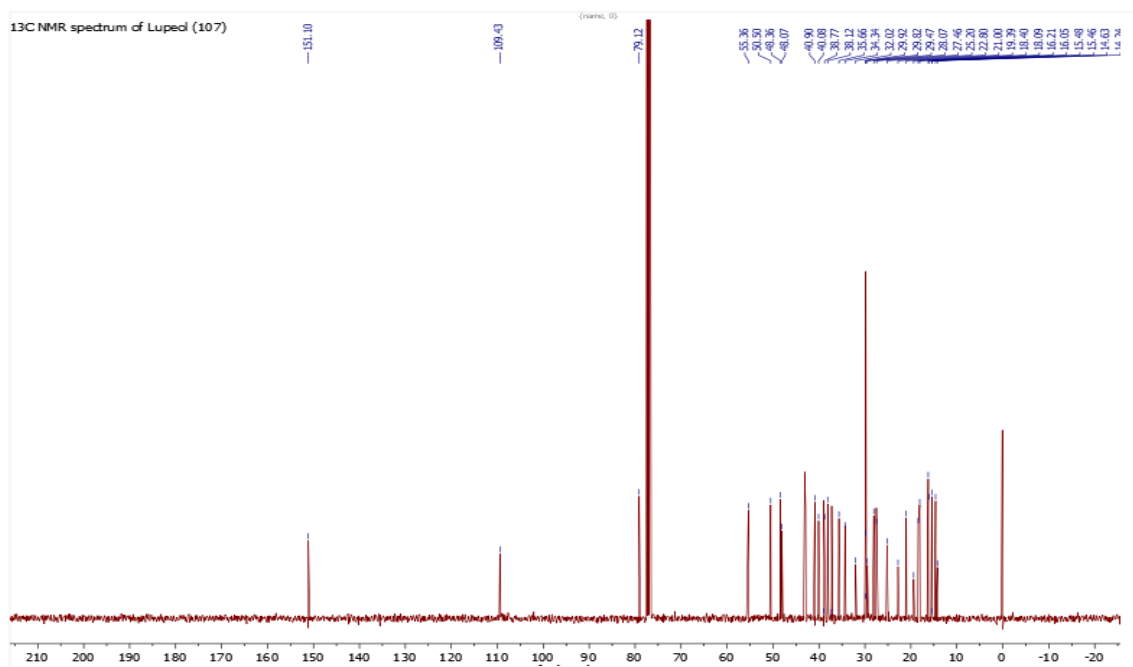
Appendix 12C: DEPT-135 (in CDCl₃) spectrum of oleic acid (**106**)



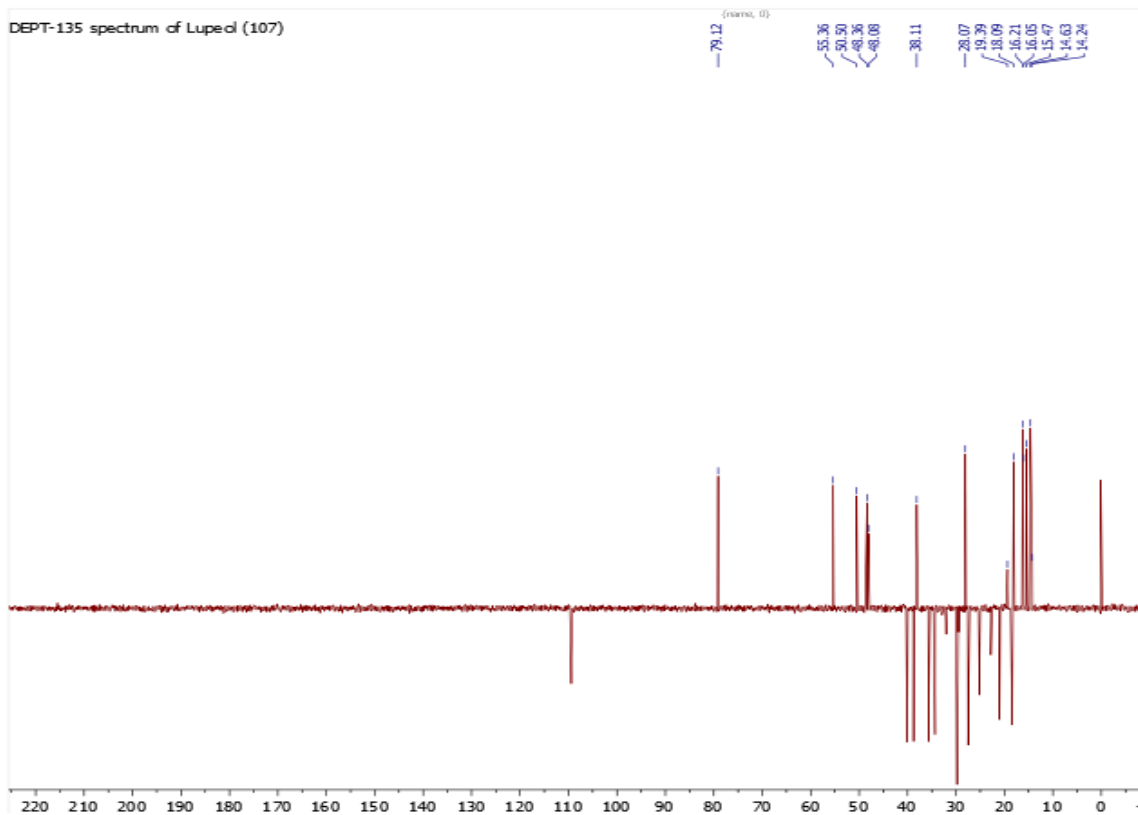
Appendix 13A: ¹H NMR (in CDCl₃) spectrum of lupeol (**107**)



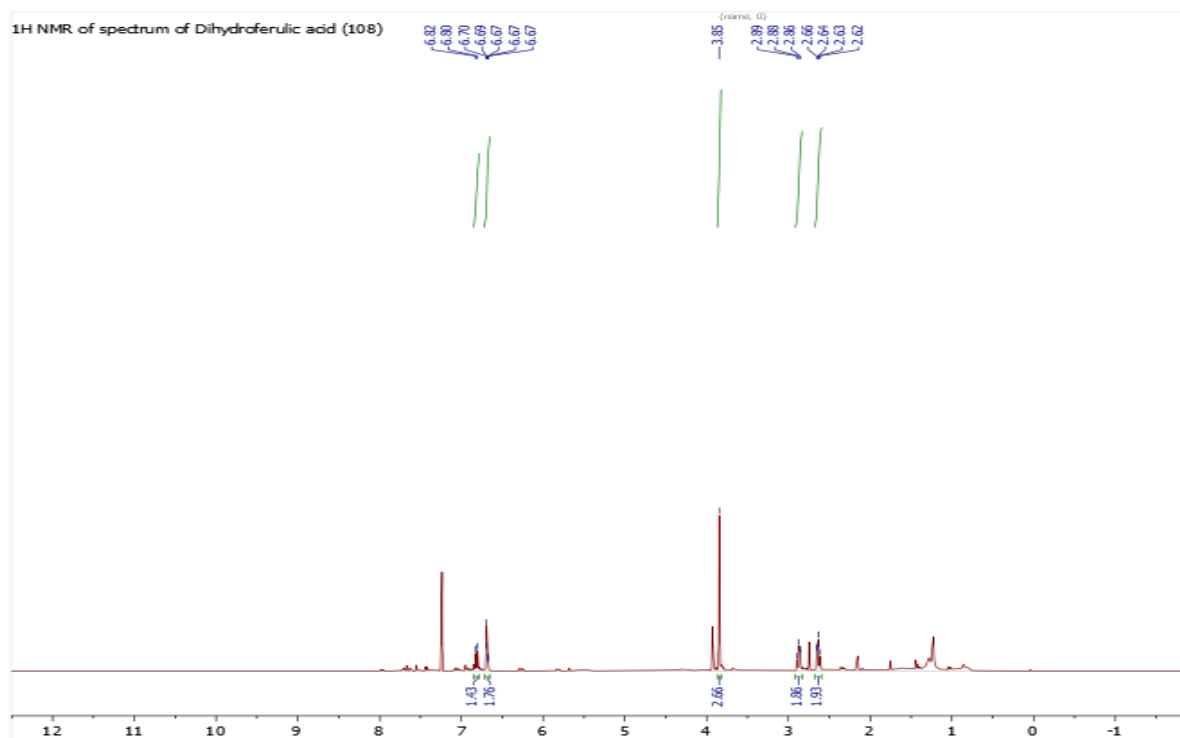
Appendix 13B: ^{13}C NMR (in CDCl_3) spectrum of lupeol (**107**)



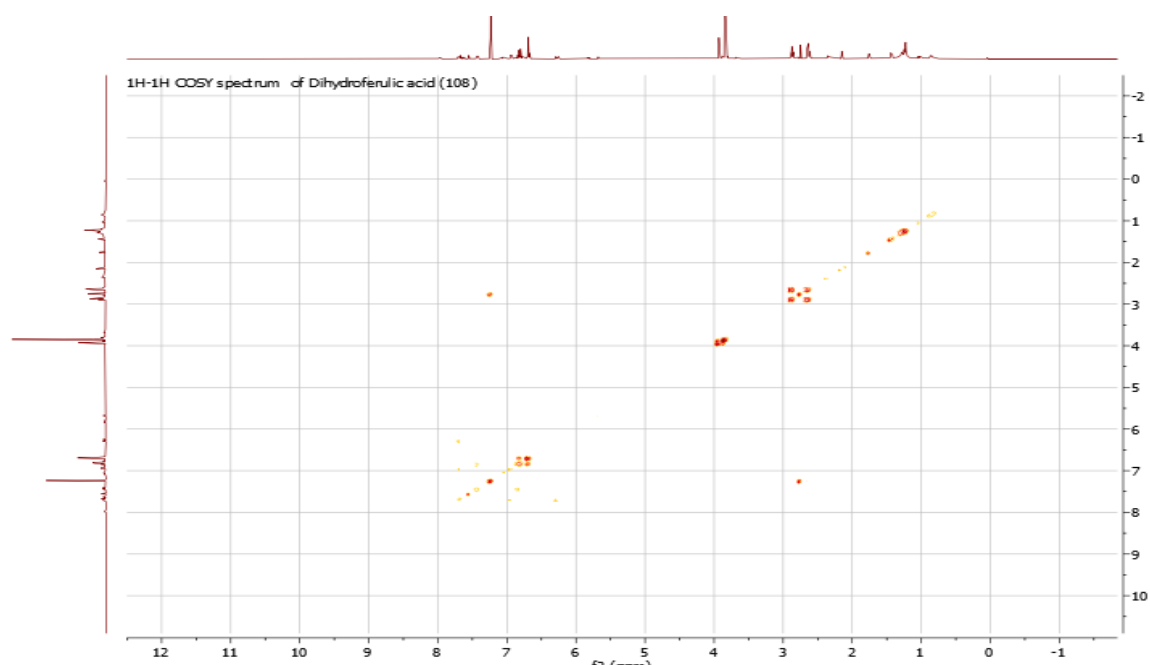
Appendix 13C: DEPT-135 (in CDCl_3) spectrum of lupeol (**107**)



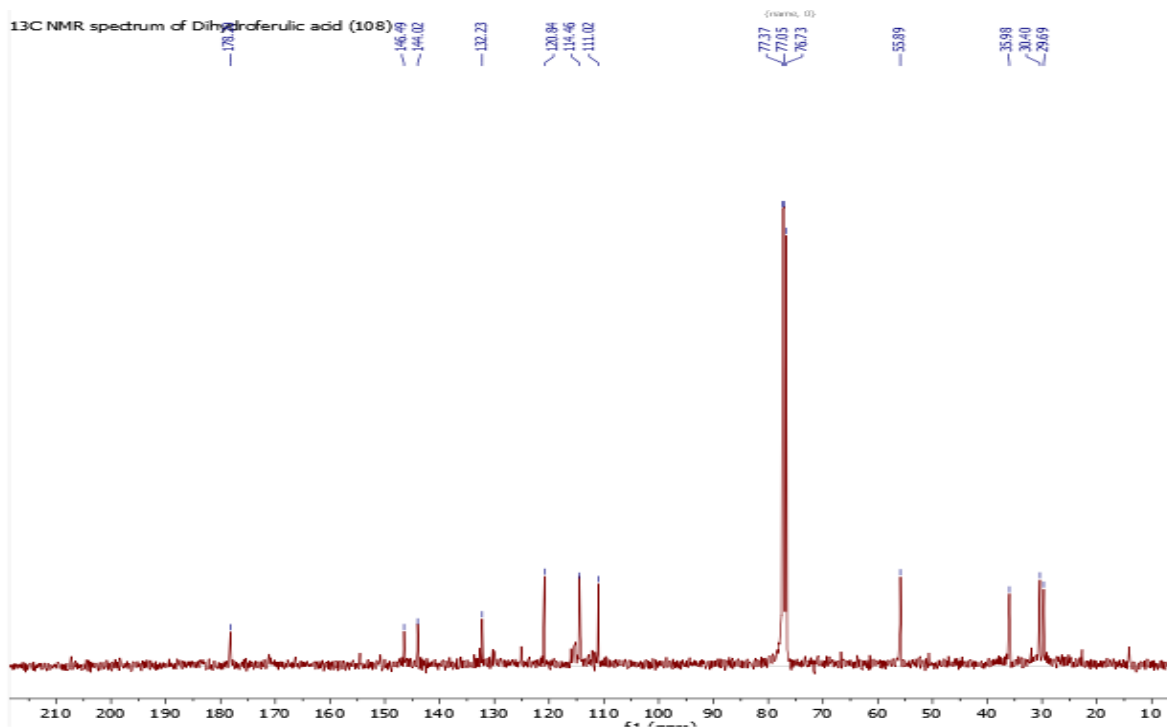
Appendix 14A: ^1H NMR (in CDCl_3) spectrum of dihydroferulic acid (**108**)



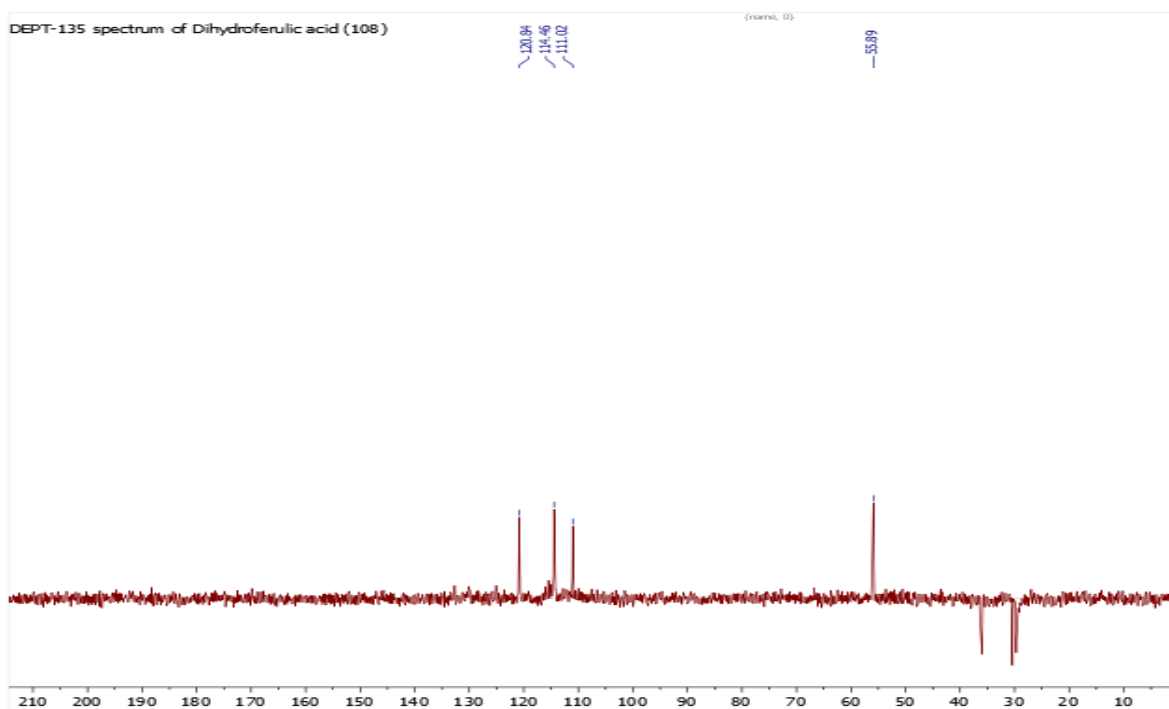
Appendix 14B: ^1H - ^1H COSY (in CDCl_3) spectrum of dihydroferulic acid (**108**)



