Phytochemical Investigation, Antibacterial and Antioxidant Activities and Molecular Docking and ADMET Studies of Stem Bark Extracts and Isolated Compounds of *Lobelia rhynchopetalum* Hemsl

Bikiltu Abera Tolasa

Thesis Submitted To Department of Applied Chemistry

School of Applied Natural Science

In Partial Fulfillment of the Requirements for Master of Science Degree in Applied Chemistry (Medicinal Chemistry)

Office of Graduate Studies

Adama science and Technology University

June, 2024

Adama, Ethiopia

Phytochemical Investigation, Antibacterial and Antioxidant Activities and Molecular Docking and ADMET Studies of Stem Bark Extracts and Isolated Compounds of *Lobelia rhynchopetalum* Hemsl

Bikiltu Abera

Advisor: Dr. Hailemichael Tesso

A Thesis Submitted to the Department of Applied Chemistry

School of Applied Natural Science

Office of Graduate Studies

Adama Science and Technology University

June, 2024

Adama, Ethiopia

Declaration

I declare that this master's thesis entitled "**Phytochemical Investigation, Antibacterial and Antioxidant Activities and Molecular Docking and ADMET Studies of Stem Bark Extracts and Isolated Compounds of *Lobelia rhynchopetalum* Hemsl**" is my work and has not been submitted to any university for similar purposes. The references used in this proposal are duly recognized by proper citation.

_____	_____	_____
Name of student	Signature	Date

Recommendation

I the advisor of this thesis work, hereby certify that I have closely advised the student while developing this thesis and have read the draft thesis entitled "**Phytochemical Investigation, Antibacterial and Antioxidant Activities and Molecular Docking and ADMET Studies of Stem Bark Extracts and Isolated Compounds of *Lobelia rhynchopetalum* Hemsl**" prepared under my guidance by Bikiltu Abera. Therefore, I recommend the submission of the thesis paper to the department for further review and evaluation.

_____	_____	_____
Advisor	Signature	Date

Approval

The advisor here by certifies that the recommendation and suggestions given by the Thesis review committee are appropriately incorporated into the final thesis entitled "**Phytochemical Investigation, Antibacterial and Antioxidant Activities and Molecular Docking and ADMET Studies of Stem Bark Extracts and Isolated Compounds of *Lobelia rhynchopetalum* Hemsl** "By Bikiltu Abera

Advisor	Signature	Date

Approval of Board

We, the undersigned, members of the Board of Examiners of the final open defense by Bikiltu Abera Tolasa have read and evaluated her thesis entitled "**Phytochemical Investigation, Antibacterial and Antioxidant Activities and Molecular Docking and ADMET Studies of Stem Bark Extracts and Isolated Compounds of *Lobelia rhynchopetalum* Hemsl"** and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Masters in Medicinal Chemistry.

Chairperson	Signature	Date
Internal Examiner	Signature	Date
Zelalem Abdissa (PhD)		
External examiner	Signature	Date

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

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

ACKNOWLEDGEMENT

First, I would like to thank the Almighty God for making me healthy and strong to conduct this piece of work. I would like to express my deepest gratitude to my advisor: Dr. Hailemichael Tesso, Department of Applied Chemistry, School of Applied Natural Science, Adama Science and Technology University, for his consistent supervision and dedication in guiding and following this thesis work by devoting his time. I would also like to acknowledge Department of Chemistry, Addis Ababa University for their support in running NMR samples. I thank also of Institute of Pharmaceutical Science, Adama Science and Technology University for providing space for Anti-bacterial activity test in their laboratory. I wish to thanks Mr. Raji Abera and Samson Habtamu for their assistance while collecting the plant material and professor Silashi Namomssa, botanist at National Herbarium of Ethiopia, Addis Ababa University for authenticating the plant and Mr. Tolosa Gutema for software molecular docking study.

Finally, I thank my academic instructors and supervisors at department of Applied Chemistry, Adama Science and Technology University, who have been an example to me during my MSc studies and MaddaWalabu University for leave of absence. Last but not least my acknowledgement goes to my families and all of those who contributed an idea give a bit of suggestion and helped me in a technical way.

TABLE OF CONTENTS

CONTENTS **PAGES**

LISTS OF TABLES

LISTS OF FIGURES

LIST OF ACRONYMS AND ABBREVIATIONS

ADMET	Absorption, distribution, metabolism, excretion and toxicity
CC	Column chromatography
DNA	Deoxyribonucleic acid
DMSO	Dimethylsulfoxide
DPPH	1, 1-diphenyl-2-picryl-hydrazyl
IC_{50}	The half maximal inhibitory concentration
IR	Infrared spectroscopy
NADPH	Nicotinamide adenine dinucleotide phosphate
MHA	Mueller Hinton Agar
NMR	Nuclear Magnetic Resonance
Rf	Retention factor
TLC	Thin layer chromatography
UV-Vis	Ultraviolet Visible

ABSTRACT

Lobelia rhynchopetalum, *the giant lobelia, is a plant endemic to Ethiopia. Its habitat is the Afroalpine climate of the Bale Mountains and Semien Mountains National Park. Recent study show that it is under a threat of climate change. L. rhynchopetalum is among plants traditionally used by Arsi people for the treatment of wide array of diseases including leprosy, wound, evil eye, retained placenta, diarrhea, fever, eye disease, rabies and tumor/cancer. Considering the absence of prior scientific report that supports ethno-medicinal uses of the stem bark of the plant, this study focused on isolation of phytoconstituents, in-vitro and in-silico evaluation of the anti-bacterial activities as well as radical scavenging activities of the stem bark extract and components thereof of L. rhynchopetalum. In view of this, the stem bark was extracted by maceration with methanol and yielded 70g (20%w/w) of extract. The methanol extract, after silica gel column chromatography, afford two analogous polyacetylenic glycosides labeled as compound* ***41*** *and* ***42****.* ***41*** *is a novel compound and* ***42*** *is discovered from the plant for the first time. The structure of* ***42*** *was elucidated using 1D proton and* ^{13}C *NMR techniques and comparison with literature. NMR spectral data of* ***42*** *appears to be similar to a previously known polyacetylinic glycoside, lobetyolin. The extracts and isolated compounds were evaluated for their antibacterial activity using Agar disc diffusion against four strains of bacterial pathogens including E. coli, P. aeruginosa, S. aureus and S. pyogenes. Results showed that all extracts and isolated compounds showed inhibition diameter beyond 9 mm. Molecular docking of isolated compounds was done using AutoDock vina software. Among the investigated ligands compound* ***42*** *exhibited better docking efficiency with PqsA with binding affinity (-7.3kcal/mol). The extract and isolated compounds were also assessed for their radical scavenging activity using DPPH assay. The compound* ***42*** *displayed promising radical scavenging activity of 93% (*IC_{50} *= 7.83). The antibacterial activity presented herein supports the traditional use of this plant against bacteria. The drug likeness analysis exhibited that compound* ***41*** *and* ***42*** *obeyed Lipinski's rule of five with zero violation. Both Compounds have been shown to have good pharmacokinetics and bioavailability characteristics. Hence, the activities herein indicated as the plant has great potential as a remedy for diseases caused by bacteria and radicals.*

Key words: *Lobelia rhynchopetalum*; Polyacetylenic glycoside; Antibacterial; Antioxidant; ADMET;Molecular docking

1. INTRODUCTION

1.1. Background of the study

Plants have been used for the treatment of diseases from centuries. The discovery of new biologically active compound usually starts with searching of useful plants from different records to the development of methods for the industrial production of drugs (David, *et al.*, 2015). According to World Health Organization (WHO), 60% of the world's population relies on herbal medicine and about 80% of the population in developing countries depends almost totally on it for their primary health care needs (Khan & Ahmad, 2019). Medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals are primary and secondary compounds (Borokini & Omotayo, 2012). Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoids, alkaloids and phenolic compounds (Lobo *et al*., 2018).

The genus *Lobelia* is a large genus of trees or shrubs, belonging to the family *Campanulaceae*, which are distributed in Asian countries such as China, India, Japan, Thailand, Indonesia, Philippines and Africa. A review of the medicinal uses and chemical constituents of various *Lobelia* species including about 20 organic chemicals which have been isolated and identified from this species was reported (Tadege *et al.*, 2022). In traditional medicinal system of the worlds, *Lobelia* species are used for many medicinal applications such as anticancer, antifungal and for internal hemorrhage and bedwetting, antihypertention, dyspnea, vermicide and vermifuge, sedative, promotes secretions, diuretic, hemostatic, antiviral, antimalarial and anti-inflammatory activities. In addition, it is used as an antidote for poisons and for the treatment of edema, liver, stomach, and intestinal diseases, breast, stomach tumors and bactericidal. All parts of these plant species have been used for medicinal purposes (Bekoe *et al.*, 2018).

The study plant is *Lobelia rhynchopetalum,* commonly known as Tarura (Afan Oromo) and Jibara (Amharic) in Ethiopia. *L. rhynchopetalum*, is a plant endemic to Ethiopia. Its habitat is the Afroalpine climate of the Bale Mountains and Semien Mountains National Park (Geleta *et.al*., 2012). Recent study show that it is under a threat of climate change (*Chala et.al., 2016*). Ethiopia, traditionally the leaves and aerial parts of this plant are used for the treatment of various ailments including leprosy, wound, evil eye, retained placenta, diarrhea, fever, eye disease, rabies and tumor/cancer, among others. Despite the presence of few reports on the

chemistry of the roots parts of this species, to the best of our knowledge there is no prior report on the stem barks.

1.2. Statement of the problem

The increasing prevalence of multi drug resistant strains of bacteria to antibiotics raises the specter of untreatable bacterial infections which adds urgency to the search for novel infection fighting strategies (Aljeldah, 2022). Enhancing the knowledge of screening of traditional medicinal plants for various biological activities as well as determination of the structure of the active chemical constituents may help to obtain lead compound from natural products for further drug discovery and development. The local people of different parts of Ethiopia, including Bokoji (Galema) use the stem bark of *Lobelia rhynchopetalum for* various ethno-medicinal purposes including antitumour and to kill lice. However, there is no scientific evidence that support the traditional use of the stem bark of *Lobelia rhynchopetalum*. Therefore, this project is intended to fish out the active ingredients responsible for the antibacterial activities from the stem bark of *Lobelia rhynchopetalum.*

1.3. Objectives

1.3.1. General objective

The general objective of this study was to identify antibacterial and antioxidant compounds from the extracts of the stem bark of *Lobelia rhynchopetalum.*

1.3.2. Specific objectives

The specific objectives of study were:

- To extract the stem bark of *lobelia rhynchopetalum* using methanol.
- To isolate compounds from the methanol extract of the stem bark of *Lobelia rhynchopetalum* using silica gel column chromatography.
- To elucidate the structure of isolated compounds using spectroscopic method [IR, UV-Vis, NMR (1D)] and literature data
- To test antibacterial activity of the extracts and isolated compounds from plant stem bark via plate agar disc diffusion method against selected Gram positive bacteria (*S. aureus* and *S.pyogenes* and two Gram-negative bacteria; *E. coli* and *P.aeruginosa)*
- To assess the DPPH radical scavenging activity of the extracts and isolated compounds of the stem bark of *Lobelia rhynchopetalum*

- To assess the DPPH radical scavenging activity of the extracts and isolated compounds of the stem bark of *Lobelia rhynchopetalum*
- To investigate ligand protein docking of the isolated compounds from stem bark extract so as to strengthen the antibacterial activities evaluation of the constituents.
- To predict the ADMET of the isolated compounds

1.4. Significance of the study

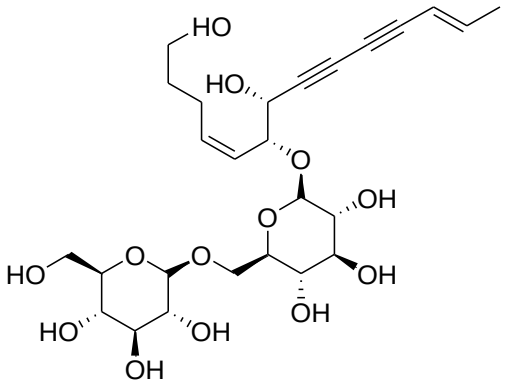
The results of the antibacterial activity and molecular docking of the extracts and constituents of the stem bark of *Lobelia rhynchopetalum* may help to arrive at lead compound for drug discovery. The result might also help as scientific evidence to support the traditional use of the stem bark of this plant by the traditional healers. The study may provide documentary information about the ethno-medicinal use and chemical constituent of the plant for anyone who is willing to do research on the plant. Above all, this work is expected to provide scientific investigation of the plant stem bark as a source of antibacterial and antioxidant compounds and substantiate the observed promising activity with molecular docking analysis on target protein of selected strain of microorganism.

2. LITERATURE REVIEW

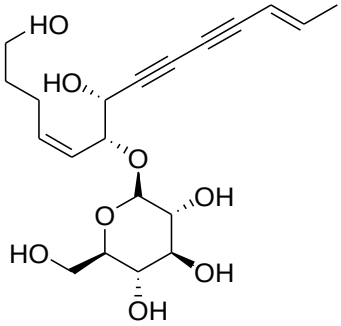
2.1. Family Campanulaceae

Campanulaceae consists of about 84 genera and almost 2400 species (Liveri *et al*., 2019). Most species are native to tropical and warm-temperate regions. Several herbaceous species are popular in gardens and thrive in moist, shady and semi-shady locations (Shaheen *et al*., 2017). Campanulaceae encompasses a wide range of plant forms, including annuals, perennials, shrubs, and even small trees (Crowl *et al*., 2016). This diversity is evident in genera such as *Campanula*, *Lobelia* and *Codonopsis*. Some species are herbaceous while others exhibit woody growth habits (Lammers, 2007). Additionally, Campanulaceae species occupy various habitats, from alpine meadows to forests, grasslands, and even rocky cliffs (Lattanzi *et al*., 2021).

