# Phytochemical Investigations, Antibacterial and Antioxidant Activities, and *In Silico* Molecular Docking Studies of Compounds Isolated From The Leaf Extracts of *Salvia leucantha CAV*.

**Urael Getachew Muleta**

**A Thesis Submitted to the Department of Applied Chemistry**
**School of Applied Natural Science**
**Presented in Partial Fulfillment of the Requirements for the Degree**
**of Master's in Applied Chemistry (Medicinal Chemistry)**

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

**November, 2024**

**Adama, Ethiopia**

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## DECLARATION

I hereby declare that this Master Thesis entitled "*Phytochemical Investigations, Antibacterial and Antioxidant Activities, and In Silico Molecular Docking Studies of Compounds Isolated from the Leaf Extracts of Salvia leucantha CAV*." is my original work. That is, it has not been submitted for the award of any academic degree, diploma, or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

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## RECOMMENDATION

I, the advisor of this thesis, hereby certify that I have read the revised version of the thesis entitled "*Phytochemical Investigations, Antibacterial and Antioxidant Activities, and In Silico Molecular Docking Studies of Compounds Isolated from the Leaf Extracts of Salvia leucantha CAV*." prepared under my guidance by Urael Getachew submitted in partial fulfillment of the requirements for the degree of Masters of Science in Applied Chemistry (Medicinal Chemistry). Therefore, I recommend the submission of a revised version of the thesis to the department following the applicable procedures.

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## APPROVAL PAGE

I, the advisor of the thesis entitled "*Phytochemical Investigations, Antibacterial and Antioxidant Activities, and In Silico Molecular Docking Studies of Compounds Isolated from the Leaf Extracts of Salvia leucantha CAV*." and developed by Urael Getachew, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

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We, the undersigned, members of the Board of Examiners of the thesis by Urael Getachew have read and evaluated the thesis entitled "*Phytochemical Investigations, Antibacterial and Antioxidant Activities, and In Silico Molecular Docking Studies of Compounds Isolated from the Leaf Extracts of Salvia leucantha CAV*." and examined the candidate during the open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied Chemistry (Medicinal Chemistry).

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AMR | Antimicrobial Resistance |
| CC | Column Chromatography |
| CDC | Centers for Disease Control and Prevention |
| $CDCl_3$ | Deuterated chloroform |
| $^{13}C$ NMR | Carbon 13-Nuclear Magnetic Resonance |
| DEPT | Distortion less Enhancement by Polarization Transfer |
| DPPH | 1-1- diphenyl-2-picrylhydrazyl |
| DMSO | Dimethyl Sulfoxide |
| EUCAST | European Committee on Antimicrobial Susceptibility Testing |
| ESBL | Extended-Spectrum Beta-Lactamase |
| FTIR | Fourier Transform-Infra-Red |
| $^{1}H$ NMR | Proton-Nuclear Magnetic Resonance |
| HAIs | Hospital-Acquired Infections |
| $IC_{50}$ | Half-maximal inhibitory concentration |
| GC-MS | Gas Chromatography-Mass Spectrometry |
| LD50 | Half-maximal Lethal Dose |
| MBC | Minimum Bactericidal Concentration |
| MDR | Multi-drug resistant |
| MIC | Minimum Inhibition Concentration |
| MRSA | Methicillin-Resistant Staphylococcus Aureus |
| MHA | Muller Hinton Agar |
| PDA | Potato Dextrose Agar |
| TLC | Thin Layer Chromatography |
| TM | Traditional Medicine |
| UV | Ultra Violet-Visible |
| WHO | World Health Organization |

## *ABSTRACT*

*Since ancient times, medicinal plants have been used around the world to treat a wide range of diseases. Traditionally, Salvia leucantha has been used as a remedy to relieve cough, chest, lung, and stomach pains. In this study, we used sequential extraction and silica gel column chromatography method for isolation of compounds. The essential oil obtained by hydro-distillation was analyzed using GC-MS. The antibacterial and antioxidant activities were evaluated using the agar disc diffusion method against four strains of bacteria and DPPH methods, respectively. The leaf of Salvia leucantha (450 g) was successively extracted with n-hexane, ethylacetate, and methanol to afford 19.4 g (4.3%), 29.1 g (6.4%), and 16.6 g (3.6%) yields, respectively. The GC-MS result revealed that the presence of 50 components, with compound* ***(40)****, identified as the most abundant constituent (36.3 %) of the oil. The methanol extract affords three compounds, identified as hesperidin* ***(44),*** *urosilic acid* ***(45)****, and β-sitosterol* ***(46)****. All compounds are reported herein for the first time from the Salvia leucantha. The structures of the isolated compounds were characterized using NMR ($^{1}H$ NMR, $^{13}C$ NMR, and DEPT-135) spectroscopic techniques. The n-hexane and ethylacetate extracts showed the highest antibacterial activity against E. coli (20.5±0.70 mm at 200 mg/mL) and S. aureus (24.5±0.70 mm at 200 mg/mL), respectively. Whereas methanol extract exhibited the highest zone of inhibition (23.0±0.00 mm at 200 mg/mL) against P. aeruginosa. Compounds* ***44*** *and* ***45*** *showed significant activity against S. aureus (24.50±0.70 mm) and S. pyogenes (*24.50±2.12 *mm) respectively. Compound* ***46*** *also exhibited promising inhibition activity against S. pyogenes (22.50±0.70 mm) each at 2 mg/mL, compared with ciprofloxacin (ranging from* 33.20±0.44–34.50±1.06 *mm at 30 μg/mL). The highest percent inhibition of the DPPH radical was observed with methanol extract ($IC_{50}$: 12.17 μg/mL) and compound* ***44*** *($IC_{50}$: 9.83 μg/mL), compared with ascorbic acid ($IC_{50}$: 0.681 μg/mL). In a molecular docking study, compound* ***44*** *and compound* ***45*** *exhibited the highest binding affinity (-9.0 kcal/mol) with the target DNA gyrase B and compound* ***44*** *exhibited (-10.3 kcal/mol) with pyruvate kinase as the target. Additionally, compounds* ***45*** *and* ***44*** *showed higher binding affinity (-10.5 and -9.9 kcal/mol respectively) against human myeloperoxidase. The drug-likeness analysis showed that compounds* ***45*** *and* ***46*** *satisfy Lipinski's rule of five with one violation. The current study generally revealed that both the extract and isolated compounds have promising antibacterial and antioxidant activity.*



***Keywords:*** *Salvia leucantha, Antibacterial, Antioxidant, Molecular docking, ADMET*

# 1. INTRODUCTION

## 1.1. Background

According to World Health Organization (WHO) estimates, there are approximately 56 million people's deaths annually worldwide. The death from infectious diseases accounts for almost one third of all deaths. The most encountered infections are caused by microorganism, particularly those caused by drug resistant bacteria and/or fungi, HIV/AIDS, malaria, and others (Marshall, 2014). Irrational use of antibiotics is an ongoing global public health problem that deserves more attention. WHO estimates that globally more than 50% of all medicines are prescribed, dispensed, or sold inappropriately (Muhie, 2019). This irrational use of antibiotics results in the development of antimicrobial resistance. Antimicrobial resistance (AMR) is defined as the inherited or acquired ability of a microorganism to halt the antimicrobial drug from working against it to the extent that it cannot be used any longer. The available drugs used to treat microbial infections become less effective or ineffective and lead to the persistence and spread of the resistant organisms causing infections (WHO Regional Office for Africa, 2021).

Rates of antimicrobial resistance among community and hospital pathogens have increased considerably. This is especially more common in clinic settings in which resistant strains are frequently found before they spread to the community (Cantón *et.al.,* 2013). The issue has received high attention from various organizations, including WHO, Infectious Diseases Society of America, who recognized antimicrobial resistance (AMR) as one of the greatest threats to human health worldwide (Llor & Bjerrum, 2014).

Highly reactive free radicals and oxygen species are found in biological systems (Sies *et.al*, 2017). The oxidative stress leads to the production of reactive oxygen species (ROS), and their removal leads to oxidative damage of cell membranes, mitochondria, lipids, and proteins. Free radical damage related to oxidative stress causes various diseases, including atherosclerosis, chronic obstructive pulmonary disease (COPD), Alzheimer's disease, and cancer. Antioxidants have been used to avoid oxidative stress (Raghu *et.al.*, 2019) (Makhaeva *et.al.*, 2017).

Medicinal plants have always been and still are a valuable source of therapeutic agents. Historically, drug discovery relied exclusively on natural products as the primary source of medicines that have significantly influenced the advances in biology and inspired drug discovery and development. Naturally derived drugs are those that contain pharmacologically

active ingredients derived from biological or mineral sources and they are used for the diagnosis, cure, mitigation, and prevention of diseases (Ayoola *et.al.,* 2008; Nuru & Hepburn, 2001).

The active components of these medicinal plants are usually secondary metabolites. These include classes of compounds such as flavonoids, terpenes, alkaloids, glycosides, saponins, resins, essential oils, etc. (Saroya, 2011)(Edeoga. *et.al.,* 1989). These compounds are generally seen as having greater "drug-likeness and biological friendliness" than completely synthetic compounds, which makes them promising candidates for further research (Kumaraswamy *et.al.,* 2008).

According to the WHO, almost 70% of the world and 80% of developing countries have relied on plant based integrated traditional medicine (TM) into their primary health care systems for both humans and animals. The WHO has set precise guidelines for the evaluation of the safety, efficacy, and quality of herbal medicines (World Health Organization (WHO), 2023).

In Ethiopia, plants have been used as a source of medicine from the time immemorial to treat different ailments. The nation has a wide biological resource, with around 6,500 species of higher plants, roughly 12% of which are indigenous, making it one of the six plant biodiversity rich regions (Tadele Henif, 2017). Due to its long history, traditional medicine has in fact become an integral part of the culture. Despite its significant contribution to society, traditional medicine experienced very little attention in modern research and development (Giday, 2001).

*Salvia leucantha* Cav is a species originally from Mexico and now found in many parts of the world (Rojas *et.al.,* 2010). It is an herbaceous shrub that can reach up to 1.20 m in height and has a pleasant smell due to the presence of a fragrant volatile fraction. The plant has been used in traditional medicine as a remedy to relieve cough, chest pain, lung pain, and stomach pains, as well as a garden plant in many parts of the world (Villalta *et.al*., 2021). In Ethiopia, *Salvia leucantha* is widely distributed in different regions and it's locally known as *'Habaaboo'* in Afan Oromo and traditionally the plant is used as an ingredient in the preparation of foods and as an ornamental plant in gardens to enhance the aesthetic environment.

## 1.2. Statement of the Problem

Since the discovery of penicillin, antimicrobials have saved millions lives and also eased the pain and suffering of human illness. However, after more than 75 years of widespread use, many antimicrobial agents are not as effective as they used to be. The WHO forecasts 13 million deaths will be attributed to infectious disease in 2050 (Guarango, 2022). The most recent estimates, a total of 700,000 people die every year from drug resistant strains of common infection organisms (Macintyre & Bui, 2017).

In America, methicillin-resistant *Staphylococcus aureus* (MRSA) alone is the significant cause of death each year more than combinations of emphysema, HIV/AIDS, Parkinson's disease, and homicide (Llor & Bjerrum, 2014). The increasing prevalence of hospital and community-acquired infections caused by multidrug-resistant (MDR) bacterial pathogens is the limiting option for effective antibiotic therapy. Moreover, this alarming spread of antimicrobial resistance has not been paralleled by the development of novel antimicrobials (Cassir *et.al.*, 2014).

In low and middle-income countries, the magnitude and burden of HAIs remains underestimated or mostly even unknown, since it needs complex diagnosis and surveillance activities to guide interventions, which require expertise and resources. In Thailand, an estimated 19,000 deaths occur each year in patients with HAI due to multi-drug resistant bacteria (*E. coli* and *K. pneumoniae* were the most common causes). Which is about 3 to 5 times larger than those of the USA and European Union countries (Lim *et.al.*, 2016).

In Africa, the overall burden of AMR is not well documented due to inadequate data. According to a recent WHO report, the results show that among gram-negative bacteria, *Klebsiella spp*. and *E. coli* reported that they have a high resistance percentage for recommended first- and second-line antibiotics: amoxicillin (24.5%), ampicillin (23.5%), cotrimoxazole (22.5%), amoxicillin/clavulanic acid (13.2%), and chloramphenicol (12.3%). Resistance to ciprofloxacin was (8.2%). Low resistance rates were reported for imipenem (0.1-3%) and meropenem (0.1-2.5%) in gram-negative bacteria. For gram-positive bacteria, *Streptococcus pneumoniae* show high resistance percentages against the key tested antibiotics: cotrimoxazole (64.3%), penicillin (23.2%), tetracycline (28.3%), amoxicillin (20.6%), ampicillin (19.3%%), chloramphenicol (19.3%), amoxicillin/clavulanic acid (17.4%), ciprofloxacin (14.8%), gentamicin (13.5%), doxycycline (1.9%), and erythromycin (1.9%) (WHO Regional Office for Africa, 2021).

According to another study, *E. coli and Klebsiella pneumoniae* which are recovered from urine and blood specimens of the community and unknown origin, had a rate of resistance to ciprofloxacin, were 67.4% and 59.3% in Ethiopia, 63.5% and 36.2% in Sudan and 47.5% and 16.7% in Uganda respectively (Alemu *et.al.*, 2021).

In Ethiopia, there is no comprehensive research that presents the whole picture. However, according to a study by Dessise *et.al. 2016* which is conducted in selected referral hospitals in Addis Ababa shows that, more than 75% Gram negative isolates were multiple antibiotic resistant. Among them, 95.7% of *Acinetobacter*, 83.3% of *E. coli*, 66.7% of *P. aeruginosa* and 60% of *K. pneumoniae* species were resistant to more than five tested antibiotics. *Acinetobacter* species (34.8%) and *E. coli (*12.5%) are Pan-antibiotic resistance. Gram positive (50%) isolates were resistant to at least one of the antibiotics tested. From Gram positive *S. aureus* (5.3%) isolates were found to be resistant to at least five of the antibiotics tested (Dessie *et.al.*, 2016).

To address this major challenge of drug resistance, it is extremely important to undergo urgent and intensive research to come up with the best quality, efficacious, safe, and affordable antibiotics from natural products particularly medicinal plants. Nowadays, medicinal plants are major sources of drugs for preventing the spread of a wide range of pathogenic carriers and also treating various diseases of human beings.

Over-production of reactive oxygen species (ROS) results in oxidative stress, which is a harmful process that damages cell structures, including lipids, membranes, proteins, and DNA (Putri *et.al.*, 2018). Oxidative stress has been culpably involved in various diseases, including atherosclerosis, cancer, cardiovascular, and neurodegenerative disorders (Raziq *et.al.*, 2017). In recent years, there have been limitations in using synthetic antioxidant compounds in food products because of their side effects. The *in vivo* carcinogenic effects of synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) have been posing another problem that many researchers also worked on. Therefore, significant effort has been spent to find out natural antioxidants over synthetic compounds and the elimination of synthetic antioxidants (Bhatti *et.al.*, 2015). As a result, natural sources have become more important to find proper and safe antioxidants.

In fact, in recent years there has been a renaissance in the consumption and demands of herbal products in both developed and developing countries. In developed countries, this may be partly due to the dissatisfaction and lack of a complete cure with conventional medicines.

Whereas, with the developing countries due to lack of medical doctors, shortage of modern medicine, and unaffordable prices of pharmaceutical products, widely acceptance, trust, and respect of traditional practitioners might be the possible causes for the revival of interest in the use of medicinal plants (Raziq *et.al.,* 2017)(Pathak & Das, 2017).

Even though *Salvia* genus is one of the most identified and studied taxa of the Lamiaceae family, the knowledge regarding the chemical composition and health-related benefits of some *Salvia* species is still scarce (Mocan *et.al.,* 2020). *Salvia leucantha* is one of these salvia species and it is a promising plant that shows different biological activity and has ethno medicinal use in different parts of the world. To the best of our knowledge, in Ethiopia *Salvia leucantha* is the less explored medicinal plant. Despite its traditional use for treatment of different disease, there is no scientific study that is conducted and reported on this medicinal plant in Ethiopia. In particular, there is no investigation done on its biological activity, isolation of its bioactive compounds and *in-silico* studies. So, it is deemed important to study the phytochemistry, biological activity and *in-silico* biological activity of *Salvia leucantha.*

## 1.3. Objectives of the Study

### 1.3.1. General objective

The overall objectives of this study were to conduct phytochemical investigation, analysis of essential oil components, extract, isolate, and characterize the chemical constituents, *in-vitro* antibacterial, and antioxidant activity evaluation, and *in-silico* biological activity studies of isolated compounds from the leaf extracts of *Salvia leucantha.*

### 1.3.2. Specific objectives

The specific objectives of this study were as follows:

- To successively extract the leaf part of *Salvia leucantha* using n-hexane, ethylacetate, and methanol.
- To isolate compounds from the n-hexane, ethylacetate, and methanol leaf extracts of *Salvia leucantha* using silica gel column chromatography.
- To extract the essential oil from the leaf parts of *Salvia leucantha* by hydrodistillation method.
- To determine the chemical composition of the essential oil by using GC-MS.
- To elucidate the structures of isolated compounds using spectroscopic techniques including UV-Vis, IR, mass, and NMR spectroscopy in comparison with literature data.

- To evaluate the antibacterial activities of the extracts and isolated compounds from the leaf parts of *Salvia leucantha* against gram negative bacteria *(Pseudomonas aeruginosa and Escherichia coli)* and gram-positive bacteria (*Staphylococcus aureus and Streptococcus pyogenes)* by using the disc diffusion method.
- To evaluate the antioxidant activity of the extracts and isolated compounds from the leaf parts of *Salvia leucantha* against DPPH.
- Conduct *in silico* molecular docking analysis of the isolated compounds using Auto-Dock 4.2.6 (MGLTools-1.5.6) software program on selected protein targets *E. coli* Gyrase B (PDB ID: 7P2W), *S. aureus* Pyruvate Kinase (PDB ID: 3T07) and Human myeloperoxidase (PDB ID: 1DNU).
- To predict in silico drug likeness and ADMET profile of the isolated compound using Swiss ADME and ProTox-II tools.

### 1.4. Significance of the Study

This study is expected to provide

- Documentary information focused on the phytochemical investigation, isolation of compounds, structural elucidation, evaluation of antibacterial, and antioxidant activity, and molecular docking with pharmacokinetics and toxicity studies of isolated compounds from the leaf part of *Salvia leucantha*.
- Valuable information observed from *in vitro* activity with *in silico* molecular docking analysis on targets proteins of selected strains of microorganisms, disease target and their pharmacokinetic & drug likeness predictions.
- Arrive at promising bioactive compounds to use as lead compounds for drug discovery.
- Baseline information for future pharmacological studies for researchers who are interested to conducting further work on the *Salvia leucantha*.

# 2. LITERATURE REVIEW

## 2.1 Lamiaceae Family

Medicinal plants and the constituents isolated from those plants were used for hundreds of years as sources of biologically active novel compounds. Statistically, around 50% of the new chemical entities are either obtained from natural products or natural product analogues(Li & Vederas, 2009). Natural products currently have considerable significance due to their unique attributes as sources of therapeutic phytochemicals and their efficacy, safety, and minimal side effects (Nn, 2015).

Lamiaceae (formerly known as Labiatae) is a widespread family, containing about 235 genera and 7170 species distributed throughout in most part of the world as annuals and perennial plants. The chemistry of the Lamiaceae is very extensive and dominated by reports concerning the volatile oils (mainly monoterpenes and sesquiterpenes) found in genera of economic importance, but chemical constituents such as diterpenes (mainly abietane and clerodane diterpenes) and triterpenes, phenolics, and others may offer great significance as taxonomic characters and biologically active compounds with a potential ecological role (Villalta *et.al.,* 2021).

## 2.2 The Genus *Salvia*

The genus *Salvia* is one of the largest genera of the Lamiaceae, with 1000 species distributed throughout the world (Mocan *et.al.,* 2020) The name Salvia is derived from the Latin "salvare," meaning "to heal or to be safe", which summarizes the folkloric belief of its "magical" therapeutic properties for many kinds of ailments and its popularity in traditional medicine. It is also known by the common name sauge (sage) in French and sawge in old English. The genus *Salvia* is widely distributed in several regions of the world including the temperate and warmer zones such as the Mediterranean, Central Asia, the Pacific Islands, Africa (in Africa many species are found in the northwest and the southern parts, whereas absent from most of western and central tropical Africa), and America (Guerrero, 2005) (Narukawa *et.al.,* 2006) (G. W. Wang *et.al.*, 2013).

Many species in the genus *Salvia L.* are noted for their brightly colored flowers, which are typically pink to red or purple to blue (G. W. Wang *et.al.,* 2013). The genus could be considered highly dynamic for a large variety of medicinal, aromatic, cosmetic, and ornamental applications, both in traditional and advanced uses (Mocan *et.al.*, 2020).

Many *Salvia* species have economic importance because they are used as herbal tea and for food flavoring, as well as in cosmetics, perfumery and the pharmaceutical industries throughout world (Coisin *et.al.*, 2012). The genus *Salvia* has recently attracted great attention due to its remarkable biological activities, so that it has been the subject of numerous chemical studies (Zengin *et.al.*, 2018)(Lu & Foo, 2002).

### 2.2.1 *Salvia leucantha*

*Salvia leucantha* commonly known as ‘‘Mexican Bush sage’’, is native to Mexico and now distributed worldwide. It is an evergreen herbaceous shrub that can reach up to 1.20 m in height; the lanceolate leaf blades are 10–20 cm long and are grayish-green with a velvety texture. Leaves are aromatic with purple chalice and purple or white corolla that are covered by thin, velvety hairs. It has slight roasted nut or chocolate scent, but is not used for culinary purposes. The plant has a pleasant smell due to the presence of a fragrant volatile fraction. *S. leucantha* can withstand being grown in a plateau region as an introductory plant at an altitude of 1400-2200 (in some papers it says 500-1300) meters above sea level. The plant is used in traditional medicine as a remedy to relieve cough, chest, lung, and stomach pains, as well as a garden plant (Aoyagi *et.al.*, 2008)(Ali *et.al.*, 2015)(Inderaja *et.al.*, 2018).

**Figure 1:** Photograph of *Salvia leucantha CAV* growing in Bokoji, Ethiopia.

### 2.2.2 Botanical Description of *Salvia leucantha CAV*

**Kingdom**: Plantae (Plants)

**Division**: Magnoliophyta (Angiosperms)

**Class**: Eudicots Magnoliopsida (Dicotyledons)

**Order**: Lamiales

**Family**: Lamiaceae

**Genus**: *Salvia*

**Species**: *Salvia leucantha*

## 2.3 Ethnobotanical Uses of the Genus *Salvia*

*Salvia* is a large and widespread genus with a diversity of ethnobotanical uses. Since ancient times, many *Salvia* species have been used in folk medicine all around the world for centuries, especially by Mexico and Chinese peoples. This is an herb that has the reputation as one that wards off evil. It was thought to be efficacious against the biting of serpents and the dispelling of evil spirits. In ancient Egypt, it was employed to increase the fertility of women (Guerrero, 2005).

Traditionally the genus has been used for treatment of coronary artery diseases (CAD), angina pectoris, myocardial infarction, cerebrovascular diseases, for various types of hepatitis, and liver fibrosis, chronic renal failure, dysmenorrhea, miscarriage, hemorrhage, sexual debility, swelling, insomnia, inflammatory diseases, tuberculosis, psoriasis, seborrheic eczemas, diarrhea, gonorrhea, hemorrhoids, eye diseases, nervous disorders, dizziness, trembling, guinea worm, and itch. It also shows antibacterial, antifungal, hallucinogenic, wound healing, antiseptic, and antispasmodic activity. The stem, roots, leaves, flowers and sometimes the aerial parts are used in the formulation of those traditional medicine (Askari *et.al.*, 2021)(Guerrero, 2005)(Juárez-Vázquez *et.al.*, 2013) (G. W. Wang *et.al.*, 2013).

In Ethiopia, even though there is no single paper reported and published on *Salvia leucantha*, the plant is widely distributed in different parts of the country. Traditionally, the plant is used as an ingredient in the preparation of food in some regions and as an ornamental plant in gardens to enhance the aesthetic environment in many parts of the country.

## 2.4 Phytochemical Studies

Phytochemicals are produced by plants via primary and secondary metabolism. The latter are produced either as a result of the organism adapting to its surrounding environment or for its own survival and defense against predators. Knowledge about the chemical constituents of a medicinal plant is important for optimizing extraction procedures, understanding pharmacological activity as well as potential toxicity and interaction with pharmaceutical drugs. Medicinal plants are rich sources of bioactive phytochemicals. Studies carried out during the past 2–3 decades have shown that these phytochemicals have an important role in preventing chronic diseases like cancer, diabetes, coronary heart disease and many more (Anode *et.al.*, 2018)(Kurmukov, 2013).

The medicinal plants from this family are also notable by their richness in secondary metabolites, especially monoterpenoids, diterpenoids (mainly with an abietane or clerodane skeleton), triterpenoids, and flavonoids (G. W. Wang *et.al.*, 2013).

The phytochemical studies of *S. leucantha* are dominated by essential oil components of the plant. According to a few studies, carried out in Asia and South America on the chemical composition of the essential oil from *S. leucantha,* sesquiterpene hydrocarbons have been identified as the major components(Negi *et.al.*, 2007).

The aerial parts of *Salvia leucantha* were collected from south Ecuador and essential oil was isolated by steam distillation method. After GC-MS and GC-FID analysis, six major compounds were identified, namely, the sesquiterpenes-6, 9-guaiadiene (19.14%), *(E)*-caryophyllene (16.80%), germacrene D (10.22%), *(E)*-β-farnesene (10.00%), and bicycle germacrene (7.52%), and the monoterpenoid bornyl acetate (14.74%). Furthermore, four pairs of enantiomers were determined by enantioselective GC-MS of the essential oil. (-)-germacrene D and (+)-α-pinene showed the highest enantiomeric excess (Villalta *et.al.*, 2021).

Another studies which was conducted in Mexico, revealed that two new diterpenoids with rearranged skeletons, salvileucantholide **(1)** and salvianduline E **(2)** were isolated along with salvigenane diterpenoid (isosalvipuberulin) (**3)** and salvifaricin (**4)** from the aerial parts of *S. leucantha* (Esquivel *et.al*., 1994)(G. W. Wang *et.al*., 2013).

According to Narukawa *et.al.(*2006) a new diterpenoid with a rearranged neoclerodane skeleton, spiroleucantholide **(5)**, along with four known diterpenoids compounds mention in the above studies (compounds **1- 4**) was isolated from the aerial parts of *S. leucantha*

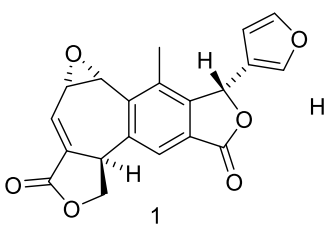
(Narukawa *et.al.*, 2006). Whereas Rajamanickam *et.al*. (2013), from the flower parts of *Salvia leucantha* a flavonoid know as quercetin-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside **(28)** isolated by using 90 % methanol (Rajamanickam *et.al.*, 2013).

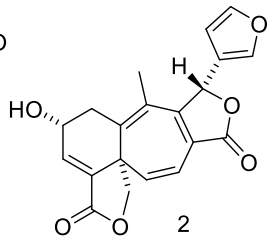
Another study conducted by Aoyagi *et.al*.(2008) able to isolate a caged polycyclic neo clerodane known as salvileucalin B **(6)** along with biosynthetically correlated salvileucalin A **(7)** from an acetone extract of the aerial parts of *Salvia leucantha* (Aoyagi *et.al.*, 2008).

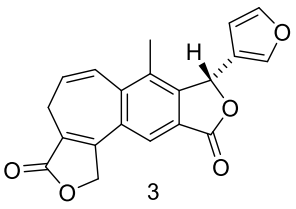
**Table 1:** Compound isolated from different *salvia* species.