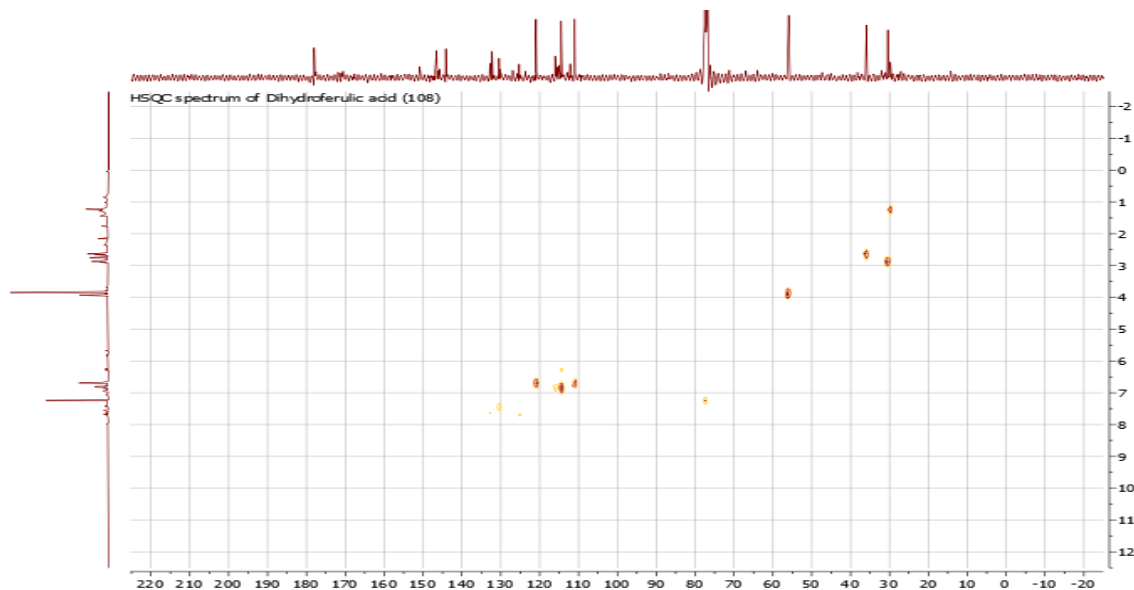
Appendix 14C: ^{13}C NMR (in CDCl_3) spectrum of dihydroferulic acid (**108**)



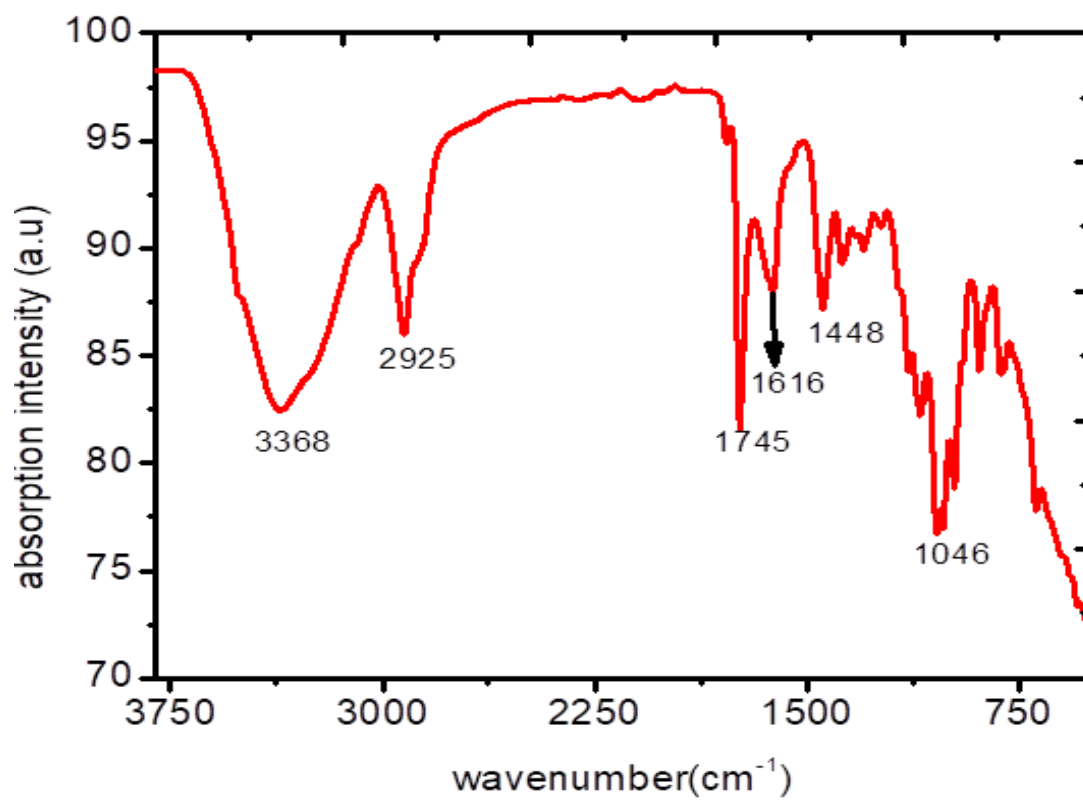
Appendix 14D: DEPT-135 (in CDCl_3) spectrum of dihydroferulic acid (**108**)



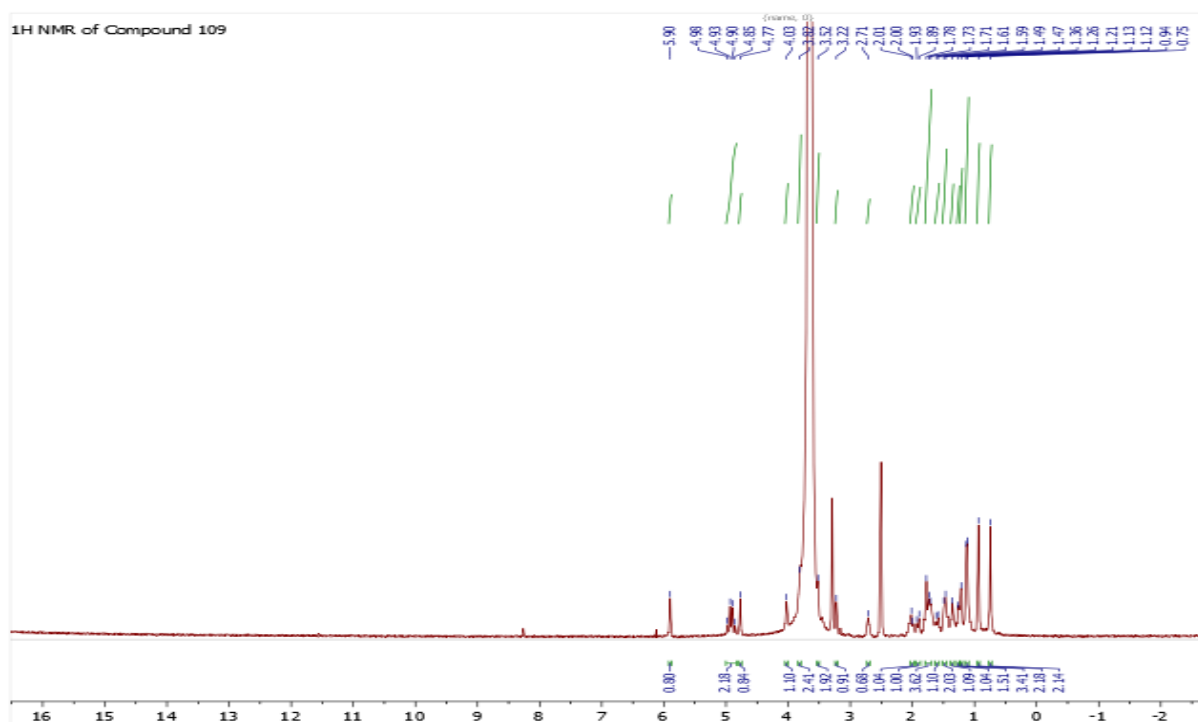
Appendix 14E: HSQC (in CDCl_3) spectrum of Dihydroferulic acid (**108**)



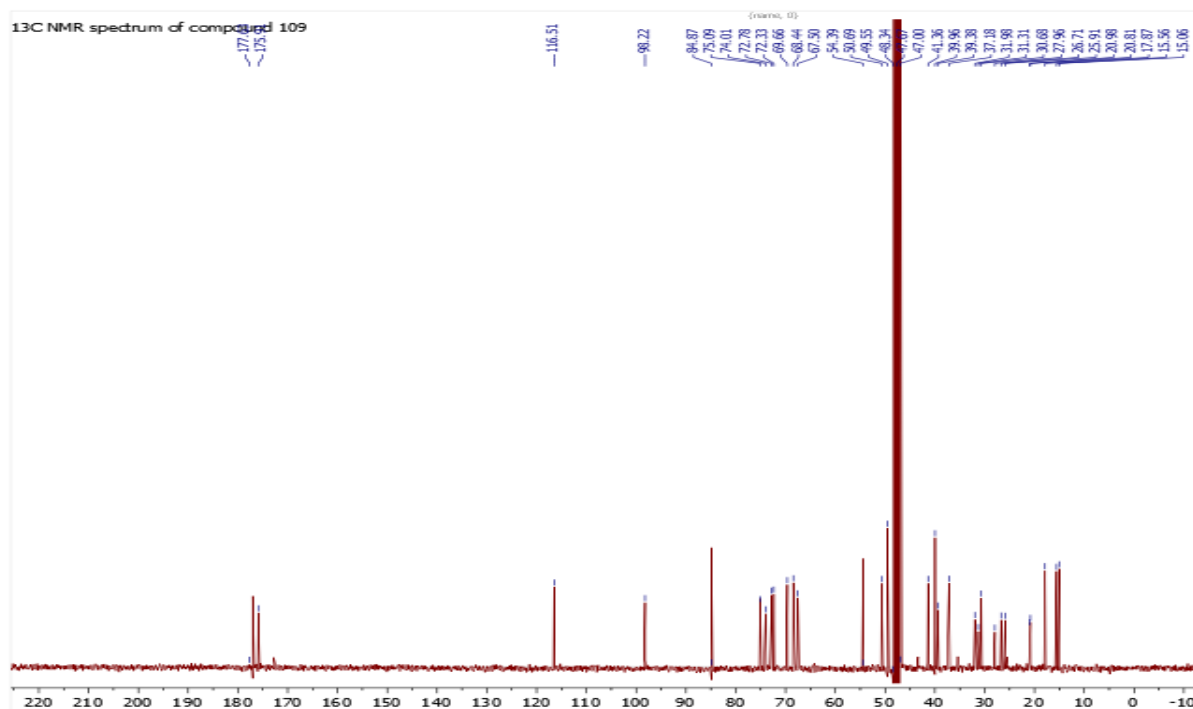
Appendix 15A: FT-IR spectrum of compound **109**



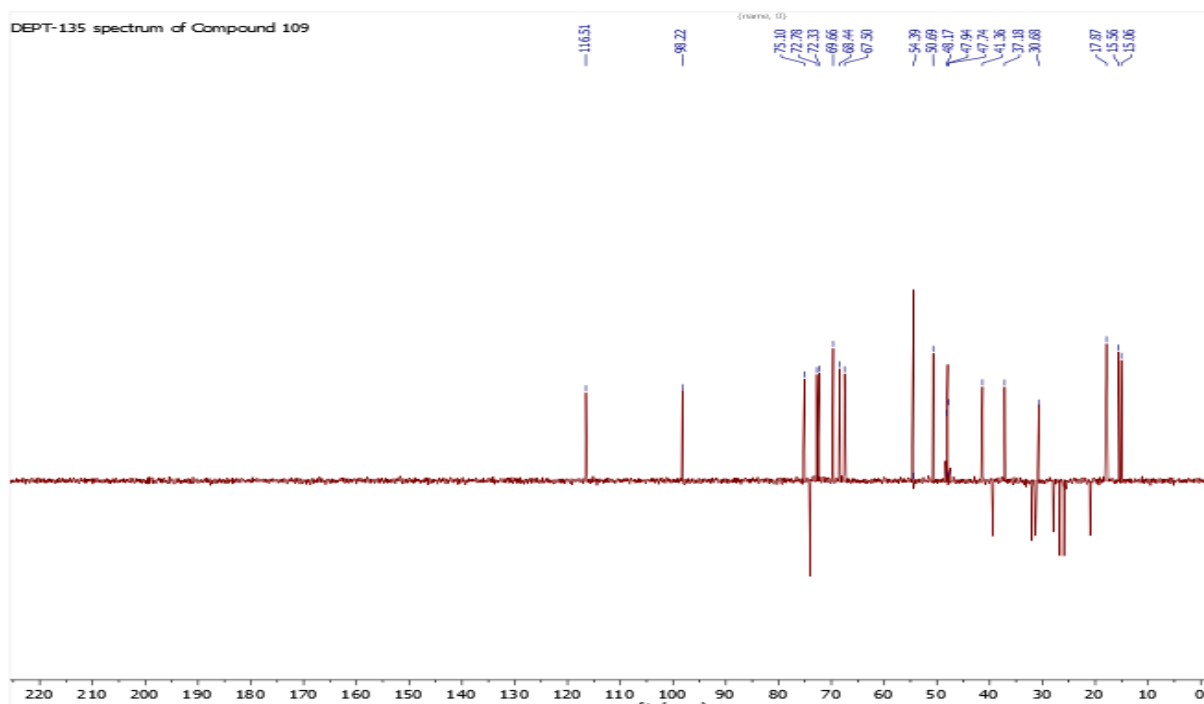
Appendix 15B: ^1H NMR (in CD_3OD) spectrum of compound **109**