A few species in the family belonging to the genus *Lobelia* have been previously analyzed with respect to their secondary metabolites and biological activities and a diverse range of compounds have been isolated to date. These include pyrolidine, piperidine, polyhydroxylated alkaloids, steroidal Saponins, triterpenoids,phenylpropanoids, flavonoids, xanthones, polyacetylenes and coumarins, some of which exhibit significant anticancer, antimicrobial and anti-inflammatory activity (Cseke *et al*., 2016). This family contains a large number of important plants which are used in the treatment of various diseases such as headache, constipation, rheumatism, evil eye, and diabetes and skin infection. Compounds such as lobetyolinin(**1**), lobetyolin(**2**), lobetyol(**3**), lupenone(**4**),taraxerol(**5**),βsitosterol(**6**) have been reported from the family (Poddar *et al*., 2020).



1



2

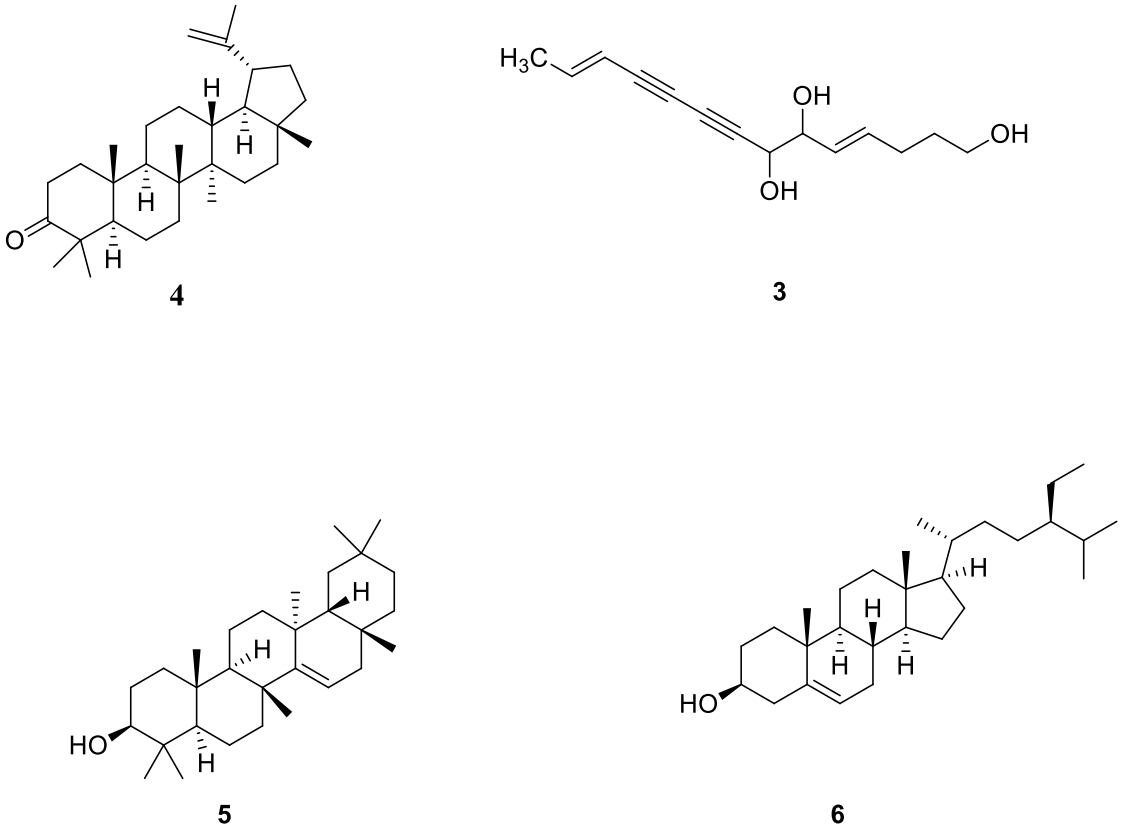
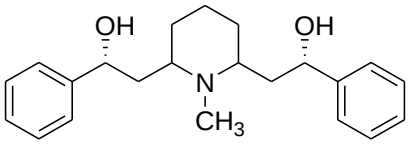


Figure 1: Isolated compounds from Family Campanulaceae

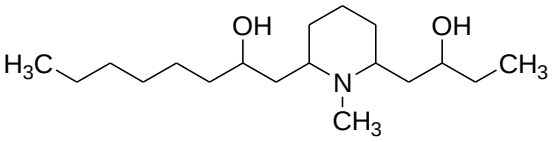
2.2. Genus *Lobelia*

The genus *Lobelia*, belonging to the family Campanulaceae, comprises a diverse array of over 400 flowering plant species known for their striking flowers (Meesakul *et al*., 2023). These plants, found across tropical, subtropical and temperate regions, exhibit a variety of growth habits, from low ground covers to tall, erect stems (Duke, 2012). *Lobelia* leaves are typically alternate and may vary in shape and size, while their flowers, arranged in terminal racemes or spikes, display vibrant hues of blue, purple, pink, and white (Clemants & Gracie, 2006). In their natural habitats, *Lobelia* species thrive in a range of environments, from moist woodlands to marshes and stream banks, adapting to conditions from wet to dry soils and both sunny and shaded locations. While some *Lobelia* species are valued for ornamental gardening due to their attractive flowers and foliage, others have been used in traditional medicine by certain indigenous cultures(Seaton *et al*., 2014). However, conservation efforts may be necessary for some species facing threats such as habitat loss or over collection (Frick *et al*., 2020). Examples of *Lobelia* species include *Lobelia cardinalis*, known for its brilliant red flowers in North

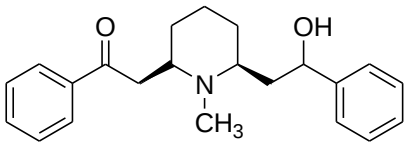
America, *Lobelia erinus*, a popular annual with compact blue, purple, or white flowers, and *Lobelia siphilitica*, featuring tall spikes of blue flowers in eastern North American wetlands(Barnes, 2012). *Lobelia* species have been used traditionally for treating various diseases, *L.inflata*, widely used in the form of powder, tincture, syrup and infusion, received more attention from scholars owing to its emetic, hypnotic, antiasthmatic and astringent properties. piperidine alkaloids such as lobeladine(**7**),lelobanidine(**8**),lobeline(**9**), norlobeladine(**10**),lobenaline(**11**),8-phenylnorlobelol(**12**),lelobanonoline(**13**),andrachcinidine(**14**) have been reported. In addition terpenoids such as β-sitosterol (**15**), stigmasterol (**16**),β-amyrin(**17**),cycloeucalenol(**18**) were also reported. Polyacetylene isolated from *Lobelia* species lobetyolin(**19**), isolobetyol(**20**), lobetyol(**3**) (Folquitto *et al*., 2019).



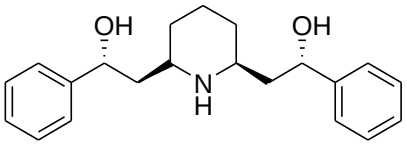
7



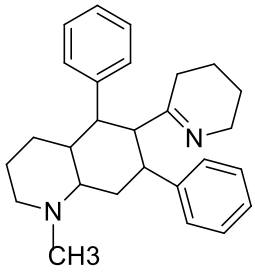
8



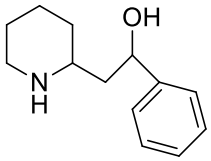
9



10



11



12

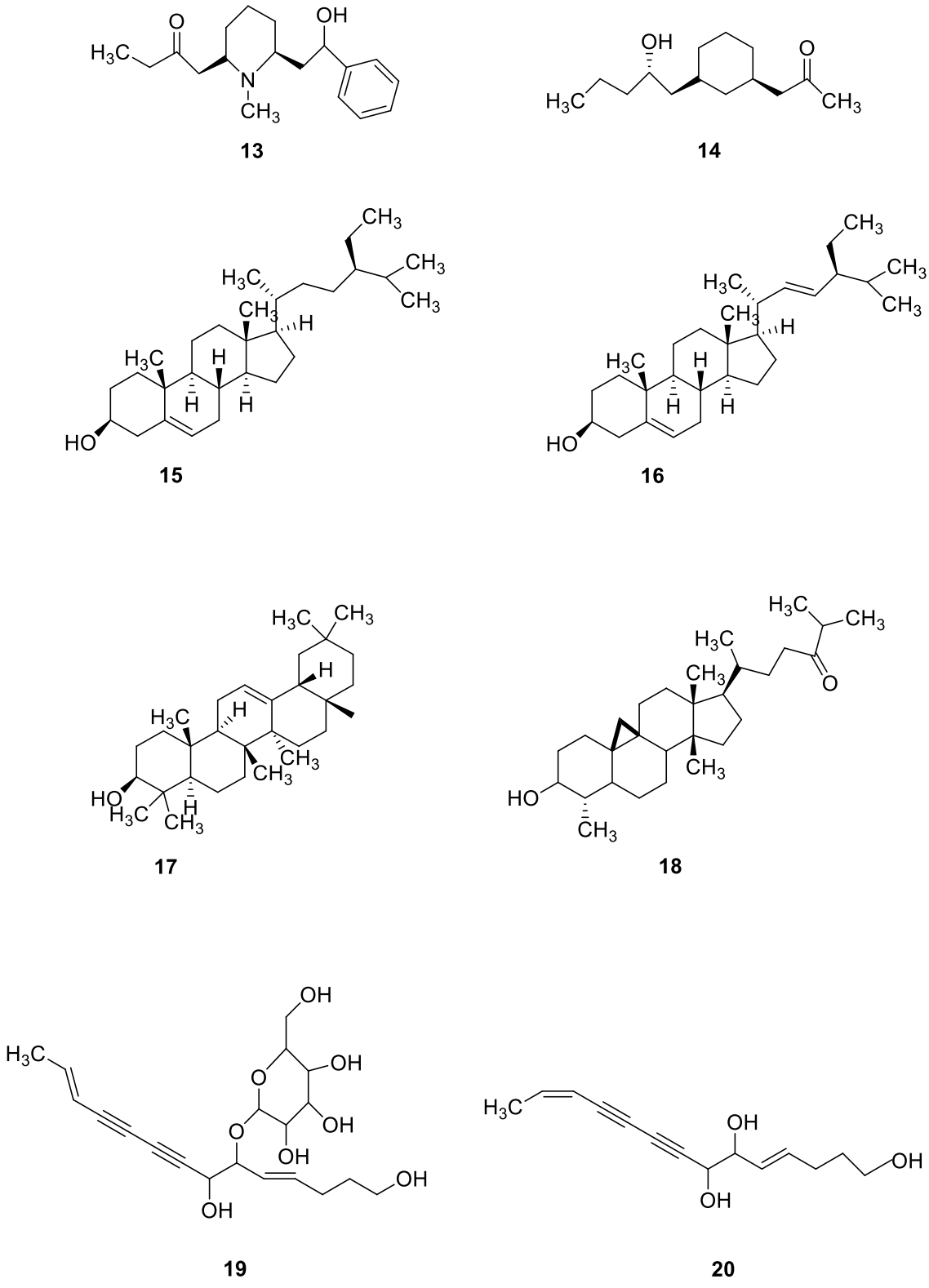


Figure 2: Structures of the compounds isolated from *Lobelia* species

2.2.1. Ethnomedicinal use of genus *Lobelia*

The genus *Lobelia* has a rich history of ethno medicinal use among various indigenous cultures around the world(Dhruv *et al*, 2019). *Lobelia* species have been used in folk medicine worldwide in the form of infusions and tinctures for the treatment of various diseases (Stolom *et al*., 2016). While the specific species and preparation methods may vary among different regions, *Lobelia* plants have been traditionally employed for a range of medicinal purposes (Sun *et al*., 2017). Here are some examples of the ethno medicinal uses of genus *Lobelia*:

1. **Respiratory Conditions:** Many indigenous tribes in North America used *Lobelia inflata*, commonly known as Indian tobacco or pokeweed, as a traditional remedy for respiratory ailments such as asthma, bronchitis, and coughs. It was often smoked or brewed into teas for its expectorant and bronchodilator properties (Mokler *et al*., 2016).
2. **Smoking Cessation:** Some Native American tribes reportedly used *Lobelia inflata* as a smoking herb to help reduce nicotine cravings and support individuals in quitting smoking. It was believed to have a similar action to nicotine on the nervous system but with reduced addictive potential (Johnson, 2018).
3. **Muscle Relaxation and Pain Relief:** *Lobelia* species have been used topically or internally by various indigenous groups to alleviate muscle tension, spasms, and pain associated with conditions like rheumatism and arthritis. The plant's muscle-relaxing properties were often exploited in traditional poultices or liniments (Pattanayak, 2018).
4. **Digestive Aid**: Certain *Lobelia* species were used as digestive aids by indigenous cultures. They were believed to stimulate digestion, relieve gastrointestinal discomfort, and alleviate symptoms such as indigestion, bloating, and stomach cramps(Lans, 2007).
5. **Sedative and Relaxant:** In some cultures, *Lobelia* plants were utilized as mild sedatives or nervines to promote relaxation, calmness, and sleep. The plant's calming effects were valued for reducing anxiety, nervous tension, and insomnia(Van Wyk & Wink, 2018).
6. **Wound Healing:** Infusions or poultices made from *Lobelia* plants were applied topically to wounds, cuts, and insect bites to promote healing and alleviate pain. The plant's purported antimicrobial and anti-inflammatory properties were thought to aid in wound care(Alamgir & Alamgir, 2017).The important genera belonging to the family are *L.rhynchopetalum**,*** *L.chinensis*, *L.cardinalis*, *L.pyramidalis, L.polyphylla, L. siphilitica, L. inflata, L. flaccida, L. nicotianaefolia and L.tupa* (Folquitto *et al*., 2019).Table 1

Table 1 : Some species of *Lobelia* with uses

Binomial	Uses	Geographic location	References
L.rhynchopetalum	arthritis, rheumatism, and muscle strains	Ethiopia,Kenya,Uganda,Tanzania	(Bussmann *et al*., 2021)
L. cardinalis	Emetic, typhoid, fever, and "love potion"	North America	(Folquitto *et al*., 2019)
L. chinensis	Diuretic, hemostatic, antimicrobial, antiviral, anti-inflammatory, edema, jaundice, liver, stomach, intestinal diseases, antitumor	China	(Singh *et al*.,2020)
L. flaccida	Analgesic, antiepileptic	South Africa	(Stolom *et al*., 2016)
L. inflata	respiratory stimulant, emetic, to tobacco smoking cessation	Eastern North America	(Folquitto *et al*., 2019; Máthé, 2020)
L.nicotianaefolia	Asthma, bronchitis, fever; eye disease; and leaves for rapid wound healing	India	(Tamboli *et al*.,2011)
L. polyphylla	Narcotic and hallucinogen	Chile	(Folquitto *et al*., 2019)
L. siphilitica	Cathartic, diaphoretic, emetic, treatment of dropsy, diarrhea, stomach complaints, syphilis and dysentery	Eastern North America	(Hosler, 1989)
L. pyramidalis	Antispasmodic, asthma, bronchitis, fever, sciatica, and back pain	India	(Folquitto *et al*. 2019; Joshi *et al*. 2011)
L. tupa	Abortifacient,hallucinogen,respiratory stimulant, to tobacco smoking cessation	Chile	(Zimare & Kakade, 2023)

2.2.2. Pharmacological activity of genus *Lobelia*

The main source of lobeline is the *Lobelia* plant. lobeline and its derivatives are valuable medicines with anti-inflammatory, antioxidative, antiviral, and antiepileptic functions (Kolap *et al*., 2021). Research into the pharmacological activity of genus *Lobelia* has revealed a spectrum of effects on the human body, attracting considerable interest in its therapeutic potential. Among the most notable pharmacological activities associated with *Lobelia* species is its impact on respiratory health (Stansbury *et al*., 2013). Studies have shown that compounds present in *Lobelia* plants, such as lobeline, exhibit bronchodilator and expectorant properties (Sindhu *et al*., 2022). These make *Lobelia* effects potentially valuable in managing respiratory conditions like asthma, bronchitis, and chronic obstructive pulmonary disease (COPD). By relaxing bronchial smooth muscles and facilitating the expulsion of mucus from the airways, *Lobelia* compounds may offer relief to individuals suffering from respiratory distress.