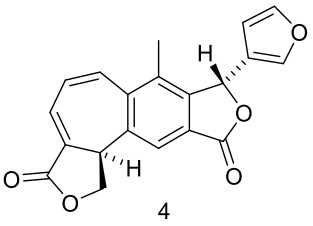
| Isolated Compounds | Species | Structure number | References |
|---|---|---|---|
| **Diterpenoids** | | | |
| Salvileucantholide | *S. leucantha* | **1** | (Esquivel *et.al.*, 1994) |
| Salvianduline E | " | **2** | " |
| Isosalvipuberulin | " | **3** | " |
| Salvifaricin | " | **4** | " |
| Spiroleucantholide | " | **5** | (Narukawa *et.al.*, 2006). |
| Salvileucalin B | " | **6** | (Aoyagi *et.al.*, 2008) |
| Salvileucalin A | " | **7** | " |
| O-methylpisiferic acid | *S.blepharochalena* | **8** | (Ulubelen *et.al.*, 2001) |
| Candelabrone | *S.palaestina* | **9** | (Wu *et.al.*, 2012) |
| Forskalinone | *S.forskahlei* | **10** | (Ulubelen *et.al.*, 1996) |
| 1-oxoferruginol | *S.napifolia* | **11** | (Ulubelen *et.al.*, 1995) |
| Methyl-12-O-methylcarnosoate | *S.africana-lutea* | **12** | (Hussein *et.al.*, 2007) |
| Inuroyleanol | *S.barrelieri* | **13** | (Kabouche *et.al.*, 2007) |
| Horminone | *S.belpharochlaena* | **14** | (Ulubelen *et.al*., 2001) |
| 7-acetylhorminone | " | **15** | " |
| Royleanone | *S.anastomosans* | **16** | (Díaz-Fernández *et.al.*, 2019) |
| Candelabroquinone | *S.candelabrum* | **17** | (Moridi Farimani *et.al.*, 2019) |
| 7-oxoroyleanone-12-methyl ether | *S.barrelieri* | **18** | (Kabouche *et.al.*, 2007) |
| Candesalvoquinone | *S.candelabrum* | **19** | (Moridi Farimani *et.al.*, 2019) |
| Candesalvone B methyl ester | " | **20** | " |

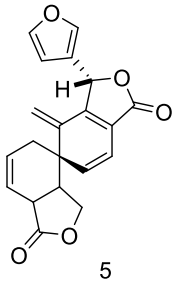
| | | | |
|---|---|---|---|
| 7,8-seco-para-ferruginone | *S.prionitis* | **21** | (X. Chen *et.al.,* 2002) |
| 8(17),12E,14-labdatrien-6,19-olide | *S.leriaefolia* | **22** | (Habibi *et.al.,* 2000) |
| **Triterpenoids** | | | |
| Oleanolic acid | *S.officinalis* | **23** | (Kumiko *et.al.,* 2007) |
| **Polyphenols** | | | |
| Salvianolic acid B | *S.miltiorrhiza* | **24** | (Tian *et.al.,* 2008) |
| Salvianolic acid A | *S.yunnanensis* | **25** | (Z. F. Zhang *et.al.,* 2008) |
| Lithospermic acid | " | **26** | " |
| Octanol ester of cis-O-methyl caffeic acid dimer | *S.forskahlei* | **27** | (Ulubelen *et.al.,* 1996) |
| Rosmarinic acid | *S.officinalis* | **28** | (Cuvelier *et.al.,* 1994) |
| **Quinone** | | | |
| Cryptotanshinone | *S.miltiorrhiza* | **29** | (Yasuhiko Koezuka, 1988) |
| Dihydrotanshinone I | " | **30** | " |
| Hydroxytanshinone II | " | **31** | " |
| Methyltanshionate | " | **32** | " |
| Tanshinone II-B | " | **33** | " |
| **Phenolic diterpenes** | | | |
| Carnosic acid | *S.officinalis* | **34** | (Cuvelier *et.al.,* 1994) |
| Carnosol | " | **35** | " |
| Rosmanol | " | **36** | " |
| Epirosmanol | " | **37** | " |
| **Flavonoid** | | | |
| Quercetin-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside | *S. leucantha* | **38** | (Rajamanickam *et.al.,* 2013) |
| Quercetin-3-O-α-L-rhamnopyranoside-2″-gallate | " | **39** | (Manivannan *et.al.,* 2014) |

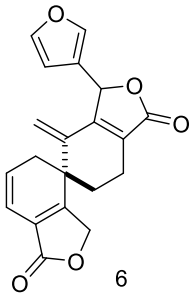


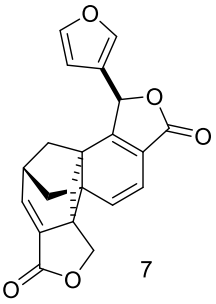



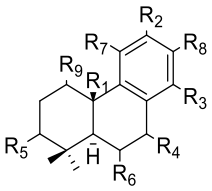



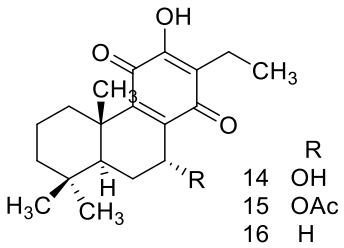



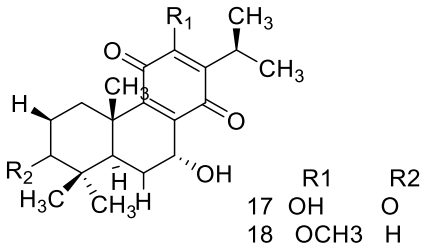



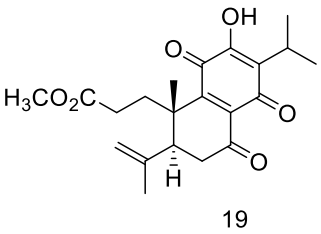



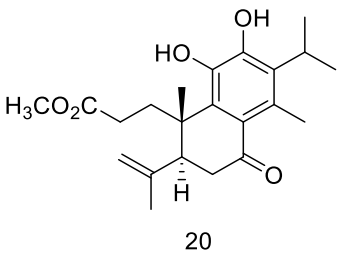



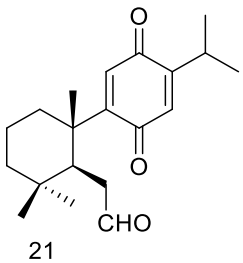
| | R-1 | R-2 | R-3 | R-4 | R-5 | R-6 | R-7 | R-8 | R-9 |
|---|---|---|---|---|---|---|---|---|---|
| 8 | CO2H | OCH3 | H | H | H | H | H | *i*-Pr | H |
| 9 | CH3 | OH | OH | O | O | H | OH | *i*-Pr | H |
| 10 | CH2OH | OCH3 | OH | O | H | O | OH | *i*-Pr | H |
| 11 | CH3 | OH | H | H | H | H | H | *i*-Pr | O |
| 12 | CO2CH3 | OCH3 | H | H | H | H | OH | *i*-Pr | H |
| 13 | CH3 | OCH3 | OH | O | H | H | OH | *i*-Pr | H |



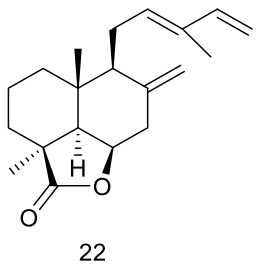




**Figure 2:** The chemical structure of diterpinodes compounds (**1-22**) isolated from *salvia* genus.

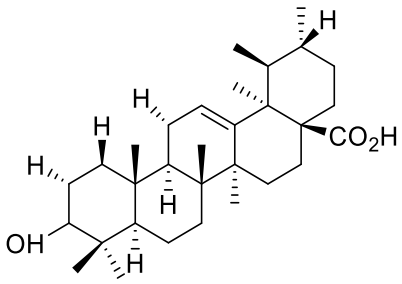



**Figure 3:** The chemical structure of compound **23** isolated from *salvia* genus.

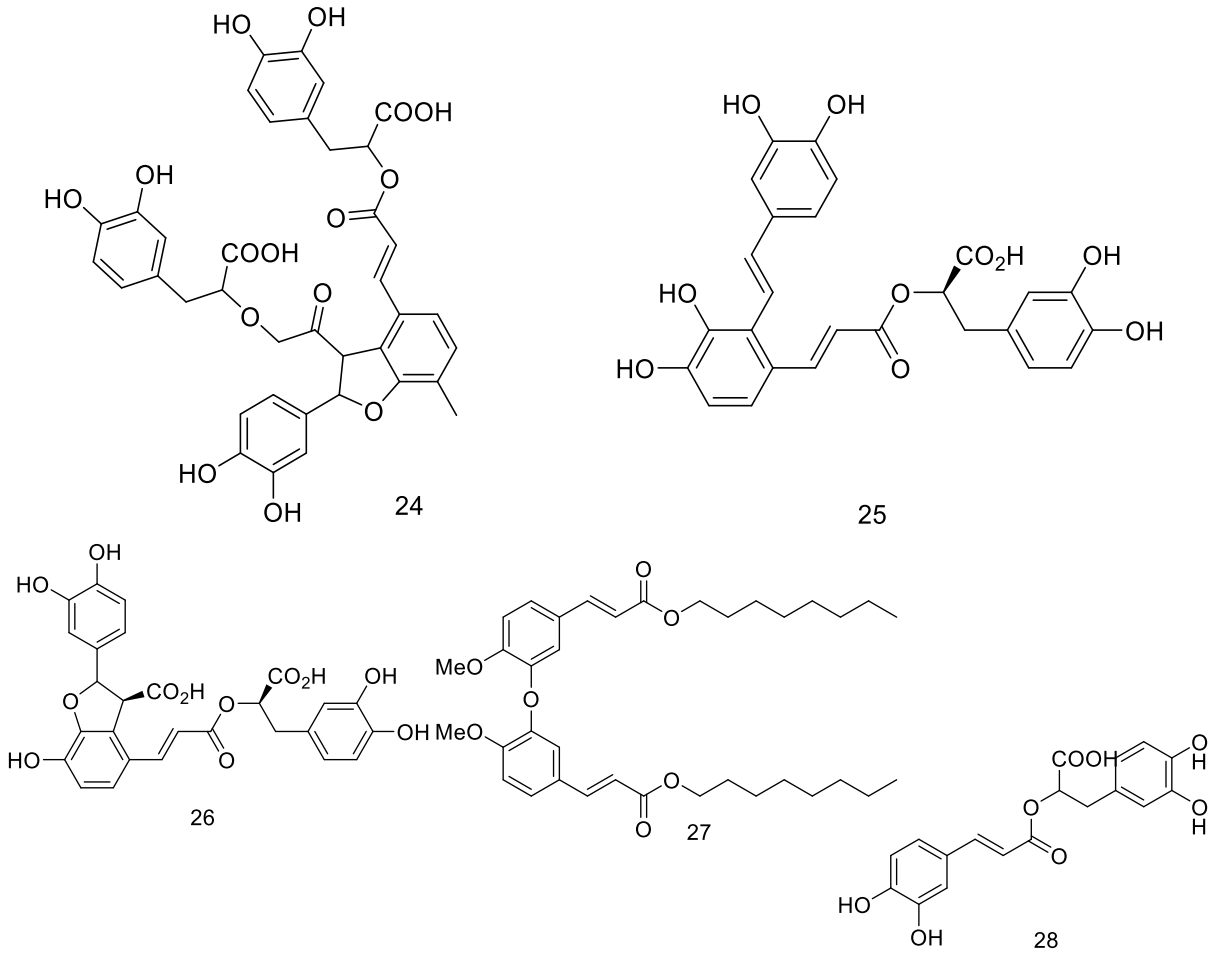



**Figure 4:** The chemical structure of polyphenols compounds (**24**-**28**) isolated from *salvia* genus.

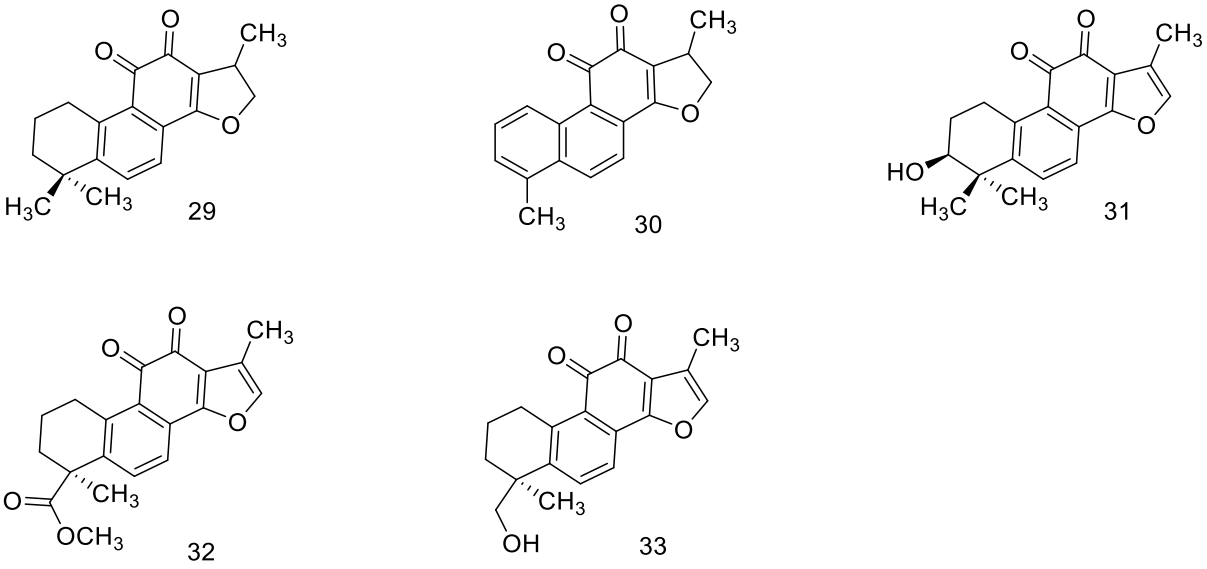



**Figure 5:** The chemical structure of quinone compounds (**29-33**) isolated from *salvia* genus.

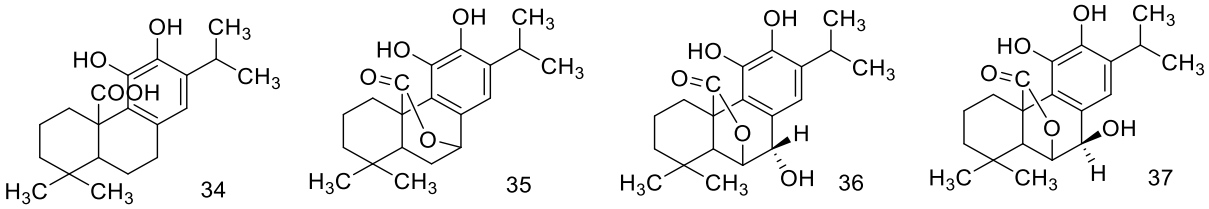



**Figure 6:** The chemical structure of phenolic diterpenes compounds (**34-37**) isolated from *salvia* genus.

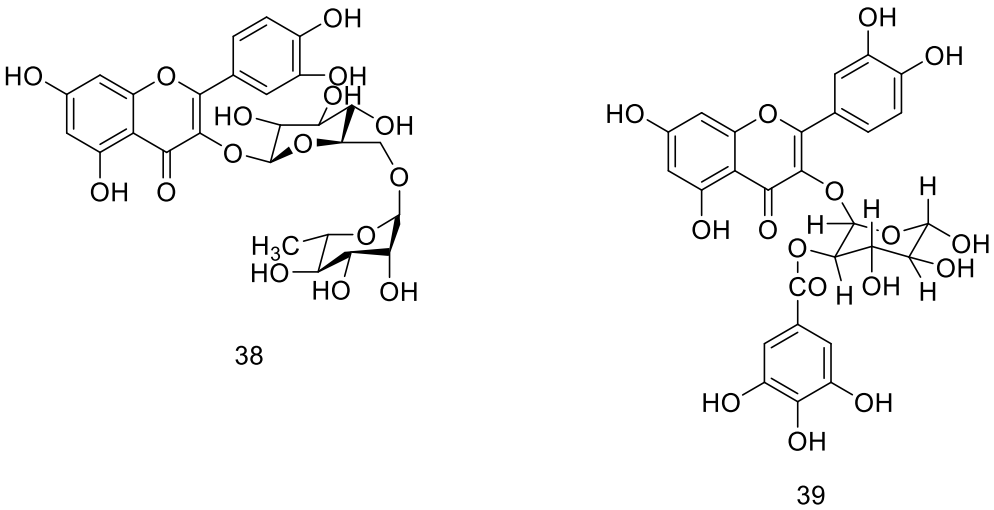



**Figure 7:** The chemical structure of glycosylated flavonoids compounds (**38 & 39**) isolated from *salvia* genus.

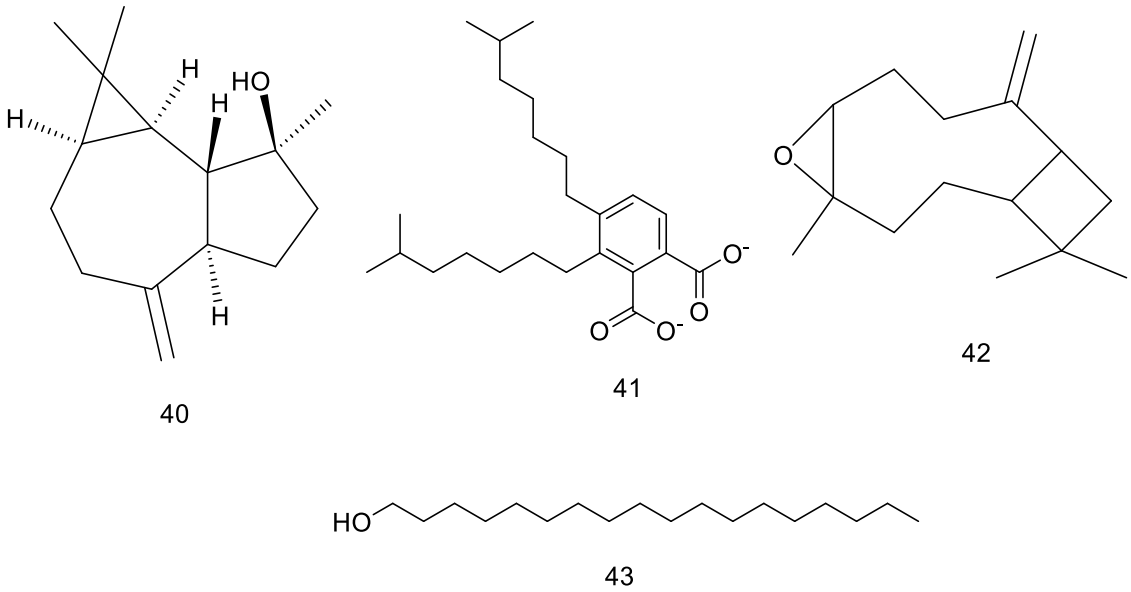



**Figure 8:** The chemical structure of essential oil components (**40-43**) isolated from leaf of *Salvia leucantha*.

## 2.5 Biological Activities of the Genus *Salvia*

Several Lamiaceae have a high pharmacological value by producing of a wide range of secondary metabolites with antioxidant, antiinflammatory, antimicrobial, antiviral, and anticancer properties (Carović-Stanko *et.al.*, 2016). Many diterpenoids isolated from this *salvia* genus have shown various biological activities, which include antioxidant, antifeedant, antibacterial, antimutagenic, anti-inflammatory, antiplatelet aggregation activities and cytotoxic properties (G. W. Wang *et.al.*, 2013).

In the past few decades, *Salvia* constituents have attracted considerable attention from medicinal chemists and clinicians as antimicrobial, antioxidant, antitumor, and antifeeding agents. Many natural Salvia constituents from different species, as well as semisynthetic derivatives, have been tested by many research groups. A few have shown very potent activity against bacteria and tumor cell lines (Carović-Stanko *et.al.*, 2016)(G. W. Wang *et.al.*, 2013)(Xu *et.al.*, 2018).

The in vitro assay from the essential oil of *Salvia leucantha* demonstrated an interesting inhibitory activity of the enzyme butyrylcholinesterase, with an $IC_{50}$ = 32.60 µg/mL, which is the highest determined for a *Salvia* species. In contrast, the oil was weakly active against acetylcholinesterase with an $IC_{50}$ > 250 µg/mL. The essential oil of S. *leucantha* can therefore be considered as a source of bioactive phytochemicals and phytotherapeutics for humans due to its potential anti-Alzheimer activity (Villalta *et.al.*, 2021).

### 2.5.1 Antibacterial Activity

Extensive literature on the antimicrobial potency of the *Salvia* genus reveals a broad variability with regard to microorganisms' sensitivity and efficiency (measured as minimal inhibitory concentration) of tested compounds when different species are considered. Essential oils with volatile monoterpenoids, which are the major constituents of this genus, are reported to have antibacterial activity in those *Salvia* species that are rich in essential oils (Guerrero, 2005).

According to a study in Egypt, essential oils of six *salvia* spices (sage, rosemary, caraway, cumin, clove, and thyme) and their basic ingredients were tested for their inhibitory effect against three strains of Gram-negative bacteria and four strains of Gram-positive bacteria. The result showed that at very low concentrations (0.25 - 12 mg/ml) of, the various essential oils were sufficient to prevent microbial growth. Thyme and cumin oils possessed very strong antimicrobial activity compared with the other essential oils. And the data also suggest that the essential oils under study and in particular thyme and clove oils, can be applied practically as antimicrobial agents and as food treatments that will prevent the deterioration of stored foods (Lisin *et.al.*, 1999).

According to Deans and Ritchie (1987), they tested fifty essential oils from *S. officinalis L* against 25 genera of bacteria. All 50 plant essential oils were showing inhibitory activity to at least one test bacterium in the undiluted form. 41 of them (oils) were inhibitory to 5 or more genera, while 33 oils were inhibitory to 10 or more of the 25 test organisms. The result revealed that the essential oil (undiluted) was moderately effective against the growth of *Bacillus subtilis, Brevibacterium linens, Micrococcus luteus,* and *Seratia marcescens* bacteria (Deans & Ritchie, 1987).

Significant antibacterial activity was observed towards gram-negative bacteria *Klebsiella pneumoniae* (400 µg/ml) and against gram-positive bacteria *Bacillus subtilis* (300 µg/ml) and *Staphylocoecus aureus* (200 µg/ml) from diterpene acid fraction extract of *S. apiana* compounds (carnosic acid, 16-hydroxycarnosic acid and their derivatives). Whereas its essential oil (composed primarily from 1, 8-cineole and camphor) as well as a mixture of oleanolic and ursolic acid were inactive even at 1000 µg/ml against tested organisms (Dentali & Hoffmann, 1992).

The crud methanol extract of *S. officinalis*, dissolved in DMSO (50 mg/ml DMSO), inhibited the growth of Gram-positive bacteria *Staphylococcus aureus* at a concentration of 100 µg/ml,

although no antibacterial activity against Gram-negative bacteria *Escherichia coli* or *Pseudomonas aeruginosa* strains was observed (Guerrero, 2005).

The medicinal mixture known as tanshinone, as well as components of the mixture such as cryptotanshinone (**29)**, dihydrotanshinone I (**30)**, hydroxytanshinone II-A (**31)**, methyltanshi-onate (**32)** and tanshinone II-B (**33)**, were shown to have bacteriostatic activity, especially on *Staphylococcus aureus* strains. Tanshinones didn't show any antimicrobial activity against gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Citrobacter freundii,* and *Serratia marcescens*) at a concentration of 100 µg/ml (Yasuhiko Koezuka, 1988). Tablets and ointment of tanshinone provided satisfactory clinical results in 455 cases of infection, mainly with *Staphylococcus aureus* (Guerrero, 2005).

Also, some flavonoids proved to be active against Gram positive and/or Gram-negative bacteria. Cirsimaritin, a flavonoid isolated from the leaves of *S. palaestina*, showed a high activity against standard strains of *Staphylococcus aureus* (MIC=31.25 µg/ml; MBC=125 µg/ml), *Staphylococcus epidermidis* (MIC=62.5 µg/ml ; MBC=125 µg/ml), *Escherichia coli* (MIC=45 µg/ml ; MBC=90 g/ml), *Pseudomonas aeruginosa* (MIC=31.25 µg/ml; MBC=125 µg/ ml), *Proteus vulgaris* (MIC=31.25 µg/ml ; MBC=125 µg/ml) and *Klebsiella pneumoniae* (MIC=45 µg/ml ; MBC=90 µg/mJ) (Miski *et.al.,* 1983).

Another abietane diterpene, forskalinone **(10)** and the dimeric cinnamic acid ester (**27)** were isolated from the roots of *S. forskahlei L.*, were tested against standard bacterial strains. The results showed that forskalinone was moderately active against *S. epidermidis* (670 µg/mL) and slightly active against *Enterococcus faecalis* (168 µg/mL). Dimeric cinnamic acid ester showed a slight activity against *Candida albicans* (156 µg/ mL) (Ulubelen *et.al.,* 1996).

Compounds (**12)** which were isolated from *S. africanalutea* exhibited MICs at 28 µM against *M. tuberculosis.* (Hussein et al., 2007). Potent antibacterial activity was exhibited by horminone (**14)** and 7-acetylhorminone (**15)** against *S. aureus* (6.5 and 10 µg/mL), *S. epidermidis* (1.5 and 6 µg/mL), and *Bacillus subtilis* (1.5 and 3 µg/mL). Horminone was also found to be active against *Enterococcus faecalis* (14 µg/mL). whereas O-Methyl- pisiferic acid **(8)** was active only against *Bacillus subtilis* (G. W. Wang *et.al.,* 2013).

1-Oxoferruginol (**11)** showed activity against *B. subtilis* (15.6 µg/mL), *S. aureus* (15.6 µg/mL), and *S. epidermidis* (15.6 µg/mL) and a modest activity against *P. mirabilis* (>250 µg/mL) (Ulubelen *et.al.,* 2001). Sclareol showed high activity against *S. aureus* (15.6 µg/

mL), *S. epidermis* (15.6 μg/mL), *E. coli* (62.5 μg/mL), *Proteus vulgaris* (62.5 μg/mL), and *Pseudomonas aeruginosa* (31.3 μg/mL) (Miski *et.al.,* 1983).

Compound (**22)** was found to possess antibacterial activity against *Staphylococcus aureus* (Habibi *et.al.,* 2000). Salvipisone and aethiopinone, which were isolated from the hairy rot of *Salvia sclarea,* showed antibacterial activity against *S. aureus* (MIC range, 18.75-37.5 μg/mL) and *S. epidermidis* (9.37-75.0 μg/mL) (Kuźma *et.al.,* 2007).

Compound (**21)** showed antimicrobial activities against two Gram-positive organisms, *Staphylococcus aureus* and *Micrococcus luteus*, with MIC values of 20.0 and 15.0 μM, respectively (G. W. Wang *et.al.,* 2013). Compound (**23)** and ursolic acid were isolated from the leaf part of *S.officinalis* and their antimicrobial activity (against vancomycin-resistant enterococci and methicillin-resistant staphylococcus aureus) was tested. The minimum inhibitory concentrations (MICs) of oleanolic acid **(23)** and ursolic acid were 8 and 4 μg/mL, respectively. These two compounds showed antimicrobial activity against *Streptococcus pneumoniae* and methicillin-resistant *Staphylococcus aureus* (Horiuchi *et.al.,* 2007)**.**

A flavonoid from flower parts of *S. leucantha* has been found to contain a quercetin-3 -O-a-L-rhamnopyranosyl-(1→6)-b-D-glucopyranoside **(38)**. The antibacterial activity of this quercetin glycoside was assayed by the agar diffusion method and exhibited as zones of inhibition against *S. aureus* (27 mm) and *E. coli* (22 mm) at a concentration of 200 μg/ml (Jassbi *et.al.,* 2016).

Flavonoids (Quercetin-3-O- α-L- rhamnopyranoside -2''- gallate) **(39)** were isolated from flowers of *Salvia leucantha* using chromatography separation techniques. This study also evaluate the antibacterial activity against gram positive and gram negative microorganisms by using agar diffusion methods. The results revealed that the highest inhibitory zone was observed in *S. aurous* (28mm), and E. coli (24mm) (Manivannan *et.al.,* 2014).

### 2.5.2 Antioxidant Activity

Within the last decade, the importance of free radicals in the etiology of disease has been increasingly recognized and has led to the development of new approaches in biochemical evaluation of events associated with mutagenesis, tumorigenesis, and/or cancer promotion. Biomembranes (microsomes, plasma membrane, etc.) are rich in polyunsaturated fatty acids, which are very sensitive to the peroxidative damage induced by free radicals. Free radicals are generated by metabolic pathways within the body or can also be caused by the

transformation of specific xenobiotic molecules and by ecological pollutants. Dietary supplies of natural antioxidants act as protective agents against such free radicals and can, in sufficient amounts, act as efficient scavengers of free radicals before any tissue damage occurs (Deighton *et.al.,* 1993).

In recent years, there has been limitation to using synthetic antioxidant compounds in food products because of their side effects. On the other hand, natural sources have become more important to find proper and safe food antioxidants (Lourenço *et.al.*, 2019). Salvia species can be one of the natural sources for this purpose with good culinary qualities, and their extracts are commonly used to increase the shelf life of foods (Deans & Ritchie, 1987).

Leaves of *S. officinalis* L. are well known for their phenolic structure-based antioxidative potency. Commercially available extracts of sage are mainly utilized by the food processing industry but may be applicable in human health. The main sage phenolic diterpene, that show high antioxidative activity are carnosic acid (**34)**, which is known for its instability, and its degradation derivatives, carnosol (**35)**, rosmanol (**36)**, its isomer epirosmanol (**37)**, 7-methyl-epirosmanol as well as rosmanol 9-ethyl ether (Cuvelier *et.al.,* 1994)(Determination *et.al.,* 1992). Rosmarinic acid (**6)** also accounts for the antioxidant activity of sage (Marković *et.al.,* 1996).

The antioxidant activities of the methanol extracts of six *Salvia* species (*S. caespitosa, S. hypargeia, S. euphratica, S. sclarea, S. candidissima, and S. aethiopis*) from Turkey were examined. In the DPPH free radical-scavenging test system, the most active plant was *S. euphratica*, with an $IC_{50}$ value of 20.7 ± 1.22 μg/ mL, followed by *S. sclarea* ($IC_{50}$ = 23.4 ± 0.97 μg/mL) among the polar subfractions. In the β-carotene/linoleic acid test system, the polar extract of *S. hypargeia* was superior to the polar extracts of other *Salvia* species studied (69.2% ± 1.90%). This activity was followed by *S. sclarea* with 63.5% ± 4.24% inhibition rate (Kuźma *et.al.,* 2007).

Candesalvoquinone **(19)**, candelabroquinone (**17)**, candesalvone B methyl ester **(20)**, and candelabrone **(9)** were evaluated for antioxidant activity in enzyme-dependent ($IC_{50}$ values 3.49-10.42 μg) and enzyme-independent ($IC_{50}$ values 1.40-13.40 μg) systems of lipid peroxidation. So, all the compounds have somewhat more pronounced effect on the enzyme-dependent lipid peroxidation. Which indicates that moderate direct enzyme inhibitory activity as a component of the total antioxidant effect (Toni *et.al.,* 1999).

The antioxidant activities of the sage polyphenols, consisting of flavone glycosides and a range of rosmarinic acid derivatives, were evaluated for their capacity to scavenge DPPH and superoxide anion radicals. The antioxidant activity of the flavonoids was variable, and those with a catechol B-ring (luteolin glycosides) were more active than those without (apigenin glycosides) (Lu & Foo, 2002).

Among abietane diterpenes, inuroyleanol **(13)** showed the highest DPPH scavenging activity as well as the highest inhibition on lipid peroxidation in the β-carotene-linoleic acid system. In contrast, inuroyleanol revealed the lowest superoxide anion scavenging activity, while abietane 7-oxoroyleanone-12-methyl ether **(18)** showed the highest activity with an equal antioxidant potency to the L-ascorbic acid, even more active than BHT and α- tocopherol. Royleanone **(16)** showed higher antioxidant activity in the two systems than both horminone **(14)** and 7-acetylhorminone **(15)**. Considering these results in both DPPH and β-carotene-linoleic acid systems, it can be concluded that quinonoids or fully substituted C ring phenolic abietane diterpenoids having no substituent other than a keto group at C-7 would have decreased activity (Miura *et.al.,* 2002)(Kabouche *et.al.,* 2007).