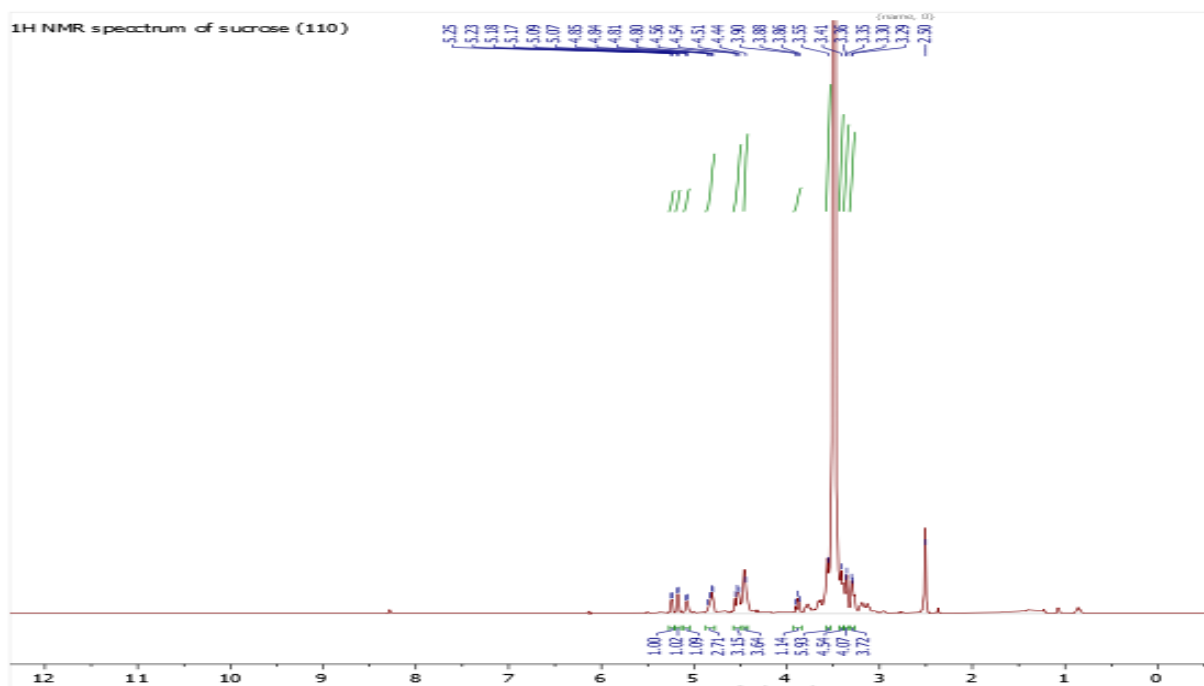
Appendix 15C: ^{13}C NMR (in CD_3OD) spectrum of compound **109**



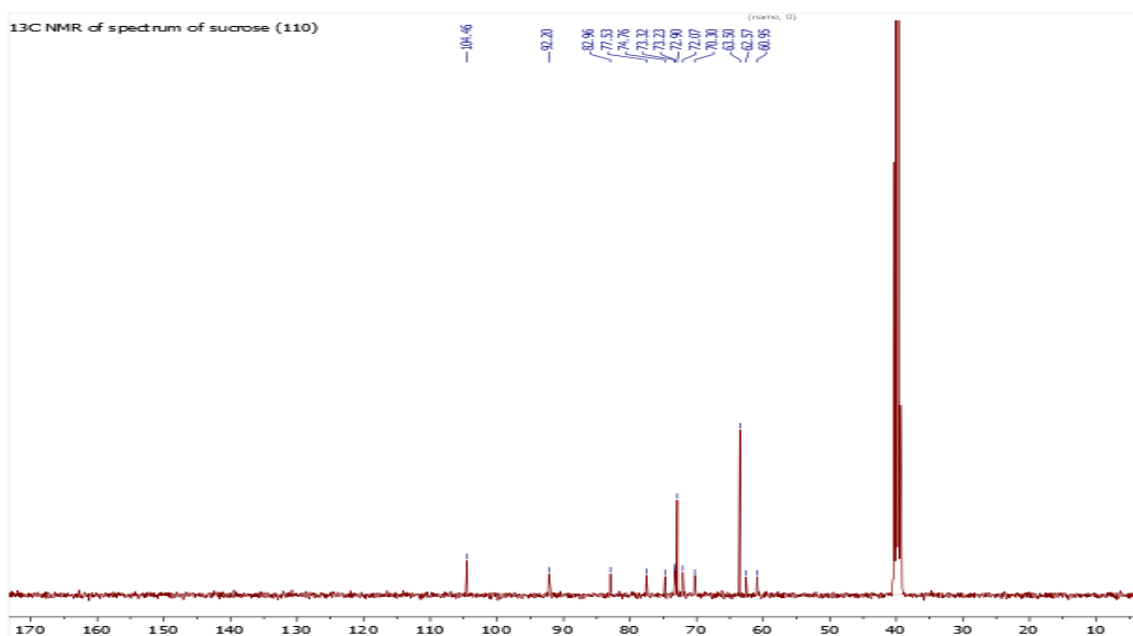
Appendix 15D: DEPT-135 (in CD₃OD) spectrum of compound **109**



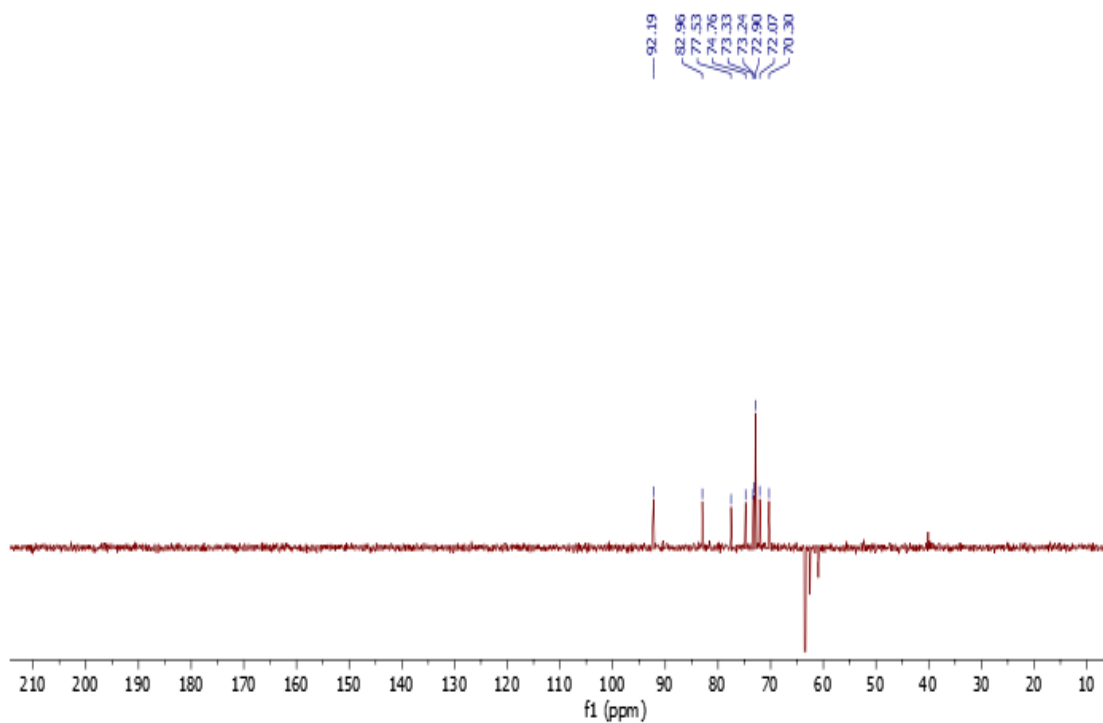
Appendix 16A: ¹H NMR spectrum (in CD₃OD) of sucrose (**110**)



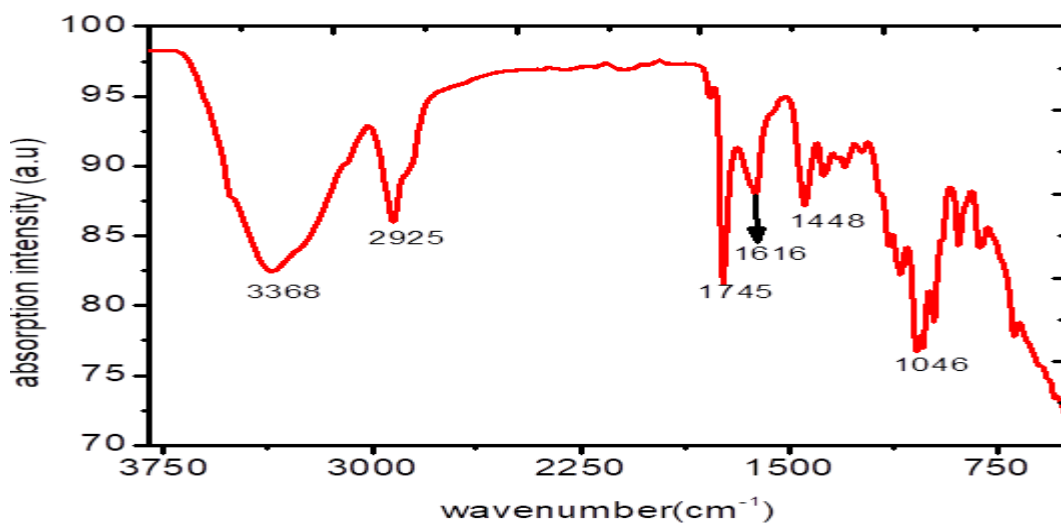
Appendix 16B: ^{13}C NMR (in CD_3OD) spectrum of sucrose (**110**)



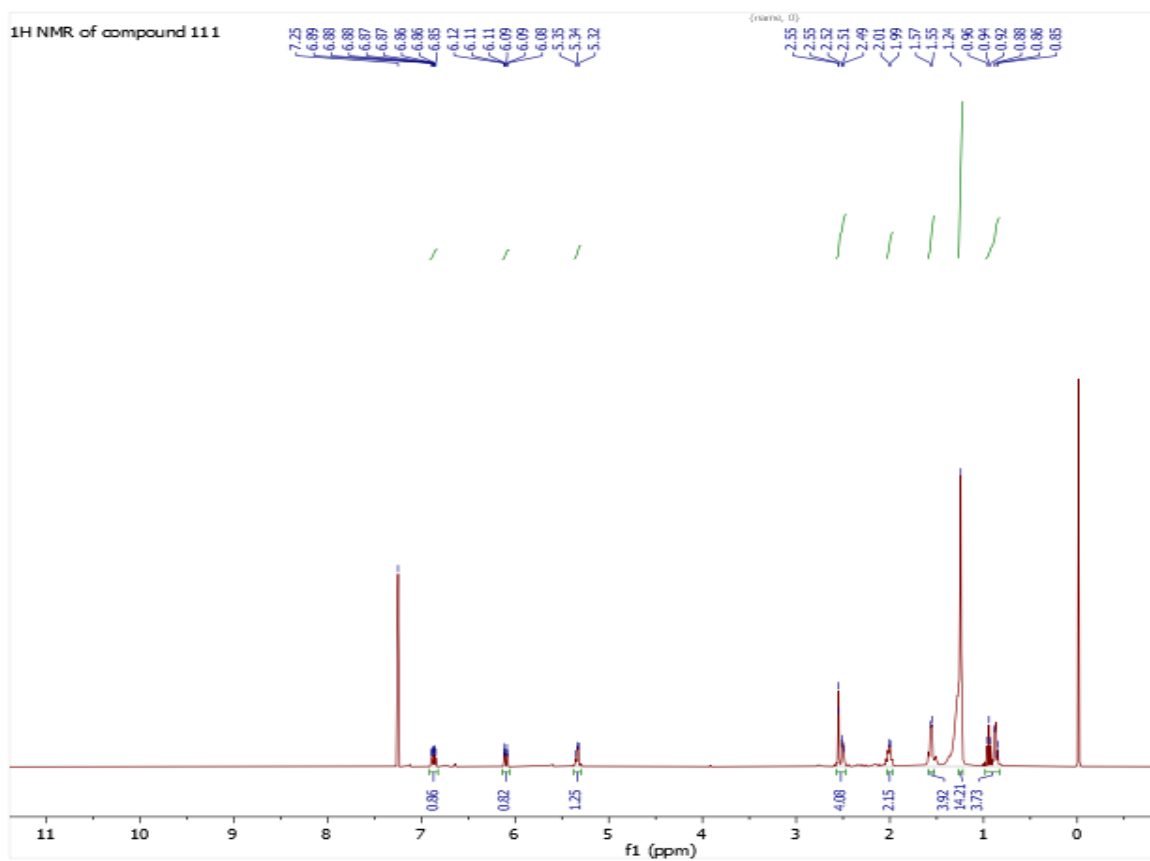
Appendix 16C: DEPT-135 (in CD_3OD) spectrum of sucrose (**110**)



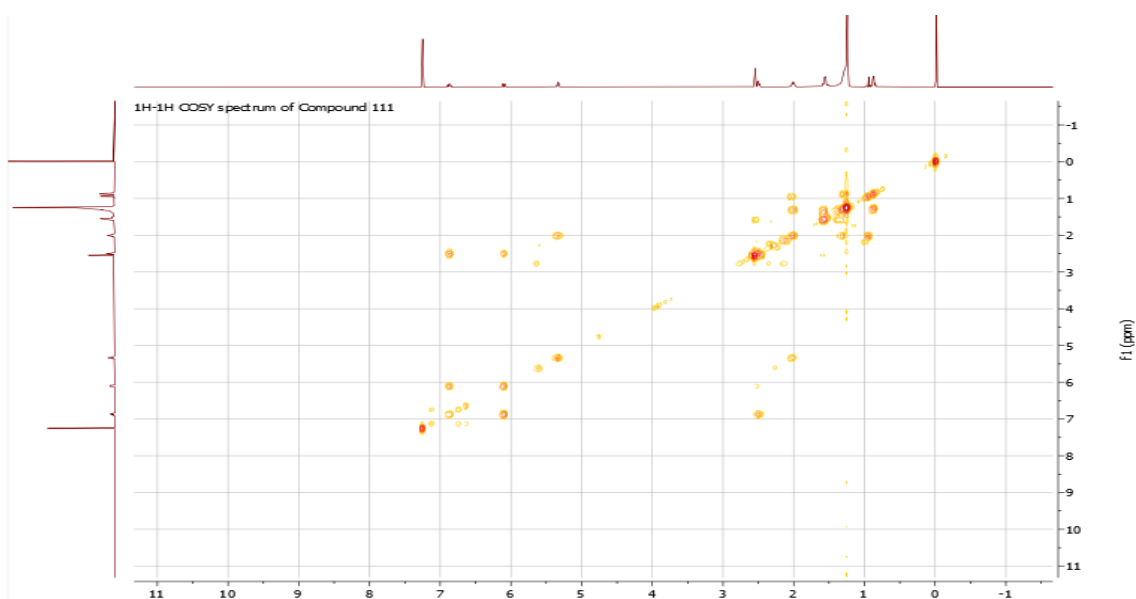
Appendix 17A: FT-IR spectrum of compound **111**



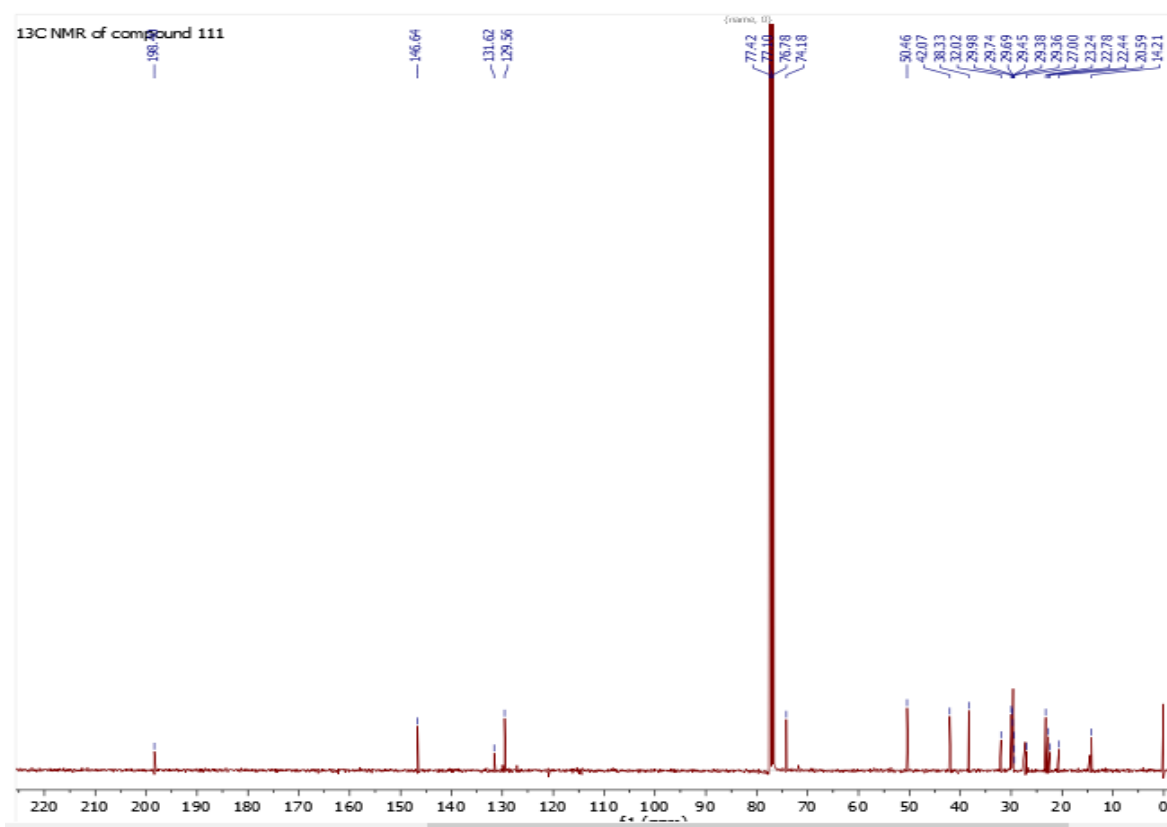
Appendix 17B: ^1H NMR (in CDCl_3) spectrum of compound **111**