Furthermore, research has explored the neurological effects of *Lobelia* species, particularly focusing on compounds like lobeline. Studies suggest that lobeline interacts with neurotransmitter systems in the central nervous system, exhibiting both stimulant and depressant effects on neuronal activity (Dwoskin & Crooks, 2002). This property has prompted investigations into *Lobelia* potential therapeutic applications for conditions such as Parkinson's disease, attention deficit hyperactivity disorder (ADHD), and nicotine addiction(Zheng *et al*., 2021). Additionally, *Lobelia* extracts have demonstrated analgesic and anti-inflammatory effects in preclinical studies. These properties suggest potential applications in managing pain and inflammation associated with various conditions, including arthritis, rheumatism, and musculoskeletal injuries. By modulating inflammatory pathways and reducing the release of pro-inflammatory mediators, *Lobelia* compounds may offer therapeutic benefits for individuals experiencing discomfort due to inflammatory conditions (Zheng *et al*., 2021).Moreover, some studies have reported cardiovascular effects associated with *Lobelia* species, including hypotensive and vasodilatory effects. These effects suggest potential benefits for individuals with hypertension or cardiovascular disease, although further research is needed to understand the mechanisms underlying these effects fully (Joshi *et al*., 2011).

Lastly, traditional uses of *Lobelia* species suggest potential gastrointestinal effects, including stimulation of digestive function and relief of gastrointestinal discomfort. Phytochemicals

present in *Lobelia* plants may modulate digestive processes and exert protective effects on the gastrointestinal mucosa, offering therapeutic potential for conditions such as indigestion, bloating, and gastritis (Poddar *et al*., 2020).Overall, the pharmacological activity of genus *Lobelia* underscores its potential as a source of novel therapeutic agents for a wide range of health conditions. While preliminary research is promising, further studies, including clinical trials, are necessary to validate the efficacy and safety of *Lobelia*-based treatments and elucidate the underlying mechanisms of action.

2.2.3. Phytochemistry of genus *Lobelia*

The phytochemistry of genus *Lobelia* is characterized by a diverse array of secondary metabolites, including alkaloids, flavonoids, terpenoids, and phenolic compounds(Kolap *et al*., 2021). These phytochemicals contribute to the pharmacological properties and traditional uses of *Lobelia* species(Stolom *et al*., 2016). Here's an overview of the key phytochemicals identified in genus *Lobelia*:

1. Alkaloids: Alkaloids are the most studied class of compounds in *Lobelia* species. Lobeline is one of the most well-known alkaloids found in *Lobelia* plants, particularly in *Lobelia inflata*(Brown *et al*., 2016). It exhibits a variety of pharmacological effects, including respiratory stimulation, neuromuscular blockade, and potential interactions with neurotransmitter systems (Bittner & Martyn, 2019).

2. Flavonoids: Flavonoids are another group of phytochemicals present in *Lobelia* plants. These compounds have antioxidant, anti-inflammatory, and potential cardiovascular protective effects(Paschoal *et al*., 2020). These flavonoids may contribute to the medicinal properties of *Lobelia* plants, including their anti-inflammatory and analgesic effects(Dillard & German, 2000).

3. Terpenoids: Terpenoids are secondary metabolites found in many plant species, including *Lobelia*. These compounds have diverse biological activities, including antimicrobial, antiviral, and anticancer properties. Some *Lobelia* species contain terpenoids such as triterpenes and sesquiterpenes, which may contribute to their traditional medicinal uses and pharmacological effects.(Alamgir & Alamgir, 2018)

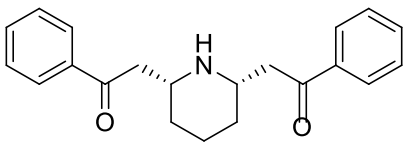
4. Phenolic Compounds: Phenolic compounds are abundant in *Lobelia* plants and are known for their antioxidant and anti-inflammatory properties (Zhang *et al*., 2017). These compounds include phenolic acids, such as caffeic acid and chlorogenic acid, as well as flavonoids and

lignans (Oluwole *et al*., 2022). Phenolic compounds in *Lobelia* species may contribute to their therapeutic effects, including their ability to scavenge free radicals and reduce oxidative stress (Li, Ho *et al*., 2015).

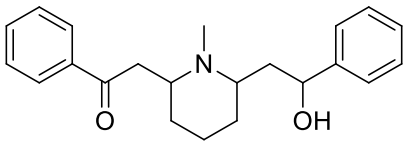
5. Other Constituents: In addition to the aforementioned classes of phytochemicals, *Lobelia* species contain other bioactive constituents, such as saponins, tannins, and volatile oils. These compounds may also contribute to the pharmacological properties of *Lobelia* plants, although their specific roles are less well-characterized compared to alkaloids and flavonoids (Kichu *et al*., 2015).Overall, the phytochemistry of genus *Lobelia* is complex and diverse, encompassing a wide range of secondary metabolites with potential pharmacological activities. Further research is needed to elucidate the individual contributions of these phytochemicals to the medicinal properties of *Lobelia* species and to explore their potential therapeutic applications in modern medicine.

2.2.3.1. Phytochemical constituent of *Lobelia Chinensis*

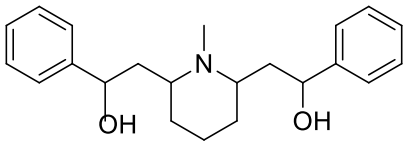
Lobelia chinensis Lour (Campanulaceae) is a popular herb that has been widely used as folk medicine in China for the treatment of fever, lung cancer, and inflammation for hundreds of years *(Li et al., 2015)*. This plant is dispersed widely throughout East Asia, including China, Korea, and Japan. The main constituents of *L. chinensis* are piperidine alkaloids and flavonoids; other components include terpenes, alkynes, coumarins and amino acids. The main piperidine alkaloids contributing to the anticancer effects are dominated by norlobelanine (**22**), lobeline (**23**), lobelanidine (**24**), lobelanine (**25**), radicamine A (**26**) and radicamine B (**27**), which are shown in Figure 3. In addition, the flavonoids are comprised of apigenin (**28**), luteolin (**29**), quercetin (**30**), linarin (**31**), and luteolin 3', 4'-dimethylether-7-*O*-D-glucoside (**32**) (Figure 3) . Among the flavonoids, apigenin and luteolin have been demonstrated as antitumor effective compounds (Mei-Wan *et al*., 2014) .



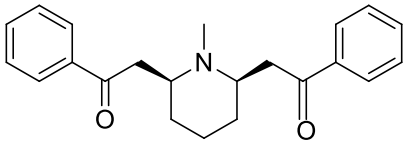
22



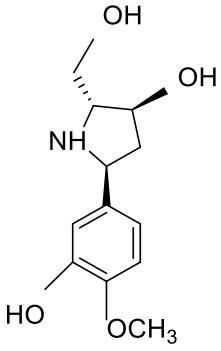
23



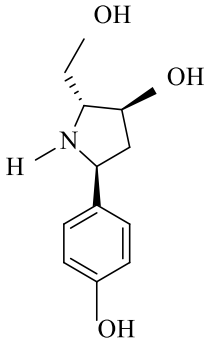
24



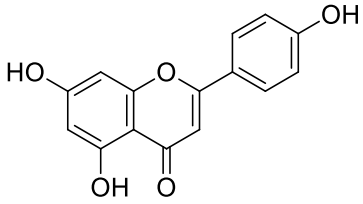
25



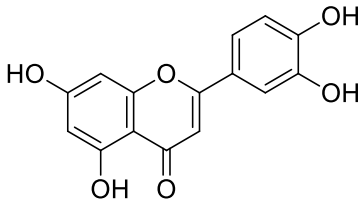
26



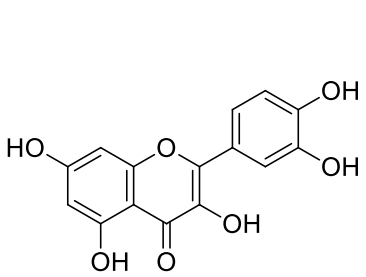
27



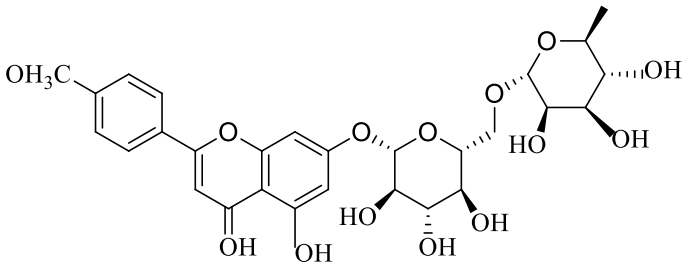
28



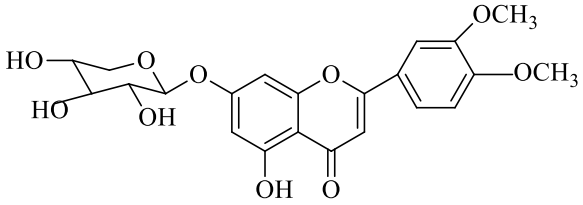
29



30



31



32

Figure 3: Structure of the flavonoids isolated from *Lobelia chinensis*

2.2.3.2. Phytochemical constituent of *Lobelia inflata*

Lobelia inflata belongs to family Campanulaceae. Commonly known as asthma weed, Indian tobacco, puke weed and vomitwort (Henkel, 1904). The species used most commonly in modern herbalism is *Lobelia inflata* (Indian tobacco). It is used mainly as a powerful antispasmodic herb in the treatment of respiratory and muscle disorders. The plant is also taken internally for the treatment of asthma, bronchitis, whooping cough and pleurisy. It also serves as a respiratory stimulant and relaxant. From the aerial parts of ***L.****inflata*, the following alkaloids were identified; 8-propyl-10-phenyl lobelionol **(34**), 3-hydroxy-3-phenyl-propanoic nor allosedamine **(22)**, and 3-hydroxy-3 phenyl-propanoic allosedamine **(23)** (Máthé, 2020)**.**

Antidepressant activity

The leaves of *Lobelia inflata*, which have been used as a remedy for spasmodic asthma, reportedly contain numerous piperidine type alkaloids. Pharmacologically active substances in medicinal plants, much attention has been given to the occurrence of natural products possessing antidepressant activity. They have found that methanol extract of the leaves of *L.inflata* showed

antidepressant activity in the forced swimming test with mice **(33)**. This was reported isolation and structural elucidation of an antidepressant substance from the plant (Subarnas *et al.*,1992).