Caffeic acid and its polymers show free radical scavenging activity. The tetrameric lithospermic acid B and its $Mg^{2+}$ salt show the strongest activity; the trimeric lithospermic acid **(26)** and dimeric rosmarinic acid show lower but similar efficacy, whereas the monomeric caffeic acid shows the lowest activity. Rosmarinic acid, salvianolic acid A **(25)**, and salvianolic acid B **(24)** exhibit strong antilipoperoxidant activity, acting by scavenging superoxide anion radicals ($O^{2-}$) (G. W. Wang *et.al.,* 2013).

## 3. MATERIALS AND METHODS

### 3.1. Plant Material Collection and Identification

The plant material was collected based on their ethnobotanical description from forestry regions of Bekoji (boqojjii) Arsi zone, south east Ethiopia (with latitude $7^0$35'N $39^0$10'E and longitude 7.583$^0$N 39.167$^0$E). The area is located about 223 km to the east of Addis Ababa with 2810 m elevation above sea level (Assefa & Getachew, 2015). The identification of the plant material was done with the help of Botanist Melaku Wendaferash at the Department of Biology, National Herbarium of Ethiopia, Addis Ababa University (voucher code: UG001).

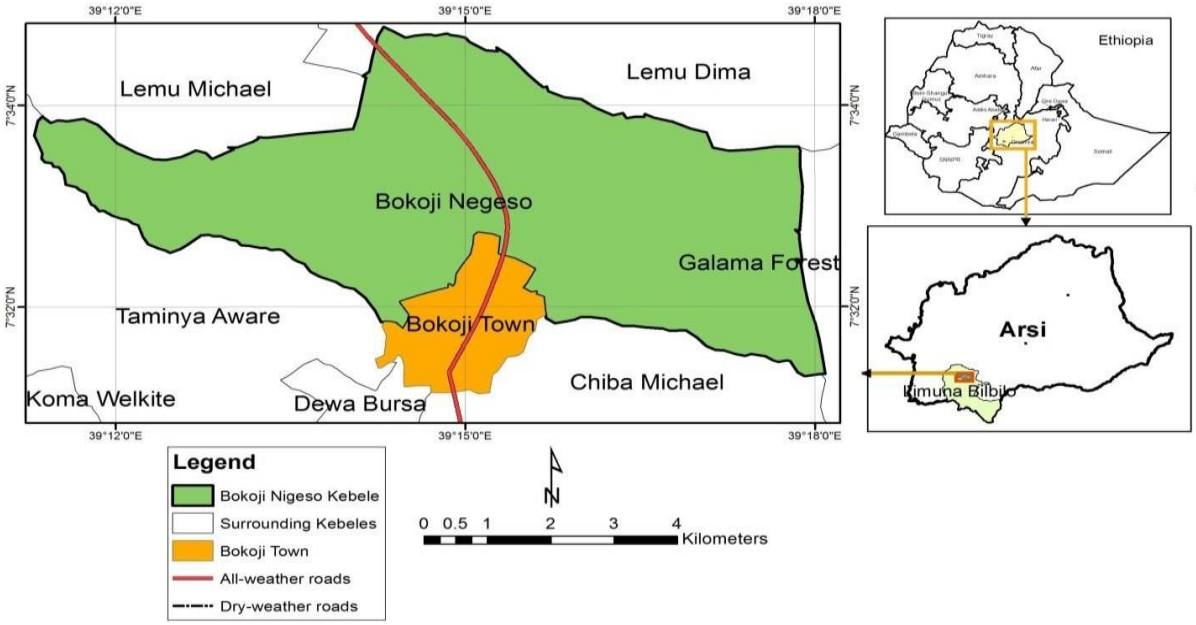



**Figure 9:** Map of Bokoji town…source (Kebede *et.al.*, 2012).

### 3.2. Chemicals and Materials

The chemicals and reagents that were used for extraction, isolation, phytochemical analysis, and other activities were silica gel (Loba Chemie PVT. LTD., 200-400 mesh), ethanol (Favor trading PLC, 99.9%), methanol (Loba Chemie PVT.LTD., 99.8%), chloroform (CDH (P) Ltd., 99.8%), n-hexane (Loba Chemie PVT.LTD., 99%), ethyl acetate (Loba Chemie PVT. LTD., 99.5%), dichloromethane (Loba Chemie PVT. LTD., 99.5%), ammonium solution (Avi chem, 25%), ferric chloride (Sigma Aldrich, 97%), sulfuric acid (Loba Chemie PVT. LTD., 98%), hydrochloric acid (Loba Chemie PVT. LTD., 35.4%), DPPH, DMSO (Srlchem, 99.5%), Potassium Iodide (Samir Tech-Chem PVT. LTD., 99.8%), Iodine (Alpha Chemika, 98.5%), and distilled water.

The materials used in this study were: chromatographic chamber and cuvette, separatory funnel, oven, Whatman filter paper No.1, analytical mills (mortar and pestle), pipettes (different size), water bath, UV lamp, beakers (different size), UV light cabinet, electronic balance, conical flask, flasks, digital measuring balance, measuring cylinder, Rota vapor, Erlenmeyer flasks, Thin Layer Chromatography (TLC) was performed using pre-coated aluminum-backed supported silica gel 60F254 (0.2 mm thickness), mass and NMR spectroscopy machine.

### 3.3. Preparation of Plant materials and Extraction

#### 3.3.1. Preparation of plant materials

The collected leaf part of the plant was cut into smaller pieces and air dried under shade. Then it was pulverized into a fine powder by using the analytical mills (mortar and pestle). Then the powder was weighed and stored in a polyethylene bag to prevent it from being attacked by certain environmental conditions (moisture, air, and other dust material) until further experimentation.

#### 3.3.2. Extraction

Sequential extraction method was used in this studies. The leaf powder of *Salvia leucantha* (450 g) was soaked into n-hexane (2.25L) and shaken with a mechanical shaker at room temperature for 72 hours. The solution was filtrated using Whatman No.1 filter paper and concentrated using a rotary evaporator at around 40$^{o}$C to afford n-hexane crude extract. The whole procedure was repeated two times. Then the marc left was soaked with ethylacetate (2.25L) and shaken with a mechanical shaker at room temperature for 3 days. The mixture was filtered and concentrated to afford ethylacetate crude extract. Again, the whole procedure was repeated twice.

Finally, the marc left was further soaked in methanol (2.25L) and shaken with a mechanical shaker at room temperature for 72 hours. Followed by filtration using Whatman No.1 filter paper and concentrated using a rotary evaporator to afford methanol crude extract. Then percentage yield was calculated for each extract. The extracts TLC profiles were analyzed to identify appropriate eluent for column chromatographic separation.

### 3.4. Phytochemical Screening

The phytochemical tests were carried out for the leaf extract of *S. leucantha* using standard procedures to identify secondary metabolites (Kashyap & Hait, 2021)(Saranya *et.al.*, 2019).

**Tests for alkaloids**

*Mayer's Test:* 0.5 mg of the extract was treated with Mayer's Reagent with one or two drops. Formation of white precipitate ratifies the presence of alkaloids.

**Test for steroids**

*Libermann burchard reaction:* this test was used to determine the presence of steroids. 2 mL extract were mixed with chloroform. To this mixture, 2 mL acetic anhydride and 2 drops concentrated sulphuric acid were added from the side of test tube. Formation of red, blue and green color indicates the presence of steroid.

**Test for Glycoside**

*Keller-Killiani Test:* 0.5 mg of the extracts was treated with 2 mL of glacial acetic acid and a drop of ferric chloride solution in a test tube. This was carefully under laid with 1 mL concentrated sulphuric acid. A brown ring at the interface will indicate the presence of deoxy sugar characteristic of cardenolides. A violet ring appeared below the ring while in the acetic acid layer, a greenish ring formation ratifies the presence of glycoside.

**Test for Terpenoids**

*Libermann Burchard Test:* 0.5 mg of the extract was treated with 2 mL of acetic anhydride then boiled and cooled. After that, concentrated sulphuric acid was added along the sides of the test tube. A brown ring at the junction of two layers with green upper layer showed the presence of steroids and formation of red color indicated the presence of triterpenoids.

**Test for Flavonoids**

*Ferric Chloride Test:* 0.5 mg of the extracts was treated with 2 mL of ferric chloride solution. Appearance of intense green color indicated the presence of flavonoids.

**Test for Tannins**

*Ferric Chloride Test:* 0.5 mg of extract was dissolved in distilled water. To this solution 2 mL of 5% ferric chloride solution was added. Formation of blue green color indicated the presence of tannins.

**Test for Saponins**

*Foam test:* 2 mL of extract was diluted with 6 mL of water in a test tube. The mixture was shaken vigorously and the formation of persistent foam which confirmed the presence of saponins.

**Test for Coumarins**

The aqueous plant extracts were added in 3 mL of 10% NaOH. Then the mixture was shaken and formation of yellow color indicated the presences of coumarins(Le Thi *et.al.*, 2021).

### 3.5. Extraction of Essential Oil by Hydrodistillation

The dried leaf of *S. leucantha* (100 g) was subjected to hydrodistillation using a Clevenger-type apparatus for 4 hours. The oil was extracted with $CHCl_3$ (100 ml) and dried over anhydrous $Na_2SO_4$ (1 g), then filtered and concentrated on a rotary evaporator. The oil was stored in a vial at -4°C until GC-MS analysis. The amount of extracted oil yield (w/w) was calculated by the following equation (Elyemni *et.al.*, 2019)(Karakoti *et.al.*, 2022).

$$\textbf{Yield (\%)} = \frac{\textbf{Amount of extracted oil (mg)}}{\textbf{Amount of dry plant matter mass(g)}} * \mathbf{100}$$

### 3.6. GC-MS Analysis of Essential Oil

The chemical composition of essential oil extracted from the leaf of *S. leucantha* by hydro-distillation was analyzed by GC-MS using the following method. The analysis was performed by GC (7890B, Agilent Technologies, USA) coupled with an MS (5977A Network, Agilent Technologies). The GC had an HP5MS column (30x 250 µm internal diameter (i.d) and 0.25 µm). Helium was used as a carrier gas (flow rate 1mL/min). The initial oven temperature was 100ºC for 2 min and raised up from 100 to 200 ºC at the speed of 10 º C/min (inlet 250 º C, split less injection/purge time 1 min), solvent delay 4 min. Mass spectra were recorded in electron impact mode, with ionization energy of mode at 70 ev, scanning the 33-550 m/z range. Identification of essential oil components was done by comparing their relative

retention index (RI) values with those compounds reported in the literature and mass spectra NIST (NIST version 2.1) and WILEY (7th edition) libraries, and by matching the fragmentation pattern of the mass spectral data (Karakoti *et.al.,* 2022).

### 3.7. Isolation of Compounds

After observing the TLC profiles of all crude extracts (n-hexane, ethylacetate, and methanol), the crude extract of methanol was showing a good TLC profile. The methanol crude extract (10 g) was adsorbed on to 10g of silica gel (mesh size 200-400), and subjected to silica gel column chromatography (150 g of silica gel) using *n*-hexane for initial packing. Elution was carried out with an increasing gradient of ethylacetate in *n*-hexane followed by an increasing gradient of methanol in ethylacetate. A total of 251 fractions (50 mL each) were collected. Fractions that showed identical $R_f$ values and the same characteristic color on TLC were combined.

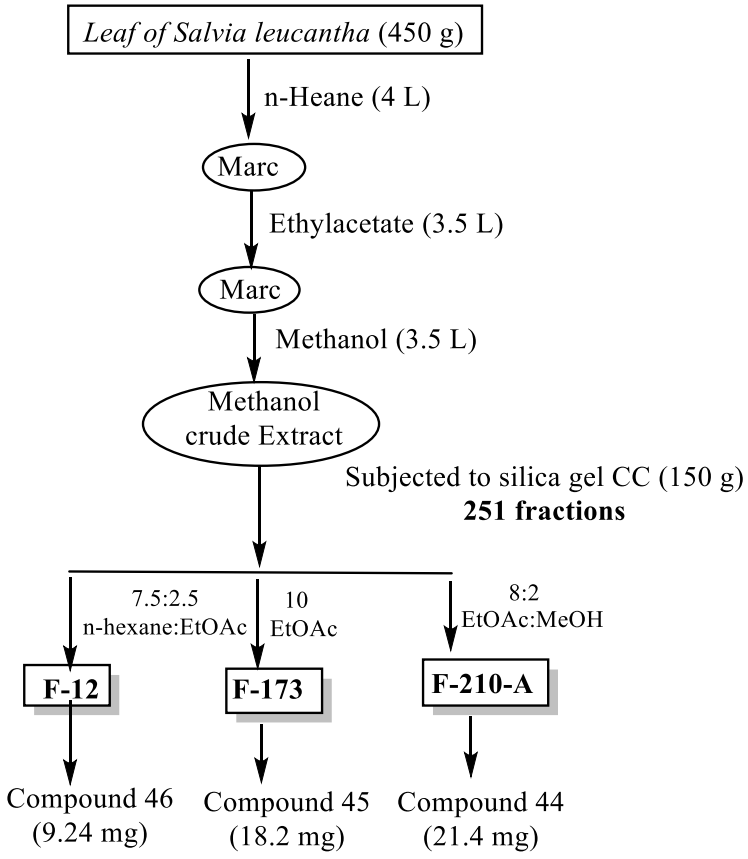



**Figure 10:** Scheme flow chart diagram showing extraction procedure.

Fraction 12 (eluted with 75% *n*-hexane in 25% EtOAc) was washed with chloroform and methanol to afford compound **46** (9.24 mg) as a white crystalline needle like shape. Fraction 173 (eluted with 100% EtOAc) was decanted and washed with *n*-hexane and chloroform,

respectively to afford compound **45** (18.2 mg) as a slightly yellow crystalline powder. Fraction 210 (eluted with 80% EtOAc in 20% methanol*)* was formed precipitate in the flask and decanted with methanol and chloroform to afford 210A and 210B fractions respectively. Fraction 210A, after washed with *n*-hexane, affords compound **44** (21.4 mg) as a light-yellow needle like shape.

**Table 2:** Summary of fractionation of methanol crude extracts.

| Solvent System | Ratio | Fractions | Solvent System | Ratio | Fractions |
|---|---|---|---|---|---|
| *n*-hexane | 100 | F1 | EtOAc:MeOH | 90:10 | F196 – F208 |
| *n*-hexane:EtOAc | 95:5 | F2 | | 80:20 | F209 – F222 |
| | 90:10 | F3 –F5 | | 70:30 | F223 – F234 |
| | 85:15 | F6, F7 | | 60:40 | F235 – F238 |
| | 80:20 | F8 – F11 | | 50:50 | F239 – F246 |
| | 75:25 | F12 – F15 | MeOH | 100 | F247 – F251 |
| | 70:30 | F16 – F44 | | | |
| | 65:35 | F45 – F62 | | | |
| | 60:40 | F63 – F76 | | | |
| | 55:45 | F77 – F84 | | | |
| | 50:50 | F85– F106 | | | |
| | 40:60 | F107 – F113 | | | |
| | 30:70 | F114 – F133 | | | |
| | 20:80 | F134 – F149 | | | |
| | 10:90 | F150 – F171 | | | |
| EtOAc | 100 | F172 – F195 | | | |

### 3.8. Structural Elucidation

The NMR spectroscopic techniques were employed to elucidate the structure of isolated compounds. The $^{1}H$ NMR, $^{13}C$ NMR, and DEPT-135 were obtained using Bruker Avance 500 MHz spectrometer at the Department of Medicinal Chemistry, University of South Australia, Australia. DMSO (*d*- 6) as a solvent was utilized for the analysis.

### 3.9. Biological Activity Testing

#### 3.9.1. Antibacterial Activity

In *vitro* antibacterial activities of the crude extracts and isolated compounds of *S. leucantha* were studied against four pathogenic bacterial strains retrieved from Adama Public Health Research and Referral Laboratory Center. The strains used in the experiment were two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Streptococcus pyogenes* ATCC 19615) and two Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) in comparison with ciprofloxacin as the standard drug and DMSO as the negative control. The bacteria were selected based on their availability and considering the likely bacterial strains that can cause bacterial infections.

The concentration of samples was prepared by dissolving required amounts in DMSO to give sets of four dilutions, 200, 100, 50, and 25 mg/mL for extracts and dilutions at 2, 1, 0.5, and 0.25 mg/mL for isolated compounds. Likewise, the reference antibiotics (ciprofloxacin) were used at 30 µg/mL and DMSO as an inert negative control (CLSI, 2012).

The bacterial cultures of each test organism were prepared by taking 100 µL of bacterial subcultures and inoculating them into 10 mL of sterile nutrient broth (as inoculation medium) and grown overnight at 37°C. After incubation, the turbidity of each bacterium was adjusted to 0.5 McFarland standards using 0.85% saline solution (w/v). The prepared Muller Hinton Agar (MHA) media solution was poured (approximately 20 mL) into sterile petri dishes aseptically and allowed to solidify. Then, the strains of bacteria were inoculated into the pre-labeled MHA plates using sterile cotton swabs by streaking swabs over the entire sterile agar surface and the procedure was repeated two more times, rotating the plates at 60˚ each time to ensure even distribution. A triplicate plate of each organism was prepared. Sterile filter paper disks impregnated with test compounds and standard drug with a diameter of 6 mm were placed over each MHA plate. Finally, the plates were incubated at 37°C for 18 hours (Manivannan *et.al.,* 2014)(Rajamanickam *et.al.,* 2013)(Biondi *et.al.,* 1993).

The resulting diameters of zones of inhibition produced by the plant extracts, isolated compounds and standard antibiotic (ciprofloxacin) were measured using a ruler and expressed in millimeters. The experiment was performed in triplicate for each bacterium. The mean zones of inhibition were calculated and expressed as mean ± SEM of triplicate (**Table 8** and **Table 9**).

### 3.9.2. Antioxidant Activity

Antioxidant activities of the extracts and isolated compounds were measured based on their free radical scavenging activity, which was determined by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. DPPH solution (0.04%) was prepared in methanol by dissolving 0.004 g DPPH in 100 mL methanol. The solution was kept in darkness for 30 min to complete the reaction. Serial dilutions were carried out with the stock solutions of 2 mg/mL for the plant extract and 0.4 mg/mL of isolated compounds to obtain concentrations of 1000, 500, 250, 125, & 62.5 µg/mL and 400, 200, 100, 50, & 25 µg/mL, respectively. The solutions were prepared using methanol as solvent. Each concentration of sample (1 mL) was placed in a clean test tube and DPPH solution (1 mL) was added to the same test tube.

The resulting solutions were placed in the dark for 30 minutes and then subjected to a UV-Vis spectrophotometer to record absorbance at 517 nm. Ascorbic acid with a similar concentration of test samples was used as the positive control. The DPPH radical scavenging activity of each of the tested compounds was reported by percentage inhibition using the following formula (Brand-Williams *et.al.*, 1995).

$$\text{\% of radical scavenging activity } (\mathbf{\%RSA}) = \frac{(\mathrm{Ac}-\mathrm{As})}{\mathrm{Ac}} \text{ X } 100$$

*Where Ac is the absorbance of the control and As is the absorbance of the sample.*

The $IC_{50}$ value is defined as the concentration of a substrate that causes 50 % loss of the DPPH activity. The lower the $IC_{50}$ value, the higher is the scavenging potential (Riaz *et.al.*, 2012). The $IC_{50}$ values of all samples were calculated by using GraphPad prism version 8, using nonlinear regression analysis of the percentage radical scavenging activity against the concentration of the tested extracts or compounds.

### 3.10. In *silico* Molecular Docking Studies of Isolated Compounds

A molecular docking study was carried out in order to evaluate the binding affinity of the isolated compounds (**44-46**) and positive controls (ciprofloxacin and ascorbic acid) against the active site of target proteins using Auto Dock Vina software and a standard protocol (Seeliger & De Groot, 2010)(Allouche, 2012). The chemical structures of isolated compounds (**44-46**) were drawn with the ChemOffice tool (Chem Draw 16.0) and assigned with the proper 2D orientation, and the energy of each molecule was minimized with ChemBio3D. The energy-minimized ligand molecules were then fed into AutoDock Vina to

perform the docking simulation. The crystal structure of receptor molecules *E. coli* DNAgyrase B (PDB ID 7P2W), *S. aureus* pyruvate kinase (PDB ID: 3T07) and human myeloperoxidase (PDB ID 1DNU) were downloaded from the protein data bank.

The protein preparation was done using the reported standard protocol by removing the co-crystallized ligand, selected water molecules and cofactors. The target protein file was prepared by leaving the associated residue with protein by using Auto Preparation of target protein file Auto Dock 4.2.6 (MGL tools 1.5.6).

The grid box for docking simulations was set using the graphical user interface program. The grid was designed to surround the region of interest in the macromolecule. To find the best-docked conformation between ligand and protein, the docking algorithm provided with AutoDock Vina was used. For each ligand, a maximum of nine conformers were considered during the docking process. Conformations with the most favorable (least) free binding energy were selected for analyzing the interactions between the target receptor and ligands by Discovery Studio visualizer and PyMOL.

The molecular docking studies were carried out using AutoDock Tools (Allouche, 2012), which is a free graphic user interface (GUI) for the AutoDock Vina program. The ligands were represented by different colors, while the H-bonds and interacting residues were represented by stick models (Narramore *et.al.*, 2019).

### 3.11. In *Silico* Drug-Likeness and Toxicity Predictions

The *in-silico* pharmacokinetic properties (absorption, distribution, metabolism, and excretion) of the isolated compounds (**44**, **45**, & **46**) and standard drug (ciprofloxacin) were conducted on the SwissADME online tool at default settings by using the SMILES of each compound. SwissADME provides information about the chemical compounds physicochemical and pharmacokinetics properties (Daina *et.al*., 2017). The isolated compounds were subjected to computational studies to predict their drug-likeness property via Lipinski's rule of five (Lipinski *et.al.,* 1997), Veber rule (Veber *et.al.,* 2002), and topological polar surface area (TPSA) using SwissADME (Daina *et.al.,* 2017). Furthermore, an online program ProTox-II (Banerjee *et.al.,* 2018) was used to predict the oral toxicity profile of the isolated compounds and standard drugs. The cLogP and TPSA values of the compounds were plotted to predict human intestinal absorption (HIA) and blood brain barrier (BBB) access through the BOILED-Egg model (Daina & Zoete, 2016).

### 3.12. Statistical Analysis

The data on antibacterial and antioxidant activities were the averages of triplicate analyses. Data were recorded as mean ± standard error of mean (SEM). The results for antioxidant activity expressed as 50% inhibition concentration ($IC_{50}$). The sample concentration that provides 50% activity ($IC_{50}$) was calculated from a nonlinear regression analysis of percent inhibitory activity versus sample concentration. All data were analyzed with the aid of Excel, Microsoft Office, and the statistical package program Graphpad Prism version 8 for Windows (Graphpad Software Inc., San Diego, USA).

# 4. RESULTS AND DISCUSSION

## 4.1 Extraction Yield

The plant materials (leaf) of *Salvia leucantha* were successively extracted with *n*-hexane, ethylacetate, and methanol to afford 19.4 g (4.3%), 29.1 g (6.4%), and 16.6 g (3.6%) yields, respectively. The methanol extract was found to be dark brown; the middle one was black and the first one was dark yellow in color and very sticky in its nature. The extract obtained by using etylacetate as a solvent had a higher extraction yield as compared to *n*-hexane and methanol extracts.

## 4.2 Phytochemical Screening

Following standard experimental protocols, phytochemical screening tests were performed to determine the class of chemicals present in the crude extracts. The test results revealed the presence of terpenoids, tannins, steroids, saponins, glycosides, and flavonoids in methanol extracts. On the other hand, flavonoids, tannins, steroids, and saponins are found in ethylacetate extracts. While the n-hexane extract revealed the presence of terpenoids, alkaloids, steroids, and saponins. **Table-3** below summarizes the results of various phytochemical identification tests that were done on the leaf extracts of *Salvia leucantha.*

**Table 3:** Phytochemical screening tests result from the leaf extract of *S. leucantha.*

| **Secondary Metabolites** | **Test** | **Extracts** | | |
|---|---|---|---|---|
| | | ***n*-Hexane** | **Ethylacetate** | **Methanol** |
| **Alkaloids** | Mayer's test | + | - | - |
| **Coumarins** | | - | - | - |
| **Flavonoids** | Ferric Chloride Test | - | + | + |
| **Glycosides** | Keller-Killiani Test | - | - | + |
| **Steroids** | Libermann burchard reaction | + | + | + |
| **Saponins** | Foam test | + | + | + |
| **Terpenoids** | Libermann Burchard Test | + | - | + |

| | | | | |
|---|---|---|---|---|
| **Tannins** | Ferric chloride Test | - | + | + |

*+ shows Presence - shows Absence*

The presence of these secondary metabolites might be attributed to the traditional uses of plants to treat various diseases such as microbial infections (Sharma & Mishra, 2020), leprosy, snake bite (Jana & Shekhawat, 2011), epilepsy (Belayneh & Bussa, 2014), cancer (Ayele, 2018) and gout and tumor (Tesfaye *et.al.*, 2020).

The study undertaken by Rajamanickam *et.al.* (2013) from the flower parts of *Salvia leucantha* confirmed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, phenol, and terpenoids while steroids, coumarin, and cardiac glycosides are absent in 90% methanol extract (Rajamanickam *et.al.*, 2013). Even though the part of plant and solvent (percentile) used was different, this study agrees with our results by revealing the presence of terpenoids, tannins, saponins, flavonoids, and glycosides in methanol extracts.

Generally, phytochemicals such as tannins, flavonoids, saponins, phenolic compounds, and essential oils are hypothesized to be responsible for plant antibacterial activities (Rahman *et.al.*, 2011). Flavonoids have been linked to numerous biological functions, including antibacterial, antioxidant, and anti-inflammatory properties. They are known to protect the plants from diseases (Hafidh, 2011) (Dhiman *et.al.*, 2012). Tannins, flavonoids, alkaloids, and saponins have been shown to destroy pathogens by a synergistic action (Nwankwo *et.al.*, 2014). Therefore, the presence of this class of compounds in *Salvia leucantha* confirms its potential for the discovery of lead compounds that belong to these classes.

### 4.3 Characterization of Isolated Compounds

Compound **44** (21.4 mg) was isolated as a light yellow, needle like shape. It has a melting temperature between 253 $^{o}$C and 254 $^{o}$C, with $R_f$ value of 0.4 (when 90% ethylacetate is used in 10% methanol as eluent). Its $^{1}$H-NMR spectrum (500 MHz, DMSO) (**Appendix-1**) revealed the existence of five aromatic protons signal at δ 6.15 (d, 1H), 6.13 (d, 1H), 6.89 (d, 1H), 6.95 (d, 1H) and 6.91 (d, 1H). Two hydroxyl protons signals were observed at δ 12.04 (s, 1H) and 9.11 (s, 1H) in the aromatic ring. A three protons singlet on an oxygenated carbon was observed at 3.79 (s, 3H), which is attributed to the methoxy group. There were methylene proton signals at δ 3.23 and 2.78 (m, 2H) and methine proton signal at δ 5.52 (m, 1H) observed. There are two anomeric proton signals at δ 4.99 and δ 4.53 indicating the presence

of two sugar moieties within the structure of the compound. These anomeric protons signals appear to be due to glucose and rhamnose groups because the chemical shift values correlate with the literature values for glucose and rhamnose.

The $^{13}$C-NMR spectrum (126 MHz, DMSO) (**Appendix-2**) in combination with DEPT-135 (**Appendix-3**) displayed 28 carbon signals, of which the peaks at δC 56.1 and δC 18.3 belong to methoxy and terminal methyl carbon, respectively. Whereas a ketone carbonyl carbon appeared at δC 197.5. Twelve aromatic carbon signals at δC 165.6, 163.5, 162.9, δC 148.4, 146.9, 131.3, 118.4, 114.9, 112.4, 103.7, 96.8, and 95.9 appeared. From those aromatic carbon signals, in DEPT-135 signals at δC 165.6, 163.5, 162.9 148.4, 146.9 131.3, and 103.7 disappeared, suggesting these peaks belong to quaternary carbons. Methylene peaks signals at δC 66.4 and 42.5 pointed down in DPET-135 spectra, which are also in good agreement with the $^{13}$C NMR spectral data.

In particular, the oxo peak at δ197.5, the aromatic quaternaries at δ165.6, 163.5, 162.9, 148.4, 146.9, 131.3, and 103.7 in conjugation with the only upfield methylene signal at δ42.5 strongly suggested a flavanone skeleton with *meta* dihydroxylations on the A ring at C-5 and C-7 as well as *ortho* dihydroxylations on the B-ring at C-3' and C-4'. This is further corroborated by the shielded aromatic methine signals at δ95.9 and 96.8 corresponding to the C-6 and C-8 of the A ring of the flavanone, respectively. Furthermore, the presence of an oxygenated methylene signal at δ66.4, two anomeric carbons signals at δ99.8 and 101.1 and several oxygenated methine carbon signals at δC 78.6, 72.5, 75.9, 70.7, 76.7, 71.1, 70.0, 73.4, and 68.7 clearly indicated the presence of a disaccharide moeity within the compound. The presence of only one oxygenated methylene group and one methyl group at δ18.3 strongly suggested that one of the sugars is most likely to be a glucose unit and the second a rhamnose unit. Based on this experimental evidence and literature review, the indicated structure was proposed for the compound, which is supported by the mass spectrum of the compound (see next).