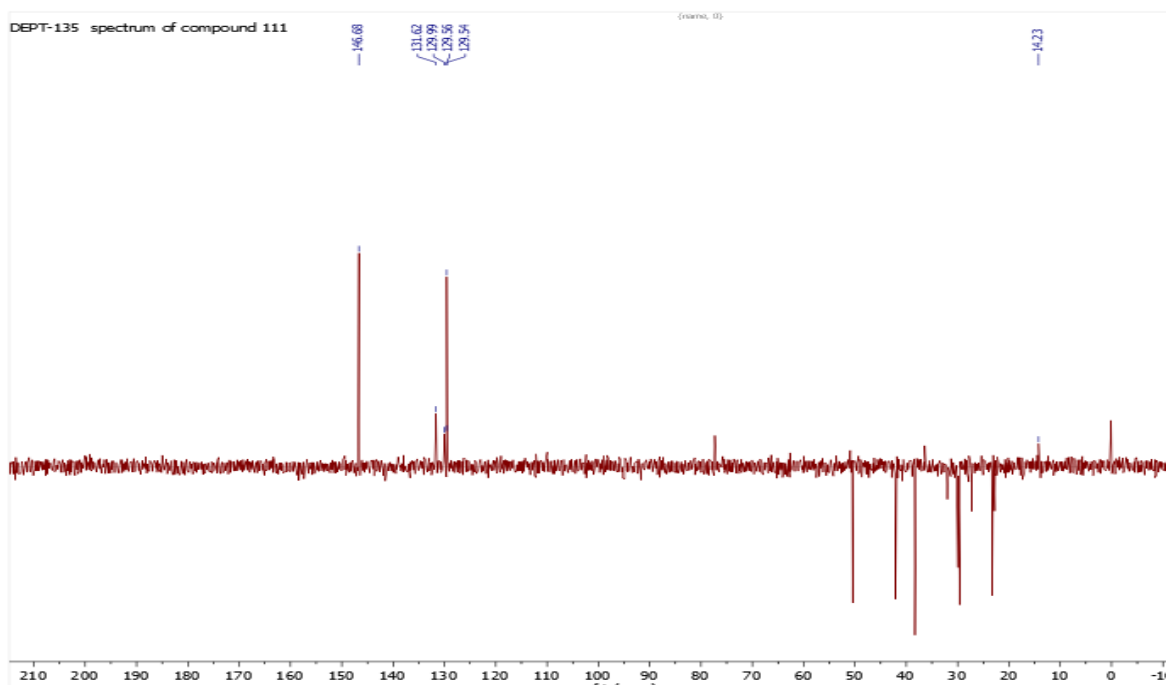
Appendix 17C: ^1H - ^1H COSY (in CDCl_3) spectrum of compound **111**



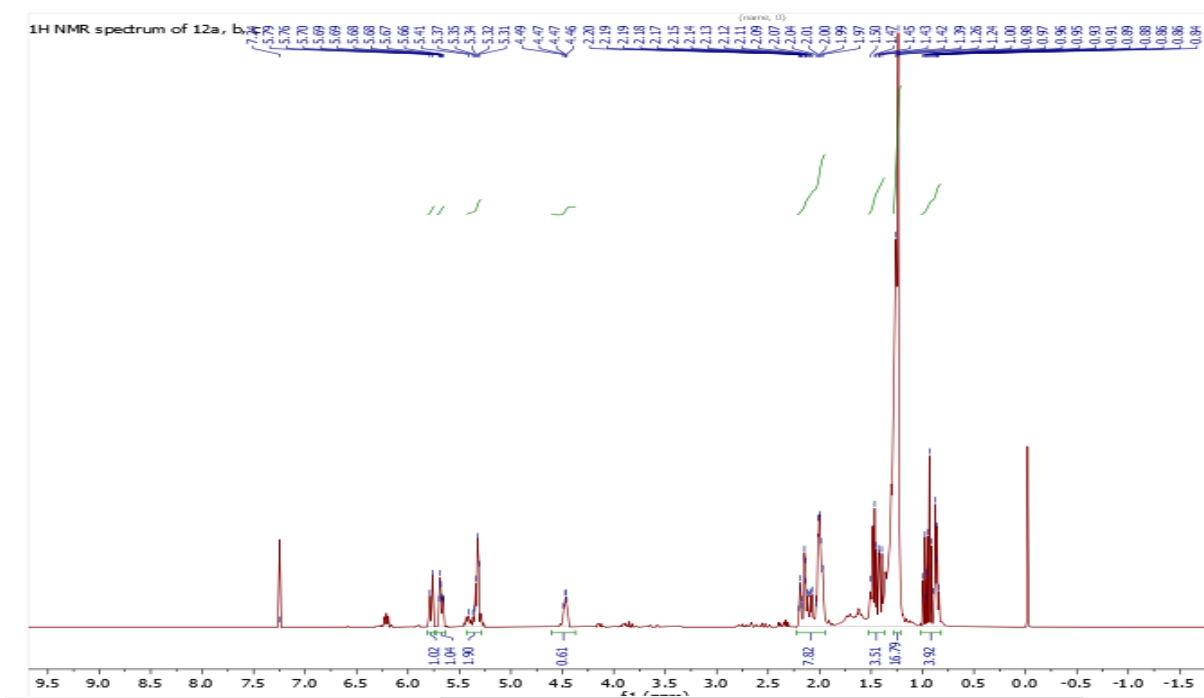
Appendix 17D: ^{13}C NMR (in CDCl_3) spectrum of compound **111**



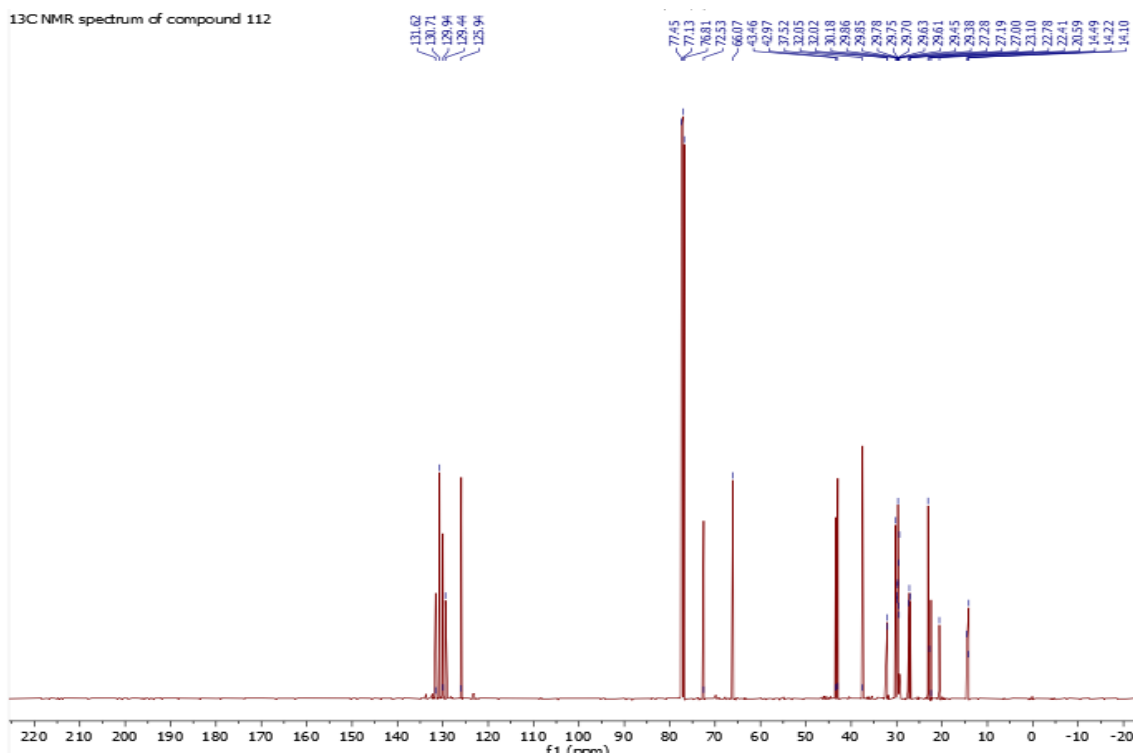
Appendix 17E: DEPT-135 (in CDCl₃) spectrum of compound **111**



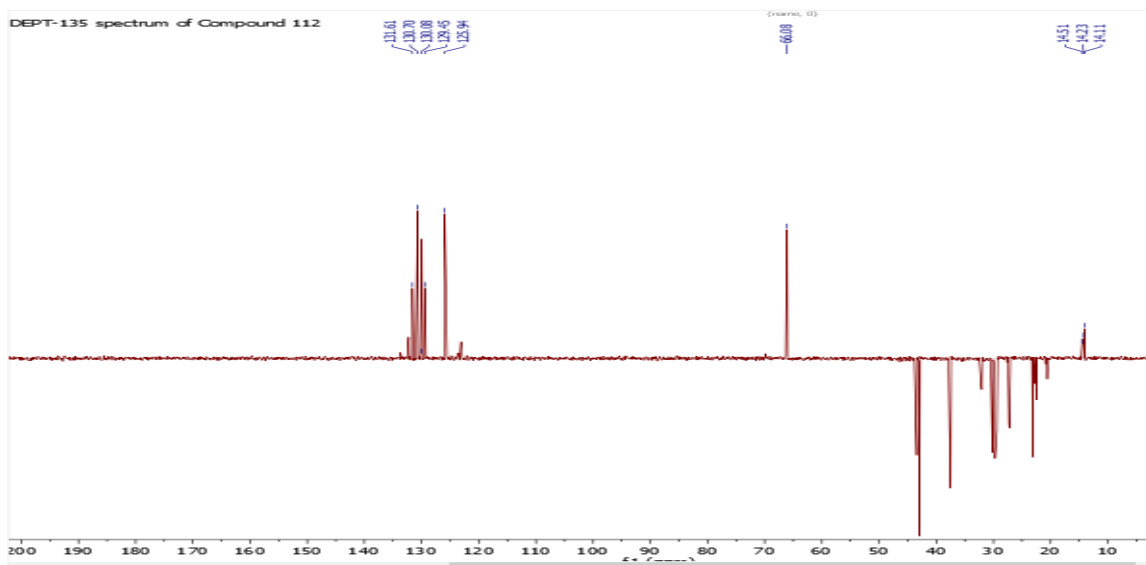
Appendix 18A: ¹H NMR (in CDCl₃) spectrum of compound **12a, b and c**.



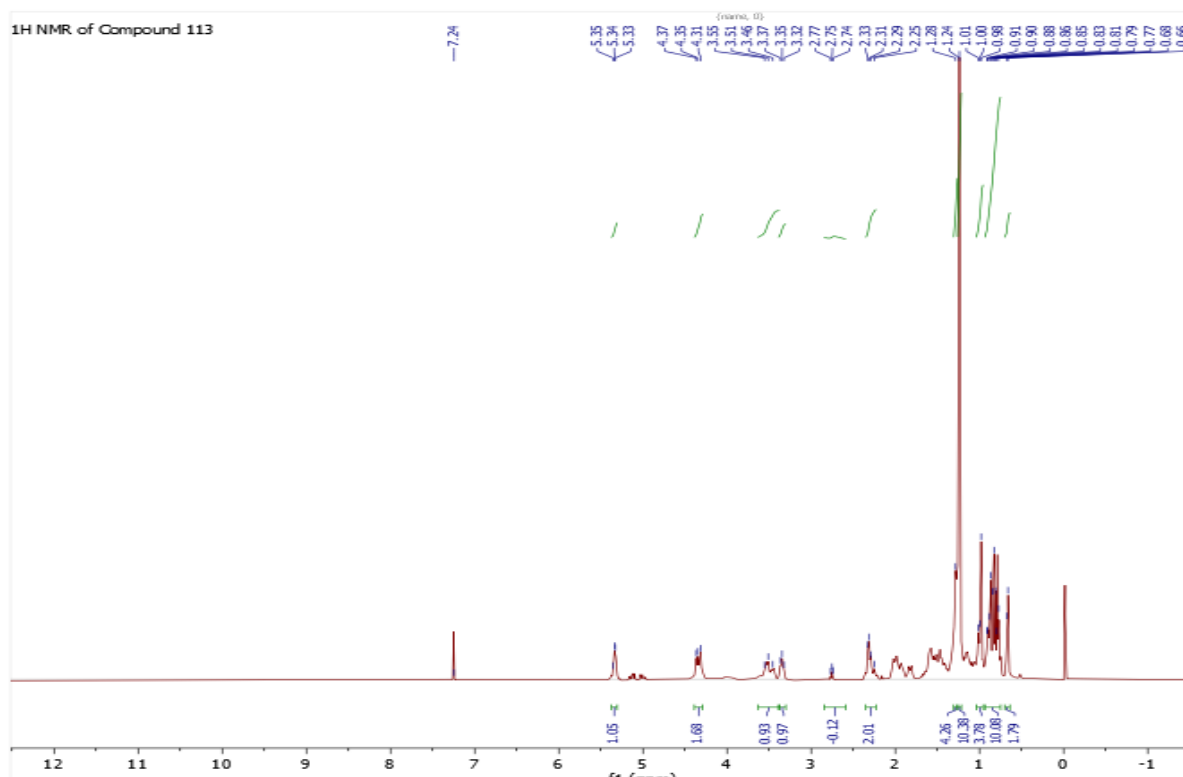
Appendix 18B: ^{13}C NMR (n CDCl_3) of compound **12a**, **b** and **c**.