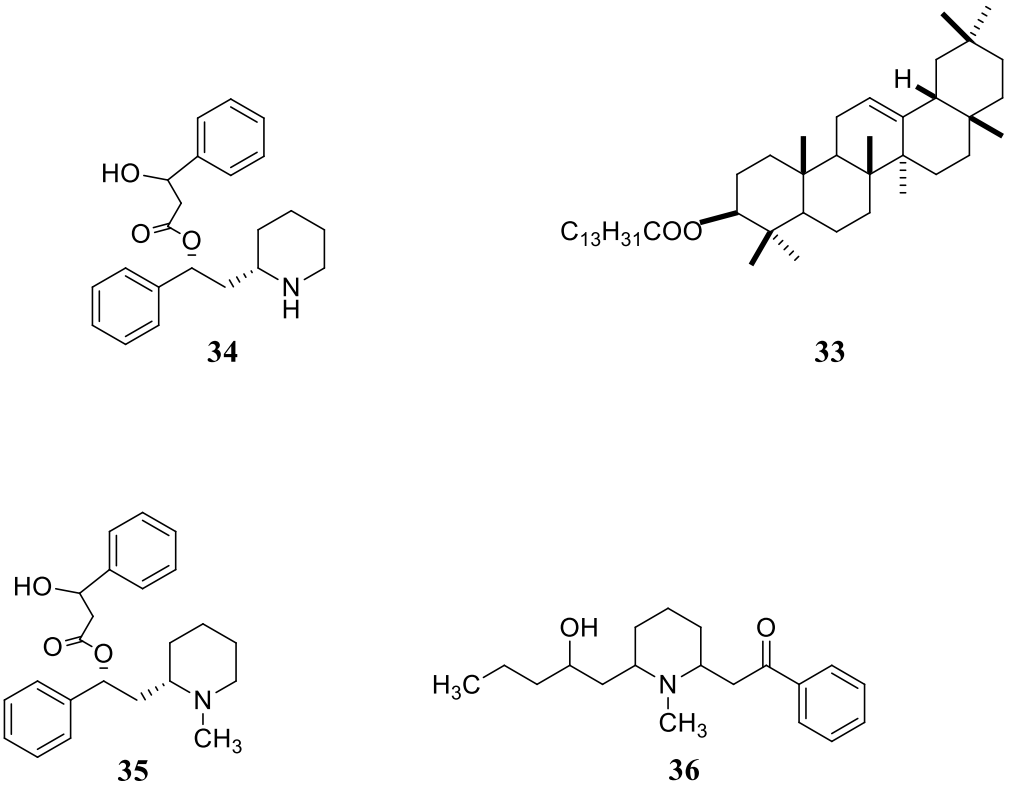


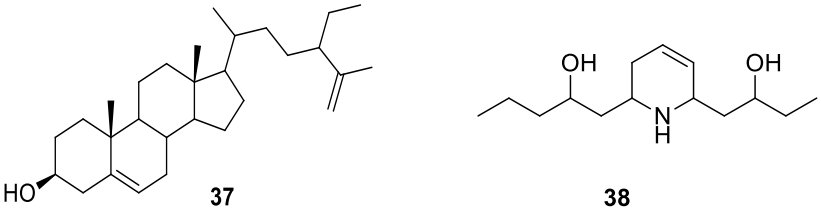
Figure 4: Isolated compound from *Lobelia inflata*

2.2.3.3. Phytochemical constituent of *Lobelia rhynchopetalum*

Ethanomedicinal plant survey in Ethiopia and other parts of the world indicated that among many medicinal plants, one of the species identified in this study is *Lobelia rhynchopetalum* has been used traditionally for many therapeutic uses. The bark and root of *Lobelia rhynchopetalum* is crushed mixed with little water and sniffed at the sickness time by nasal to relieve evil eye (Wubetu *et.al.*, 2018). In Ethiopia, *Lobelia rhynchopetalum* has a vernacular name Tarura (in Afan Oromo) and Jibara (in Amharic). The seed of *Lobelia rhynchopetalum is* crushed, powdered, mixed with little water and drunk orally to treat heart problem (Ayalew *et.al.*, 2022) .

Figure 5: *Lobelia rhynchopetalum* (picture was taken by Bikiltu on December, 2022)

Elucidation of methanol root extracts of *Lobelia rhynchopetalum* (Campanulaceae) on the basis of its spectral data(UV,1NMR and MS) and comparision with data published on literature resulted in the partial structure for compound lobetyol (2,10-tetradecadien-4,6-diyne-8,9,14-triol)(**3**),24-ethyl-3-hydroxylcholesta-5,25-diene**(37)**,aspertin-A**(38)**(reported from Abu.j,2021).



37 38

Figure 6: Isolated compound from root of *L. rhynchopetalum*

3. MATERIALS AND METHODS

3.1. Description of the sample collection site

The stem barks of *Lobelia rhynchopetalum* were collected in February, 2023 from Galema which is located in the Arsi Zone of the Oromia Region. It has latitude and longitude of (7°35′N39°10′E) with an elevation of 2810 m (Shaki & Areas, 2022). Bokoji and the Galema area are characterized by their high altitude, agricultural productivity, and rich cultural heritage.

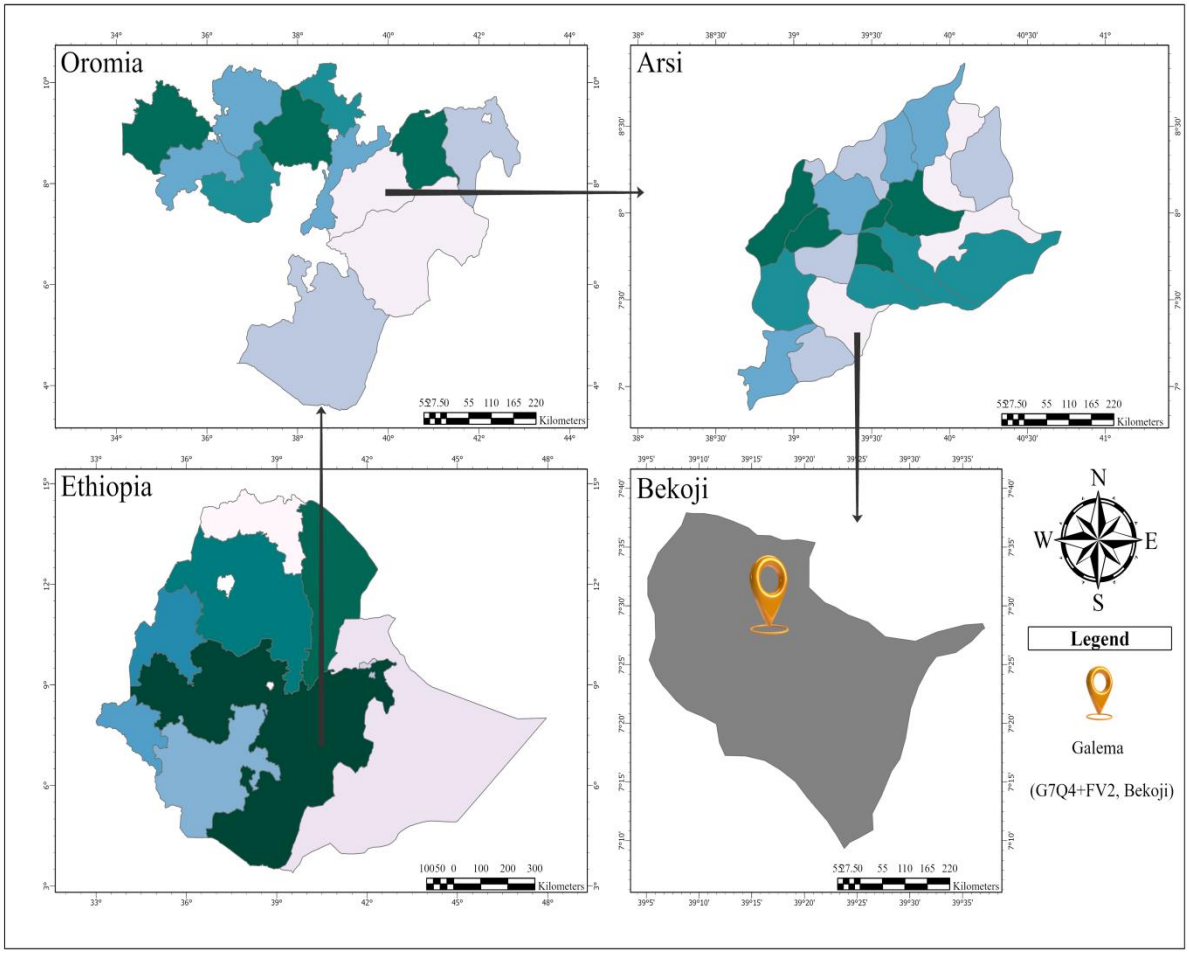


Figure 7 : Geographical map of the study area (drawn using ArcGIS Pro 3.0.0)

3.2. Materials and chemicals

Electrical grinder, Rotary evaporator, digital measuring balance, 1000 mL Erlenmeyer flask, 100 mL beaker, TLC plates (pre-coated aluminum sheet, 20 × 20 cm), silica gel (60 F254), UV lamp (254 nm and 365 nm) were used. Techniques employed for purification of compounds were

silica gel column chromatography (CC) which was performed using silica gel (100-200 mesh size) as stationary phase and organic solvent as mobile phase. Vanillin in H_2SO_4 was also employed for visualization purpose.^{1}H and^{13}C-NMR spectra were recorded using a Bruker Avance 400MHz NMR spectrometer, Dimethylsulfoxide (DMSO) was used as solvent in the NMR laboratory, Addis Ababa University, Ethiopia.

IR-spectroscopy: FT-IR spectra were obtained on a Perkinelmer apparatus. The spectra were measured in KBr pellets and presented in cm^{-1}.

UV-Vis spectroscopy: λ max for each purified compounds were recorded on PG instrument UV-Vis double beam spectrometer (T80+ model) having scanning range from 190-2100 nm.

3.3. Plant materials collection and identification

Representative samples of plant material (stem bark, leaves and root) for botanical identification of *Lobelia rhynchopetalum* (Campanulaceae) were collected during full flowering stage season at Arsi (Galema) Mountains, Oromia Region, Ethiopia. The plant material was identified with the help of Professor Sileshi Nemomissa and voucher specimen (AJ-001) was deposited at the National Herbarium of Addis Ababa University, Ethiopia.

3.4. Extraction

The air dried and powdered stem bark of (350g) were macerated in methanol (2L) for 72 hrs. At the end of the 72 hours maceration, soaked sample was subjected to final enough agitation before the solution was filtered using filter paper (What man, No 1). The filtrate was concentrated under reduced pressure and a temperature range of 40 ºC, over rotary evaporator to give the crude extracts. For further 35g methanol crude used for washed by n-hexane, ethyl acetate, chloroform respectively. All crude extract were stored in refrigerator at 4^{o}C until required for further analysis. The extracts were analyzed with TLC for further fractionation silica-gel column to select the best possible solvent system for the column.

3.5. Phytochemical Screening Tests of extracts

Phytochemical screening is a crucial step in assessing the chemical composition of plant extracts. It helps in identifying the presence of various classes of secondary metabolites, which can provide insights into the potential medicinal properties and bioactivity of the extract(Berhanu, 2017).

Test for flavonoids: 4mg of extracts was treated with conc. H_2SO_4 solution. The formation of a yellowish orange color indicates the presence of flavonoids.

Test for saponins (Foam test): 4mg of extracts was diluted with 5mL of distilled water, shake vigorously and was observed for a stable persistent froth.

Test for phenolic compounds (Ferric chloride test): 4mg of extracts was treated with diluted $FeCl_3$ solution. Appearances of a violet color indicate the presence of phenol like compounds.

Test for tannins: To a 4mg of extracts, a few drops of 10% ferric chloride solution were added. The appearance of a green or blue color indicates the presence of tannins.

Test for steroids: 4mg of extract was mixed with 1mL of chloroform and 2-3 drops of conc. H_2SO_4 was added to it. The appearance of a pink or red color indicates the presence of steroids.

Test for Terpenoids (Salkowski test): 4mg extracts were mixed with 2ml of chloroform and 3mL of conc. H_2SO_4 solution. A reddish-brown color at the interphase indicates the presence of terpenoids.

Test for alkaloids (Wagner' test): 4mg of the extract was treated with diluted HCl, filtered followed by addition of Wagner's reagent (1.27 g of iodine and 2g of KI along with 100mL of distilled water) and observed for the formation of reddish-brown colored precipitate.

3.6. Isolation of compounds

The TLC profile of the EtOAc and MeOH extracts were found to be similar. The Methanol extract (5 g) of the stem bark of *Lobelia rhynchopetalum* was dissolved and subjected to silica gel column chromatography over silica gel, (150 g) using *n*-hexane for packing. Elution was carried out with increasing polarities of EtOAc in *n*-hexane to afford 128 fractions (Each 100 mL). The eluent, ratio of solvents used for fractionation, volume of eluent collected and amount obtained are depicted in Table 2.

Table 2: Column chromatography fractionation of extracts

Fraction	eluent (ratio	Volume (ml) amount	Remark
	Hexane 100%	100	no spot
	Hexane:EtOAc(95:5)	100	" "
	Hexane:EtOAc(90:10)	100	" "

	Hexane:EtOAc(85:15)	100	“ ”
	Hexane:EtOAc(80:20)	100	“ ”
	Hexane:EtOAc(75:25)	100	“ ”
	Hexane:EtOAc(70:30)	100	“ ”
	Hexane:EtOAc(65:35)	100	“ ”
	Hexane:EtOAc(60:40)	100	“ ”
	Hexane:EtOAc(55:45)	100	“ ”
F_1-F_6	Hexane:EtOAc(50:50)	100	
F_7-F_{15}	Hexane:EtOAc(45:55)	100	
F_{16}-F_{21}	Hexane:EtOAc(40:60)	100	
F_{22}-F_{27}	Hexane:EtOAc(35:65)	100	
F_{28}-F_{33}	Hexane:EtOAc(30:70)	100	
F_{34}-F_{40}	Hexane:EtOAc(25:75)	100	
F_{41}-F_{57}	Hexane:EtOAc(20:80)	100	One single spot labeled as 41
F_{58}-F_{63}	Hexane:EtOAc(15:85)	100	
F_{64}-F_{70}	Hexane:EtOAc(10:90)	100	
F_{71}-F_{79}	Hexane: EtOAc(5:95)	100	
F_{80}-F_{87}	Hexane: EtOAc (100%)	100	
F_{88}-F_{96}	EtOAc:Methanol(90:10)	100	
F_{97}-F_{111}	EtOAc:Methanol(80:20)	100	One single spot labeled as 42
F_{112}-F_{120}	EtOAc:Methanol(70:30)	100	
F_{121}-F_{128}	EtOAc:Methanol(60:40)	100	

3.6. Anti-bacterial activities

3.6.1. Disc diffusion Assay

Anti-bacterial activities of the crude extracts and isolated compounds were investigated using agar disc diffusion method against four bacterial strains (two Gram-positive bacteria; *S. aureus* and *S.pyogenes* and two Gram-negative bacteria; *E. coli* and *P.aeruginosa)* obtained from

Biology laboratory of Adama Science and Technology University. Appropriate colonies of required bacterial strains were selected and picked using an inoculating loop and Inoculum suspension was prepared and standardized with 0.5 McFarland standards. The surface of the medium plate was inoculated with standardized suspension by streaking back and forth from edge to edge and the discs containing crude extract and isolated compounds of concentration (200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml), standard drugs (ciprofloxacin) of concentration 25µg/ml and negative control (DMSO) were applied within 15 minutes of inoculation. It was incubated in ambient air at 35°C for 16-18 hours. The inhibition zones were measured with a ruler and compared with the positive control disk (disk containing Ciprofloxacin) of the same concentration. All of the experiments were performed in triplicate and the results (millimeters of the inhibition zone) were expressed as mean values.