Apparently, the mass spectrometry (**Appendix-4**) of compound **44** (molecular formula is $C_{28}H_{34}O_{15}$ and molecular weight is 610.572) seems to be in agreement with the compound reported by Jiao *et.al.* as hesperidin (Jiao *et.al.*, 2020). The above $^{1}$H-NMR, $^{13}$C-NMR, DPET-135, and mass spectra data led to the proposed flavanone glycoside structure for compound **44**. Which is very similar to an already known flavanone glycoside known as hesperidine, which was isolated herein for the first time from *Salvia leucantha.*

**Table 4:** $^{1}$H NMR and $^{13}$C NMR spectral data of compound **44** and the reference hesperidin

| Carbon Position | $^{1}$H-NMR data of compound **44** | $^{13}$C-NMR data of compound **44** | $^{1}$H-NMR data of Hesperidin (Jadeja *et.al.*, 2017) | $^{13}$C-NMR data of Hesperidin (Lahmer & Belboukhari, 2015) | Types of carbons |
|---|---|---|---|---|---|
| -O-$CH_3$ | 3.79,(s) | 56.1 | 3.77,(s) | 55.8 | $CH_3$ |
| 2 | 5.52,(m) | 78.8 | 5.54,(dd) | 78.6 | CH |
| 3 | 3.23,(m)<br>2.78,(m) | 42.5 | 3.24,(dd)<br>2.79,(dd) | 42.2 | $CH_2$ |
| 4 | - | 197.5 | - | 197.2 | Carbonyl Carbon |
| 5 | - | 163.5 | - | 163.2 | C |
| 6 | 6.95,(s) | 96.8 | 6.95,(s) | 96.5 | CH |
| 7 | - | 165.6 | - | 165.5 | C |
| 8 | 6.92,(s) | 95.9 | 6.93,(s) | 95.7 | CH |
| 9 | - | 162.9 | - | 162.7 | C |
| 10 | - | 103.7 | - | 103.5 | C |
| 1’ | - | 131.3 | - | 131.1 | C |
| 2’ | 6.15,(d) | 118.4 | 6.16,(d) | 118.1 | CH |
| 3’ | 6.13,(d) | 112.4 | 6.13,(d) | 112.2 | CH |
| 4’ | - | 148.4 | - | 148.1 | C |
| 5’ | - | 146.9 | - | 146.6 | C |
| 6’ | 6.91,(s) | 114.9 | 6.89,(s) | 114.3 | CH |

| | | | | | |
|---|---|---|---|---|---|
| 1'' | 4.99,(d) | 99.8 | 4.99,(d) | 99.6 | CH |
| 2'' | 3.57,(t) | 72.5 | 3.57,(t) | 72.2 | CH |
| 3'' | 3.32,(t) | 75.9 | 3.32,(t) | 75.7 | CH |
| 4'' | 3.42,(t) | 70.7 | 3.43,(t) | 70.9 | CH |
| 5'' | 3.64,(m) | 76.7 | 3.65,(dd) | 76.5 | CH |
| 6'' | 3.82,(m), 3.44,(m) | 66.4 | 3.44,(dd) 3.82,(dd) | 66.2 | $CH_2$ |
| 1''' | 4.53,(d) | 101.1 | 4.54,(d) | 100.8 | CH |
| 2''' | 3.39,(m) | 71.1 | 3.39,(q) | 69.7 | CH |
| 3''' | 3.18,(t) | 70.0 | 3.19,(t) | 70.5 | CH |
| 4''' | 3.25,(t) | 73.4 | 3.25,(t) | 73.2 | CH |
| 5''' | 3.16,(t) | 68.7 | 3.17,(t) | 68.5 | CH |
| 6''' | 1.10,(d) | 18.3 | 1.10,(d) | 18.1 | $CH_3$ |

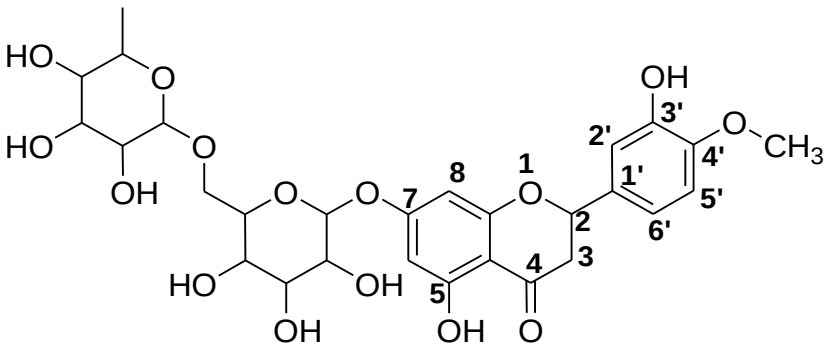



**Figure 11:** Proposed chemical structure of compound **44**

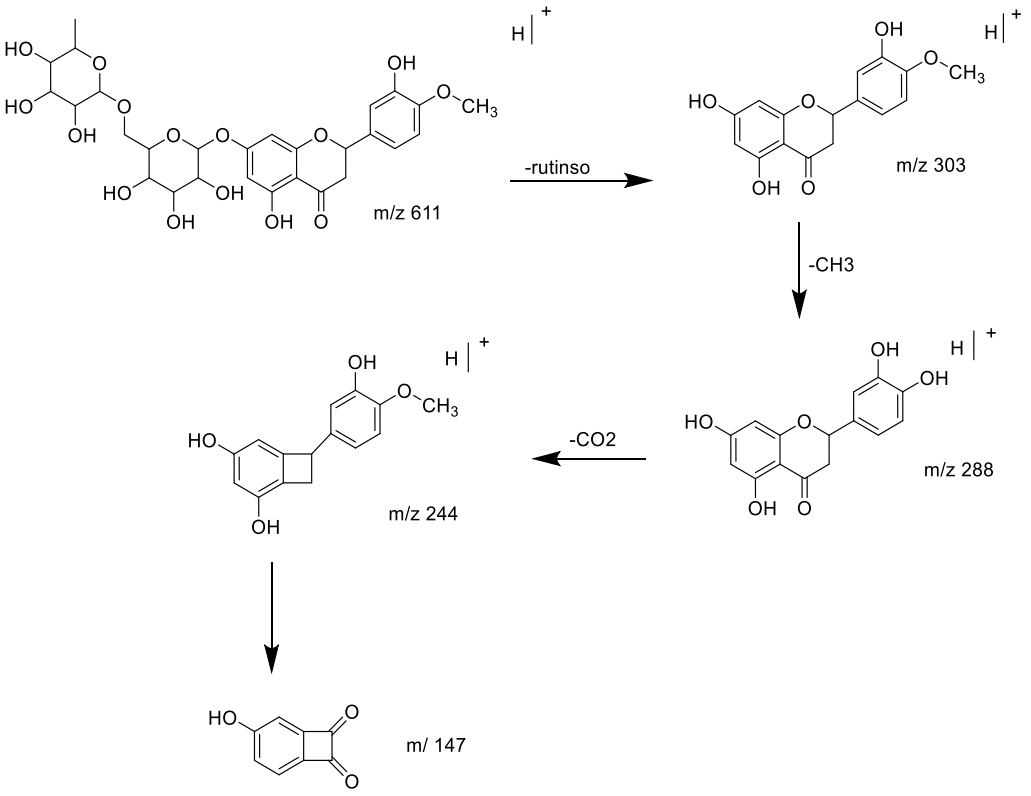



**Figure 12:** Proposed mass fragmentation structure of compound **44**

Compound **45** (18.2 mg) was isolated as slightly yellow crystalline powder. It has a melting temperature between 280 ºC and 281 ºC, with $R_f$ value of 0.7 (when 100% ethylacetate is used as eluent). Its $^1$H-NMR spectrum (500 MHz, DMSO) (**Appendix-5**) revealed signal at δ 5.13 (m, 1H) corresponds to a vinyl proton (=CH), indicating the presence of a double bond. While signal at δ 0.94 (m, 5H), 0.80 (s, 3H), 0.75 (s, 3H), 0.86 (s, 3H), 1.04 (s, 3H), 0.89 (d, 3H), and 0.81 (d, 3H) represent distinct methyl protons attached to seven terminal methyl carbons. The methylene protons are prominently featured with signals at δ 1.86 (m, 2H), 1.59 (m, 4H), 1.82 (m, 1H), and 1.59 (m, 4H), 1.96 (m, 1H), 0.94 (m, 5H), 1.23 (m, 1H), 1.76 (m, 2H), 1.46 (m, 4H), and 1.31 (m, 2H). Whereas signal at δ 3.02 (m, 1H), 0.67 (d, 1H), 1.46 (m, 4H), 2.11 (d, 1H), 1.33 (m, 1H), and 0.98 (m, 1H) revealed the existence of methine protons.

The $^{13}$C-NMR spectrum (126 MHz, DMSO) (**Appendix-6**) showed 30 carbon signals, corresponding to which the peaks at δ C 177.6 belong to carbonyl carbon. Quaternary carbons are represented by signals at δ C 137.5, 46.4, 41.0, 38.36, 37.7, and 35.6. Nine methylene carbons signals at δ C 37.5, 26.9, 20.4, 32.0, 22.2, 27.6, 26.3, 29.5, 35.8, and seven methine carbon signals at δ C 76.2, 54.1, 46.18, 123.9, 51.7, 37.8, and 37.7 are shown in $^{13}$C-NMR. Whereas seven terminal methyl carbon signals at δ C 28.1, 15.4, 14.5, 17.3, 23.2, 16.3, and 22.6 appear in the $^{13}$C-NMR spectrum.

The above $^{1}$H-NMR and $^{13}$C-NMR spectral data together with references suggest that the compound is urosilic acid, which was isolated herein for the first time from *Salvia leucantha*.

**Table 5:** $^{1}$H NMR and $^{13}$C NMR spectral data of compound **45** and the reference, ursolic acid.

| Carbon Position | $^{1}$H-NMR data compound of **45** | $^{13}$C-NMR data of compound **45** | $^{1}$H-NMR of Ursolic acid (Gnoatto *et.al.*, 2008) | $^{13}$C-NMR of Ursolic acid (Shaheen *et.al.*, 2011) | Types of carbons |
|---|---|---|---|---|---|
| 1 | 1.59 (m, 4H) | 37.5 | 1.58 *(m, 4H, $CH_2$(Hb) 1, $CH_2$(Ha)-15, $CH_2$(Hb)-16, $CH_2$(Hb) 21)* | 38.32 | $CH_2$ |
| 2 | 1.31 (m, 2H) | 26.9 | 1.33 *(m, 3H, $CH_2$-2, CH-19)* | 27.49 | $CH_2$ |
| 3 | 3.02 (m, 1H) | 76.2 | 3.20*(dd, 1H)* | 76.57 | CH |
| 4 | - | 37.7 | - | 38.20 | C |
| 5 | 0.67 (d, 1H) | 54.1 | 0.68 *(d, 1H)* | 54.59 | CH |
| 6 | 1.46 (m, 4H) 1.76 (m, 2H) | 20.4 | 1.47 *(m, 4H, $CH_2$(Hb)-6, $CH_2$(Hb)-7, $CH_2$(Ha)-9, $CH_2$(Ha)-21),* 1.67 *(td, 1H, $CH_2$(Ha)-6)* | 17.80 | $CH_2$ |
| 7 | 1.23 (m, 1H) | 32.0 | 1.21 *(m, 1H, $CH_2$(Ha)-7)* | 32.49 | $CH_2$ |
| 8 | - | 38.36 | - | 39.23 | C |
| 9 | 1.46 (m, 4H) | 46.18 | | 46.98 | CH |
| 10 | - | 35.6 | - | 36.46 | C |
| 11 | 0.94 (m, 5H) | 22.2 | | 22.70 | $CH_2$ |
| 12 | 5.13 (m, 1H) | 123.9 | 5.40 *(m, 1H)* | 124.17 | =CH |
| 13 | - | 137.5 | - | 137.76 | =C |
| 14 | - | 41.0 | - | 41.50 | C |
| 15 | 1.96 (m, 1H) 1.59 (m, 4H) | 27.6 | 1.96 *(td, 1H, $CH_2$(Hb)-15)* | 28.30 | $CH_2$ |
| 16 | 1.82 (m, 1H) 1.59 (m, 4H) | 26.3 | 1.81 *(td, 1H, $CH_2$(Ha)-16)* | 26.67 | $CH_2$ |
| 17 | - | 46.4 | - | 45.53 | C |
| 18 | 2.11 (d, 1H) | 51.7 | 2.13 *(d,1H)* | 52.19 | CH |
| 19 | 1.33 (m, 1H) | 37.8 | | 38.95 | CH |
| 20 | 0.98 (m, 1H) | 37.7 | 0.98 *(m, 1H)* | 38.66 | CH |
| 21 | 1.46 (m, 4H) | 29.5 | | 30.17 | $CH_2$ |

| | | | | | |
|---|---|---|---|---|---|
| | 1.59 (m, 4H) | | | | |
| 22 | 1.86 (m, 2H) | 35.8 | 1.86 *(dd, 2H, $CH_2$-22)* | 36.40 | $CH_2$ |
| 23 | 0.94 (m, 5H) | 28.1 | 0.93 *(m, 4H, $CH_3$-23, $CH_2$-11)* | 29.76 | $CH_3$ |
| 24 | 0.80 (s, 3H) | 15.4 | 0.76 *(s, 4H, $CH_3$-24, $CH_2$(Ha)-1)* | 15.29 | $CH_3$ |
| 25 | 0.75 (s, 3H) | 14.5 | 0.73 *(s, 3H)* | 14.92 | $CH_3$ |
| 26 | 0.86 (s, 3H) | 17.3 | 0.87 *(s, 3H)* | 16.61 | $CH_3$ |
| 27 | 1.04 (s, 3H) | 23.2 | 1.04 *(s, 3H)* | 23.68 | $CH_3$ |
| 28 | - | 177.6 | - | 179.22 | Carbonyl Carbon |
| 29 | 0.89 (d, 3H) | 16.3 | 0.90 *(d, 3H)* | 16.52 | $CH_3$ |
| 30 | 0.81 (d, 3H) | 22.6 | 0.81 *(d, 3H)* | 23.00 | $CH_3$ |

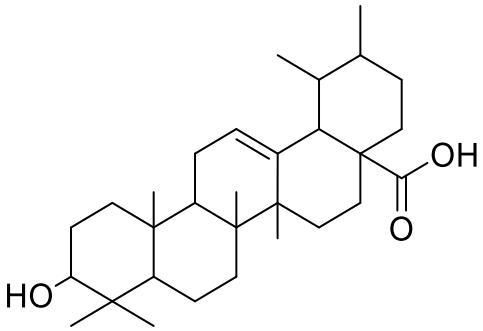



**Figure 13:** Proposed chemical structure of compound **45**

Compound **46** (9.24 mg) was isolated as a white crystalline needle like shape with a melting point temperature between 136 $^oC$ and 137 $^oC$. It has $R_f$ value of 0.36 (when 10% ethylacetate in 90% *n*-hexane is used as eluent). $^1H$-NMR (300 MHz, $CDCl_3$) (**Appendix-7**) spectrum of the compound varied between 0.68 ppm to 5.36 ppm. Signals at δ 5.36 and δ 3.53 revealed the existence of one olefinic proton and hydroxyl group, which appeared as multiplets, respectively. The complex multiplets signal at δ 2.28 and δ 2.38 revealed that the two $CH_2$ protons adjacent to carbon attached to a hydroxyl group. From six methyl signals, signals at δ 0.68, δ 0.82, and δ 0.91 appeared as methyl sinlgets, whereas signal at δ 0.85 and δ 0.92 appeared as methyl doublets and signal at δ 0.84 appeared as methyl triplet.

The $^{13}C$-NMR spectrum (75 MHz, $CDCl_3$) (**Appendix-8**) in combination with DEPT-90 and DEPT-135 (**Appendix-9)** revealed 29 carbon signals. The signal at δ 140.8 and δ 121.74 indicated the presence of olefinic carbon. Meanwhile, the chemical shift at δ 71.9 showed that a tertiary carbon was attaching to a beta hydroxyl group.

The DEPT-90 and DEPT-135 data revealed that there were three quaternary carbons, nine methine carbons, eleven methylene carbons and six methyl carbon atoms. From the six methyl carbons, signals at δ 19.83, 19.03 18.79, and 11.99 were terminal methyl's, whereas signals at 19.41 and 11.87 correspond to angular methyl carbons. The HSQC spectrum supported the data on the DEPT 135 spectrum, where there were only 26 carbon atoms directly bonded to proton atoms. There were many cross-peaks in the COSY spectrum.

The above $^{1}$H-NMR, $^{13}$C-NMR, DPET-90, and DPET-135 spectra data, together with references suggest that the compound is β-sitosterol, which was isolated herein for the first time from *Salvia leucantha.*

**Table 6:** $^{1}$H NMR and $^{13}$C NMR spectral data of compound **46** and the reference, β-sitosterol

| Carbon Position | $^{1}$H-NMR data of compound **46** | $^{13}$C-NMR data of compound **46** | $^{1}$H-NMR data of β-sitosterol (Hernandi *et.al.*, 2022) | $^{13}$C-NMR data of β-sitosterol (Arjun *et.al.*, 2010) | Types of carbons |
|---|---|---|---|---|---|
| 1 | 1.08; 1.01(m) | 37.25 | 1.07; 1.02(2H, dd) | 37.28 | $CH_2$ |
| 2 | 1.48; 1.44 (m) | 31.66 | 1.44; 1.48(2H, dt) | 31.69 | $CH_2$ |
| 3 | 3.53(m) | 71.83 | 3.51(1H, m) | 71.82 | CH |
| 4 | 2.29; 2.23(m) | 42.29 | 2.22; 2.29(2H, m) | 42.33 | $CH_2$ |
| 5 | - | 140.75 | - | 140.70 | C |
| 6 | 5.36(t) | 121.74 | 5.35(1H, br) | 121.72 | CH |
| 7 | 2.00; 1..84(m) | 29.71 | 2.01; 1.85(2H, dt) | 31.69 | $CH_2$ |
| 8 | 1.56(m) | 31.91 | 1.57(1H, m) | 31.93 | CH |
| 9 | 0.93(m) | 50.13 | 0.93(1H, m) | 50.17 | CH |
| 10 | - | 36.51 | - | 36.52 | C |
| 11 | 1.49; 1.40 (m) | 21.09 | 1.49; 1.42(2H, m) | 21.10 | $CH_2$ |
| 12 | 1.15; 1.98(m) | 39.77 | 1.15; 1.98(2H, d) | 39.80 | $CH_2$ |
| 13 | - | 42.32 | - | 42.33 | C |
| 14 | 0.99(m) | 56.77 | 1.00(1H, m) | 56.79 | CH |
| 15 | 1.06; 1.58(m) | 24.31 | 1.57(2H, m) | 24.37 | $CH_2$ |
| 16 | 1.84(2H, m) | 28.26 | 1.84(2H, m) | 28.25 | $CH_2$ |
| 17 | 1.10(m) | 56.05 | 1.09(1H, dt) | 56.09 | CH |
| 18 | 0.68(s) | 11.87 | 0.68(3H, s) | 11.86 | $CH_3$ |
| 19 | 0.82(s) | 19.41 | 1.01(3H, s) | 19.40 | $CH_3$ |
| 20 | 1.33(m) | 36.15 | 1.36(1H, m) | 36.52 | CH |
| 21 | 0.92(d) | 18.79 | 0.92(3H, d) | 18.79 | $CH_3$ |
| 22 | 1.33(m) | 33.94 | 1.38(2H, m) | 33.98 | $CH_2$ |
| 23 | 1.50(m) | 26.05 | 1.54(2H, m) | 26.14 | $CH_2$ |

| | | | | | |
|---|---|---|---|---|---|
| 24 | 0.92(m) | 45.83 | 0.93(1H, m) | 45.88 | CH |
| 25 | 1.66(m) | 29.14 | 1.66(1H, m) | 28.91 | CH |
| 26 | 0.85(d) | 19.83 | 0.84(3H, d) | 19.80 | $CH_3$ |
| 27 | 0.91(s) | 19.03 | 0.92(3H, d) | 18.79 | $CH_3$ |
| 28 | 1.25(m) | 23.06 | 1.26(2H, m) | 23.10 | $CH_2$ |
| 29 | 0.84(t) | 11.99 | 0.83(3H, t) | 11.99 | $CH_3$ |

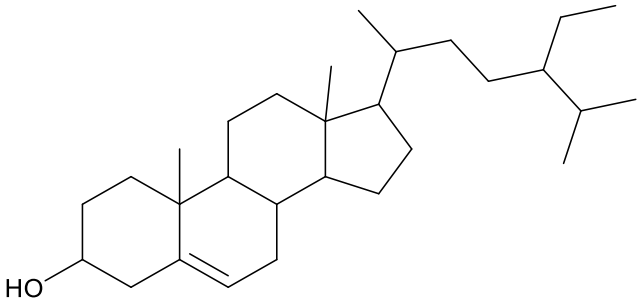



**Figure 14:** Proposed chemical structure of compound **46**

### 4.4 Analysis of Essential Oils

The dried leaf part of *Salvia leucantha* was subjected to hydrodistillation for 4 hours to isolate essential oil using a Clevenger-type apparatus. The oil yield (w/w) was 0.064 % (64 mg/100 g). Generally, the available evidence of the GC-MS result indicates that a total of 50 components are identified, in the essential oils of *Salvia leucantha* Cav (leaf part). From those 50 components identified only trans-β-ionone, caryophyllene oxide, α-calacorene, isospathule-nol and dibutyl phthalate were previously reported from this plant. Whereas 45 compounds are reported for the first time from the essential oils of *Salvia leucantha* leaf.

The secondary metabolite classes represented in the essential oils of *Salvia leucantha* are terpenes and terpenoids (including monoterpenes, sesquiterpenes, and its derivatives), alkaloids, phenolics (including phenolic compounds and chalcones), and other compounds (like fatty acids and their derivatives, phthalates, siloxanes, ketones, alkanes, and esters). From this class of compounds, terpenes are the most abundant component of the essential oils (73.3213 %), followed by other compounds (23.5298%), phenols (2.4200%), and alkaloids (0.7289%).

The most abundant compound is 1H-Cycloprop[e]azulen-7-ol decahydro-1,1,7-trimethyl-4-methylene-, 1ar-(1a. α-.,4a. α-,7. -β, 7a. -β, 7b. α-)] **40** (36.3 %) followed by diisooctylphthal ate **41** (6.9 %) and caryophyllene oxide **42** (5.3 %). Whereas stearyl alcohol P721 **43** (0.37%) was the less abundant compound.

**Table 7:** Essential oil composition of the leaf of *Salvia leucantha*

| No | Compound Name | Molecular Formula | RI | % Composition |
|---|---|---|---|---|
| **1** | 6,7-Dimethoxy-2-methyl-3,4-dihydro[1-D]isoquinolinium ion | $C_{12}H_{15}DNO_2$ | 4.689 | 0.728908156 |
| **2** | trans-.beta.-Ionone | $C_{13}H_{20}O$ | 25.306 | 0.42316244 |
| **3** | 2,6-Di-tert-butyl-4-methyl-phenol | $C_{15}H_{24}O$ | 26.101 | 1.316776267 |
| **4** | Cyclohexanecarboxylic acid, 3-fluorophenyl ester | $C_{13}H_{15}FO_2$ | 26.302 | 0.585937635 |
| **5** | cis-Calamenene | $C_{15}H_{22}$ | 26.41 | 1.110530772 |
| **6** | 1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol | $C_{15}H_{26}O$ | 26.668 | 0.626573751 |
| **7** | 1-((1S,2R,3R)-2-(3-Isopropylfuran-2-yl)-3-methylcyclopentyl)ethanone | $C_{15}H_{22}O_2$ | 26.931 | 1.537411876 |
| **8** | Cadala-1(10),3,8-triene | $C_{15}H_{22}$ | 27.017 | 3.582115679 |
| **9** | .alpha.-Calacorene | $C_{15}H_{20}$ | 27.606 | 1.542841818 |
| **10** | Isospathulenol | $C_{15}H_{24}O$ | 27.732 | 0.418443928 |
| **11** | 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)] | $C_{15}H_{24}O$ | 28.075 | 36.33835875 |
| **12** | Caryophyllene oxide | $C_{15}H_{24}O$ | 28.23 | 5.269154754 |
| **13** | Pethylbrene | $C_{15}H_{24}$ | 28.522 | 2.127464269 |
| **14** | (3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol | $C_{15}H_{26}O$ | 29.289 | 0.459133881 |
| **15** | 1.beta.,4.beta.H,10.beta.H-Guaia-5,11-diene | $C_{15}H_{24}$ | 29.42 | 1.066748982 |
| **16** | Tau-Cadinol acetate | $C_{17}H_{28}O_2$ | 29.849 | 0.707619093 |
| **17** | 1,1,7-trimethylspiro[2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[e]azulene-4,2'-oxirane] | $C_{15}H_{24}O$ | 29.998 | 0.922028728 |
| **18** | 1(2H)-Naphthalenone, 3,4,4a,5,6,7-hexahydro-4a,5-dimethyl-3-(1-methylethenyl)-, [3S-(3.alpha.,4a.alpha.,5.alpha.)]- | $C_{15}H_{22}O$ | 30.101 | 0.630838485 |
| **19** | .tau.-Muurolol | $C_{15}H_{26}O$ | 30.221 | 1.768522812 |
| **20** | Ylangenal | $C_{15}H_{22}O$ | 30.376 | 2.247503669 |
| **21** | Bis[3,3,4,7-tetramethyl-1,3-2H-benzofuran-1-yl] ether | $C_{24}H_{30}O_3$ | 30.456 | 0.454419215 |
| **22** | (1R,7S,E)-7-Isopropyl-4,10 dimethylenecyclodec-5-enol | $C_{15}H_{24}O$ | 30.942 | 0.837410853 |
| **23** | (-)-Isolongifolol, methyl ether | $C_{16}H_{28}O$ | 31.251 | 0.522666508 |
| **24** | Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)- | $C_{15}H_{24}O_2$ | 31.394 | 0.765260309 |
| **25** | Linoleic acid @P785 | $C_{18}H_{32}O_2$ | 31.778 | 1.910654999 |
| **26** | .alpha.-Vetivol | $C_{15}H_{24}O$ | 32.012 | 1.624544752 |
| **27** | Murolan-3,9(11)-diene-10-peroxy | $C_{15}H_{24}O_2$ | 32.47 | 0.779354315 |
| **28** | Ylangenol | $C_{15}H_{24}O$ | 32.991 | 0.591571392 |
| **29** | Retinal | $C_{20}H_{28}O$ | 34.009 | 0.700231757 |

| | | | | |
|---|---|---|---|---|
| **30** | 1-Propyl-3-(propen-1-yl)adamantine | $C_{16}H_{26}$ | 34.209 | 0.891183428 |
| **31** | 2-Butyloxycarbonyloxy-1,1,10-trimethyl-6,9-epidioxydecalin | $C_{18}H_{30}O_5$ | 34.913 | 0.6152947 |
| **32** | 2-Pentadecanone, 6,10,14-trimethyl- | $C_{18}H_{36}O$ | 34.999 | 1.677882778 |
| **33** | Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R-(1.alpha.,2.alpha.,3.beta.,6.alpha.)]- | $C_{15}H_{26}O$ | 35.308 | 0.400162098 |
| **34** | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | $C_{16}H_{22}O_4$ | 35.474 | 2.981157256 |
| **35** | Undec-10-ynoic acid, tridec-2-yn-1-yl ester | $C_{24}H_{40}O_2$ | 35.897 | 0.511187488 |
| **36** | 2,4,7,14-Tetramethyl-4-vinyl-tricyclo[5.4.3.0(1,8)]tetradecan-6-ol | $C_{20}H_{34}O$ | 36.287 | 0.429196136 |
| **37** | Palmitic acid ME P721 | $C_{17}H_{34}O^2$ | 36.435 | 0.731750031 |
| **38** | Palmitic acid P639 | $C_{16}H_{32}O_2$ | 36.968 | 1.044160117 |
| **39** | Dibutyl phthalate | $C_{16}H_{22}O_4$ | 37.025 | 2.329721909 |
| **40** | Silikonfett | | 37.311 | 1.459000748 |
| **41** | 2-Propen-1-one, 1-(2-hydroxyphenyl)-3-phenyl- | $C_{15}H_{12}O_2$ | 37.402 | 0.679950232 |
| **42** | Octadecane P630 | $C_{18}H_{38}$ | 38.026 | 0.468405545 |
| **43** | Stearyl alcohol P721 | $C_{18}H_{38}O$ | 38.581 | 0.370235889 |
| **44** | 9-Octadecenoic acid (Z)-, methyl ester | $C_{19}H_{36}O_2$ | 38.804 | 1.424117602 |
| **45** | 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- | $C_{20}H_{40}O$ | 38.947 | 2.248380458 |
| **46** | 11,13-Dimethyl-12-tetradecen-1-ol acetate | $C_{18}H_{34}O_2$ | 39.869 | 0.497708772 |
| **47** | Tetracosamethyl-cyclododecasiloxane | $C_{24}H_{72}O_{12}Si_{12}$ | 40.693 | 1.632558915 |
| **48** | Butyl 9-tetradecenoate | $C_{18}H_{34}O_2$ | 41.768 | 0.543117237 |
| **49** | cis-9-Tetradecenoic acid, isobutyl ester | $C_{18}H_{34}O_2$ | 42.615 | 0.56010311 |
| **50** | Diisooctylphthalate @P1404 | $C_{24}H_{38}O_4$ | 43.084 | 6.908535707 |

According to studies in India, essential oil from the leaves of *Salvia leucantha* was determined by GC and GC-MS. Forty-two compounds were identified, which accounted for 94.3% of oil composition. The sesquiterpenoids made up 57.6% of the oil, major constituents being spathulenol (12.1%), β-caryophyllene (10.7 %), α-himachalene (10.5%) and γ-cadinene (6.5%). Monoterpenoids constituted 35.7% of the oil, with bornyl acetate (27.8%) as the single major compound (Upadhyaya *et.al.,* 2009). This result agrees with our result since terpenes are the most abundant component of the essential oils.

Six major compounds namely, the sesquiterpenes 6.9-guaiadiene (19.14%), (E)-caryophyllene (16.80%), germacrene D (10.22%), (E)-β-farnesene (10.00%), and bicyclogermacrene (7.52%), and the monoterpenoid bornyl acetate (14.74%) were reported from the essential oil of *Salvia leucantha* isolated by steam distillation from the aerial parts collected in the south of Ecuador (Villalta *et.al.,* 2021). Another study in Venezuela also revealed that an aerial parts of *S. leucantha* (collected at 1400 M above sea level) 0.05% of average oil yield and thirty compounds were identified in the oil, which account for 95.9% of

the total. The major constituents were bornyl acetate (24.1%), β-gurjunene (14.8%), β-caryophyllene (14.1%), dillapiol (11.0%), bicyclogermacrene (8.9%), and germacrene-d (6.6%) (Rojas *et.al.*, 2010).