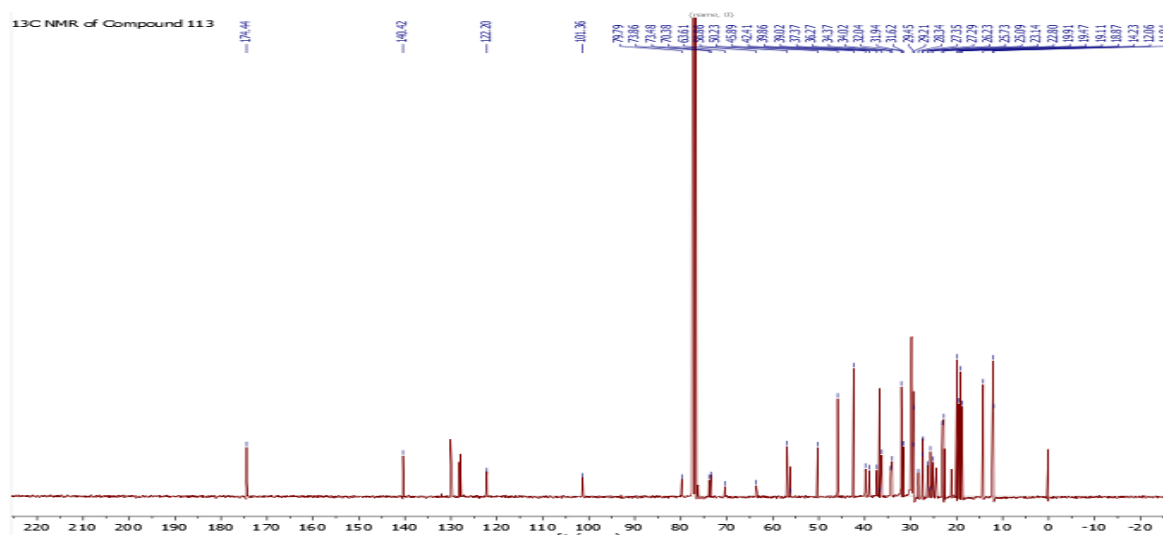
Appendix 18C: DEPT-135 spectrum of compound **12a**, **b** and **c**



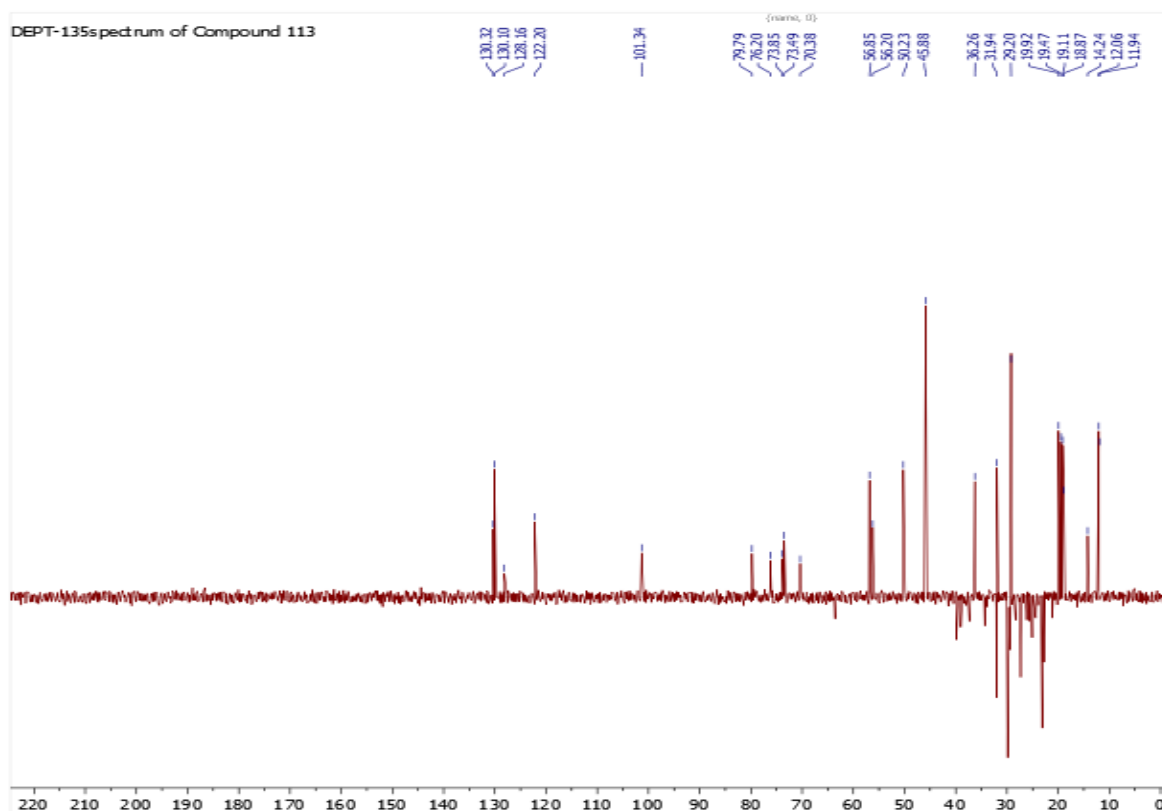
Appendix 19A: ^1H NMR (in CDCl_3) spectrum of compound **113**



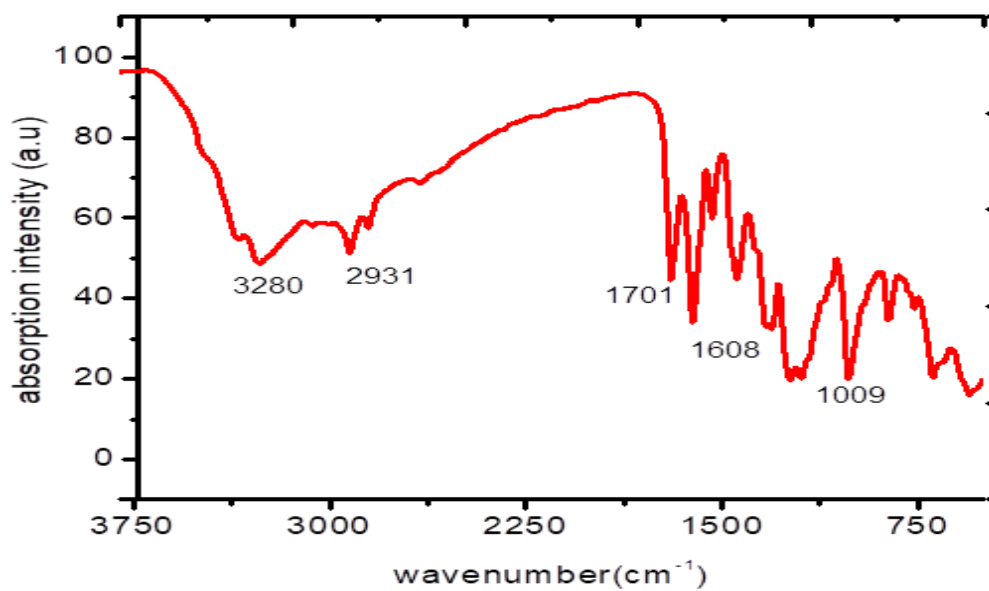
Appendix 19 B: ^{13}C NMR (in CDCl_3) spectrum of compound **113**



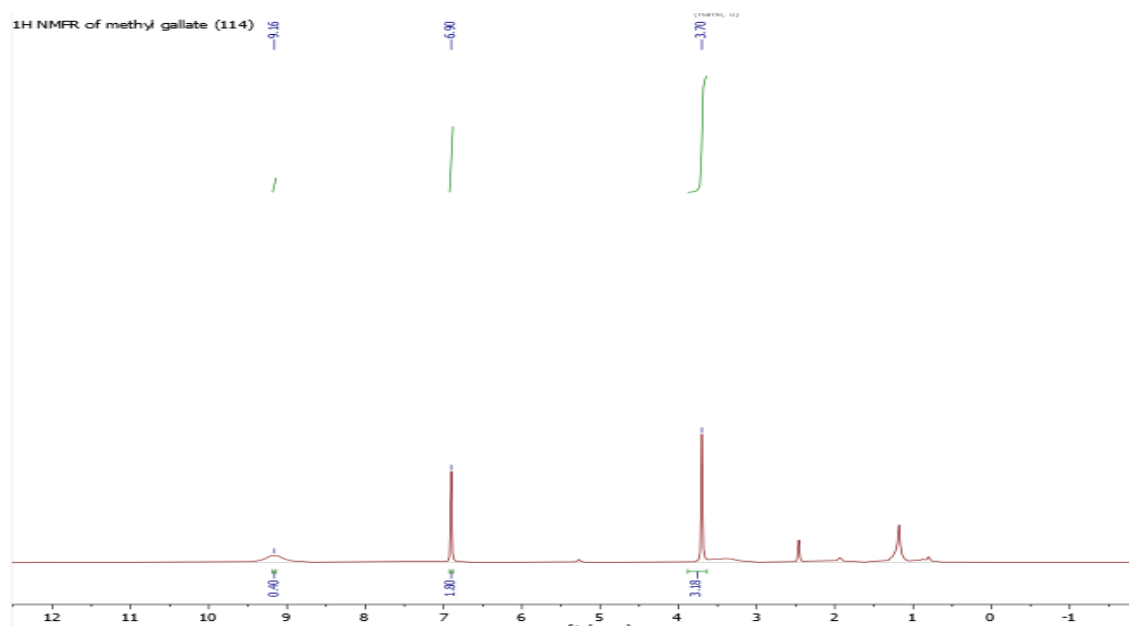
Appendix 19C: DEPT-135 (in CDCl₃) spectrum of compound **113**



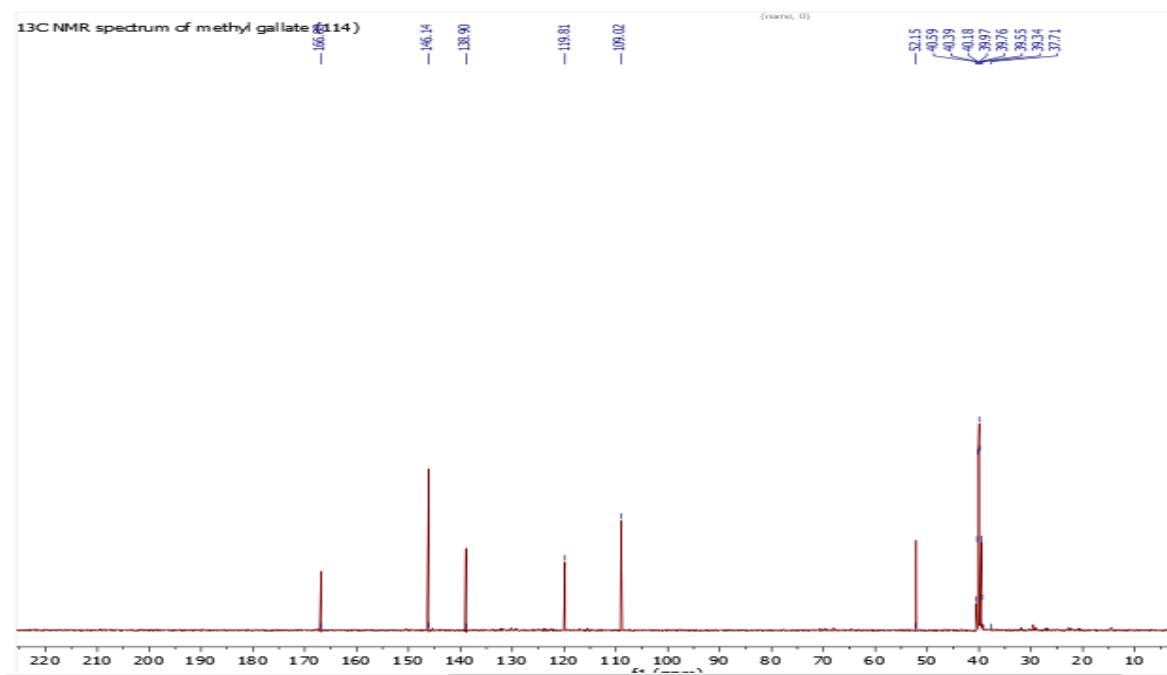
Appendix 20A: FT-IR spectrum of methyl gallate (**114**)



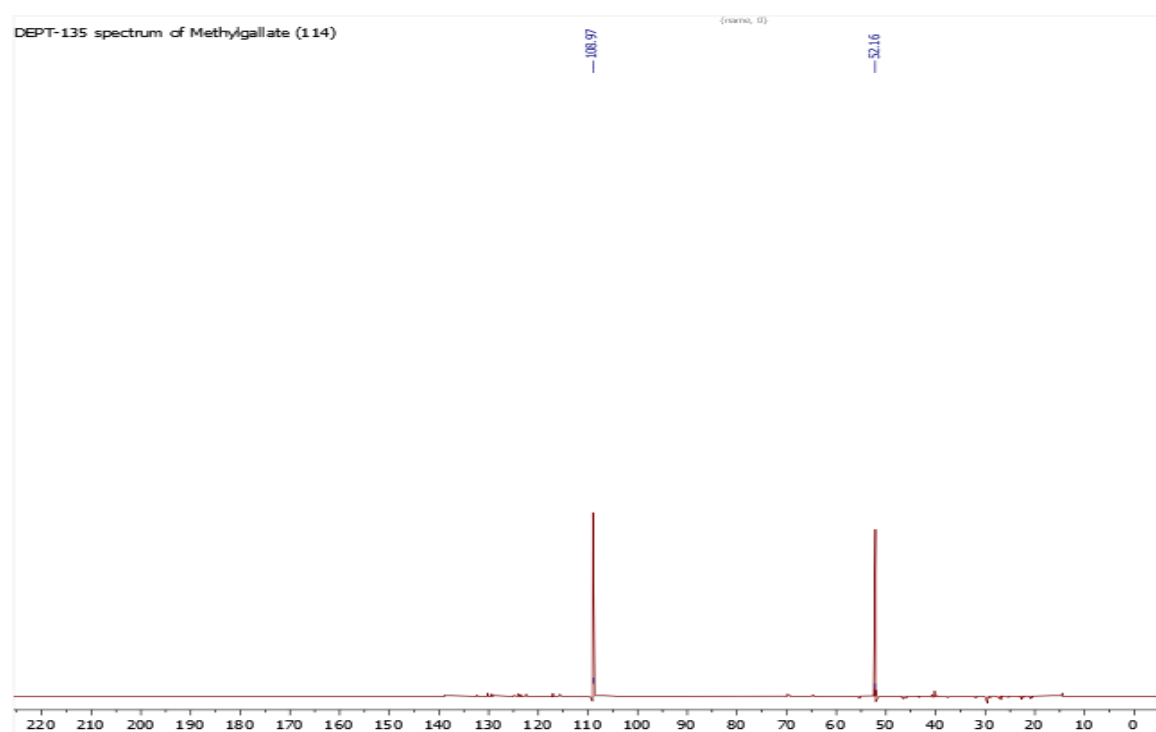
Appendix 20B: ^1H NMR (in $\text{DMSO-}d_6$) spectrum of methyl gallate (**114**)



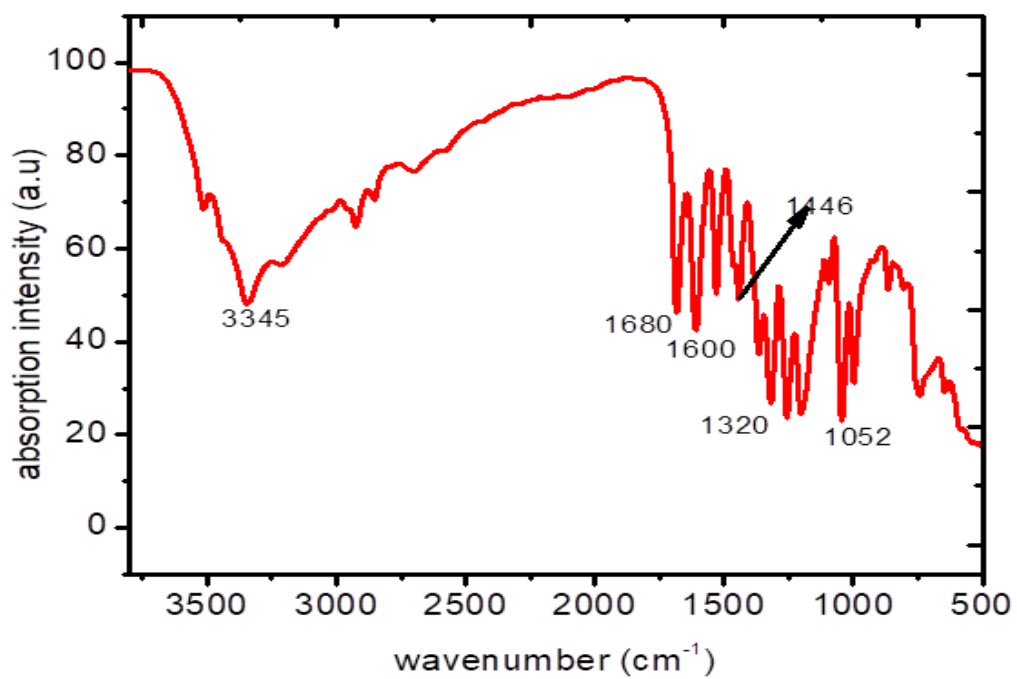
Appendix 20C: ^{13}C NMR (in $\text{DMSO-}d_6$) spectrum of methyl gallate (**114**)