3.6.2. Molecular Docking study of isolated compounds

The procedure described (Rizvi *et al*., 2016)was followed to model the docking of isolated compounds with various model proteins. In detail, ChemDraw 22.0 was used to sketch the compound structures, which were then stored in the. mol file format. The data were then saved in the .pdb file format and optimized by Discovery Studio Visualizer 12.1 (Oliveira *et al*., 2023) The model protein structures of *E. coli* DNA gyrase B (PDB ID: 7P2M), *Pseudomonas* quinolone signal A (PqsA, PDB ID: 5OE4), pyruvate kinase M2 (PKM2; PDB ID: 4G1N), and human topoisomerase IIβ (PDB ID: 3QX3) were obtained and stored in a .pdb file format from the web database, protein data bank (Duguma *et al*., 2023). The active (binding) sites were then located, and the proteins were cleaned using the Discovery Studio Visualizer program as well. Using AutoDock Vina along with auxiliary programs including MGLtools 1.5.6 and Python 3.10.9, docking simulations were performed. By default, nine conformers were used for each run. To see the interactions between the ligand and target as 2D and 3D structures, from all conformers, the conformer showing the best docking score (least binding affinity) was chosen to be examined. The antibacterial agent ciprofloxacin, the anticancer agent etoposide (a topoisomerase II inhibitor), and the anticancer agent PKM2-IN-1 (a pyruvate kinase M2 inhibitor) were used as positive controls in simulations for comparison.

3.6.3. *In silico* Pharmacokinetics and Toxicity Study of the Isolated Compounds

The canonical simplified molecular-input line-entry systems (SMILESs) of the isolated compounds were obtained from the PubChem database. Compounds that do not have SMILES were sketched with ChemDraw 22.0 and stored as .mol files. Then, using Discovery Studio 12.1 software, they were transformed into a SMILES file and submitted to the SwissADME online tool to estimate their physicochemical properties and drug-likeness (Lipinski rule) (Degfie, 2022). To estimate oral acute toxicity, organ toxicity, and toxicity endpoints, the SMILES files of the test compounds were uploaded to the Pro Tox 3.0 online tool(Bhat & Chatterjee, 2021). Ciprofloxacin, PKM2-IN-1, etoposide, and data were also generated for comparison purposes.

3.7. Evaluation of the DPPH radical scavenging activity

Free radical scavenging activity of crude extract and isolated compounds from the stem barks of *Lobelia rhynchopetalum* were measured by 1-1- diphenyl-2-picryl hydrazyl (DPPH). Sample was prepared by dissolving 4 mg of DPPH in 100 mL of MeOH. Four different concentrations of the samples were mixed with DPPH solution to furnish 25, 50,100 and 200µg/ml. The mixture was shaken vigorously and incubated at 37^{o}C for 30 min. Then, absorbance was measured at 517 nm by using UV-Vis spectrophotometer. The % inhibition was calculated using the following formula from literature (Capson-Tojo *et al*., 2020).

Inhibition % = Ac-As/Ac×100 where Ac is the absorbance of the control and As is the absorbance of the sample.

4. RESULTS AND DISCUSSION

4.1. Extract yield

350 g of dried and powdered stem bark of *L. rhynchopetalum* was extracted with methanol and yielded a 70g crude methanol extract (20% w/w) upon drying under vacuum using rotary evaporator. The relatively high percentage yield of the methanol extract was an indication of the presence of fairly large amounts of polar phytoconstituents in the stem bark of the plant. In order to separate phytoconstituents which could be less polar or of intermediate polarity, half of the crude methanol extract (35g) was subjected to successive dissolution in n-hexane, ethyl acetate and chloroform and each fraction was dried under vacuum. The n-hexane fraction yielded 2g crude extract (5.7% w/w), ethyl acetate gave 4g, (11.4% w/w), and chloroform gave 5g (14.2% w/w). The results showed that with increasing polarity of the solvents more material was obtained suggesting that more polar compounds were present in the methanol extract of the stem bark of the plant.

4.2. TLC Analyses of the Extracts

Each of the extracts obtained following the above described extraction protocol was subjected to normal phase thin layer chromatographic analysis (TLC) to determine their chemical compositions (Figure 9). The hexane extract was analyzed using hexane-ethylacetate solvent system in a 3:2 (V:V) ratio, the ethylacetate and methanol extracts were analyzed using ethylacetate-methanol solvent system in a 4:1 (V:V) ratios. Since the separations of the components of the crude extracts on the TLC were not visible to the naked eyes, the TLCs were sprayed with vanillin in sulphuric acid which resulted in the TLC profiles shown in Figure 9. As can be seen from the figures, each TLC showed the presence of several phytoconstituents that could be isolated by appropriate chromatographic techniques such as column chromatography. Thus, the methanol crude extract was selected for further column chromatographic analysis and separations based merely on the relatively its higher amount and presence of several constituents.

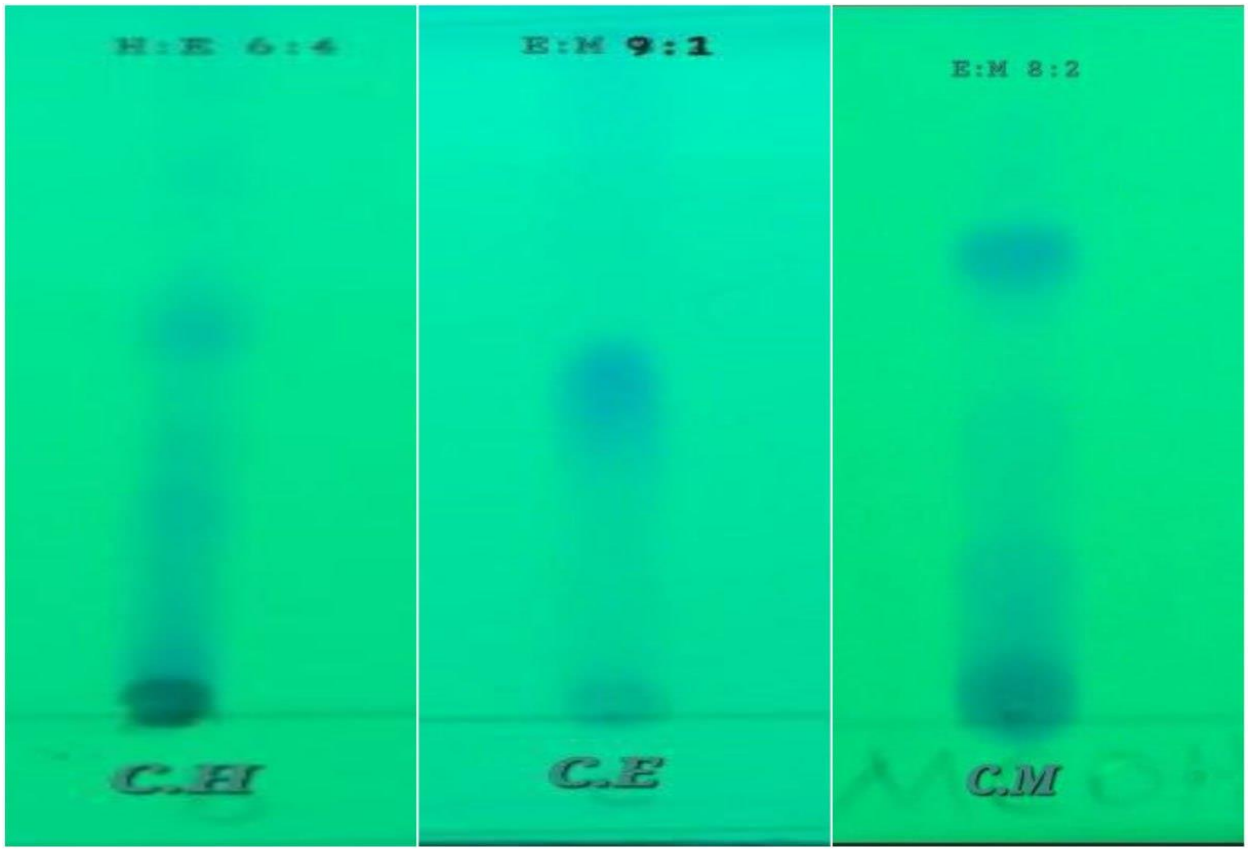


Figure 8 : TLC profile of the extracts of the stem bark of the *L.rhynchopetalum*

4.3. Preliminary Phytochemical Analysis

The distribution of different phytochemical constituents in methanol extract of *L. rhynchopetalum* was evaluated qualitatively using phytochemical screening tests. They were identified by characteristic color changes based on standard procedures as described previously and the obtained results are presented in Table 3.The phytochemical screening tests were undertaken to determine the class of secondary metabolites that are biosynthesized in the plant and that could be extracted into the menstruum, in this case, the methanol. It is generally agreed that any of the biological activities or the pharmacological or medicinal effects displayed by crude drugs obtained from any part(s) of plants results from these secondary metabolites biosynthesized by the plants (Mahomoodally *et al*., 2005). The result revealed that flavonoids, terpenoids, phenolics, alkaloids and tannins were presents in the stem bark extracts of MeOH. However steroids and saponins were not detected in this extract.

Table 3: Phytochemical analysis of *L.rhynchopetalum* extract

Phytochemical screening	Types of tests	Methanol extract
Test for alkaloids	Wagner test	+
Test for Saponins	Foam test	-
Test for terpenoids	Salkowski's test	+
Test for flavonoids	Shinoda test	+
Test for phenolics	Ferric chloride test	+
Test for tannins	-	+
Test for steroids	-	-

Notice that: + indicates presence, - indicates absence.

4.4. Isolation and Characterization of the isolated compounds

In the course of this work, the methanol extract of the bark of the plant was subjected to normal phase column chromatographic analyses on silica gel columns eluted with n-hexane, ethylacetate and methanol mixtures in appropriate combinations, as described under the experimental section, to separate the components of the extract into pure compounds. As a result, two pure compounds, compound **41** and compound **42** could be isolated from the methanol stem bark extracts of *L. rhynchopetalum* and their structures were characterized. The detailed characterizations of these compounds are presented below.

4.4.1. Characterization of Compound 42 (BL2)

The methanol extract of *L. rhynchopetalum* was subjected to normal phase column chromatographic fractionations using n-hexane, ethylacetate and methanol mixtures with increasing polarities as eluents and several fractions were collected. Thus, compound **42** was obtained as reddish oil (40mg) from fraction 97-111 upon removal of the solvent via evaporation. The compound was analyzed on normal phase TLC for its purity using methanol-ethyl acetate in a 1:4 (V/V) ratio as eluent. The TLC displayed a single spot with Rf value of 0.49 (Figure 10).

Figure 9 : TLC profile of compound **42**

The structure of compound **42** was elucidated through careful and detailed interpretation of its ^{1}H-NMR, ^{13}C-NMR and DEPT-135 NMR (Table 4) spectral data and comparison with literature. In the ^{1}H NMR spectrum of compound **42**, (appendix 1.3.), a proton signal at δ 4.15 (m, 1H) indicated the presence of an anomeric proton, there by indicating the presence of a sugar unit in the compound, suggesting that **42** is a glycoside. In addition, the proton signals at δ1.79 (dt, *J* = 7.0, 1.9 Hz, 3H) were indicative of the presence of a methyl group in proximity to olefinic carbons. The presence of two pairs of *trans* coupled olefinic proton signals at δ 6.38 (dqd, *J* = 15.6, 6.8, 1.8 Hz, 1H) and 5.37 (ddd, *J* = 15.5, 7.6, 1.8 Hz, 1H), and δ 5.82 (dt, *J* = 14.4, 6.7 Hz, 1H), and 5.69 (dt, *J* = 16.4, 2.1 Hz, 1H) were indicative of the presence of two sets of double bonds, i.e. four olefinic methines each pair arranged in a trans relationships due to the presence of large vicinal coupling constant (14 - 16 Hz) in **42**. Further, signals at δ 3.66 (d, *J* = 11.9 Hz, 2H) was indicative of hydroxylated methylene group of the sugar unit while the signal at 3.11 (t, *J* = 8.2 Hz, 2H) indicated the presence of a second hydroxymethylene group in the compound

next to an aliphatic methylene group. The signals at δ 1.50 and δ 2.07 and each were integrated to two protons that were attributed to be CH_2 protons.

The ^{13}C NMR spectrum (Appendix 1.5.) showed a total of twenty carbons signal. The DEPT-135 spectrum (Appendix 1.7.) Showed carbon signals of sixteen carbons only indicating that four of the carbons in ^{13}C NMR were quaternary carbons. Those quaternary carbons, whose signals were missing from the DEPT-135 spectrum, appeared at δ 83.21, 77.57, 72.42, and 69.57 in the proton decoupled ^{13}C NMR. These chemical shift values unmistakably indicated that these four quaternary carbons are part of one conjugated diyne system, *i.e.* two conjugated triple bonds. Four olefinic methine signals at δ 145.5, 136.8, 125.4 and 109.6 confirmed the presence of four unsaturated carbons, namely, two separate double bonds that were displayed in the ^{1}H-NMR discussed above. Further, both ^{13}C and DEPT-135 spectra displayed the presence of seven oxygenated methine signals at δ100.4, 79.9, 77.4, 77.3, 73.7, 70.6, and 65.0. This clearly showed that the compound must contain a sugar unit in which five of the methine signals were due to a glucose moiety, which is further substantiated by the presence of an anomeric carbon signal at δ100.4. However, the remaining two oxygenated methine signals must be extra and belong to the remaining part of the structure. Further both ^{13}C and DEPT-135 spectra displayed the presence of two oxygenated methylene groups, which appeared as negative signals in the DEPT-135,at δ60.6 and 61.5 in which one of them belonged to the glucose moiety as shown in the ^{1}H-NMR signal as well. This supports the fact that at least one of the oxygenated methylenes belongs to the sugar groups and that compound **42** was a fourteen carbon, polyacetylenic, diene, diol glucoside spectra displayed the presence of a single aliphatic methyl signal at δ19.1 and two aliphatic methylene signals, which appeared as negative signals in the DEPT-135 at 28.9 and 32.5 ppm.