According to studies in India from EO of *S. leucantha,* forty-eight components were identified which contributed about 94.4% of the oil. The oil was found rich in sesquiterpene hydrocarbons (64.0%) and monoterpenoids (26.1%). Bornyl acetate constituted 23.9% of the oil. This is the first detailed report on the essential oil composition based on capillary GC and GC/MS analyses (Negi *et.al.*, 2007).

Generally, when we compare our result with other published data even though terpenes (73.3213 %) is the major component of the oil, only five components of the essential oils are reported previously from *S. leucantha.* In conclusion, the chemical profiles of the EOs isolated from *S. leucantha* growing in different countries are similar. However, they are not identical and thus characterize each oil. On the other hand, the different percentages of the major constituents of the different EOs could depend on several factors such as origin, age of plant, plant part, climate, place of growth, soil conditions, temperature, extraction methods and storage (Villalta *et.al.*, 2021).

### 4.5 Antibacterial Activity

The antibacterial activity of crude extracts and isolated compounds was evaluated by measuring the size of the zone of inhibition against the test organisms (**Table 8 and 9**). The results revealed that the extracts displayed a promising growth inhibitory effect at the concentration of 200 mg/mL against the selected bacterial strains. The *n*-hexane extract showed the highest zone of inhibition (20.5±0.70 mm) against *E. coli,* followed by inhibition against *S. aureus* and *S. pyogenes* with zone of inhibition values of 17.5±0.70 and 17.50±2.12 mm, respectively. Whereas the least inhibition zone was measured against *P. aeruginosa* at 13.5±0.70 at 200 mg/mL and no effect at 50 and 25 mg/mL. This finding was promising in comparison to the standard drug (ciprofloxacin). Ciprofloxacin showed inhibition zones of 33.33±1.25 mm, 37.33±1.25mm, 32.66±0.28mm and 33.16±2.02mm at 30 µg/mL against *E. coli, P. aeruginosa, S. pyogenes, and S. aureus,* respectively.

However, the ethylacetate extract had only activity against *S. aureus*. The inhibition zones were maximum at 200 mg/mL (24.5±0.70 mm) and minimum at 25 mg/mL (14.0±1.41 mm). The methanol extract showed the highest zone of inhibition (23.0±0.00 mm) against *P. aeruginosa,* followed by inhibition against *S. pyogenes* and *S. aureus,* with zone of inhibition

values of 19.5±0.70 mm and 18.50±0.70 mm, respectively. Whereas the least inhibition zone was measured against *E. coli* 8.5±0.70 mm at 200 mg/mL and no effect at 100, 50, and 25 mg/mL.

In comparison to the ethylacetate and methanol extracts (at smaller concentration of 25 mg/mL), the *n*-hexane extract showed the highest mean inhibition zone of (10.50±0.70 mm) against *S. aureus* (8.00±0.00 mm) followed by *E. coli and S. pyogenes*. All extracts have no activity at a smaller concentration (25 mg/mL) against *P. aeruginosa.*

As far as we know, this is the first scientific study that is conducted and reported on the antibacterial activity of *S. leucantha* crude extracts by using *n*-hexane, ethylacetate, and methanol as solvent.

**Table 8:** Antibacterial activity of *S. leucantha* leaf extracts against selected bacterial strains.

| Extract | Conc (mg/ml) | *E. coli* | *P. aeruginosa* | *S. Pyogenes* | *S. aureus* |
|---|---|---|---|---|---|
| ***n*-Hexane** | 200 | 20.5±0.70 | 13.5±0.70 | 17.50±2.12 | 17.5±0.70 |
| | 100 | 18.0±1.41 | 10.0±1.41 | 15±1.41 | 15.5±0.70 |
| | 50 | 15.5±0.70 | NE | 11.5±0.70 | 12.5±2.12 |
| | 25 | 8.0±0 | NE | 8.00±0 | 10.5±0.70 |
| **Ethylacetate** | 200 | NE | NE | NE | 24.5±0.70 |
| | 100 | NE | NE | NE | 22.0±1.41 |
| | 50 | NE | NE | NE | 18.5±0.70 |
| | 25 | NE | NE | NE | 14.0±1.41 |
| **Methanol** | 200 | 8.5±0.70 | 23.0±0 | 19.5±0.70 | 18.5±0.70 |
| | 100 | NE | 20.5±0.70 | 16.0±0 | 13.0±0.70 |
| | 50 | NE | 17.0±1.41 | 8.5±0.70 | NE |
| | 25 | NE | NE | NE | NE |
| **Ciprofloxacin** | 30 μg/mL | 33.33±1.25 | 37.33±1.25 | 32.66±0.28 | 33.16±2.02 |

*NE= No Effect*

Among compounds isolated from the leaf extract of *S. leucantha*, compound **44** exhibited strong antibacterial activities against *S. aureaus, S. pyogenes, E. coli,* and *P. aeruginosa* with inhibition zones of 24.50±0.70 mm, 22.00±1.41 mm, 21.00±1.41 mm, and 18.00±0.00 mm (at 2 mg/mL), respectively, compared to standard drug ciprofloxacin (34.2±1.25 mm, 33.8±1.25 mm, 34.5±1.06 mm, and 32.2±0.44 mm at 30 μg/mL).

Compound **45** also showed strong antibacterial activities (22.00±1.41, 24.50±2.12, 20.0±2.82, and 21.00±1.41 mm, each at 2 mg/mL) against *S. aureaus, S. pyogenes, and E. coli* and *P. aeruginosa* respectively, which was comparable to compound **44** and ciprofloxacin.

However, compound **46** only showed strong antibacterial activity against *S. pyogenes* and *E. coli* (22.50±0.70 mm and 21.50±2.12 mm at 2 mg/mL, respectively) and moderate antibacterial activity against *S. aureaus* and *P. aeruginosa* (13.00±2.82 mm and 10.50±0.70 mm at 2 mg/mL, respectively) in relative to other isolated compounds (**44** and **46**) and the standard drug ciprofloxacin.

The isolated compound from the leaf extract of *S. leucantha* displayed a remarkable inhibitory effect at a lower concentration when compared to the methanol extract. Whereas the antibacterial activity observed with the methanol extract might be attributed to its bioactive compounds content.

**Table 9:** The antibacterial activities of isolated compounds (**44-46**) against selected bacterial strains

| Compound | Concentration (mg/mL) | Inhibition Diameter in mm (mean ± SEM) | | | |
|---|---|---|---|---|---|
| | | ***S. aureus*** | ***S. pyogenes*** | ***E. coli*** | ***P. aeruginosa*** |
| **44** | 2 | 24.50±0.70 | 21.00±1.41 | 22.00±1.41 | 18.0±0.00 |
| | 1 | 21.50±2.12 | 17.50±0.70 | 18.50±0.70 | 14.50±0.70 |
| | 0.5 | 18.00±1.41 | 14.00±1.41 | 16.50±2.12 | 10.50±0.70 |
| | 0.25 | 15.50±0.70 | 9.50±2.12 | 14.00±1.41 | 9.00±1.41 |
| **45** | 2 | 22.00±1.41 | 24.50±2.12 | 20.00±2.82 | 21.00±1.41 |
| | 1 | 18.50±0.70 | 22.50±2.12 | 16.00±1.41 | 16.50±2.12 |
| | 0.5 | 16.00±0.00 | 16.50±2.12 | 12.50±0.70 | 13.5±2.12 |
| | 0.25 | NE | 13.50±0.70 | NE | 11.00±1.41 |
| **46** | 2 | 13.00±2.82 | 22.50±0.70 | 21.50±2.12 | 10.50±0.70 |
| | 1 | 11.00±2.82 | 18.50±0.70 | 18.50±0.70 | 8.50±0.70 |
| | 0.5 | 8.00±1.41 | 15.50±2.12 | 15.50±2.12 | NE |
| | 0.25 | NE | 12.50±2.12 | 12.50±2.12 | NE |
| Ciprofloxacin (μg/mL) | 30 μg/mL | 34.20±1.25 | 33.80±1.25 | 34.50±1.06 | 33.20±0.44 |

***S. aureaus*** *= Staphylococcus aureus,* ***S. Pyogenes*** *= Streptococcus pyogenes,* ***P. aeruginosa*** *= Pseudomonas aeruginosa,* ***E. coli*** *= Escherichia coli,* ***NE****= No Effect*

Chaisin *et.al*. reported that hesperidin shows minimal inhibitory concentration at 16 mg/mL and 8 mg/mL against *E. coli* and *S. aureus*, respectively. Whereas the minimal bactericidal concentration was at 32 mg/mL and 16 mg/mL against *E. coli* and *S. aureus*, respectively (Chaisin *et.al.,* 2021). In another study hesperidin, which was isolated from citrus fruits, demonstrates antibacterial activity against *E. coli* (Hassan *et.al.,* 2018).

A comparative study was conducted by Choi *et.al.,* to compare the in vitro antioxidant, anti-inflammatory, and antibacterial activities of hesperetin, hesperidin, and hesperidin glucoside. The result revealed that hesperidin has minimal inhibitory concentration (1000, >2000 and

2000 µg/mL) and minimal bactericidal concentration (>2000, >2000 and >2000 µg/mL) for *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa,* respectively (Choi *et.al.,* 2022). Hesperidin has potential not only against common bacteria but also against more resilient pathogens like *Mycobacterium tuberculosis* (Elghani *et.al.,* 2023).

Earlier structure activity related studies on the antimicrobial activity of flavonoids generally suggested that the hydroxyl group at positions C-5 and C-7 is important for antibacterial activity. A hydroxyl at C-4 was required for activity against *P. aeruginosa* (Ngabaza *et.al.,* 2017) (Teffo *et.al.,* 2009). This structural activity relationship is also true for compound **44** antibacterial activity.

According to Collins & Charles (1987) at the highest concentration(150µg/mL) ursolic acid shows inhibition zone of 18.1 mm and 20.3 mm in diameter against *S. aureus* and *E. coli* respectively (Collins & Charles, 1987). Another study revealed that ursolic acid shows inhibition zone diameter of (mm) 24.6±0.4, 22.0±1.0, and 19.9±0.6 against *S. aureus,* 9.2±0.5, 8.0±0.3, and 7.1±0.3 against *E. coli,* 10.2±0.5, 8.0±0.1, and 7.8±0.2 against *P. aeruginosa* at concentrations of 2000, 1000, and 500 µg/mL respectively (Ghasemzadeh *et.al.,* 2022).

Liu *et.al*. demonstrated that ursolic acid exhibits significant antibacterial activity against *S. aureus*, with minimum inhibitory concentrations (MIC) as low as 39 µg/mL for 98% pure ursolic acid. This study also revealed that ursolic acid disrupts the bacteria's cell wall integrity and protein synthesis while promoting reactive oxygen species production within *S. aureus* cells, indicating a multifaceted mechanism of action (Liu *et.al.,* 2024). While Song *et al*. reported that ursolic acid and its derivatives possess enhanced antibacterial properties against methicillin-resistant *Staphylococcus aureus* (MRSA). Ursolic acid shows minimum inhibitory concentrations at 64 µg/mL for MRSA HK01 strains and 128 µg/mL for MRSA ATCC 33592 strains (Song *et.al.,* 2019).

Sycz *et.al.* investigated UA's effects on uropathogenic *Escherichia coli* in biofilms, finding that ursolic acid significantly reduced bacterial survival and biofilm formation. The compound was particularly effective against young biofilms, indicating that the use of preparations containing ursolic acid would be appropriate early intervention of urinary tract infections (Sycz *et.al.,* 2022).

Ursolic acid and its derivatives showed activity against six bacterial strains, and the best result was found against *S. aureus* (ATCC 6538) and *E. coli* (ATCC 25922) with a minimal inhibitory concentration value of 32 μg/mL and 64 μg/mL. Ursolic acid was also effective against *K. pneumoniae* and *S. flexneri* with a MIC value of 64 μg/mL (Do Nascimento *et.al.,* 2014). Ursolic acid isolated from *Hedyotis corymbosa* at concentration (100 μg/ disc) showed significant activity against *Staphylococcus aureus* (21 mm) and moderate activity against *Pseudomonas aeruginosa* (10 mm) when compared with standard Kanamycin (Sultana *et.al.,* 2010).

Kurek *et.al.* found that ursolic acid enhanced the susceptibility of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Listeria monocytogenes* to the β-lactam antibiotics ampicilin and oxacillin, but no synergy was observed for *P. aeruginosa.* The results from this study indicate that ursolic acid may be useful when administered in combination with β-lactam antibiotics to combat bacterial infections caused by some Gram-positive pathogens (Kurek *et.al.*, 2012). In another study, ursolic acid showed a synergistic effect with ampicillin and tetracycline against both *Bacillus cereus* and *S. aureus* but was limited to Gram-positive bacteria (C. Wang *et.al.,* 2016). As we mentioned before, compound **45** exhibited strong activity against both Gram-positive and Gram-negative bacteria, so those studies are in agreement with our result.

β-Sitosterol shows antibacterial activity against *Salmonella typhi* and *Escherichia coli* with zones of inhibition of 20 and 35 mm respectively, at 50 μg/mL concertation. The values of minimum inhibitory concentration and minimum bactericidal concentration were as low as 6.25 and 12.50 μg/ml against *Escherichia coli* respectively (Nweze *et.al.,* 2019). Another study demonstrated that β-sitosterol isolated from the stem of *Terminalia mantaly* shows a minimum inhibitory concentration of 0.5 mg/mL against both Gram-positive (*S. aureus* and *S. pyogenes*) and Gram-negative (*S. dysenteriae* and *E. coli*) bacteria (Hernandi *et.al.*, 2022). Similarly, β-sitosterol from *Odontonema strictum* exhibited MIC and MBC values of 1.24 mg/mL and 2.208 mg/mL respectively, against *S. aureus*, indicating its potent antibacterial properties (Pierre Luhata & Usuki, 2021).

β-sitosterol isolated from *Momordica charantia* shows antimicrobial activity against *E. coli* (14 mm), *S. aureus* (13 mm), and *P. aeruginosa* (11 mm) inhibition zone (Sen *et.al.*, 2012). Hoskeri *et.al*, reported that β-sitosterol inhibited the growth of *E. coli* (14.5 ± 1.84 mm), *S. aureus* (17.83 ± 0.58 mm), and *P. aureginosa* (16.11 ± 1.41). Whereas the minimum inhibitory concentrations for β-sitosterol were 5 mg/ml, 10 mg/ml and 20 mg/ml against *S.*

*aureus, P. aureginosa, and E. coli* respectively (Hoskeri *et.al.,* 2012).

The result from compound **46** was in agreement with the reported paper regarding the antibacterial activity of β-sitosterol. The slight differences in the β-sitosterol antibacterial activity to generate inhibition zones are presumably dependent on the condition of the antimicrobial assay. The different antimicrobial assay conditions have different sample solvents and microbial concentrations (Zuluaga *et.al.,* 2009). The β-sitosterol has been registered as a non-toxic, safe supplement, and it has potential bioactivities, including antibacterial activity (Kurniawan *et.al.,* 2021).

### 4.6 Antioxidant Activity

Oxidative stress can be incited in our cells by a high level of reactive oxygen species, causing cellular damage by enhancing oxidative impairment of the cell components and initiating various other conditions. Oxidative stress extremely affects the pathogenesis of chronic diseases such as neurological disorders, cancers, cardiovascular diseases, age-related eye diseases, and diabetes. When oxidative stress destroys body antioxidant capacity, physiological functions may be impaired; thus, human diseases can be activated. Therefore, using additional sources of antioxidants can defer these detrimental effects (Ghasemzadeh *et.al.,* 2022).

Multiple techniques can be used to test antioxidant activity in vitro and in vivo. Because of its simplicity, speed, and low cost, the DPPH-based technique is likely the most widely used in vitro assay (Gulcin & Alwasel, 2023). The stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) can be decreased by transferring hydrogen from other molecules. In the DPPH antioxidant assay, the ability of DPPH to decolorize in the presence of antioxidants is employed. The deep purple color is caused by the odd electron in the DPPH radical. DPPH decolorizes when it accepts an electron from an antioxidant molecule (Kumarasamy *et.al.,* 2008).

The DPPH radical scavenging activities of the crude extracts and compounds isolated from the leaves of *Salvia leucantha* were examined by comparison with ascorbic acid as a positive control. The $IC_{50}$ values of all samples were calculated and the results are presented in (**Table 10** and **11**) and **(Figure 14** and **15**). The results generally revealed that activity dropped as the concentration of the samples decreased.

Generally, the methanol extract exhibited the highest percentage inhibition of DPPH radical as compared to the other extracts. It showed 76.62 % and 65.81 % inhibition of the DPPH radical at a concentration of 1000 µg/mL and 62.5 µg/mL. The methanol extract $IC_{50}$ was found to be 12.17 µg/mL, compared to the positive control ascorbic acid, having an $IC_{50}$ of 1.315 µg/mL. The lowest radical scavenging activity was found in *n*-hexane extract, with an inhibition value of 83.01% at 1000 µg/mL and $IC_{50}$ value of 241.2 µg/mL. Whereas the radical scavenging activity and $IC_{50}$ value of the ethaylacetate extract were found to be 74.46% at 1000 µg/mL and 21.62 µg/mL respectively.

Compared to the standard ascorbic acid, the DPPH radical scavenging activities of the extracts were found to be medium for methanol ($IC_{50}$ = 12.17 µg/mL) and ethaylacetate ($IC_{50}$ = 21.62 µg/mL) extracts. While the *n*-hexane ($IC_{50}$ = 241.2 µg/mL) extract revealed low DPPH radical scavenging activity.

**Table 10**: DPPH radical scavenging activities (%) of *S. leucantha* leaf extract

| **Conc (µg/ml)** | **Control** | **Positive control** | | **Extracts** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Ascorbic Acid* | | *Hexane* | | *Ethaylacetate* | | *Methanol* | |
| | | A | %RSA | A | %RSA | A | %RSA | A | %RSA |
| **1000** | 0.971 | 0.057 | 94.13 | 0.165 | 83.01 | 0.248 | 74.46 | 0.227 | 76.62 |
| **500** | 0.971 | 0.063 | 93.51 | 0.245 | 74.77 | 0.279 | 71.27 | 0.244 | 74.87 |
| **250** | 0.971 | 0.068 | 92.99 | 0.407 | 58.08 | 0.286 | 70.55 | 0.253 | 73.94 |
| **125** | 0.971 | 0.072 | 92.58 | 0.689 | 29.04 | 0.384 | 60.45 | 0.325 | 66.53 |
| **62.5** | 0.971 | 0.077 | 92.07 | 0.752 | 22.55 | 0.412 | 57.57 | 0.332 | 65.81 |
| **$IC_{50}$(µg/mL)** | | 1.315 | | 241.2 | | 21.62 | | 12.17 | |

***A**-Absorbance, **RSA**-Radical Scavenging Activity, **$IC_{50}$** – 50% Inhibitory Concentration*

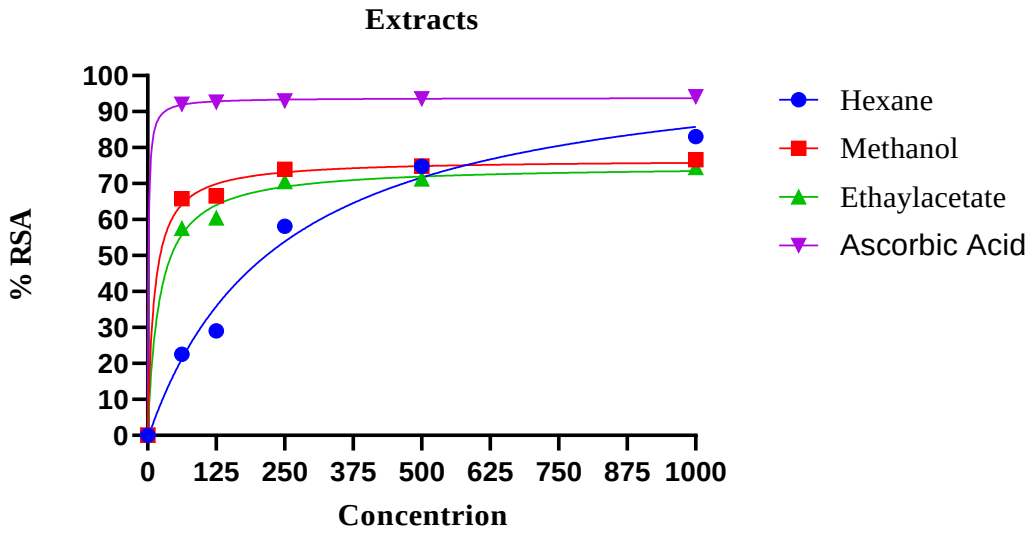



**Figure 15**: DPPH scavenging activity (%) of *S. leucantha* extracts and positive reference

The DPPH assay also revealed varying degrees of antioxidant activity for the isolated compounds. The $IC_{50}$ values were found to be 9.83 µg/mL, 21.38 µg/mL, and 38.01 µg/mL for compounds **44**, **45**, and **46** respectively. The result showed that compound **44** exhibited the highest DPPH radical scavenging activity ($IC_{50}$ = 9.83 µg/mL), because this compound had two conjugated ring systems and many hydroxyl groups. The results are expressed relative to the ascorbic acid, a reference standard having an $IC_{50}$ = 0.11 µg/mL.

When compared to the standard ascorbic acid, the DPPH radical scavenging activities of isolated compounds were generally lower than the standard compound (**Table 11**).

**Table 11**: DPPH radical scavenging activities (%) of isolated compounds **44-46**

| **Conc (µg/ml)** | | **Positive control** | | **Compounds** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Control** | **Ascorbic Acid** | | **44** | | **45** | | **46** | |
| | | A | %RSA | A | %RSA | A | %RSA | A | %RSA |
| **400** | 0.971 | 0.066 | 93.2 | 0.467 | 51.9 | 0.481 | 50.5 | 0.482 | 50.4 |
| **200** | 0.971 | 0.069 | 92.9 | 0.512 | 47.3 | 0.508 | 47.7 | 0.580 | 40.3 |
| **100** | 0.971 | 0.074 | 92.4 | 0.594 | 38.8 | 0.584 | 39.8 | 0.668 | 31.2 |
| **50** | 0.971 | 0.081 | 91.6 | 0.597 | 38.5 | 0.648 | 33.2 | 0.697 | 28.2 |
| **25** | 0.971 | 0.089 | 90.8 | 0.599 | 38.3 | 0.667 | 31.3 | 0.721 | 25.7 |
| **IC50(**µg/mL) | | 0.6816 | | 9.83 | | 21.38 | | 38.01 | |

***A**-Absorbance, **RSA**-Radical Scavenging Activity, **IC$_{50}$** – 50% Inhibitory Concentration*

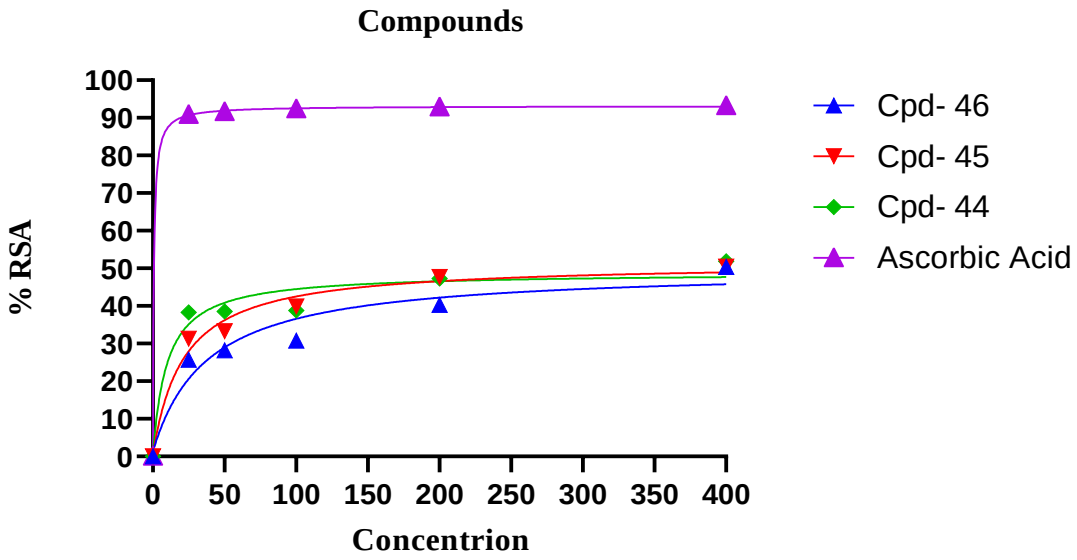



**Figure 16:** DPPH scavenging activity (%) of isolated compounds (**44-46**) and positive reference

Choi *et.al*, (2022) found that hesperetin showed a higher effect than hesperidin and hesperidin glucoside, whereas hesperidin and hesperidin glucoside did not show a significant difference. The mean scavenging concentration ($SC_{50}$, representing 50% of scavenging) was 525.18 ± 1.02 µM for hesperetin, 896.21 ± 0.15 µM for hesperidin, and 911.00 ± 0.14 µM hesperidin glucoside, respectively, while the $SC_{50}$ of ascorbic acid as a positive control was 61.78 ± 0.02 µM (Choi *et.al.,* 2022).

A study by Huang *et.al*. tried to investigate the effects of hesperidin on $H_2O_2$-induced oxidative stress in bovine mammary epithelial cells and the underlying molecular mechanism. This study suggests that hesperidin effectively protected against $H_2O_2$-induced oxidative damage in bovine mammary epithelial cells (bMECs) by inhibiting cell apoptosis, reducing reactive oxidative stress overproduction and malondialdehyde formation, as well as enhancing the levels of Catalase (Huang *et.al.,* 2024).

In addition to its vast health benefits, the antioxidant properties of hesperidin are utilized in the food industry, where it serves as a natural preservative. For example, when added to meat and eggs, hesperidin improves oxidative stability, thereby prolonging shelf life and reducing spoilage. This application not only improves the quality of food but also contributes to minimizing food waste, showcasing the practical benefits of hesperidin in everyday life (Pereira *et.al.,* 2024).

Hesperidin antioxidant activity is widely researched in the pharmaceutical industry for its anti-inflammatory, anticancer, antiviral, anti-aging, neuroprotective, and cardioprotective effects (Pereira *et.al.,* 2024). Hesperidin shows strong antioxidant properties, which help to neutralize harmful free radicals in the body. This antioxidant activity helps our cells to protect from oxidative damage, which is associated with various chronic diseases, including cardiovascular diseases, neurodegenerative disorders, and cancer. Several mechanisms have been proposed to explain the antioxidant effect of hesperidin. Which includes radical scavenging activity, metal chelation, induction of antioxidant enzymes, modulation of cellular signaling pathways, protection against lipid peroxidation, and regeneration of other antioxidants (Eur *et.al.,* 2023).

The antioxidant property (hydrogen radical scavenging effect, iron chelating activity, and reducing power of oxidants) of the hesperidin is superior to antioxidants such as alpha-tocopherol, ascorbic acid, butylated hydroxytoluene, and butylated hydroxyanisole (Suarez *et.al.,* 1998). This finding was not true in our case because compound **44** did not show superior antioxidant activity than ascorbic acid. Except for this specific research, other

research supports that compound **44** (hesperidin) has strong antioxidant activity. Overall, the multidimensional antioxidant effects of hesperidin make it a valuable compound across various fields, including pharmaceuticals and food preservation.

From the roots of *Potentilla fulgens*, five triterpene acids, namely ursolic acid, euscaphic acid, corosolic acid, fulgic acid A, and fulgic acid B were isolated and their antioxidant activity was evaluated. Ursolic acid was the most potent, with $IC_{50}$ of 5.3 ± 0.2 μM and 7.8 ± 0.4 μM in the DPPH· and ABTS+· assay, respectively. Whereas the standard drug ascorbic acid had $IC_{50}$ of 3.6 ± 0.7 μM and 6.2 ± 0.2 μM in DPPH· and ABTS+· assay respectively (Choudhary *et.al.,* 2013).

According to Do Nascimento *et al.,* ursolic acid showed antioxidant activity by inhibiting DPPH. The compounds strongly scavenged DPPH radical, with $IC_{50}$ values of 0.0597 ± 0 mg/mL. Whereas Trolox and vitamin C, used as positive controls, showed $IC_{50}$ values of 0.026 ± 0.019 and 0.043 ± 0.019 mg/mL, respectively (Do Nascimento *et.al.,* 2014). Ghasemzadeh *et.al,* used the DPPH technique for assessing the antioxidant effects of the purified ursolic acid. They found that ursolic acid at concentrations greater than 625 μg/mL showed significant antioxidant effects ($p < 0.05$) (Ghasemzadeh *et.al.,* 2022).

According to Zhang *et.al*, the radical scavenging ability of β-sitosterol was determined by the DPPH assay. The study shows that the scavenging rate of the free radicals increased with an increasing β-sitosterol concentration in the experimental concentration range. This study concluded that for β-sitosterol at a concentration of 100 μg/mL, the scavenging rate was 43%, indicating that β-sitosterol had a strong ability to scavenge DPPH (P. Zhang *et.al.,* 2023).

In the study aims to evaluate the in-vitro antioxidant potential of β-sitosterol, the result shows that, at 25 μg/ml, 50 μg/m, 75 μg/ml, 100 μg/ml, 125 μg/ml, and 150 μg/ml concertation β-sitosterol demonstrated inhibition percentages of 10.84 ±1.27, 31.83 ±1.31, 46.42 ±1.28, 57.64 ±0.78, 71.82 ±1.26, and 84.29 ±1.24 respectively (Arivarasu, 2023).

### 4.7 In silico Molecular Docking Study

Molecular docking is a powerful computational modeling that facilitates the prediction of the preferred binding orientation of one molecule (eg. ligands/compounds) to another (eg. receptors), when both interact with each other in order to form a stable complex. Molecular docking studies are generally employed to investigate the binding energy and to further validate the molecular mechanisms for ligands at the active site of a protein (Mehrotra, 2016).

Information gained from the preferred orientation of bound molecules may be employed to predict binding affinity, H-bond, hydrophobic, and Van der Waals interactions of ligands. This can be done using the scoring function of molecular docking.

The molecular docking study was carried out to assess the binding affinities and binding interactions of isolated compounds (**44-46**) toward target proteins *E. coli* DNAgyrase B (PDB ID 7P2W), *S. aureus* pyruvate kinase (PDB ID: 3T07) and human myeloperoxidase (PDB ID 1DNU). The binding affinity and amino acid residues that are involved in hydrogen bonds, hydrophobic interactions and Van der Waals interactions at each ligand-protein complexe were mapped using Auto Dock 4.2.6 (**Tables 12** to **14**) and (**Figure 16** to **27**).