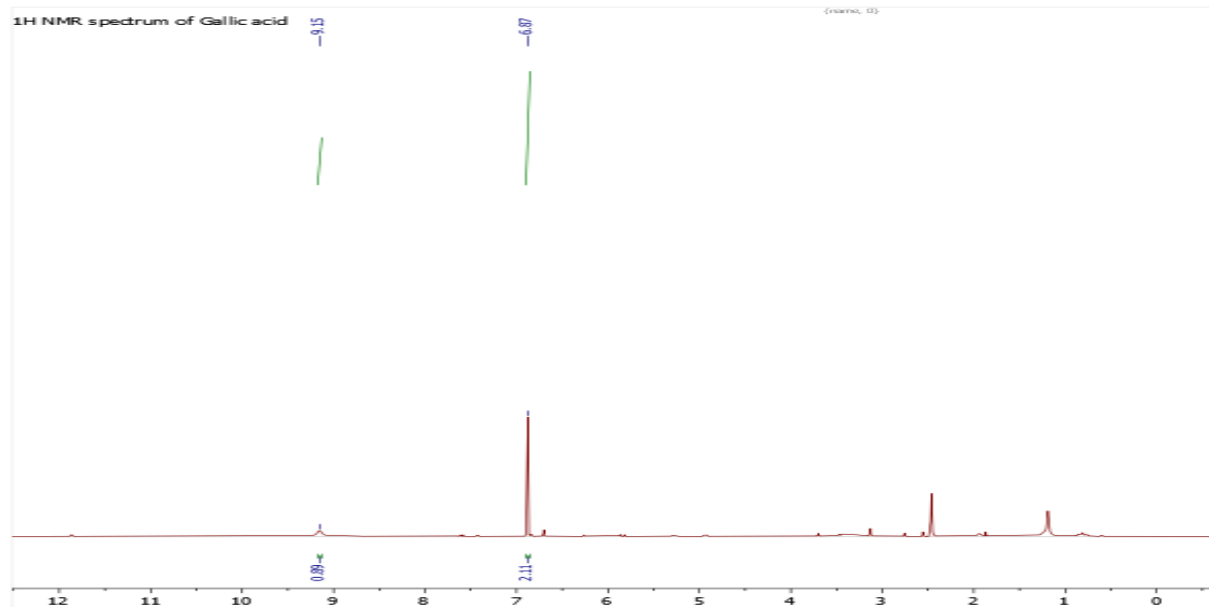
Appendix 20D: DEPT-13 (in DMSO-*d*₆) spectrum of methyl gallate (**114**)



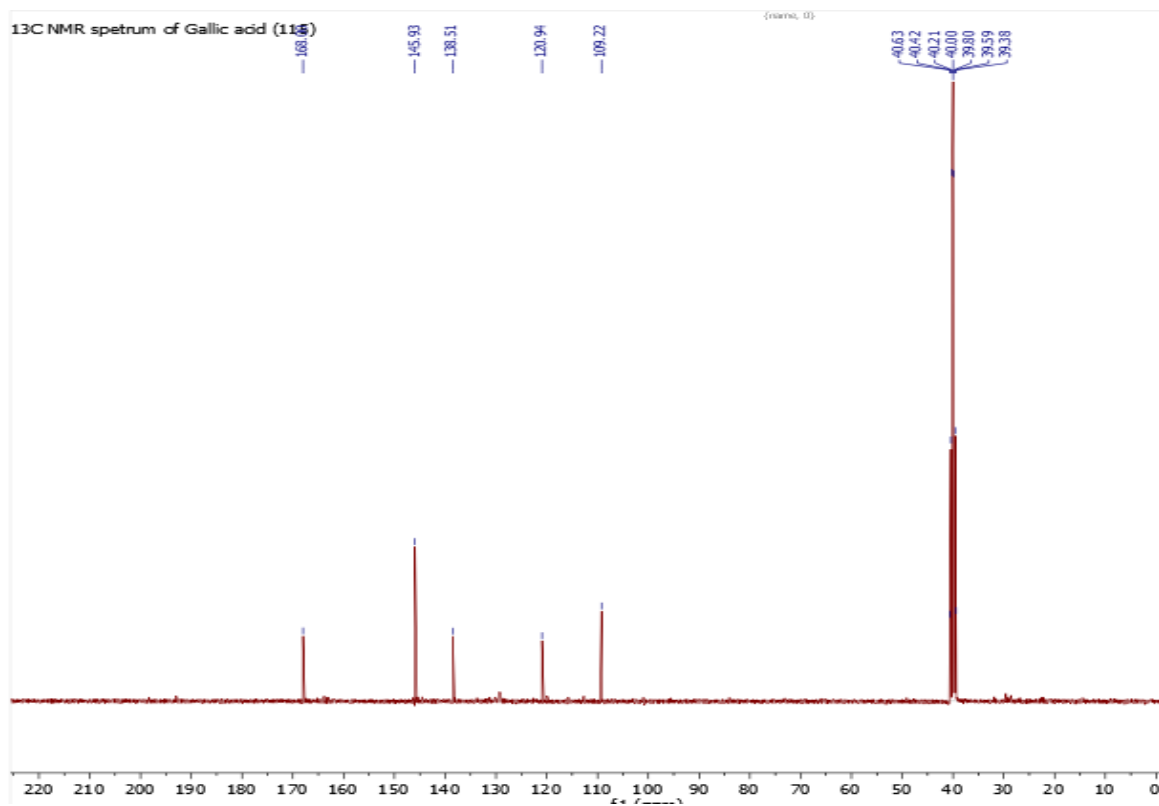
Appendix 21A: FT-IR spectrum of gallic acid (**115**)



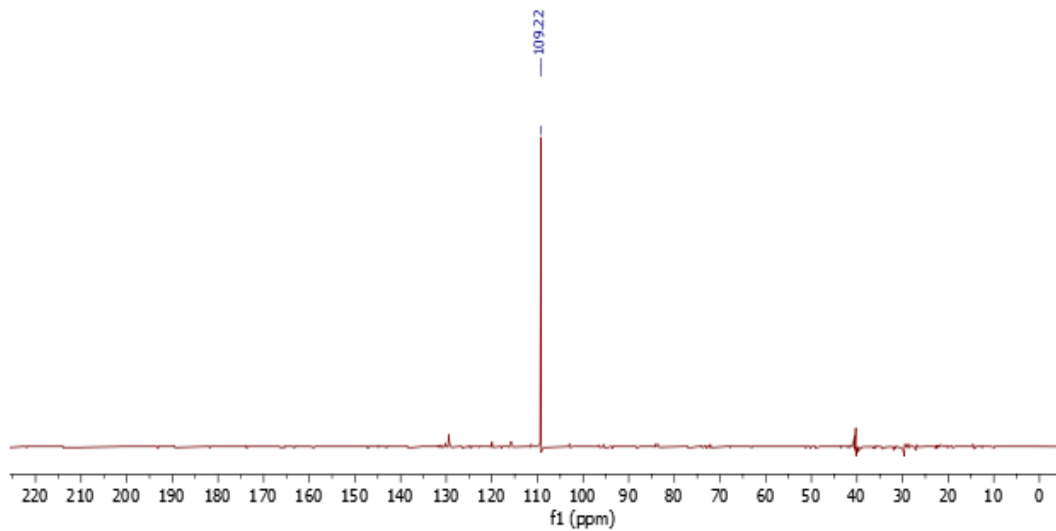
Appendix 21 B: ^1H NMR (in $\text{DMSO-}d_6$) spectrum of gallic acid (**115**)



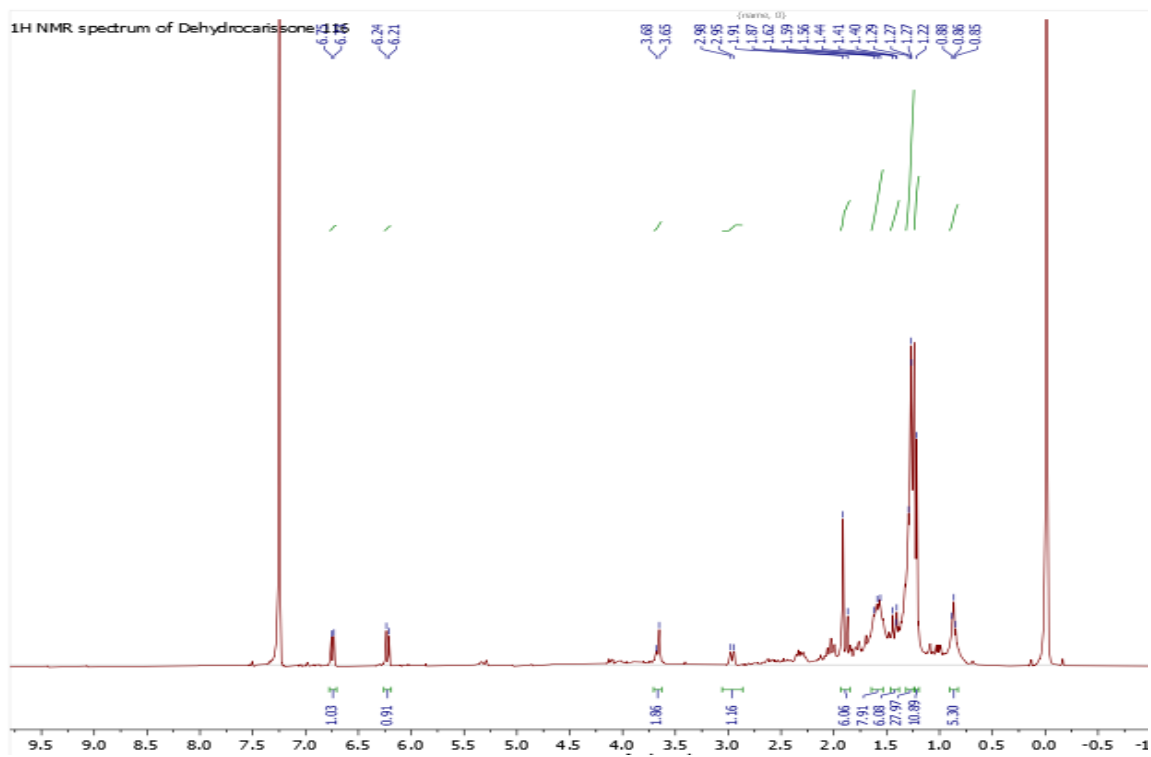
Appendix 21C: ^{13}C NMR (in $\text{DMSO-}d_6$) spectrum of gallic acid (**115**)



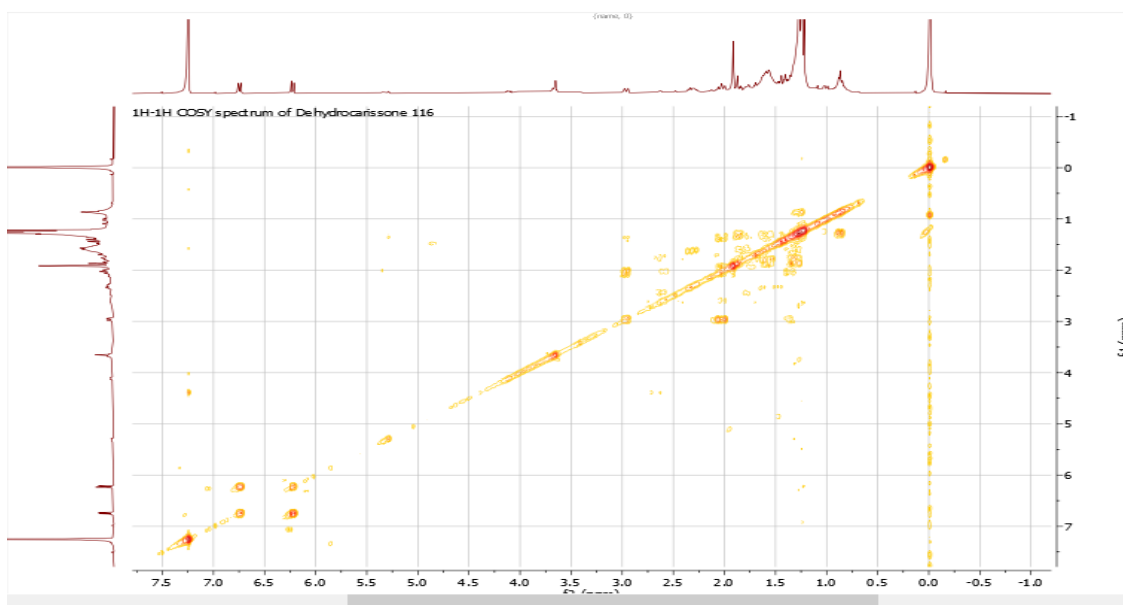
Appendix 21D: DEPT-135 (in DMSO-*d*6) spectrum gallic acid (**115**)



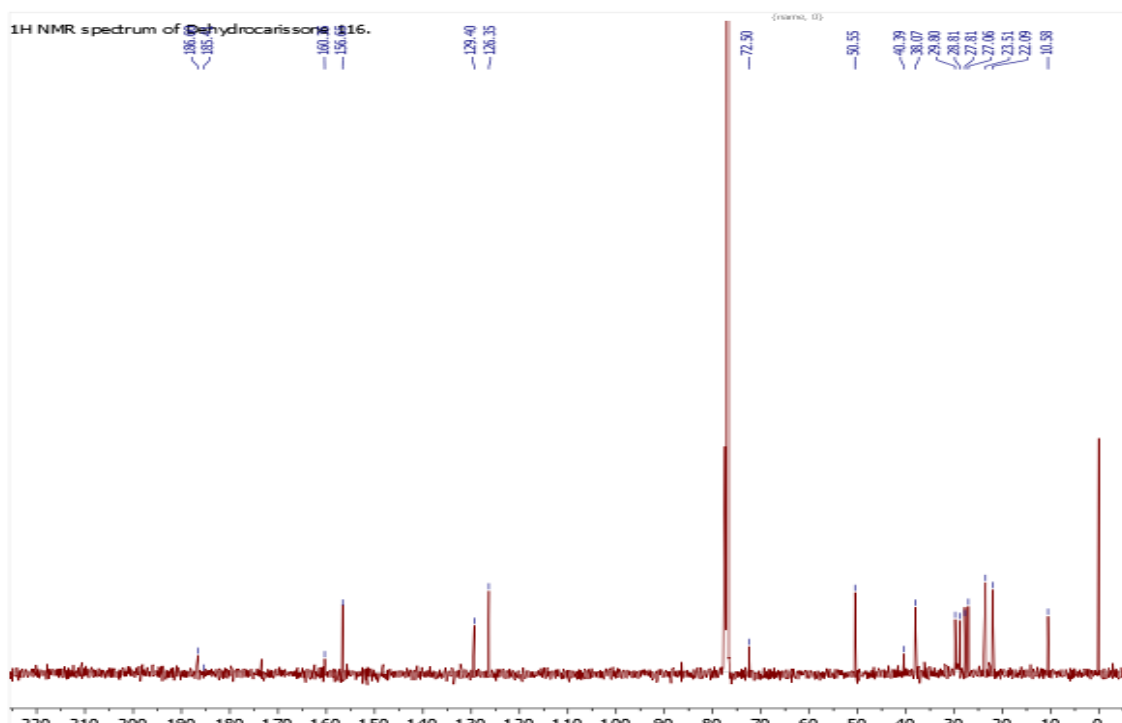
Appendix 22A: ^1H NMR (in CDCl_3) spectrum of dehydrocarissone (**116**)