Based on these data, a structure given in Figure 11, which can be named 2-((4E, 12E)-1, 7-dihydroxytetradeca-4, 12-dien-8, 10-diyn-6-yloxy)-tetrahydro-6-(hydroxymethyl)-2H-pyran-3, 4, 5-triol was proposed for the compound. Further, literature survey was carried out on the proposed structure and it was found that a similar compound, commonly known as lobetyolin, has been reported previously from plants such as *L. inflata* (Ishimaru *et.al*., 1991) *Codonopsis pilosula*, also known as *Radix codonopsis* or Dangshen (a popular Chinese medicinal plant) (Christian Bailly, 2021), and *L. giberroa* Hemsl (Tadege *et al*., 2022) from Ethiopia. However,

this is the first finding of **42** from *L. rhynchopetalum* hitherto. Interestingly, the aglycone of compound **42**, known as lobetyol, has previously been reported from the root bark of the same plant (Jeylu, MSc thesis, ASTU).

Table 4: ^{1}H NMR (DMSO) (400 MHz), ^{13}C and DEPT-135(100Mz, DMSO-d6) spectral data of compound **42**

C	42			Literature (Lobetyolin)	
	^{1}H-NMR (m, J in Hz)	**13C NMR (δC in ppm)**	**DEPT-135 (δC in ppm)**	**^{1}H**	**^{13}C**
1	1.80(3H,dt,1.9)	19.14	19.11	1.80(3H,dd,1.9,6.9)	19.12
2	6.38(1H,dqd,1.8, 6.8,15.6)	145.55	145.54	6.39(1H,dq,6.8,16.1)	145.48
3	5.69(1H,dt,16.4,2.1)	109.59	109.59	5.71(1H, dd,2.1, 15)	109.66
4	-	83.20	-	-	83.31
5	-	77.60	-	-	77.55
6	-	72.40	-	-	72.46
7	-	69.57	-	-	69.57
8	4.49(4H,d,36.9)	65.0	65.0	4.45(1H,d,5.4)	65.08
9	4.09(1H,m)	77.40	77.40	4.04(1H,t,7.1)	80.02
10	5.37(1H,ddd,15.5, 7.6,1.8)	125.45	125.45	5.37(1H,dd,7.4,15.5)	125.53
11	5.82(1H,dt,14.4,6.7)	136.72	136.72	5.95(1H,dt,15.3,7.0)	136.62
12	2.07(2H,q,7.2)	28.9	28.9	2.07(2H,q,7.2)	28.94
13	1.49(2H,dt,8.5,6.4)	32.49	32.49	1.50(2H,q,6.7)	32.55
14	3.11(2H,t, 8.2)	61.50	61.50	3.41(2H,t,6.9)	61.55
1’	4.15(H,m)	100.42	100.42	4.15(1H,d,7.7)	100.54
2’	3.01(H,q)	77.31	77.31	3.30(1H,m)	77.44
3’	3.02	73.90	73.90	3.0(1H,m)	73.75
4’	3.04	70.56	70.56	3.10(1H,t,8.3)	70.61

5’	3.06	79.97	79.97	3.88(1H,dd,1.5,3.4)	77.37
6’	3.66(2H,d,11.9)	60.57	60.57	3.65(1H,d,11.7)	60.58

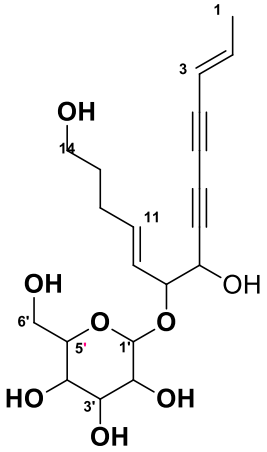


Figure 10: Proposed Structure of compound **42**

The analysis of the peaks obtained from the FTIR analysis of the compound isolated from *Lobelia rhynchopetalum* confirms the presence of functional groups related to lobetyolin. The absorption spectrum of the compound, as shown in (Appendix 1.8.) signaled the presence of O–H, C–O, C–H stretch of CH_3, C–H stretch of CH_2, and CC functional groups stretch. The broad bands at 3310 cm^{-1} and 1021 cm^{-1} showed the hydroxyl and C–O stretches respectively. The band at 1652 showed the presence of alkene. The bands at 2920 cm^{-1} and 2873 cm^{-1} confirm for the existence of sp^3 hybridized C–H stretch and the weak band at 2233 cm^{-1} indicated acetylenic carbon stretch. Therefore, the IR suggested that lobetyolin contains triple bond, alcohol, alkenes and C–O. compared to result reported (Tadege *et al*., 2022).

4.4.2. Characterization of Compound 41(BL1)

Compound **41** was isolated from fraction F_{41}-F_{57} as white crystalline substance (20mg) from the methanol extract of stem bark of *L. rhynchopetalum*. The normal phase TLC analyses of the compound indicated a single spot with Rf value of 0.5 in a solvent system of n-hexane-ethylacetate with 1:1 (V/V) ratio as a developing solvent (Figure 12).

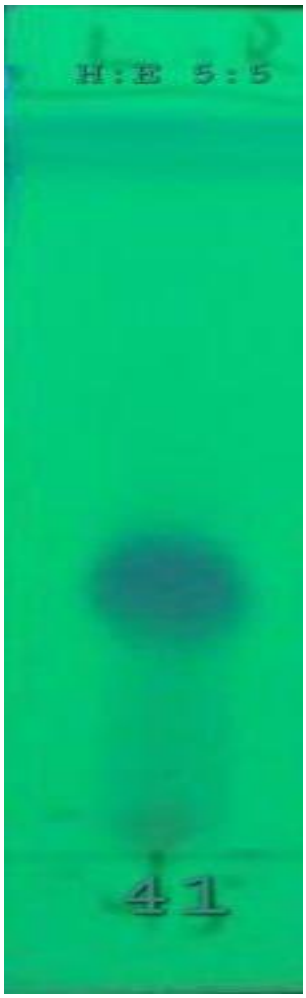


Figure 11: TLC profile of compound **41**

Structure elucidation of **41** was based mainly on its ^{1}H-NMR and its ^{13}C-NMR as well as its DEPT-135 NMR spectral data (Table 5) and comparison with compound **42** and literature. In its ^{1}H-NMR spectrum (Appendix 1.2.), the compound showed unique structural features that encompassed an olefinic proton singlet at δ6.60 whose ^{13}C signal was observed at an unusually up-field position of δ85 ppm (Appendix 1.4.) indicating this olefinic methine is in conjugation with diacetylenic system. Furthermore, the ^{1}H-NMR spectrums of **41** showed the presence of what appeared to be two fold methoxy protons singlet at δ3.75 (s, 5H). Further, the remaining signals are very much similar to the ^{1}H-NMR profile of **42**, such as the methyl protons signal at δ1.79 (dd, J = 6.9, 1.9 Hz, 3H) which were coupled to olefinic protons at δ6.38 (m) and 5.68(m), the anomeric proton signal at δ4.17 (d, J = 6.3 Hz, 2H) which also indicated that **41** is also a polyacetylenic glycoside.

The proton decoupled ^{13}C NMR spectrum of **41** ((Appendix 1.4.101 MHz, DMSO) also displayed a total of twenty two carbons signals between δ 148.35 and 19.11ppm. The multipilicities of these twenty two ^{13}C-peaks were deduced from the DEPT-135 spectrum of the compound which showed carbon signals of 17 carbons only indicating that five of the carbons in ^{13}C NMR were quaternary carbons and four appeared as negative peaks, two in the oxygenated methylenes region and two in aliphatic methylenes region in a similar manner to compound **42**. Thus, the ^{13}C-spectrum was comprised of one oxygenated olefinic quaternary carbon signal at δ 148.35, four olefinic tertiary methine carbons signals at δ 145.38, 132.70, 129.43, and 109.66, an anomeric sugar tertiary carbon signal at δ 104.02, an up-field olefinic tertiary methine carbon at δ 85.81, four conjugated acetylinic quaternary carbon signals at δ 83.73, 77.39, 72.43, and 69.43 ppm, two sugar tertiary methine peaks at δ 74.58, and 66.58, two oxygenated secondary methylene signals at δ 71.52 and 60.60, two oxygenated primary carbon signals (methoxy groups) at δ 56.45, 54.12, two aliphatic secondary carbon signals belonging to two aliphatic methylene groups at δ 32.57, and 28.80, and lastly an aliphatic primary carbon signal (an aliphatic methyl) at δ 19.11.

Based on these NMR spectral data and the close similarity of the spectra to that of **42** with some key diagnostic differences such as the olefinic singlet at δ 6.60, and its unusually up-field ^{13}C peak at δ 85.81, the presence of two methoxy signals in both of its ^{1}H and ^{13}C spectra and the oxygenated olefinic quaternary at δ 148.35 ppm, **41** was assigned a tentative polyacetylinic glycoside structure given under Figure 13. **41** represent a novel compound that is hitherto unreported from any source. However, complete elucidation of its structure requires further data, in particular, high resolution mass spectral and 2D-NMR data.

Table 5: ^{1}H NMR (DMSO) (400 MHz), ^{13}C and DEPT-135(100Mz, DMSO-d6) spectral data of compound **41**

C	2		
	δH(m, J in Hz)	**^{13}C NMR (δC in ppm)**	**DEPT-135 (δC in ppm)**
1	1.79(3H, dd,6.9, 1.9)	19.11	19.11
2	6.38(1H,m)	145.40	145.40

3	5.68(1H,m)	109.66	109.66
4	-	83.73	-
5	-	77.39	-
6	-	72.49	-
7	-	69.42	-
8	6.60(H,s)	85.80	85.80
9	-	148.5	-
10	5.47(H,dd,6.2,15.5)	129.42	129.42
11	5.75(1H,m)	132.70	132.70
12	2.06(2H,p,6.6)	28.80	28.80
13	1.49(4H,p,6.8)	32.57	32.57
14	3.88(2H,t,6.3)	71.52	71.52
15	3.75(5H,s)	54.12	54.12
1’	4.17(2H, d,6.3)	104.03	104.03
2’	3.05(1H,s)	74.58	74.58
3’	3.05(1H,s)	66.58	66.58
4’	3.05(1H,s)	71.52	71.52
5’	3.05(1H,s)	74.58	74.58
6’	3.88(2H,t,6.3)	60.59	60.59
7’	3.75(5H,s)	56.45	56.45

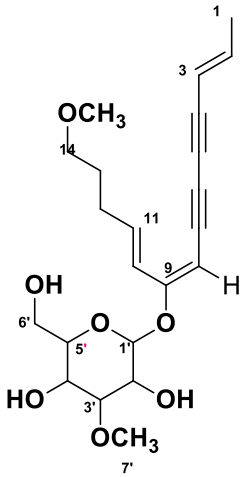


Figure 12: Proposed Structure of compound **41**

4.3. Anti-bacterial Activity

The antibacterial activity of the extracts and isolated compounds of stem bark of *L. rhynchopetalum* were investigated against two Gram positive *(S. aureus* and *S. pyogenes*) and Gram negative bacteria (*E.coli* and *P. aeruginosa*) using Agar disc diffusion methods. In the method employed, the susceptibility of the bacteria to the test extract or test compound is directly related to the diameter of the inhibition zone, the larger the diameter, the better. As a general guide, inhibition zone of less than 9 mm is considered as inactive, 9-12 mm intermediate activity, 13-18 mm active and greater than18 mm very active (Nigussie & Ashenef, 2020). The susceptibility of each test bacteria was evaluated against the isolated compounds **41**, **42** and the crude methanol extract in a concentration dependent manner at four different concentrations measured in μg/mL (25**,** 50, 100 and 200). Ciprofloxacin, a second generation broad spectrum fluoroquinolone antibiotic, was used as a positive control. The measured zone of inhibition values for the test samples displayed that susceptibility was a function of concentration; i.e. increased susceptibility were seen where concentrations are increased (Table 6).

Table 6 describes measurable zone of inhibition against all the tested bacterial strains. The susceptibility of the test bacteria to methanol crude extract and isolated compounds was significant, even at the smallest concentration used (25 μg/ml) except for the methanol extract against *E. coli*, which appears to be non-susceptible at all concentrations. In particular, all test organisms displayed remarkable susceptibility against compound **42** at all concentrations, but especially at concentration of 100 μg/ml, with observed zone of inhibitions of 21.33±0.57, 19±1, 21.67±0.57, 19.33 ±0.57 against *E. coli, P. aeruginosa, S.aureus* and *S.pyogenes* respectively. These susceptibility is closely comparable that of the reference antibiotic, ciprofloxacin. This indicates that compound **42** can become a lead compound for antibacterial drug development.

Table 6: Anti-bacterial activities of extracts and isolated compounds from the stem bark of *Lobelia rhynchopetalum*

Measurable zone of inhibition (MZI) of compounds and MeOH in mm

Compound and sample standard	Concentrati-on(μ/ml)	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pyogenes*
41(BL1)	100	15.33±0.57	16.67±0.57	15.33±0.57	18 ±1
	50	13.67±0.58	15.33±0.57	13.33±0.57	15±1
	25	10.33±0.57	12.33±0.57	12.33±0.57	12.7±0.57
	PC	23.67±0.57	27.67±0.57	28.33±0.7	29.33±1.16
42(BL2)	100	21.33±0.57	19±1	21.67±0.57	19.33 ±0.57
	50	18.33±0.57	19±1	19.67±0.57	19±1
	25	15.67±0.57	16±1.73	19±0.57	17±1
	PC	25.33±0.57	29±1	29.33±0.57	30±1
MeOH	200	8.67±0.57	15.67±0.57	16±1	17.33±0.57
	100	7.66±0.57	14.33±0.57	15.33±0.57	15±1
	50	0	13±1	14.66±0.57	13
	PC	27±1	28±1	28±1	28±1

The experiments were done in triplicates and results were presented as mean ±SD

Overall, this *in-vitro* bacterial susceptibility assay against crude extracts and isolated compounds of the stem bark of *L. rhynchopetalum* showed that the plant does contain phytochemicals with inhibitory effects on the tested bacteria. It is notable that these phytochemicals display inhibitory effects particularly against *Pseudomonas aeruginosa* bacteria, one of the difficult to treat organisms, in the health care settings.