In this study, *E. coli* DNA gyrase B (PDB ID 7P2W) was used as a target and a fluroquinolone class of antibacterial ciprofloxacin was used as a reference control for docking with the target. DNA gyrase is a topoisomerase-type enzyme that is required during bacterial DNA replication and transcription to maintain topology and integrity. It consists of four subunits (two A subunits and two B subunits) attached together to form a tetrameric holoenzyme (Janupally *et.al.,* 2015). Currently, DNA gyrase is considered one of the primary targets and has been clinically validated in most pathogenic bacteria (Narramore *et.al.,* 2019).

In this study, each of the isolated compounds (**44-46**) demonstrated better binding affinity (binding energy of -9.0 to -8.5 kcal/mol) against protein DNA gyrase than the standard drug, ciprofloxacin (-7.3 kcal/mol). The highest binding affinity (-9.0 kcal/mol) was recorded by compounds **44** and **45**, followed by compound **46** (-8.5 kcal/mol).

All the compounds have shown hydrogen bonding interactions. Compound **44** showed good H-bonding interaction with amino acid residues Arg-76, Ile-82, Asp-73, Glu-50, and Pro-84 whereas compound **45** showed moderate interaction with Arg-76 (at Dist. 2.53352 Å and Dist. 2.60602 Å), Glu-50. Compound **46** showed H-bond interaction only with His-83. Except for compound **45**, compounds **44**, **46** and the standard drug ciprofloxacin exhibited residual hydrophobic interaction. All compounds exhibited residual Van der Waals interaction more than the standard drug ciprofloxacin. Binding affinity and interactions with different amino acids are presented in **Table 12**, and 3D and 2D binding interactions are depicted in **Figure 16** to **19.**

**Table 12:** Molecular docking value of isolated compounds (**44-46**) against *E. coli* Gyrase B (PDB ID 7P2W)

| Ligands | Binding Affinity (kcal/mol) | H-bond | Residual Interactions | |
|---|---|---|---|---|
| | | | Hydrophobic and Electrostatic | Van der Waals |
| **44** | – 9.0 | Arg-76, Ile-82, Asp-73, Glu-50, Pro-84 | Gly-81, Ile-82, Pro-79, Ala-90 | Ala-47, Val-43, Asn-46, Thr-165, Val-167, Val-120, Ile-78, Val-118, His-83, Gly-101, Ile-94 |
| **45** | – 9.0 | Arg-76 (Dist. 2.53352 Å), Arg-76 (Dist. 2.60602 Å), Glu-50 | _ | Ala-53, His-55, Asn-46, Asp-49, Val-118, Ile-94, Ala-90, Pro-79, His-83 |
| **46** | – 8.5 | His-83 | Ile-78 (Dist. 5.34025 Å), Ile-78 (Dist. 4.92769 Å), Ala-90, Val-118, His-83 | Arg-76, Ile-94, Pro-84, Pro-79, Ala-47, Asp-73, Thr-165, Asn-46, Glu-50, Asp-49 |
| **Cipro-floxacin** | – 7.3 | Arg-76, Gly-77, Arg-136, Asp-73 | Glu-50, Gly-77, Arg-76,Glu-50, Val-118, Ile-94, Ile-78, Pro-79 | Thr-165, Ala-47, Asn-46, Val-120 |

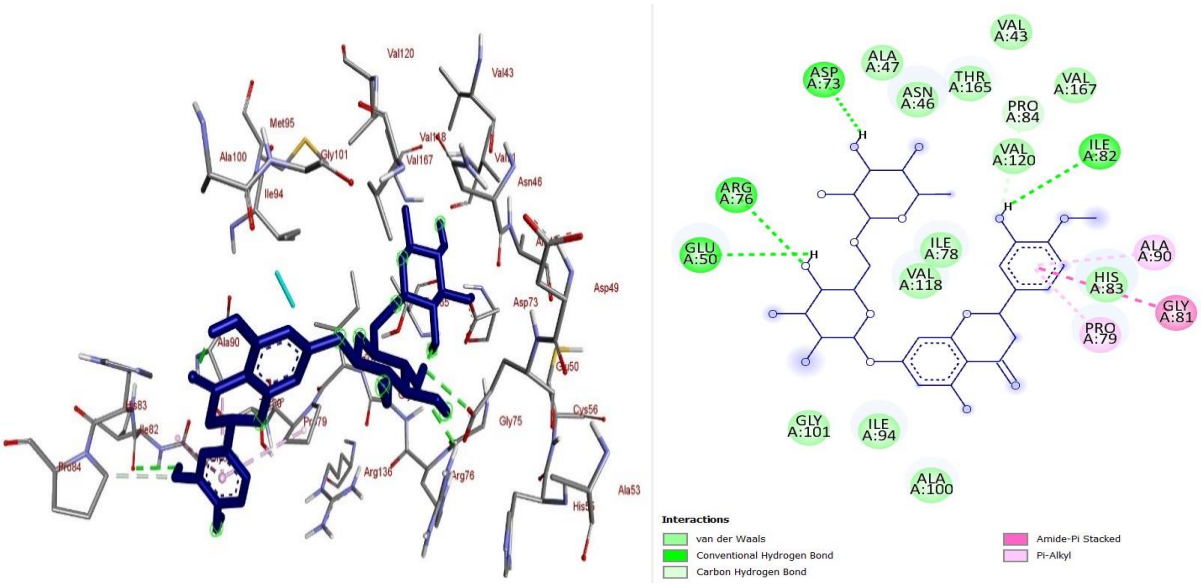



**Figure 17**: 3D and 2D representations of the binding interactions of compound **44** against *E. coli* Gyrase B (PDB ID: 7P2W).

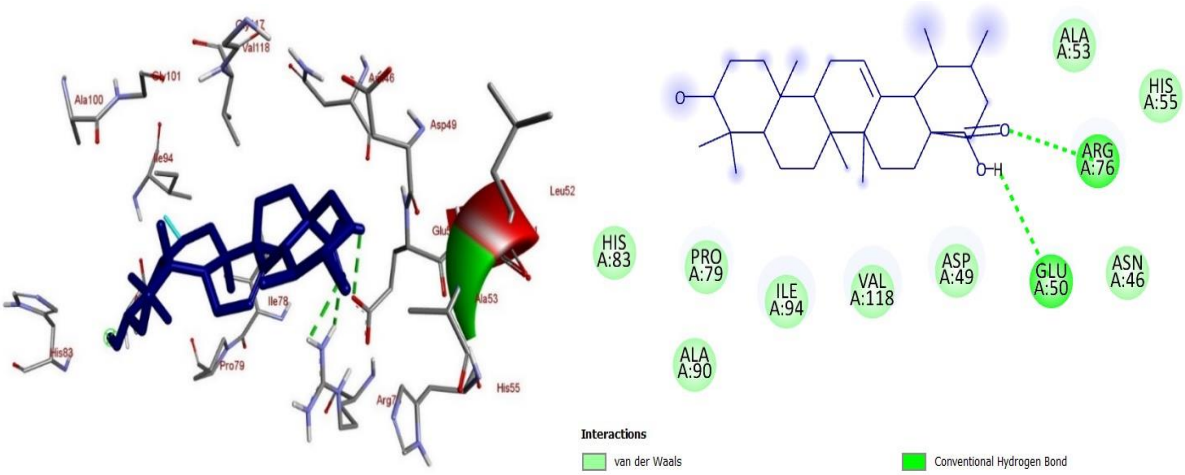



**Figure 18**: 3D and 2D representations of the binding interactions of compound **45** against *E. coli* Gyrase B (PDB ID: 7P2W).

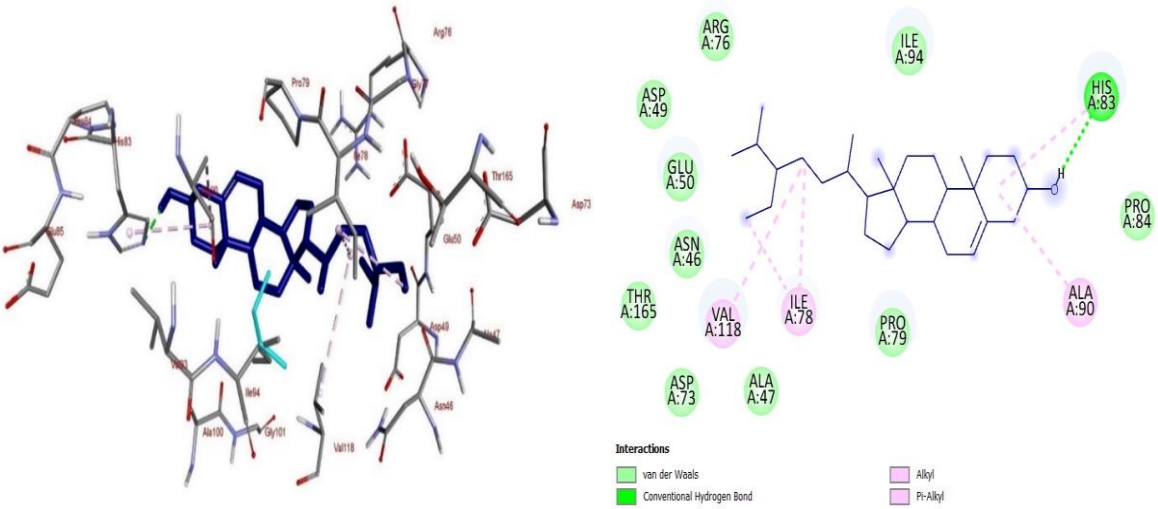



**Figure 19**: 3D and 2D representations of the binding interactions of compound **46** against *E. coli* Gyrase B (PDB ID: 7P2W).

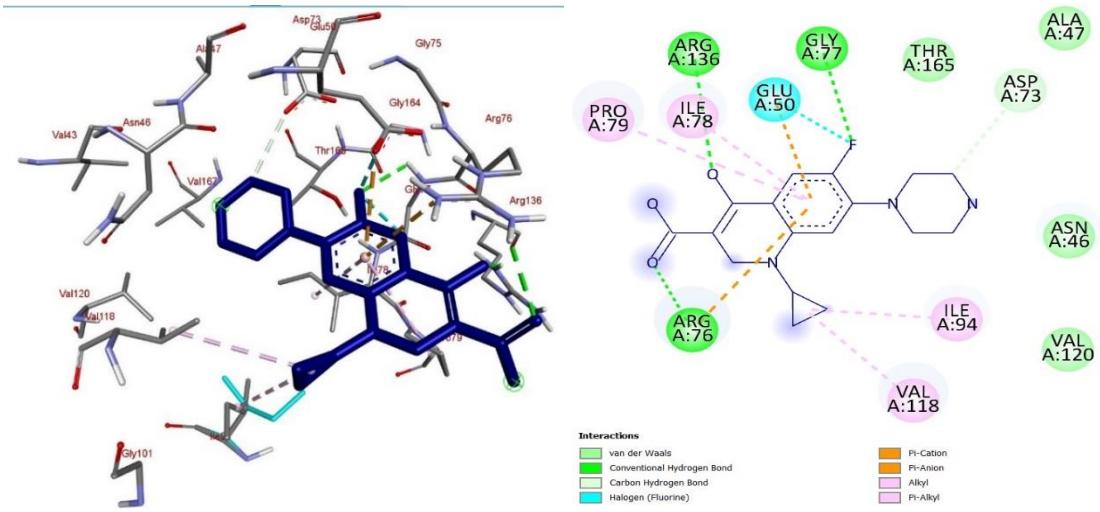



**Figure 20**: 3D and 2D representations of the binding interactions of **Ciprofloxacin** against *E. coli* Gyrase B (PDB ID: 7P2W).

Pyruvate kinase (PK) has been identified as a crucial enzyme in staphylococci and regulates their growth, antibiotic resistance, and biofilm formation. The concentration of pyruvate plays a critical role in *S. aureus*, which is primarily catalyzed by pyruvate kinase (Vasu *et.al.*, 2015). Furthermore, it was found to be structurally distinct from human homologs, and hence it provides a promising target for novel antimicrobial agents (Akunuri *et.al.*, 2022). For the docking studies, *S. aureus* PK (PDB ID: 3T07) was used as a target, and ciprofloxacin was used as a standard drug. Binding affinity and interactions with different amino acids are presented in **Table 13** and 3D and 2D binding interactions are depicted in **Figure 20** to **23.**

The result in this study demonstrated that each of the isolated compounds (**44-46**) and standard drug (ciprofloxacin) has good binding affinity (-10.3 to -7.6 kcal/mol) against pyruvate kinase. Compound **44** exhibited the highest binding affinity (-10.3 kcal/mol) and better H-bonding interaction than the standard drug (-8.0 kcal/mol). The rest of the compounds showed binding affinity lower than the standard drug. They also showed H-bonding interaction with amino acid residues Asp-339 and Gly-338 (for compound-**45**) and Asn-299 (for compound-**46**). All the compounds have exhibited residual Van der Waals interactions. Except for compound **45**, compound **44**, **46**, and the standard drug exhibited residual hydrophobic interaction.

**Table 13:** Molecular docking value of isolated compounds (**44-46**) against Pyruvate Kinase (PDB ID: 3T07)

| **Ligands** | **Binding Affinity (kcal/mol)** | **H-bond** | **Residual interactions** | |
|---|---|---|---|---|
| | | | **Hydrophobic and Electrostatic** | **Van der Waals** |
| **44** | – 10.3 | Lys-342, Lys-260, Arg-264, Leu-343, Lys-349, Asp-303, Asn-299 | Lys-342, Asp-346, Lys-342, Arg-264, Lys-342 | Ser-345, Asp-339, Gln-338, Gly-304, Tyr-302, Asn-267, Asp-303, Asn-299, Tyr-302, Lys-260 |
| **45** | – 7.8 | Asp-339, Gln-338 | – | Arg-264, Lys-260, Gln-338, Tyr-302, Lys-342, Asp-339, Leu-343, Asp-303 |
| **46** | – 7.6 | Asn-299 | Lys-342, Lys-260 | Leu-343, Asp-339, Gln-338, Arg-264, Asp-303, Tyr-302, Lys-260, Asn- |

| | | | | |
|---|---|---|---|---|
| | | | | 299 |
| **Cipro-floxacin** | – 8.0 | Asn-369, Ser-362, Ala-358 | Ile-361, His-365 | Thr-366, Thr-348, Asn-369, Ile-361, His-365 |

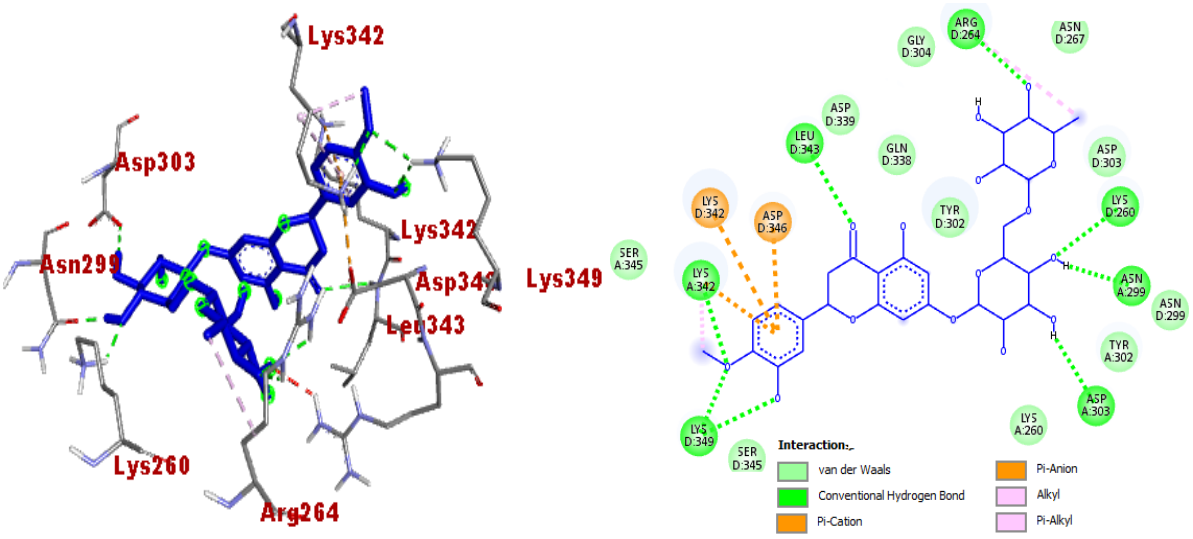



**Figure 21**: 3D and 2D representations of the binding interactions of compound **44** against *S. aureus* Pyruvate Kinase (PDB ID: 3T07).

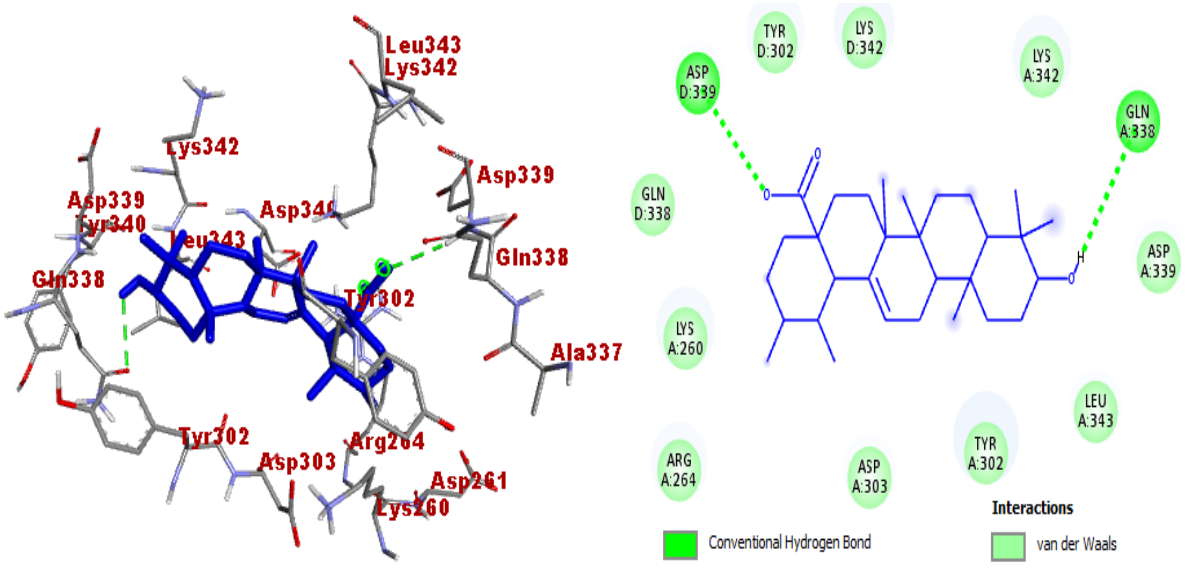



**Figure 22**: 3D and 2D representations of the binding interactions of compound **45** against *S. aureus* Pyruvate Kinase (PDB ID: 3T07).

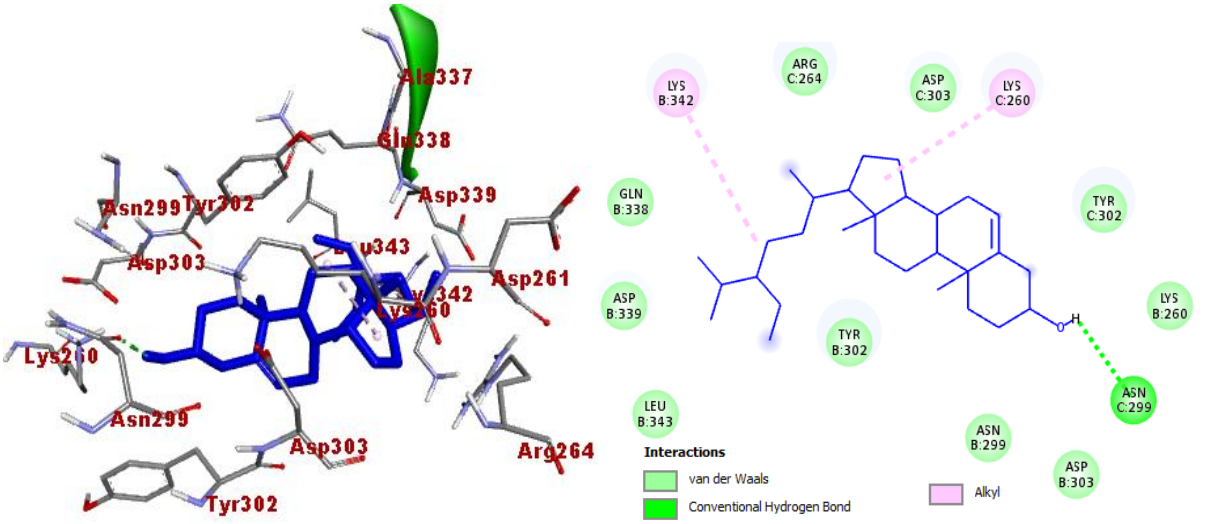



**Figure 23**: 3D and 2D representations of the binding interactions of compound **46** against *S. aureus* Pyruvate Kinase (PDB ID: 3T07).

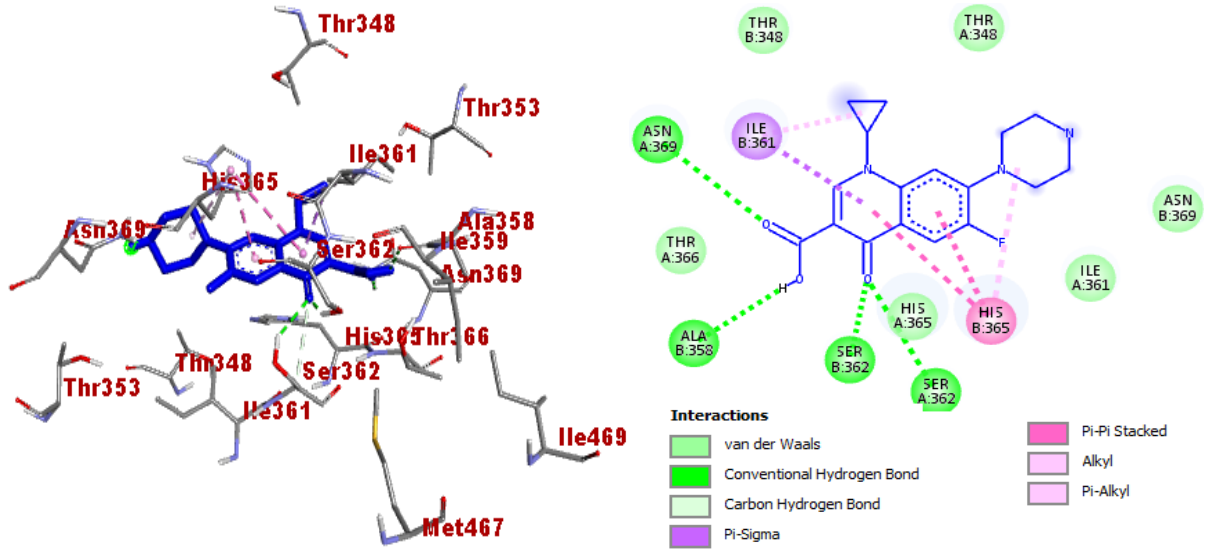



**Figure 24**: 3D and 2D representations of the binding interactions of compound **Ciprofloxacin** against *S. aureus* Pyruvate Kinase (PDB ID: 3T07).

Myeloperoxidase is a heme-containing peroxidase abundantly expressed in neutrophils and to a lesser extent, in monocytes. It includes a set of enzymes with potent oxidoreductase activity and has been implicated in the pathogenesis of several diseases (Frangie & Daher, 2022). Enzymatically active myeloperoxidase, together with hydrogen peroxide and chloride, produces the potent oxidant hypochlorous acid and is a key contributor to the oxygen-dependent microbicidal activity of phagocytes (Van Der Veen *et.al.*, 2009).

In addition, excessive generation of myeloperoxidase derived oxidants has been linked to tissue damage in many diseases, especially those characterized by acute or chronic

inflammation. Now, it has become clear that myeloperoxidase exerts effects that are beyond its oxidative properties. Depending on the local milieu, the myeloperoxidase-$H_2O_2$ system is capable of producing a wide range of oxidant species, including HOCl, chloramines, hydroxyl radicals (HO·), singlet oxygen ($^1O_2$), and ozone ($O_3$). All these products are short lived but highly reactive compounds that can potentially oxidize any oxidizable group in any substrate, including thiols, thiol-esters, heme groups, and unsaturated fatty acids (Van Der Veen *et.al.*, 2009)(Davies *et.al.*, 2008).

When docked to the human myeloperoxidase protein (PDB ID 1DNU), the isolated compounds (**44-46**) demonstrated better and comparable binding affinity (binding energy of -10.5 to -8.1 kcal/mol) against human myeloperoxidase than the standard drug, ascorbic acid (-8.1 kcal/mol).

The highest binding affinity was recorded by compound **45** (-10.5 kcal/mol) followed by compound **44** (-9.9 kcal/mol) and **46** (-8.1 kcal/mol). All compounds have shown hydrogen bonding interactions. Compound **44** showed a good H-bonding interaction comparable to standard drug ascorbic acid, while compound **45** shows a batter H-bonding interaction than compound **46**. Almost all isolated compounds have Van der Waals interaction more than ascorbic acid. Except for compound **45**, all other compounds and ascorbic acid have residual hydrophobic and electrostatic interactions.

**Table 14**: Molecular docking results of isolated compounds (**44-46**) against Human myeloperoxidase (PDB ID 1DNU)

| **Ligands** | **Binding Affinity (kcal/mol)** | **H-bond** | **Residual interactions** | |
|---|---|---|---|---|
| | | | **Hydrophobic and Electrostatic** | **Van der Waals** |
| **44** | **– 9.9** | Arg-323, Ile-160, Arg-31, Asn-162, Leu-33, Pro-34 | Arg-323, Arg-31, Ala-28 | Ala-152, Thr-159, Cys-153, Ala-28, Asn-162, Ala-152, Thr-159, Ile-160, Pro-34, Leu-33, Val-30, Trp-32, Ala-35 |
| **45** | **– 10.5** | Ile-160 | - | Ala-28, Asn-162, Arg-323, Arg-31, Val-30, Trp-30, Leu-33, Ile-160, Thr-159, Ala-152, Cys-153 |

| | | | | |
|---|---|---|---|---|
| **46** | **– 8.1** | His-336 | Leu-420, Phe-146 | Phe-99, Phe-147, Pro-145, Leu-415, Arg-424, Phe-407, Phe-366, Arg-239, Glu-242, Leu-406, Leu-417, His-95, Gln-91, Arg-333 |
| **Ascorbic Acid** | **– 8.1** | Gln-91, Thr-100, Arg-239, Arg-333 | Asp-94, Arg-333 | His-336, Phe-332, Phe-99, Thr-329, His-95, Asp-98 |

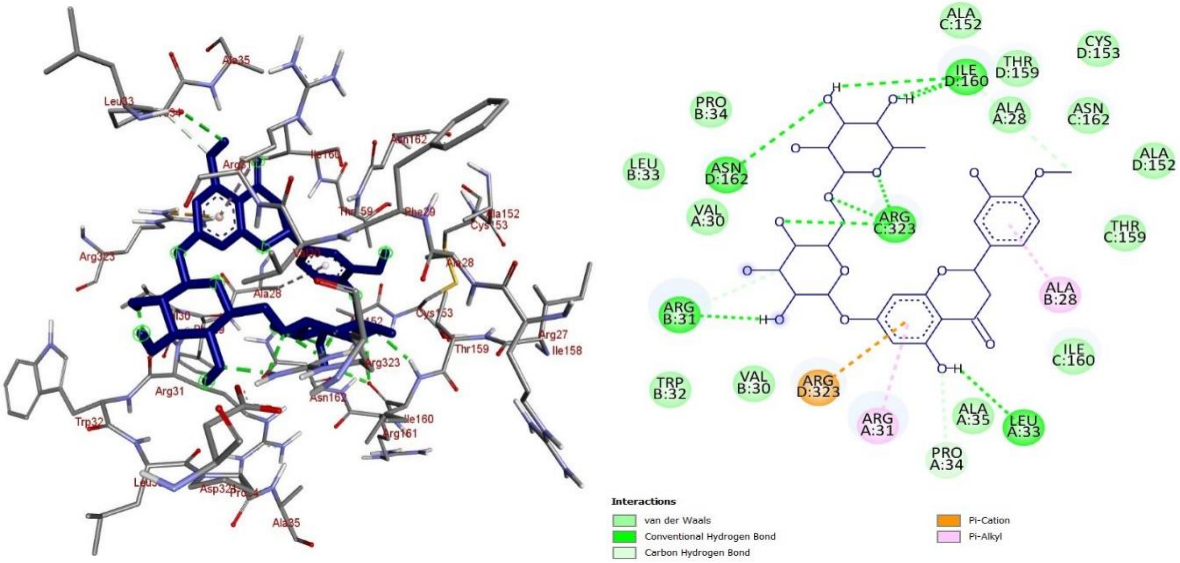



**Figure 25**: 3D and 2D representations of the binding interactions of compound **44** against Human *myeloperoxidase* (PDB ID: 1DNU).