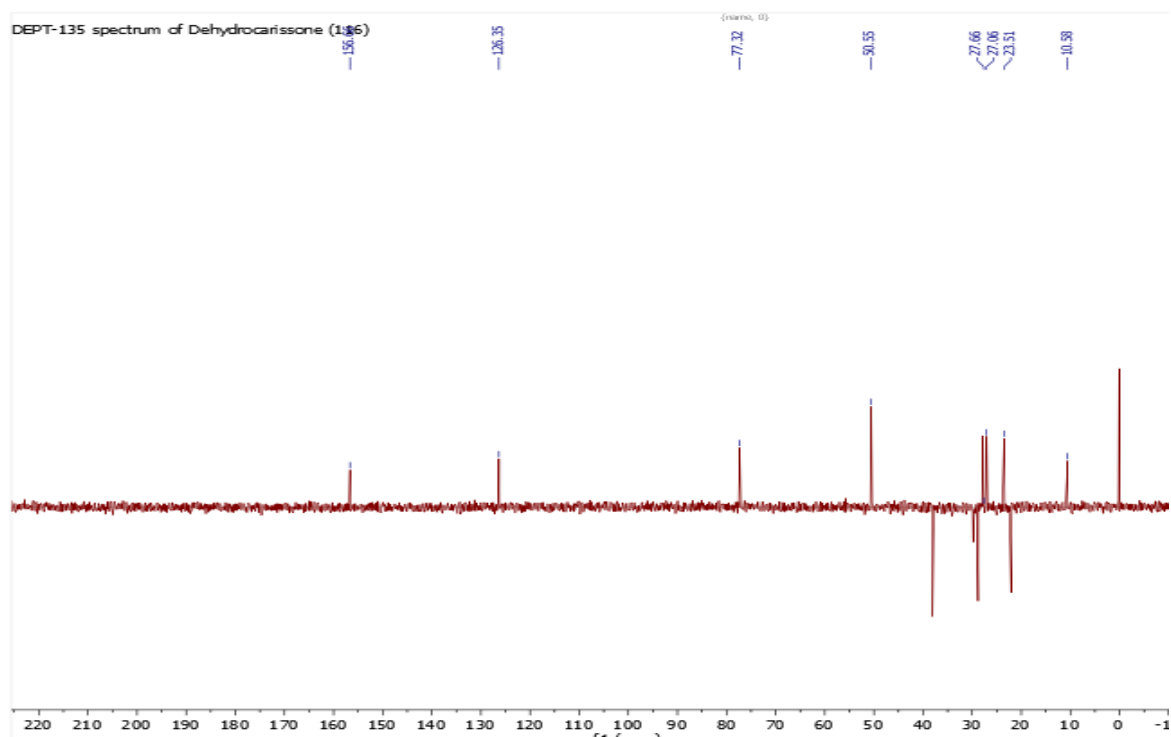
Appendix 22B: ^1H - ^1H COSY (in CDCl_3) spectrum of dehydrocarissone (**116**)



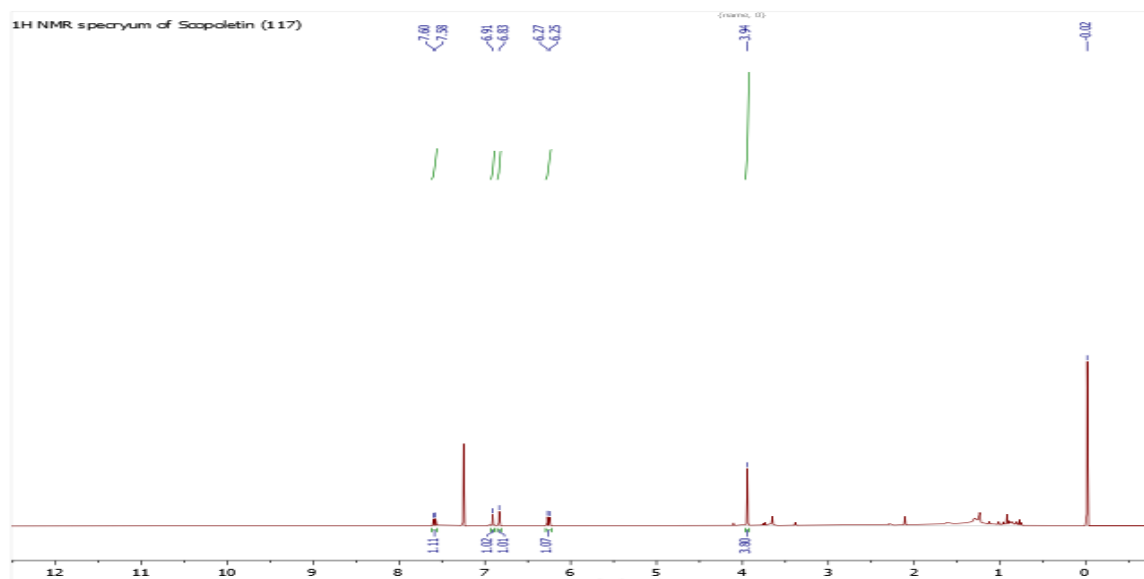
Appendix 22C: ^{13}C NMR (in CDCl_3) spectrum of dehydrocarissone (**116**)



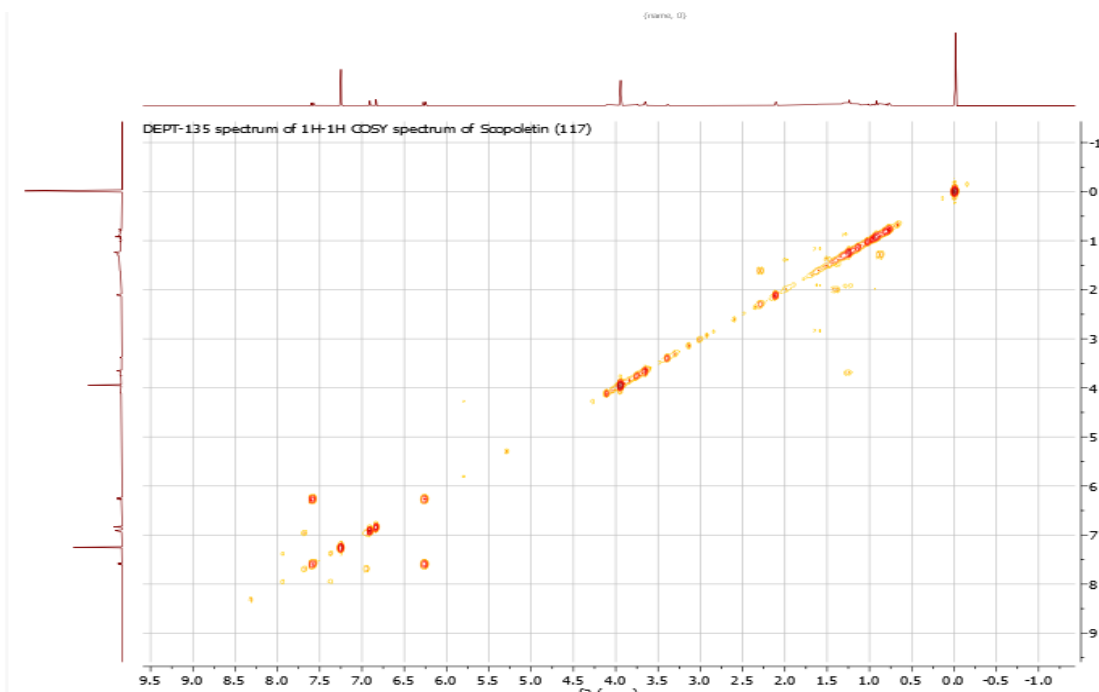
Appendix 22D: DEPT-135 (in CDCl₃) spectrum of dehydrocarissone (**116**)



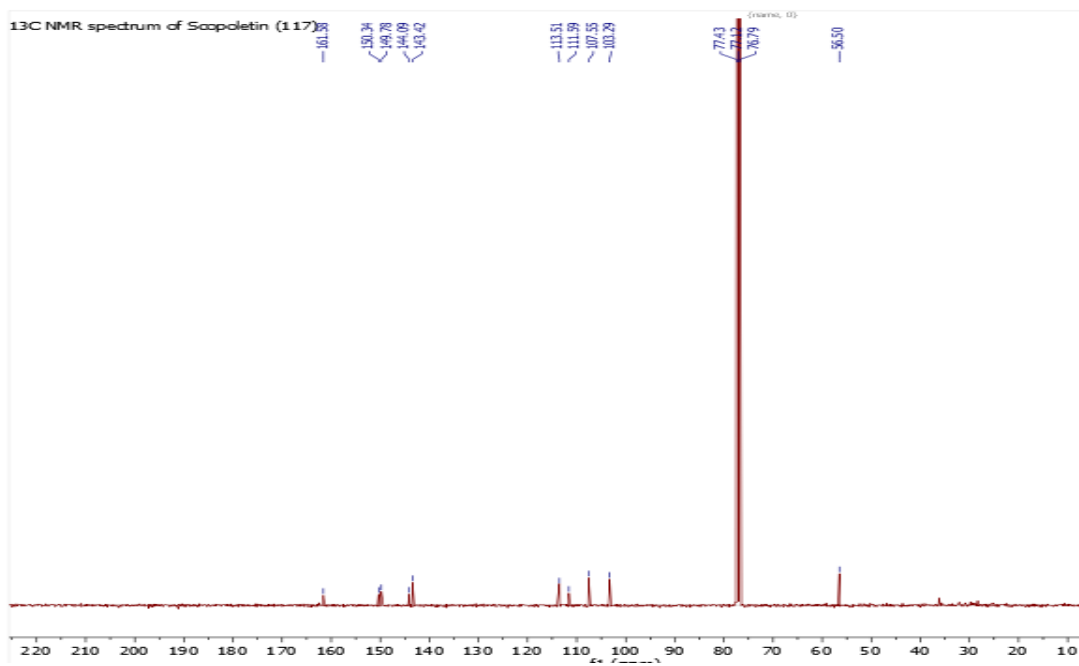
Appendix 23A: ¹H NMR (in CDCl₃) spectrum of scopoletin (**117**)



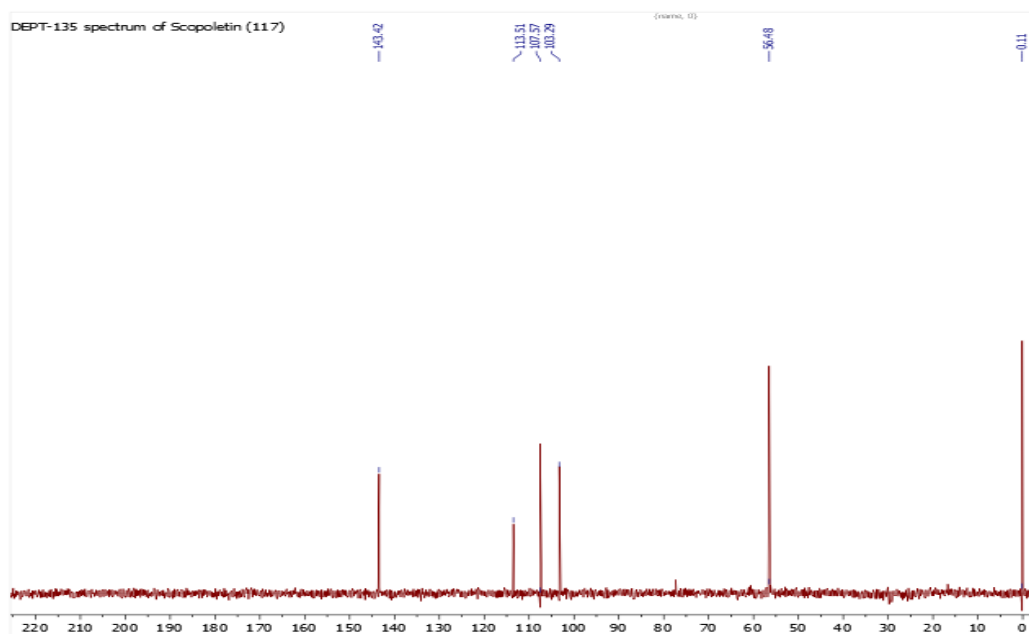
Appendix 23B: ^1H - ^1H COSY spectrum (in CDCl_3) of scopoletin (**117**)



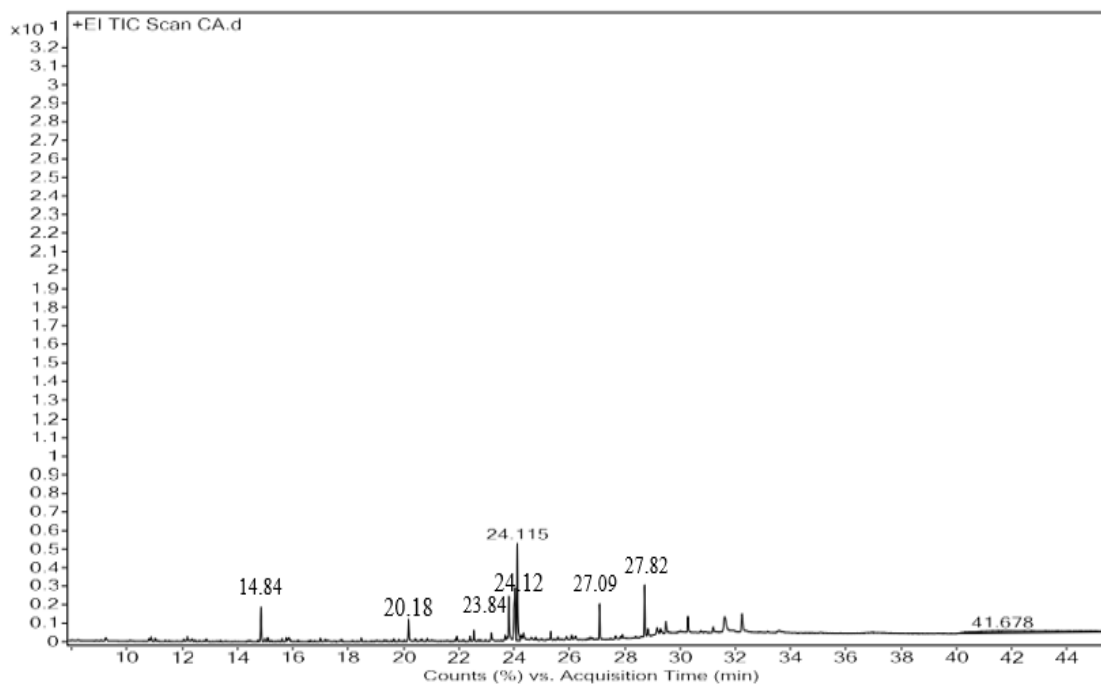
Appendix 23C: ^{13}C NMR spectrum (in CDCl_3) of scopoletin (**117**)