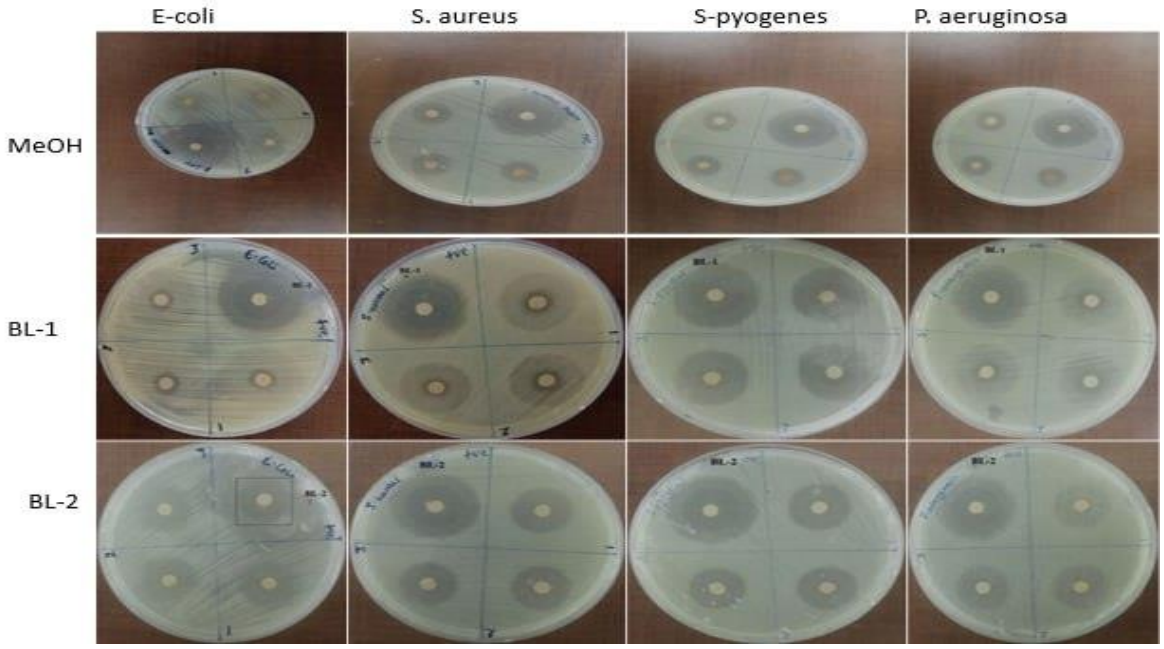


Figure 13: Inhibition zones of *L. rhynchopetalum* stem barks crude extract and compounds against bacterial strain in different concentration

4.4. Antioxidant Activity

In the present study, stem bark extracts of *L. rhynchopetalum* in solvent methanol and two isolated compounds of methanol extracts were tested for their free radical scavenging ability by using DPPH assay and it was observed that the plant extract and compounds high potency for scavenging free radicals as presented in Table 7.

Table 7: Percentage scavenging activity of the extract and isolated compounds

Concentration	**41**	**42**	Crude MeOH extract	Ascorbic Acid
25	35	37	38	45
50	68	73	55	62
100	71	84	72	75
200	85	93	81	94
IC_{50}	26.52	7.82	43.22	20.35

The extract and the compounds were tested on a concentration ranges (25-200 µg/mL) and it was found that the test samples not only displayed antioxidant activities, but also exhibited concentration dependent increase in activity. The IC_{50} value for each test sample was calculated to determine the concentration of the sample required to inhibit 50% of the radical. From among all samples analyzed, compound **42** demonstrated the highest antioxidant activity. The observed IC_{50} value of compound **42** exhibited promising antioxidant activity (93%, IC_{50} value of 7.82µg/ml). It is known that the lower the IC_{50} value, the higher the antioxidant activity of substances. Thus, according to the data, the isolated compound **42** (7.82µg/ml) was a more potent scavenger of free radicals than not only the crude methanol and compound **41**, but also the reference compound, ascorbic acid (IC_{50} = 20.35 µg/ml). The graphs were plotted against percent inhibition vs. concentration of stem bark extracts, compounds and standard drug ascorbic acid (Figure 15). IC_{50} value (the amount of antioxidant required to decrease the initial DPPH concentration by 50%). IC_{50} value for each extract, compounds and ascorbic acid was calculated automatically using graph pad prim version 8

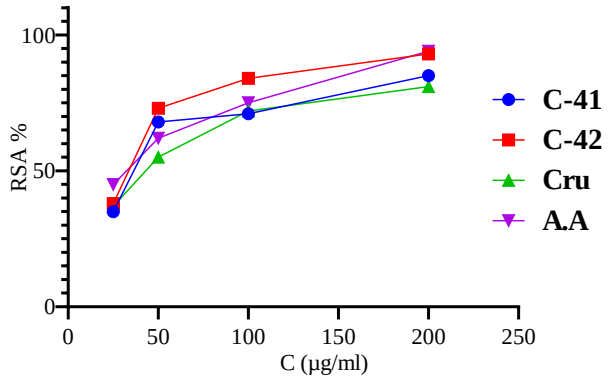


Figure 14: Antioxidant activity of extracts and isolated compounds from stem bark of *Lobelia rhynchopetalum*

4.5. Molecular Docking Studies

The currently advanced understanding of structural details of drug target proteins and protein-ligand complexes has allowed computational strategies to permeate all aspects of drug discovery today. Therefore, virtual screening techniques, such as molecular docking studies for hit identification and methods for lead optimization are becoming increasingly important tools in rational drug discovery. Compared with traditional experimental high-throughput screening (HTS), virtual screening is a more direct and rational drug discovery approach and has the advantage of low cost and effective screening.

The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level, which allow us to characterize the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes. The docking process involves two basic steps: prediction of the ligand conformation as well as its position and orientation within these sites (usually referred to as *pose*) and assessment of the binding affinity.

With this in-mind, it deemed necessary to carry out *in-silico* studies of the isolated compounds, **41** and **42**, to predict their orientation and binding affinities at the active sites of selected protein targets. For this purpose, four protein targets, whose structures are well known, were selected; *viz*. bacterial enzymes *E. coli* DNA gyrase B (PDB ID: 7P2M), and *Pseudomonas* quinolone signal A (PqsA, PDB ID: 5OE4) and cancer related enzymes pyruvate kinase M2 (PKM2; PDB

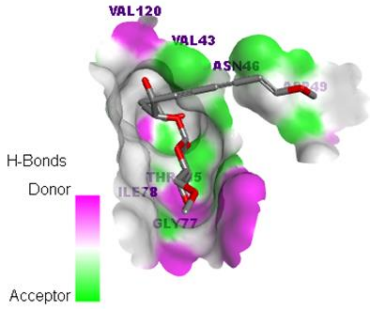
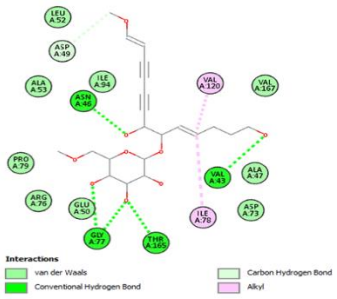
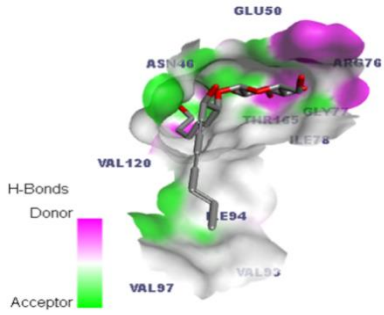
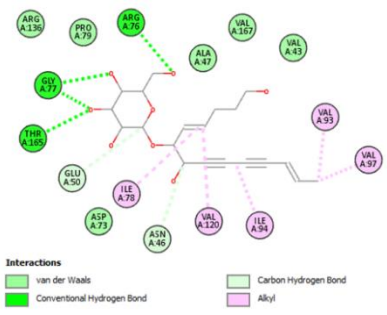
ID: 4G1N), and human topoisomerase IIβ (PDB ID: 3QX3).The active (binding) sites were then located, and using AutoDock Vina along with auxiliary programs, docking simulations were performed for each compound, **41** and **42**. By default, nine conformers were used for each run. A conformer with the least binding energy was taken as showing the best docking score and displayed in 2D and 3D models from which the interaction mechanism of each isolated compound was examined. The antibacterial agent ciprofloxacin, the anticancer agent etoposide (a topoisomerase II inhibitor) and the anticancer agent PKM2-IN-1 (a pyruvate kinase M2 inhibitor) were used as positive controls in simulations for comparison.

The binding affinity, and the intermolecular binding forces involved between the binding groups on each compound and the binding regions in the active (binding) sites of the targets are presented in Table 8 along with those of the reference drugs. Four major intermolecular binding forces are involved: H-bonding, van der Waals interactions, hydrophobic interactions and electrostatic forces. Both compounds, **41** (BE = -6.6 kcal/mol) and **42** (BE = -6.8 kcal/mol), exhibited remarkable binding affinities towards the DNA gyrase, comparable to that of ciprofloxacin (BE = -7.4 kcal/mol), although **42** demonstrated a slightly higher affinity than **41**. Corresponding to the complete structural differences between ciprofloxacin, a fluoroquinolone, and the isolated compounds, polyacetylenic glycosides, there is a large difference in the number of hydrogen bonding interactions between the ligands and the target binding sites. While each of the isolated compounds has shown four hydrogen bonding interactions, ciprofloxacin has displayed only one H-bonding interactions with the DNA gyrase. Nonetheless, the number of hydrophobic and van der Waals interactions between each of the three ligands and the active site of the target enzyme appear to be comparable.

Table 8: Binding affinity and interaction with the target of isolated compound against *E. coli* DNA Gyrase

Compound	Binding Affinity (kcal/mol)	H-bonding	Hydrophobic and Electrostatic	Van der Waals

41	-6.6	Asn-46, Gly-77, Gly-77, Thr-165,	Carbon hydrogen bond: Asp-49; Alkyl: Ile-78, Val-120	Leu-52, Ile-94, Ala-53, Pro-79, Arg-76, Glu-50, Asp-73, Ala-47,
42	-6.8	Thr-165, Gly-77, Gly-77, Arg-76	Carbon hydrogen bond: Glu-50, Asn-46; Alkyl: Ile-78, Ile-94, Val-93, Val-97, Val-120	Arg-136, Pro-79, Ala-47, Val-167, Val-43, Asp-73
Ciprofloxacin	-7.4	Arg-76	Carbon hydrogen bond: Asp-73; Halogen: Asp-73; Amide pi-stacked: Gly-77; Alkyl and pi-alkyl:Pro-79, Ile-	Arg-136, Glu-50, Thr-165, Val-120, 167, 43, Asn-46, Ala-47

3D	**2D**
	
	

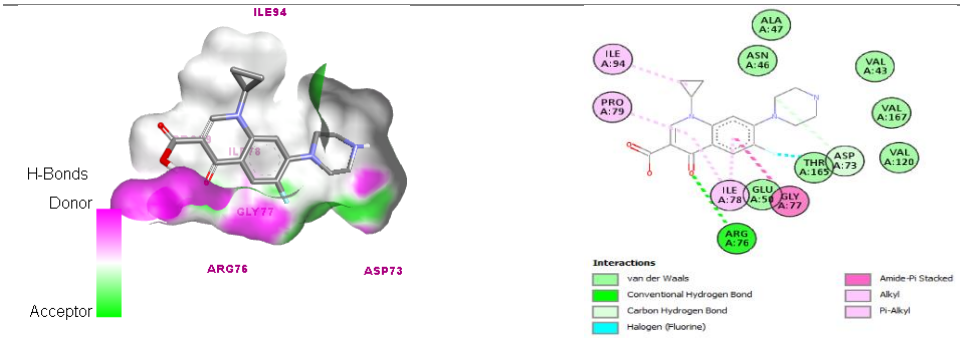


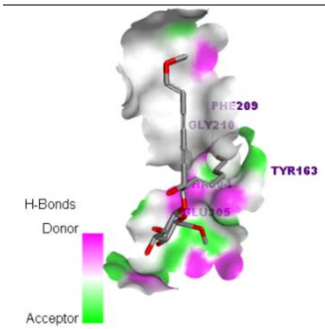
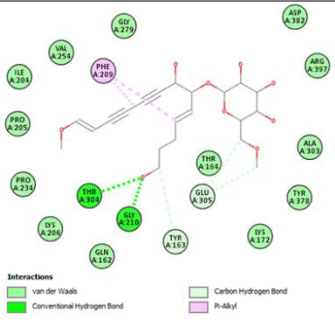
Figure 15: The 3D and 2D binding interactions of compound **41**, **42**, and ciprofloxacin against *E. coli* DNA gyrase B

In a similar approach, *Pseudomonas* quinolone signal A (PqsA, PDB ID: 5OE4) was used as a target and ciprofloxacin was used as reference control in another *in-silico* antibacterial screening test of the isolated compounds, **41** and **42**. The binding affinity, H-bonding, hydrophobic and electrostatic interactions of compounds **41** and **42** along with ciprofloxacin are presented in Table 9. The result showed a strong binding of **42** (BE = -7.3 kcal/mol) to PqsA, which is even better than that of ciprofloxacin (BE = -7.1 kcal/mol). Compound **41** also displayed significant binding affinity for the target molecule, PqsA (BE = -6.4 kcal/mol) (Table 9). The isolated compound **42** and ciprofloxacin have shown similar amino acids binding interactions with Arg-397, (hydrogen bond). Compound **42** showed the highest binding affinity score (-7.3 kcal/mol). Therefore this *in silico* pharmacodynamics prediction of isolated compounds support in vitro data of this study, in particular compound **42** have a promising antibacterial activity against PqsA with binding affinities of (-7.3kcal/mol). Binding affinity and interactions with different amino acids are presented in the (Table 9) and 3D and 2D binding interactions is shows in (Figure 17).