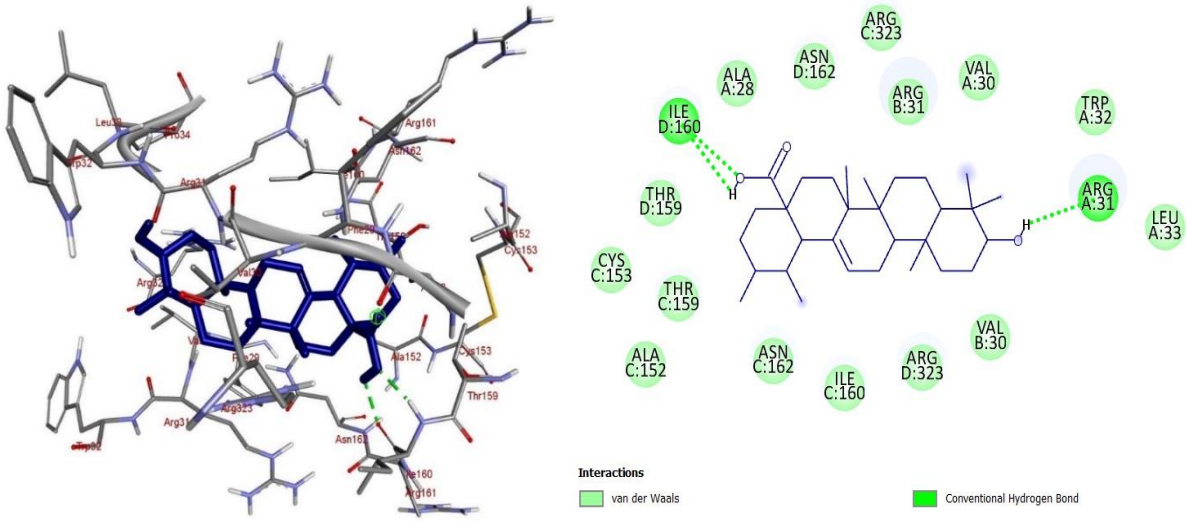



**Figure 26**: 3D and 2D representations of the binding interactions of compound **45** against Human *myeloperoxidase* (PDB ID: 1DNU).

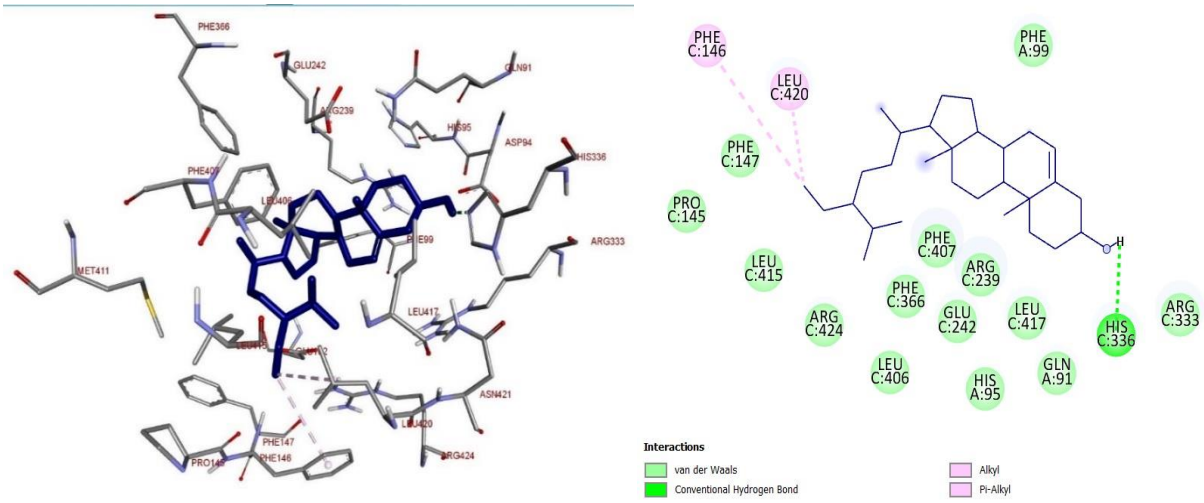



**Figure 27**: 3D and 2D representations of the binding interactions of compound **46** against Human *myeloperoxidase* (PDB ID: 1DNU).

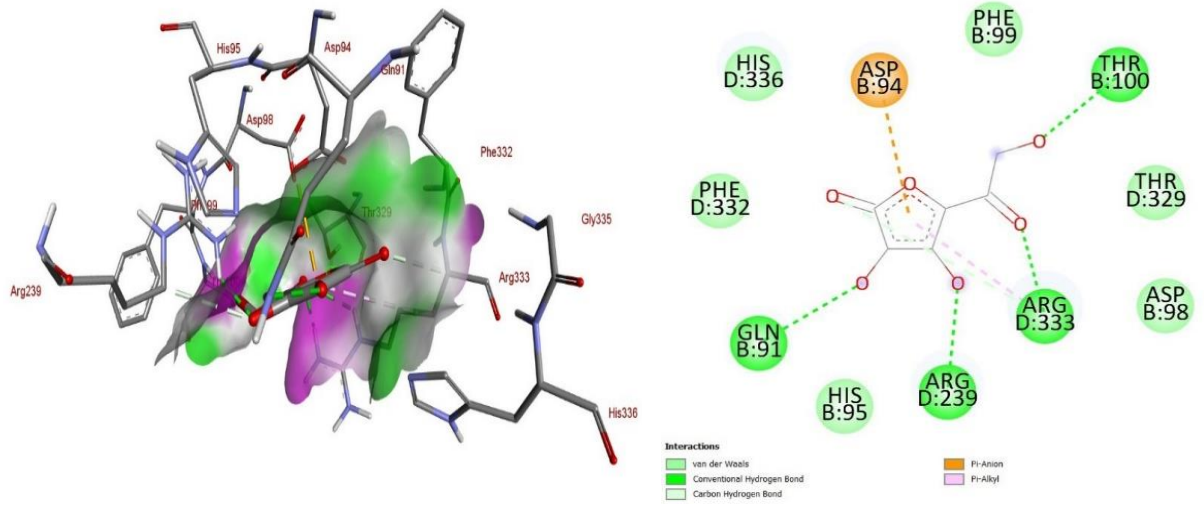



**Figure 28**: 3D and 2D representations of the binding interactions of compound **ascorbic acid** against Human *myeloperoxidase* (PDB ID: 1DNU).

### 4.8 In Silico Drug-Likeness and Pharmacokinetic Prediction Study

Drug-likeness prediction determines whether a specific organic molecule has qualities that indicate it could be an orally active drug (Lipinski *et.al.*, 2012). Lipinski's rule of five (ROF) is one of the most effective tools for predicting new chemical entities (NCE) drug-likeness, with the following criteria: MW $\leq$ 500 Daltons, cLog $P \leq 5$, H-bond donors $\leq$5, and H-bond acceptors $\leq$10 (Daina *et.al.*, 2017; Lipinski *et.al.*, 1997). The results are shown in **Table-15**. The standard and two of the isolated compounds (**45** & **46**) meet Lipinski's criteria with the exception of one violation because of their high lipophilic character (cLogP > 5). However, compound **44** did not meet the drug likeness criteria since it had three violations, including a higher molecular weight (MW >500), a number of hydrogen bond acceptors (N or O >10),

and number of hydrogen bond donors (NH or OH >5). In addition, compound **44** shows negative cLogP, the compound is likely highly soluble in water and may have difficulty crossing cell membranes.

According to Veber's parameters, the number of rotatable bonds should be ≤ 10 and the TPSA should be ≤ 140 $Å^2$ (Veber *et.al.,* 2002), this has proven a useful descriptor to estimate some ADME properties, especially with regards to biological barrier crossing such as absorption and brain access (Ertl *et.al.,* 2000). Accordingly, compounds **45**, **46**, and ciprofloxacin meet the criteria, while compound **44** had one violation because of the high value of the TPSA (TPSA >140), which indicates poor oral bioavailability and high water solubility. According to the finding, compounds **45**, **46** and the reference drug appeared to be good oral drug candidates, with an exception for compound **44 (Table 15)**.

**Table 15**: Drug-likeness predictions of compounds and standard, computed by SwissADME

| Comp-ounds | Formula | MW (g/mol) | NHD | NHA | LogP (cLogP) | Lipinski's ROF violation | NRB | TPSA ($Å^2$) | Veber's rule violation |
|---|---|---|---|---|---|---|---|---|---|
| **44** | $C_{28}H_{34}O_{15}$ | 610.56 | 8 | 15 | -0.72 | No 3 violations: MW>500, NorO>10, NHorOH>5 | 7 | 234.29 | 1 violation: TPSA>140 |
| **45** | $C_{30}H_{48}O_3$ | 456.7 | 2 | 3 | 5.88 | Yes 1 violation: MLOGP>4.15 | 1 | 57.53 | Yes |
| **46** | $C_{29}H_{50}O$ | 414.71 | 1 | 1 | 7.19 | Yes; 1 violation: MLOGP>4.15 | 6 | 20.23 | Yes |
| **CPFX** | $C_{17}H_{18}FN_3O_3$ | 331.34 | 2 | 5 | 1.1 | Yes; 0 violation | 3 | 74.57 | Yes |

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

The investigation of compounds isolated from *Salvia leucantha CAV* revealed diverse pharmacokinetic characteristics, as shown in **Table 16**. All isolated compounds exhibited low gastrointestinal absorption (GIA), while the standard drug demonstrated high GIA absorption. The reason for low GIA may account for the hydrophilic nature and large molecular size of compound **44** and the high lipophilic nature (cLogP >5) of compounds **45** and **46**, which can hinder absorption from the gastrointestinal tract. Additionally, all the test compounds were predicted with no permeability toward the blood-brain barrier (BBB).

P-glycoprotein (P-gp) is a transmembrane protein that acts as a pump, actively transporting various substances out of cells. Increased P-glycoprotein (P-gp) expression in the intestine can limit the absorption of medicines that are P-gp substrates, this can lead to decreased drug concentration at the target site and reduced therapeutic efficacy (Elmeliegy *et.al.*, 2020). The result revealed compound **44** acting as substrates of P-glycoprotein (P-gp).

The rate at which a substance penetrates the stratum corneum is measured by skin permeability (Kp). This value is commonly used to quantify the transport of molecules in the epidermal skin's outermost layer and to highlight the importance of skin absorption. The more negative the logKp, the lower the cutaneous permeability of the molecule, with a permissible range of LogKp - 8.0 to - 1.0 cm/**s** (C. P. Chen *et.al.*, 2018; Zeng *et.al*., 2021). In this study, compared to ciprofloxacin, compounds **45** and **46** showed a lesser LogKp value ranging from -2.2 to -3.87 (**Table 16**), so these compounds might have better skin permeation than ciprofloxacin. While compound **44** is predicted with a -10.12 Logkp value, lower cutaneous permeability.

Moreover, about 60% of prescribed drugs are metabolized by CYP enzymes, with $CYP3A_4$ accounting for about half of this metabolism, followed by $CYP2D_6$, $CYP2C_9$, and $CYP2C_{19}$ (Esteves *et.al*., 2021). The result for inhibitory interaction of compounds with cytochrome P450 monooxygenase enzymes computed by SwissADME revealed no inhibitor interaction, suggesting that these enzymes may metabolize those compounds. Enhancement or inhibition of CYP activities can alter the drug metabolism profile, affecting either an increase in bioavailability or a decrease in efficacy.

**Table 16**: ADME predictions of the compounds (**44-46**) and standard, computed by SwissADME and PreADMET

| **Compounds** | **GI ABS** | **BBB Permeability** | **Log Kp (cm/s)** | **P-gp substrate** | **Inhibitor Interaction (SwissADME/PreADMET)** | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | **CYP3A4 inhibitor** | **CYP2D6 inhibitor** | **CYP2C9 inhibitor** | **CYP2C19 inhibitor** | **CYP1A2 inhibitor** |
| **44** | Low | No | -10.12 | Yes | No | No | No | No | No |
| **45** | Low | No | -3.87 | No | No | No | No | No | No |
| **46** | Low | No | -2.2 | No | No | No | No | No | No |
| **CPFX** | High | No | -9.09 | Yes | No | No | No | No | No |

***GI** = Gastro-Intestinal, **ABS** = Absorption, **BBB** = Blood Brain Barrier, **P-gp** = P-glycoprotein, **CYP** = Cytochrome-P-450 enzymes*

The BOILED-Egg model is a method used to predict two key ADME parameters, i.e. passive gastrointestinal absorption (HIA) and brain access (BBB). As shown in **Figure 28**, the egg-shaped classification plot includes the yolk (i.e. the physicochemical space for highly probable BBB permeation), the white (i.e. the physicochemical space for highly probable HIA absorption), and a gray area (no HIA or BBB access). The outside gray region stands for molecules with properties implying predicted low absorption and limited brain penetration (Daina & Zoete, 2016). In addition, the points are colored in blue if predicted as actively effluxed by P-gp (PGP+) and in red if predicted as a non-substrate of P-gp (PGP−) could pass through and act on CNS (Daina & Zoete, 2016).

The result demonstrated that compound **45** is predicted as having low absorption through the intestine and limited brain penetration (outside the egg) and as a non-substrate of P-gp (PGP−). The other two compounds (**44** and **46)** were predicted not to be absorbed and not to BBB permeate because they were outside of the range of the plot (compound **44** with a TPSA of 234.29 $Å^2$ and a WLOGP of -1.48, while compound **46** with a TPSA of 20.23) and the standard ciprofloxacin is predicted to be well absorbed, but not accessing the brain (in the white) and PGP+ (blue dot).

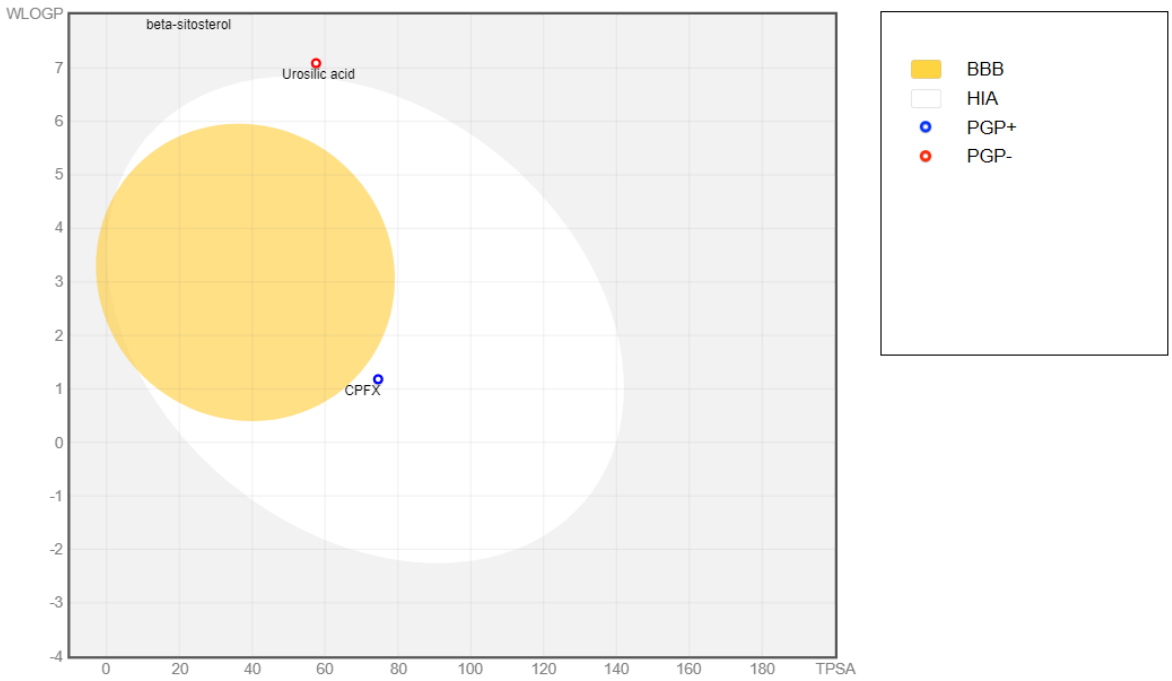



**Figure 29:** BOILED-Egg model for predicting gastrointestinal absorption and brain access.

The level of toxicity is expressed on a scale from 1 to 6, with a greater number indicating a lower level of toxicity. It is determined based on the $LD_{50}$ (mg/kg) value, which indicates a dose that will kill 50% of the test animal (Drwal *et.al.*, 2014). Compound **44** exhibited $LD_{50}$ 12000 mg/kg, which was safer than ciprofloxacin (2000 mg/kg). The predicted $LD_{50}$ (mg/kg) for the tested compounds ranges from 890 to 12000. Among all the studied compounds, compound **44** was predicted to be less/none toxic since it falls under Class 6. Compounds **45**, **46**, and the standard drug showed toxic class 4. None of the isolated compounds revealed cytotoxicity or mutagenicity. General compound **44** showed comparable in silico oral toxicity results as ciprofloxacin (**Table 17**).

**Table 17**: In silico oral toxicity prediction results for isolated compounds and standard, computed by Pro-Tox II

| **Toxicity Model** | | **Cpd-44** | **Cpd-45** | **Cpd-46** | **CPFX** |
|---|---|---|---|---|---|
| **Predicted $LD_{50}$ (mg/kg)** | | 12000 | 2000 | 890 | 2000 |
| **Predicted Toxicity Class** | | 6 | 4 | 4 | 4 |
| **Organ toxicity** | **Hepatotoxicity** | Inactive | Active | Inactive | Inactive |
| | **Neurotoxicity** | Inactive | Inactive | Active | Active |
| | **Nephrotoxicity** | Active | Inactive | Inactive | Active |
| | **Respiratory toxicity** | Active | Active | Active | Active |
| | **Cardiotoxicity** | Inactive | Active | Inactive | Inactive |
| **Toxicity end points** | **Carcinogenicity** | Inactive | Active | Inactive | Inactive |
| | **Immunotoxicity** | Active | Active | Active | Inactive |
| | **Mutagenicity** | Inactive | Inactive | Inactive | Active |
| | **Cytotoxicity** | Inactive | Inactive | Inactive | Inactive |
| | **BBB-barrier** | Inactive | Active | Active | Inactive |
| | **Ecotoxicity** | Inactive | Inactive | Active | Inactive |
| | **Clinical toxicity** | Active | Active | Inactive | Active |
| | **Nutritional toxicity** | Active | Active | Active | Inactive |

# 5. CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

In conclusion, natural products represent a significant source of new lead compounds in drug discovery research. In this study, phytochemical screening analysis was conducted on leaf extracts of *Salvia leucantha* and revealed the presence of flavonoids, terpenoids, glycosides, steroids, tannins, alkaloids, and saponins suggesting the plant has a valuable reservoir of secondary metabolites. The methanol extract after column chromatography afforded three compounds: hesperidin (**44**), urosilic acid (**45)** and β-sitosterol (**46)**. All compounds reported herein for the first time from *Salvia leucantha.*

Antibacterial activity revealed that n-hexane and ethylacetate extracts showed the highest antibacterial activity against *E. coli* (20.5±0.70 mm at 200 mg/mL) and *S. aureus* (24.5±0.70 mm at 200 mg/mL), respectively. Whereas methanol extract exhibited the highest zone of inhibition (23.0±0.00 mm at 200 mg/mL) against *P. aeruginosa*. Compound **44** exhibited promising activity against *S. aureus* (24.50±0.70 mm), *E. coli* (22.00±1.41 mm), and *S. pyogenes* (21.00±1.41 mm). Compound **45** against S. pyogenes (24.50±2.12 mm), *S. aureus* (22.00±1.41 mm), *P. aeruginosa* (21.00±1.41 mm) and compound **46** against *S. pyogenes* (22.50±0.70 mm) and *E. coli* (21.50±2.12 mm) showed strong antibacterial activity each at 2 mg/mL, compared with standard drug (ranging from 33.20±0.44– 34.50±1.06 mm at 30μg /mL). The highest percent inhibition of the DPPH radical was observed with methanol extract ($IC_{50}$: 12.17 μg/mL) and compound **44** ($IC_{50}$: 9.83 μg/mL), compared with ascorbic acid ($IC_{50}$ of 0.681 μg/mL).

A molecular docking study was done using Auto-Dock Vina, where compound **44** and compound **45** exhibited the highest binding affinity (-9.0 kcal/mol) with the target *E. coli* DNA gyrase B and compound **44** (-10.3 kcal/mole) with S. aureus pyruvate kinase as target. Additionally, compounds **45** and **44** showed higher binding affinity (-10.5 and -9.9 kcal/mol respectively) against human myeloperoxidase. The *in sillico* drug-likeness prediction revealed that compounds **45** and **46** satisfy Lipinski's rule of five with one violation. The ADMET prediction suggested that all isolated compounds possess low GI absorption and did not show BBB permeability. Compounds **45** and **46** showed a lesser LogKp value, suggesting better skin permeation than ciprofloxacin.

The $LD_{50}$ and toxicity profile prediction suggested all isolated compounds are safe and none of the isolated compounds showed mutagenicity or cytotoxicity. Only compound **45** was found to be hepatotoxic and cardiotoxic. To conclude, based on the *in vitro* studies and *in silico* molecular docking analysis, it can be concluded *Salvia leucantha* is a promising source of antimicrobial and antioxidant compounds.

## 5.2 Recommendation

In this study only 1D NMR spectrum and literature data were used for structural elucidation of compounds **44** and **45,** so we recommend 2D NMR spectroscopic techniques (HMBC, HSQC, COSY, and NOESY) and mass spectrophotometry (for compounds **45** and **46**) for further confirmation of the structure of compounds isolated from the leaves of *Salvia leucantha*.

We also recommend further antimicrobial activity tests of isolated compounds and extracts based on methods used to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) that may address the problem of antimicrobial resistance. This study recommends additional antibacterial activity on other clinically resistant bacteria, such as *methicillin-resistant S. aureus*, *vancomycin-resistant Enterococcus* and multidrug-resistant *Mycobacterium tuberculosis*.

Since *in vitro* antioxidant activities of isolated compounds (**44** and **45**) were promising, we recommend further activity tests be held by researchers by targeting diseases caused by oxidative stress (anticancer, cytotoxicity, anti-proliferative, antidiabetic, anticholinesterase activity).

Further phytochemical study is recommended on other parts of the plant, especially on the root and additional studies are also needed to investigate the pharmacokinetic profile of the promising compounds in an animal model.

## 6. REFERENCES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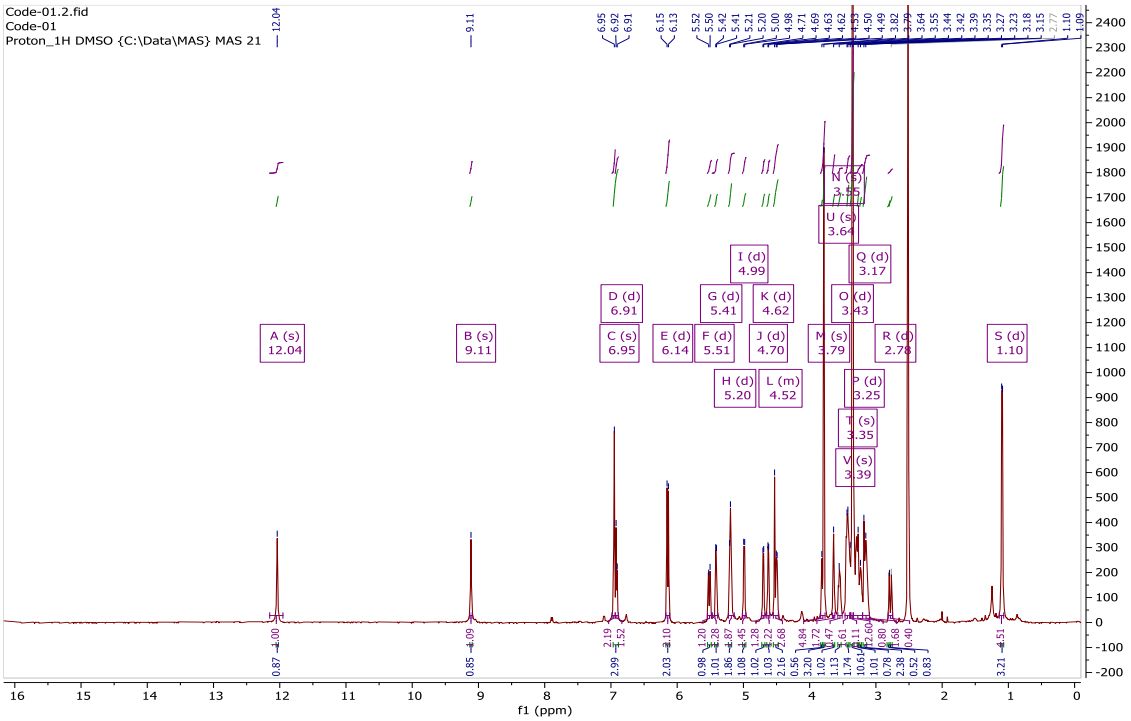
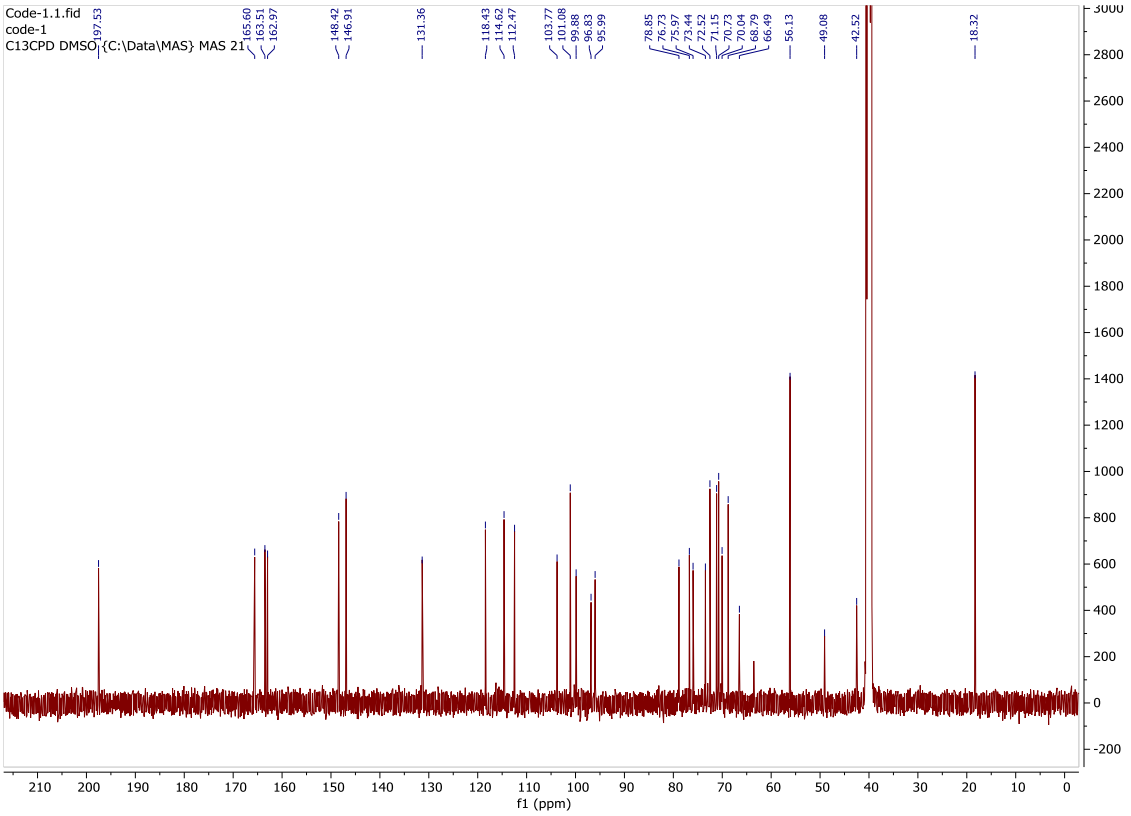
# APPENDIX

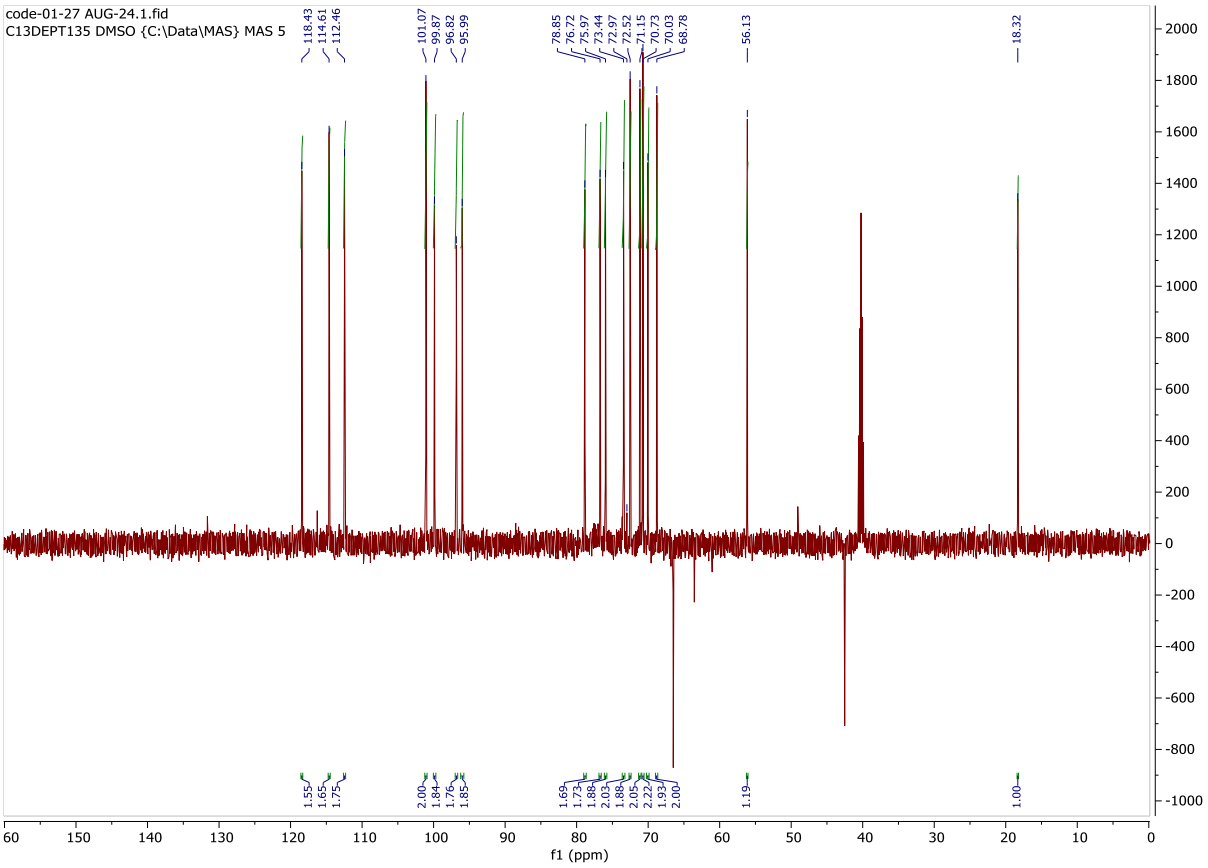
**Appendix 1:** $^{1}$H NMR (500 MHz, DMSO) spectrum of compound **44**