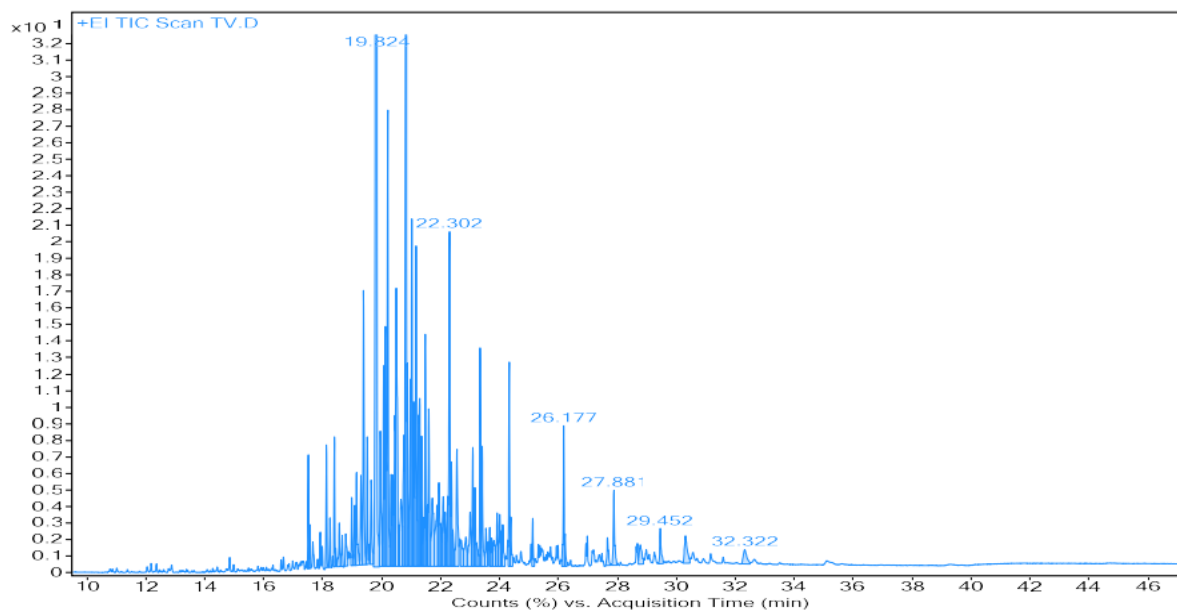
Appendix 23D: DEPT-135 (in CDCl₃) spectrum of scopoletin (**117**)



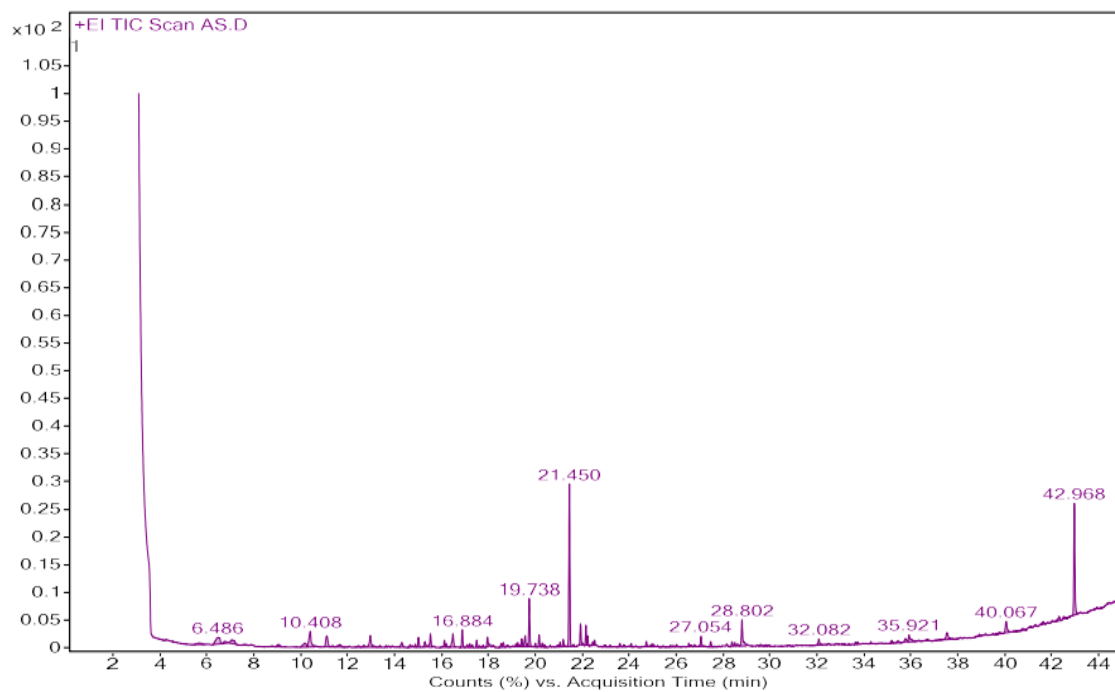
Appendix 24: GC chromatogram of essential oil from the roots of *W. uniflora*



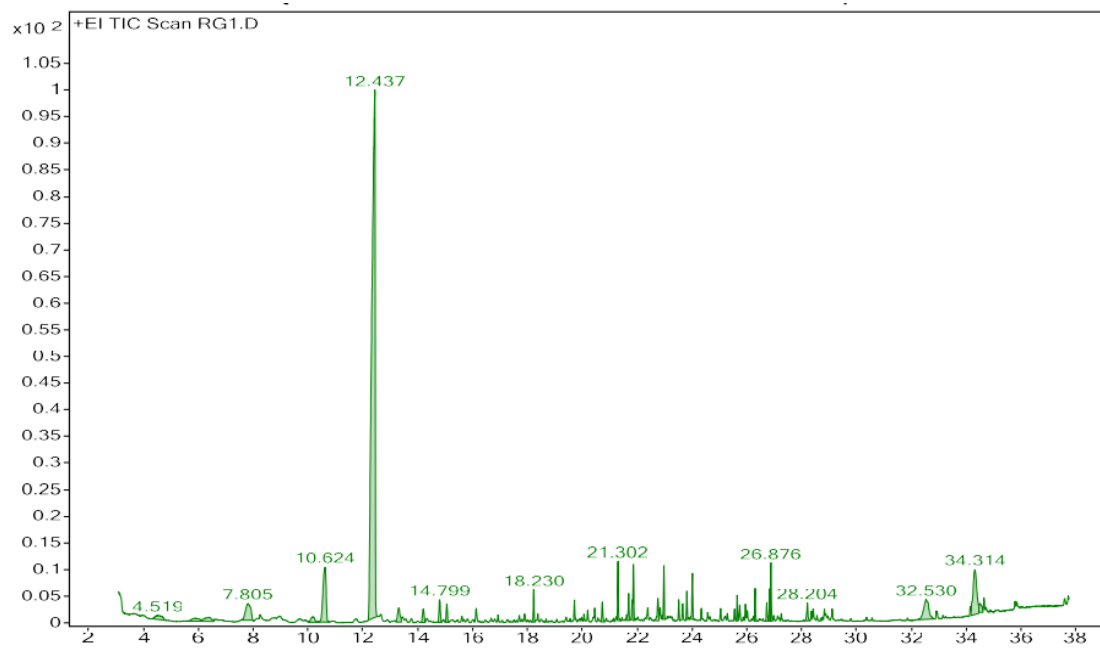
Appendix 25: GC chromatogram of essential oil from the roots of *T. vogelii*



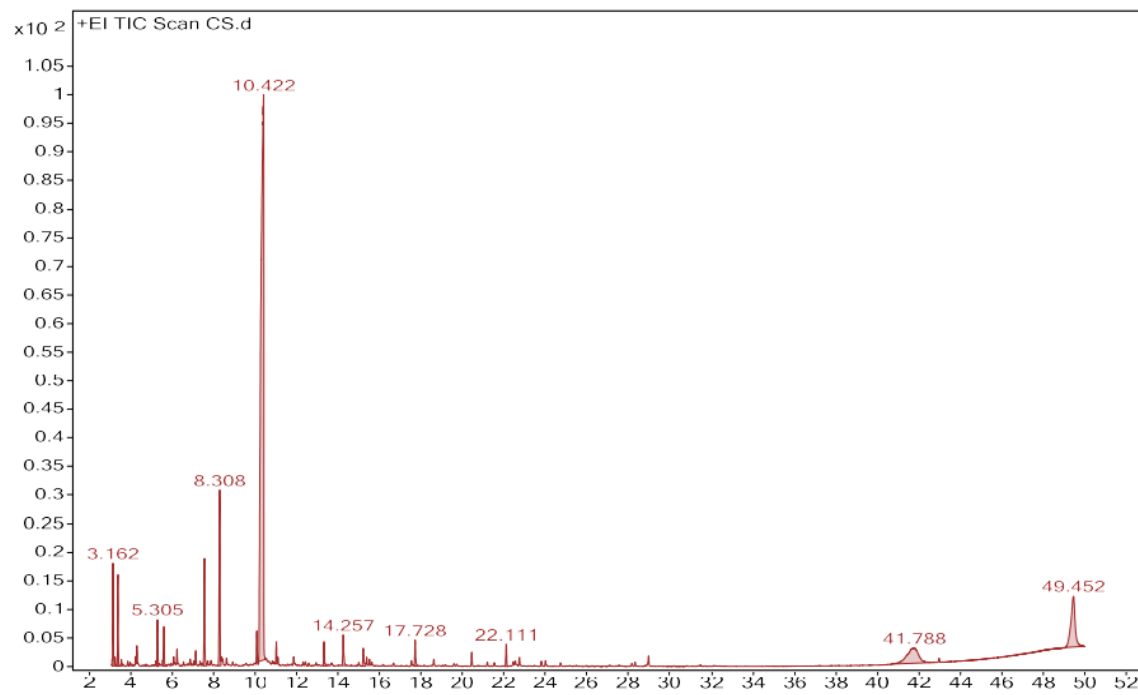
Appendix 26: GC chromatogram of essential oil from the roots of *A. schimperi*



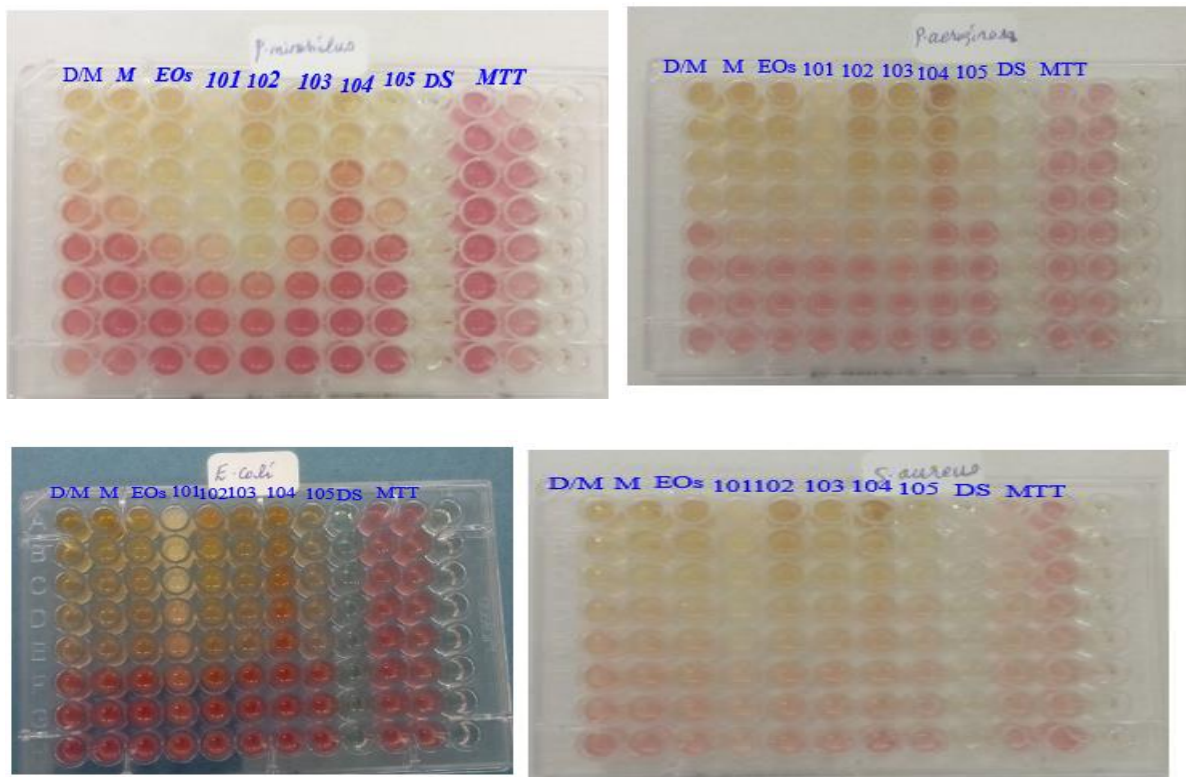
Appendix 27: GC chromatogram of essential oil from the roots of *R. glutinosa*



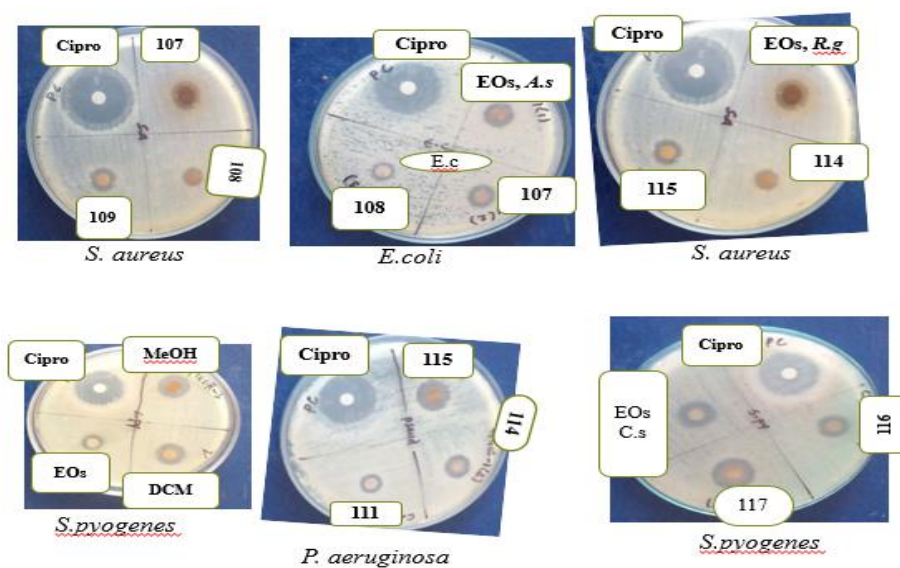
Appendix 28: GC chromatogram of essential oil from the roots of *C. spinarum*



Appendix 29: Antibacterial activities of compounds from *T. vogelii* evaluated using the broth dilution method.



Appendix 30::Antibacterial activities of some compounds evaluated using disc diffusion method



PUBLICATIONS

1. Bihon Abera, Yadessa Melaku, Kebede Shenkute, Aman Dekebo, Negera Abdissa, Milkyas Endale, Temesgen Negassa, Messay Woldemariam and Mo Hunsen, 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 (2024) 67:77, <https://doi.org/10.1186/s13765-024-00930-6>.
2. Bihon Abera, Yadessa Melaku, Kebede Shenkute, Sileshi Degu, Abiy Abebe, Worku Gemechu, Milkyas Endale*, Messay Woldemariam, Mo Hunsen, Ahmed A. Hussein and Aman Dekebo, In vitro antibacterial and antioxidant activity of flavonoids from the roots of *Tephrosia vogelii*: a combined experimental and computational study, Z. Naturforsch. 2024; aop, <https://doi.org/10.1515/znc-2024-0044>