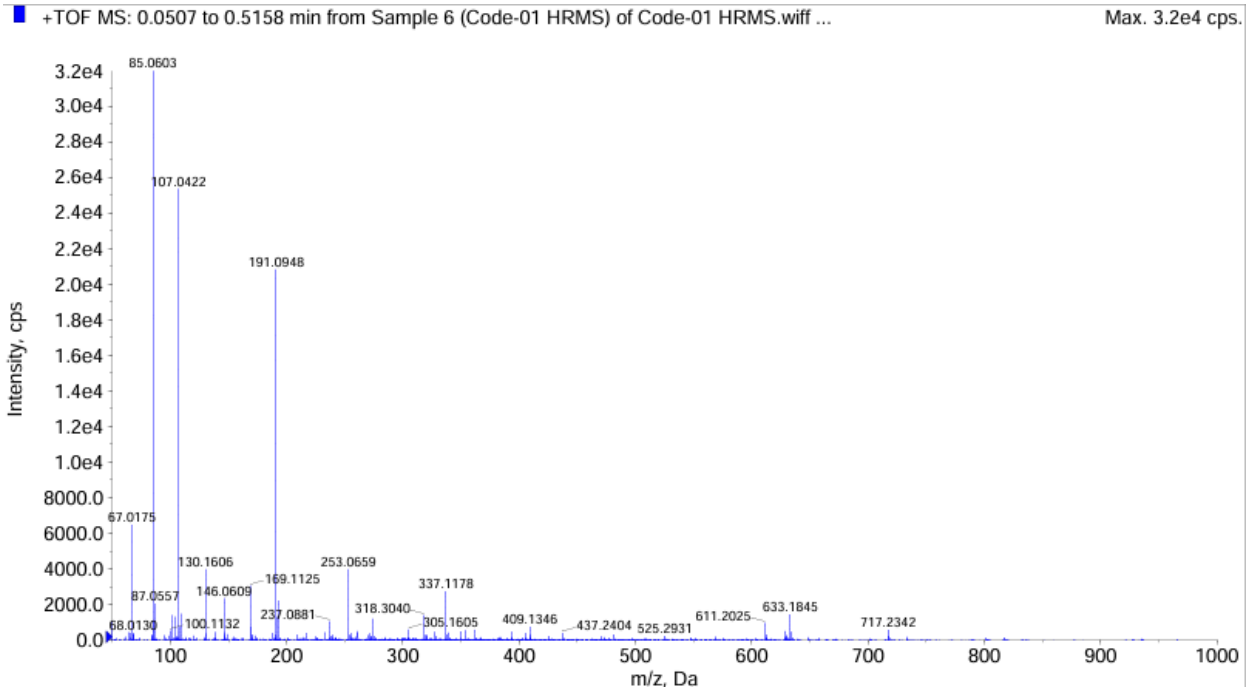

**Appendix 2:** $^{13}$C-NMR (126 MHz, DMSO) spectrum of compound **44**



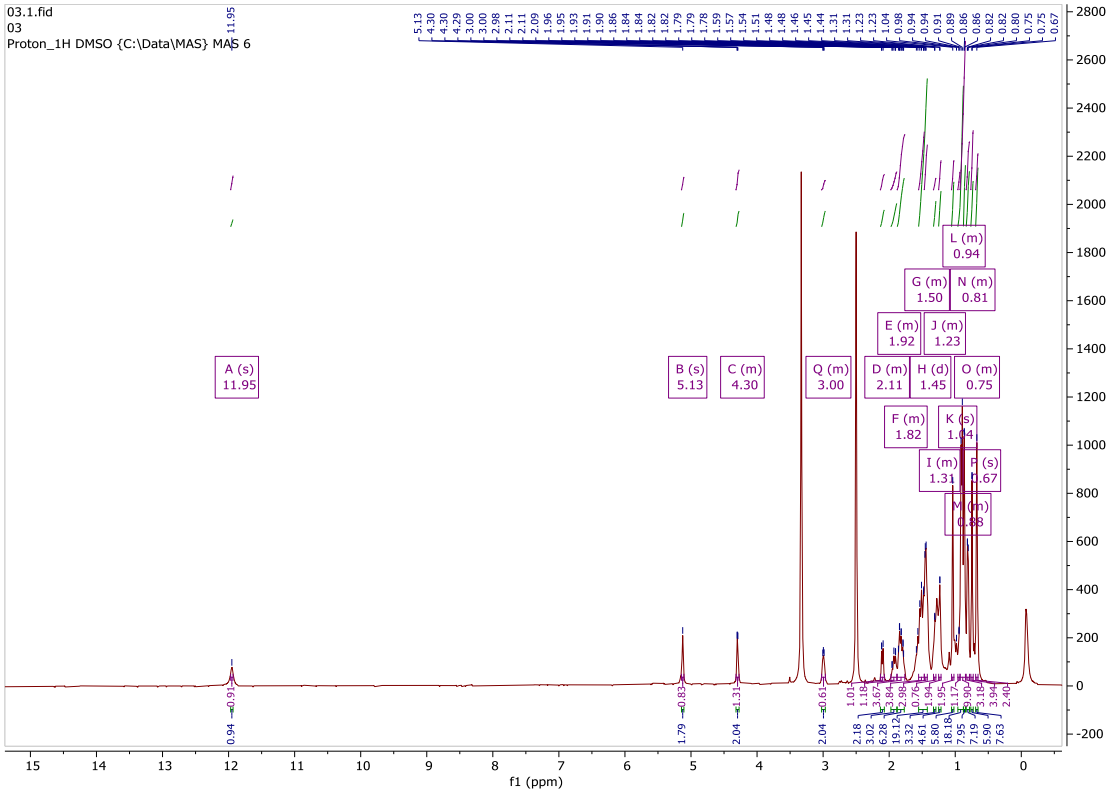
**Appendix 3**: DEPT-135 (126 MHz, DMSO) spectrum of compound **44**



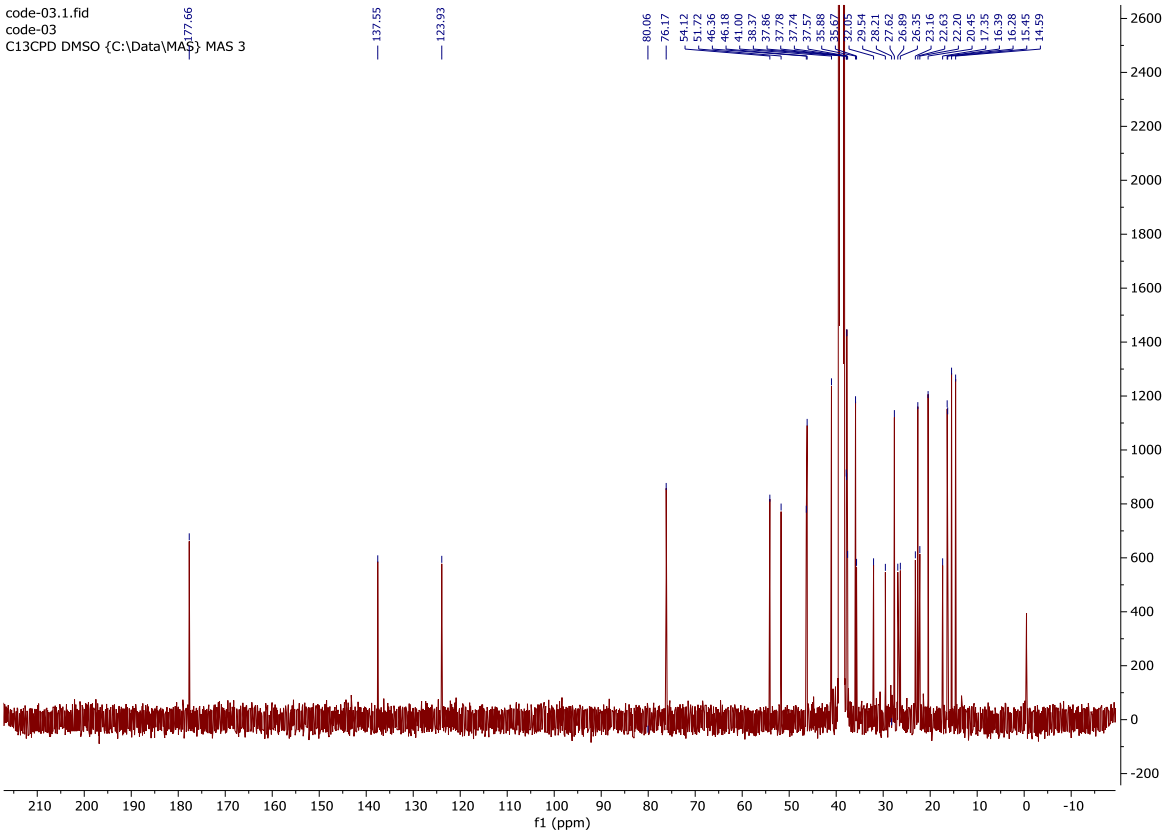

**Appendix 4**: Mass spectrum of compound **44**



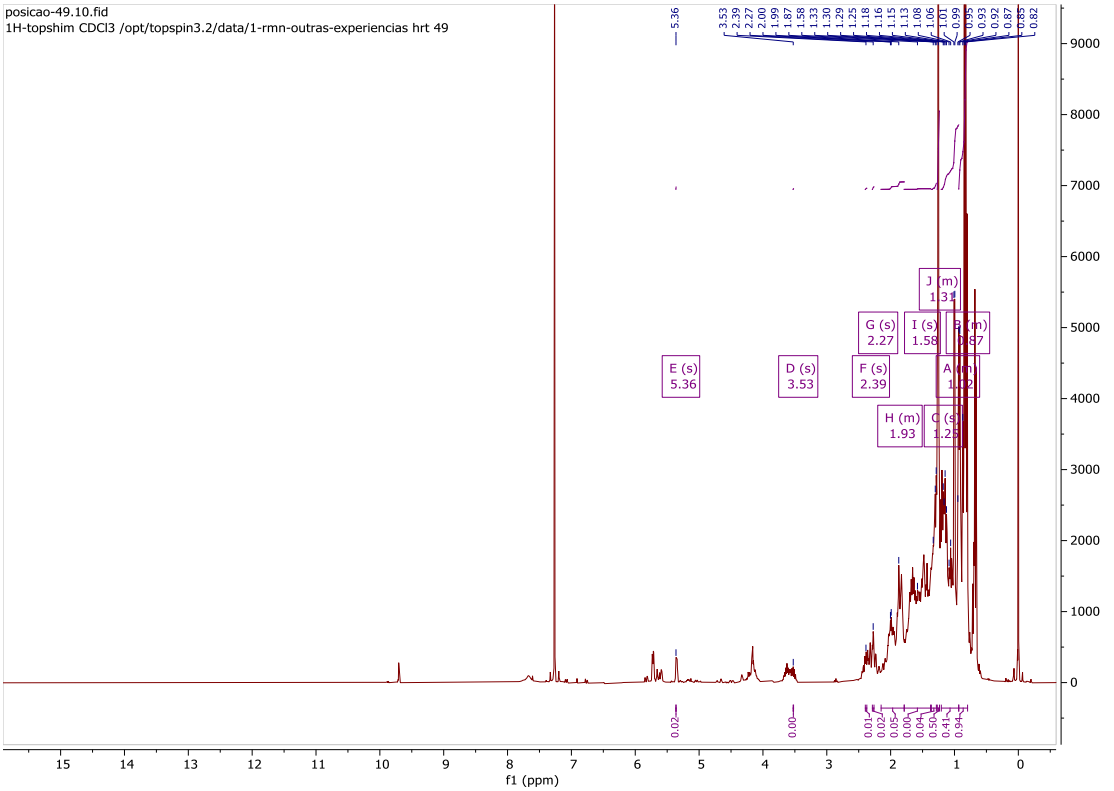
**Appendix 5**: $^{1}$H NMR spectrum (500 MHz, DMSO) of compound **45**



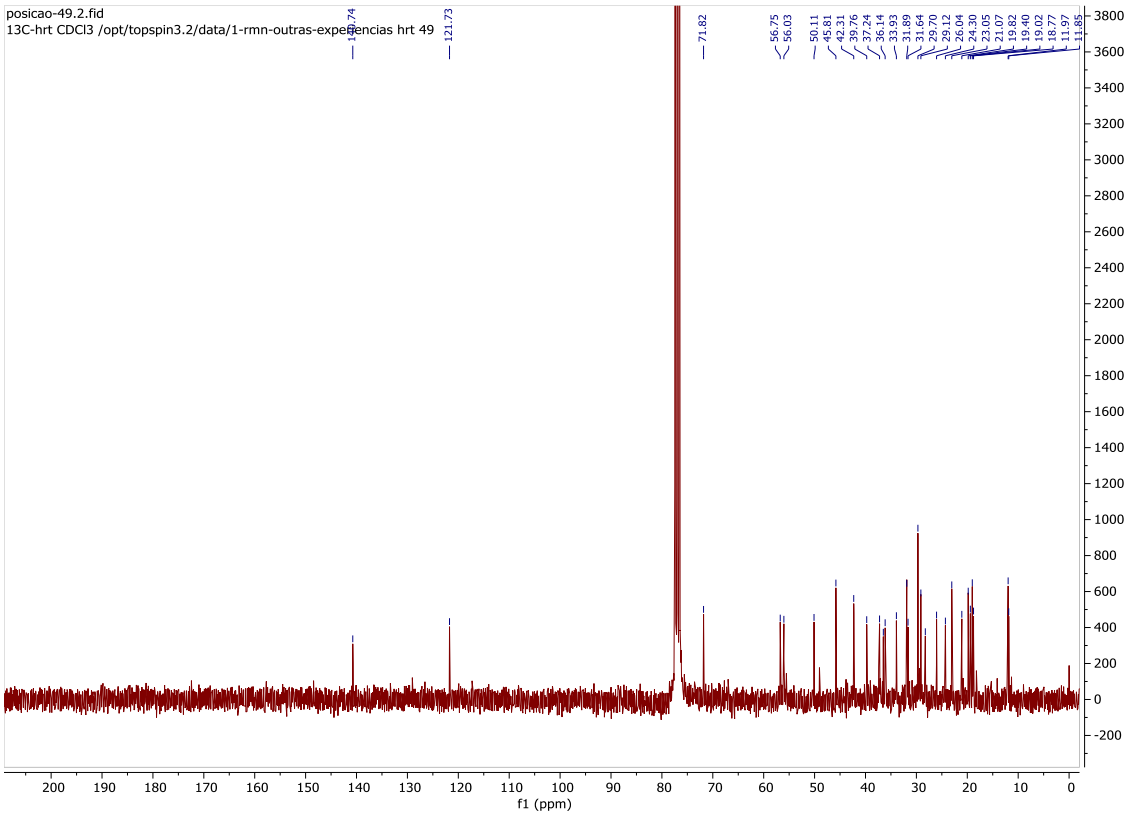

**Appendix 6**: $^{13}$C-NMR (126 MHz, DMSO) spectrum of compound **45**



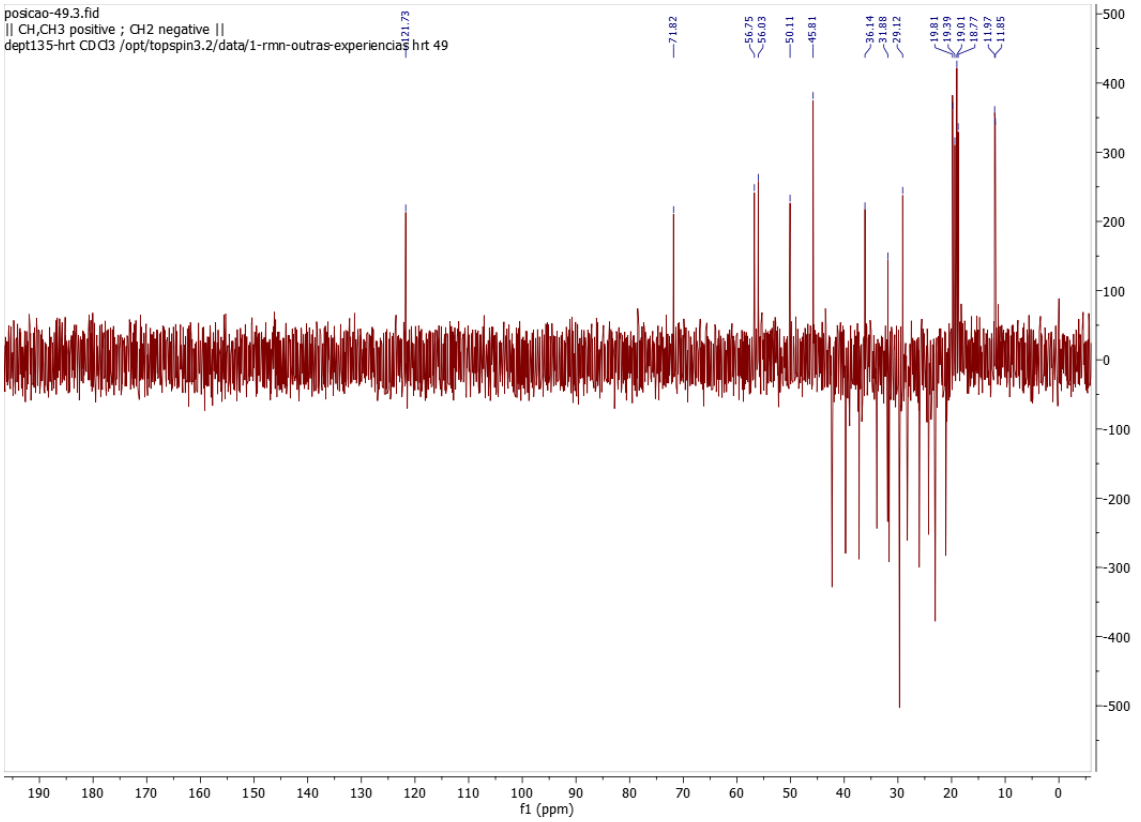
**Appendix 7:** $^{1}$H NMR spectrum (300 MHz, CDCl3) of compound **46**



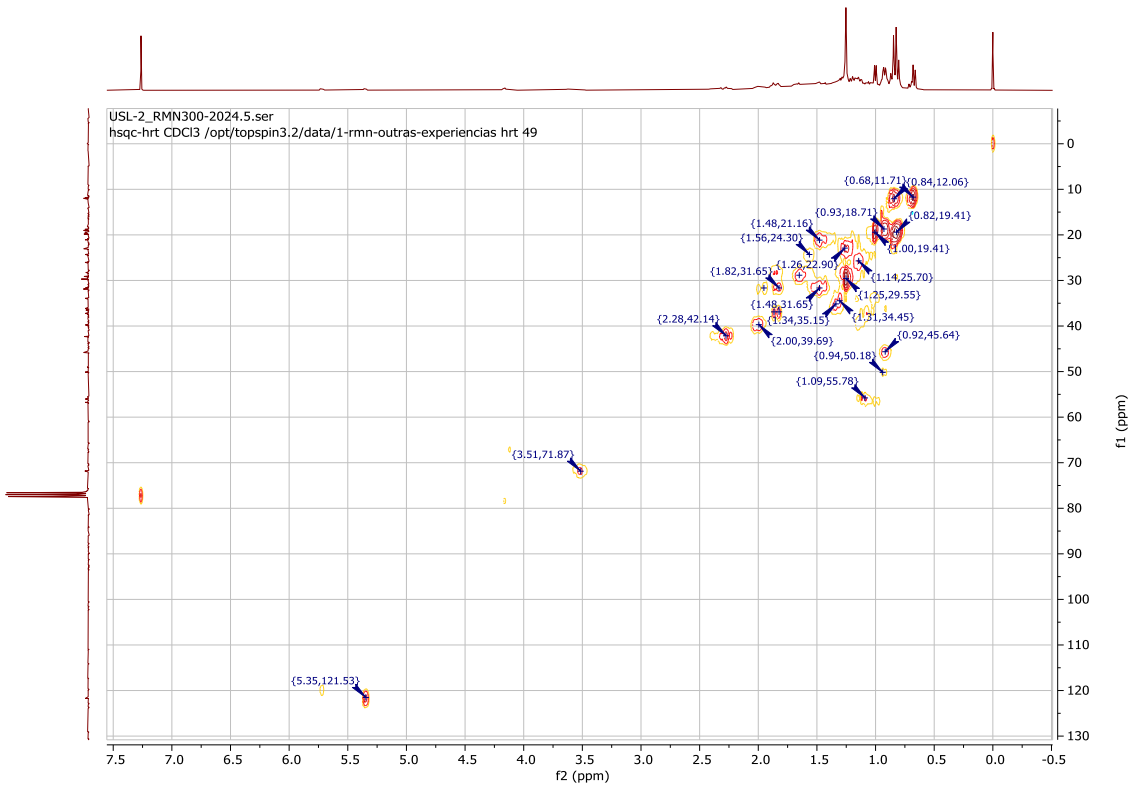

**Appendix 8:** $^{13}$C-NMR (75 MHz, DMSO) spectrum of compound **46**



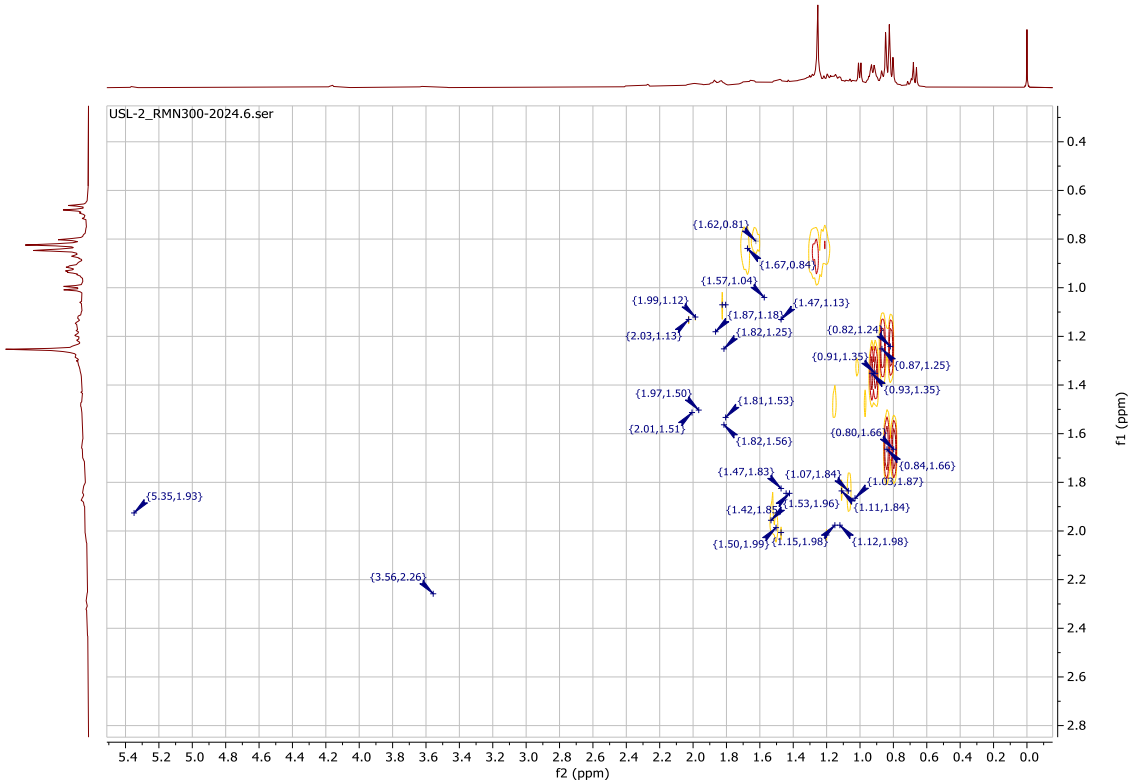
**Appendix 9:** DEPT-135 (75 MHz, DMSO) spectrum of compound **46**



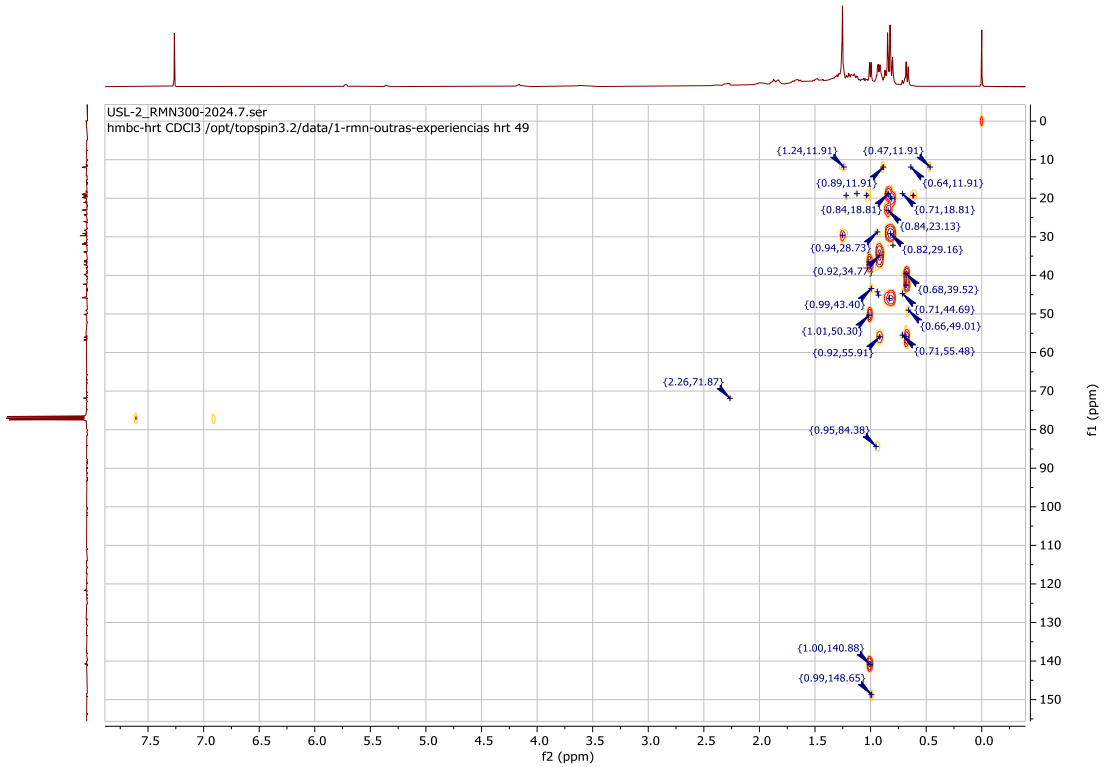

**Appendix 10:** HSQC (75 MHz, DMSO) spectrum of compound **46**



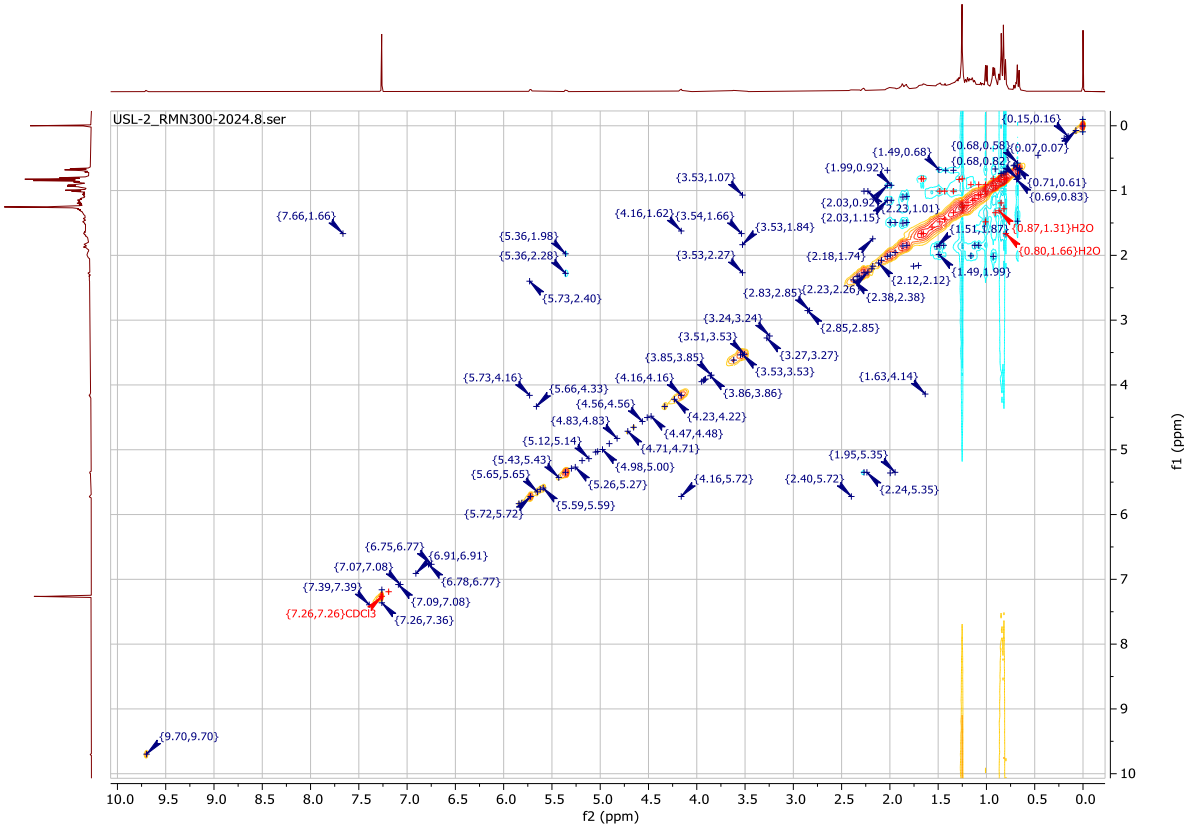
**Appendix 11:** COSY (75 MHz, DMSO) spectrum of compound **46**




**Appendix 12:** HMBC (75 MHz, DMSO) spectrum of compound **46**



**Appendix 13:** NOESY (75 MHz, DMSO) spectrum of compound **46**




**Appendix 14**: GC-MS chromatogram of essential oils of *Salvia leucantha* leaf.