Table 9: Binding affinity and interaction with the target of isolated compound against PqsA

Compound	Binding (kcal/mol)	H-bonding	Hydrophobic and Electrostatic	Van der Waals

41	- 6.4	Thr-304, Gly-210	Carbon hydrogen bond: Tyr-163, Glu-305, Glu-305; Pi-alkyl: Phe-209, Phe-209	Gly-279,Val 254, Ile-204, Pro-205, Pro-34, Lys-206, Gln-162,Thr164, Lys-172,Tyr 378, Ala-303,Arg397, Asp-382
42	-7.3	Gly-279, Gly-302, Thr-164, Tyr-378, Arg-372, Arg-397, Thr-380,	Pi-alkyl: Phe-209, Phe-209	Val-254, Ile-301, Ala-278, Tyr211, Lys-172, Glu305 Ala-303,
Ciprofloxacin	-7.1	Arg-397, Gly-302, Asp-299	Carbon hydrogen bond: Gly-300, Thr-323; Halogen: Gly-302; Pi-cation and anion:Arg-397, Asp-382; Amide pi-stacked: Gly-279; Alkyl and Pi-alkyl: Ile-301, His-394, Arg-397	Ala-278, Leu282 , Phe-384, Pro-281, Ser-280

3D	**2D**
	

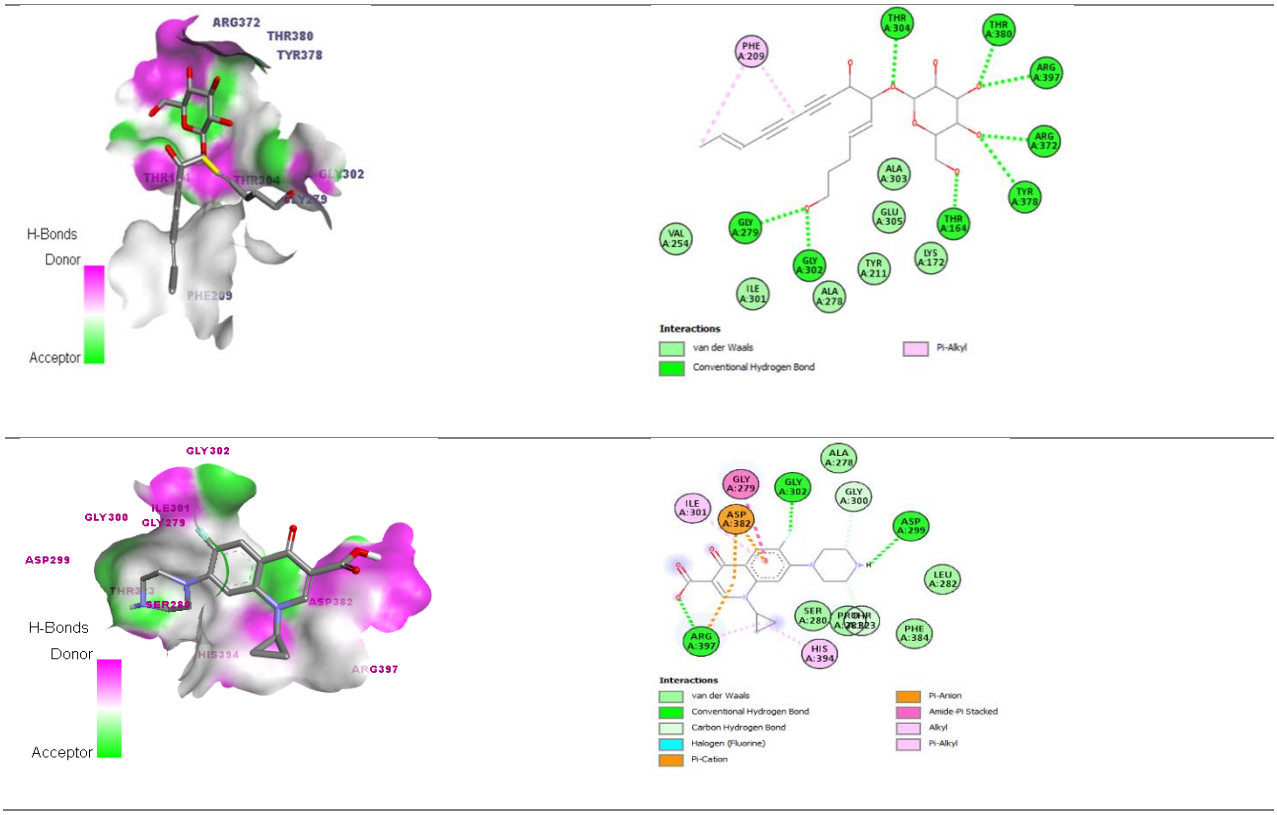


Figure 16: The 3D and 2D binding interactions of compound **41**, **42**, and ciprofloxacin against PqsA.

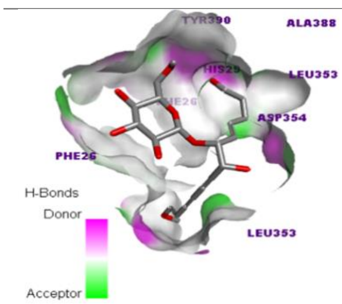
In this study PKM2 was used as a target and PKM2-IN-1 used as reference control. The binding affinity, H-bond, hydrophobic and electrostatic of compounds **41** and **42** along with PKM2-IN-1 are described in (Table 10). The result showed a binding of PKM2 of isolated compounds (**41**and **42**) were found to have minimum binding–8.0 and–8.4 kcal/mol, respectively (Table 10).The isolated compound **42** amino acids binding interactions with Tyr-390(hydrogen bond). Compound **42** showed the highest binding affinity score (8.4kcal/mol). Compound **42** lesser binding affinity than standard.

Table 10: Binding affinity and interaction with the target of isolated compound against PKM2

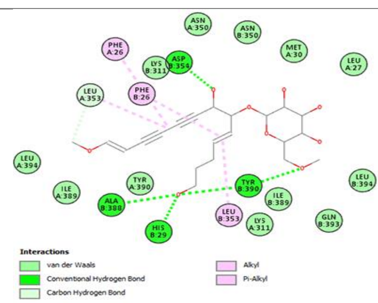
Compound	Binding (kcal/mol)	H-bonding	Hydrophobic and Electrostatic	Van der Waals

41	-8.0	Ala-388, His-29, Tyr-390, Asp-354	Carbon hydrogen bond: Leu-353; Alkyl and Pi-alkyl: Leu-353, Phe-26, Phe-26, Leu-353	Leu-394, Ile-389, Tyr-390, Lys-311, Ile-389, Gln-393, Leu-394, Leu-27, Met-30, Asn-350, Asn-350, Lys-311
42	-8.4	Tyr-390	Carbon hydrogen bond: Leu-353; Alkyl and Pi-alkyl: Leu-394, Ile-389, Phe-26, Leu-353, Phe-26, Leu-353	Tyr-390, Lys-311, Asp-354, Ile-389, Ala-388, Met-30, Leu-27, Leu-394, Glu-397, Gln-397
PKM2-IN-1	-9.6	Lys-311	Pi-sigma: Leu-353; Pi-sulfur: Phe-26; Pi-pi T-shaped: Phe-26; Alkyl and Pi-alkyl: Leu-353, 353, Phe-26	Asp-354, 354,Lys-311, Tyr-390, 390, Ala-388, 388, Ile-389, 389, Met-30, His-29

3D



2D



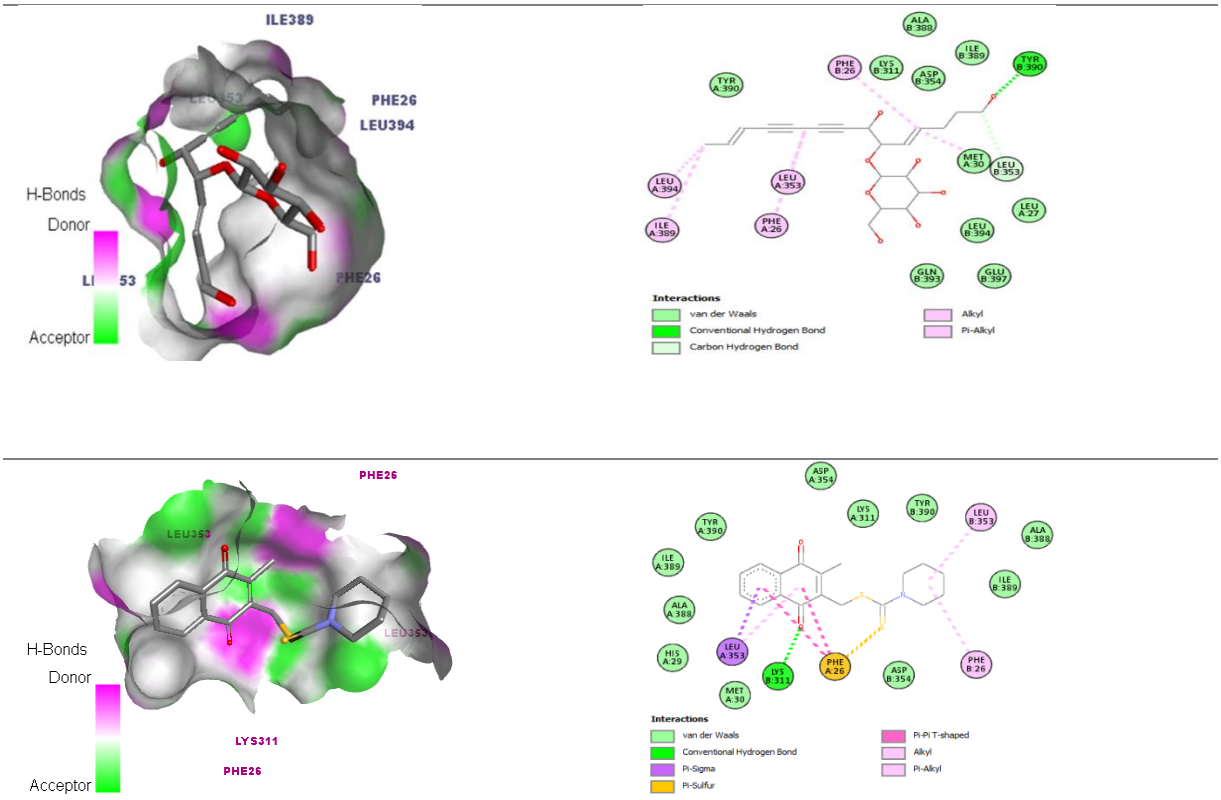


Figure 17 : The 3D and 2D binding interactions of compound **41**, **42** and PKM2-IN-1 against PKM2

In this study topoisomerase II (TOP2) enzyme is a nuclear enzyme that plays crucial functions during DNA processes such as, replication, transcription, and repair via catalysis of the relaxation and unwinding of double-stranded DNA (Atkin *et al*., 2019). Recent studies showed that TOP2β is an important target for many anticancer agents including etoposide (EVP) (Assefa *et al*., 2023). In the present study human TOP2β (PDB ID: 3QX3) is used as a target and EVP is used as a reference control. Binding affinity and interactions with different amino acids are presented in the Table 11, 3D and 2D binding interactions is depicted in Figure 19. Among the isolated compounds, **42** showed highest binding affinity (-3.9 kcal/mol), whereas standard EVP exhibited -5.3kcal/mol. EVP showed H-bonding interactions with showed similar interaction with Gln-778 these amino acids standard. Compound **41**showed lesser binding affinities compared to other compounds and standard.

Table 11: Binding affinity and interaction with the target of isolated compound Pyruvate kinase M2 (PKM2) and human topoisomerase II*β*

Compound	Binding	H-bonding	Hydrophobic and Electrostatic	Vaander Waals
41	-3.7	Arg-503, Lys-505, Gly-776, Ala-778	Carbon hydrogen bond: Arg-503	Gly-504, His-775, Gln-778
42	-3.9	Lys-505, Gly-776, Gln-778	Carbon hydrogen bond: Gly-776; Alkyl: Arg-503	Gly-504, Asp-561, His-775
Etoposide	-5.3	Arg-503, Gln-778	Alkyl and Pi-Alkyl: Ala-779	Gly-776

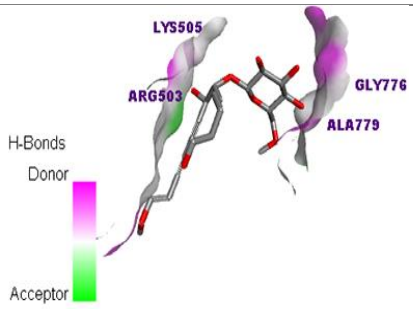
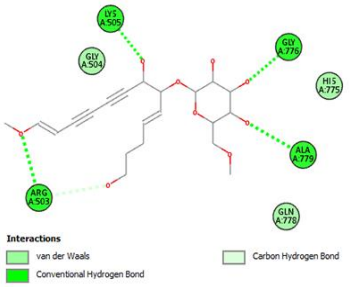
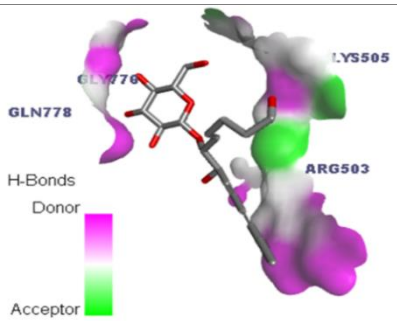
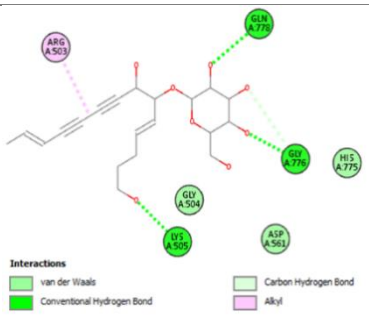
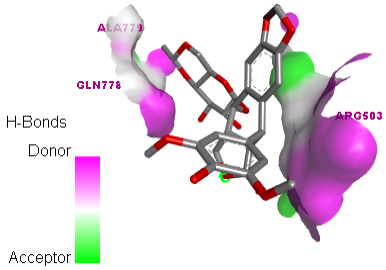
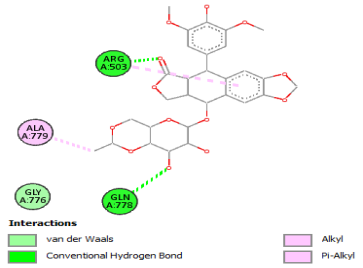
3D	**2D**
	
	
	

Figure 18: The 3D and 2D binding interactions compound **41**, **42**, and etoposide against human topoisomerase II*β*

4.6. *In silico* Pharmacokinetics and Toxicity Study of the Isolated Compounds

Pharmacodynamics is the study of how a drug binds to its target binding site and produces a pharmacological effect. However, a drug capable of binding to a particular target *in-vitro* is not necessarily going to be useful as a clinical agent or medicine. For that to be the case the drug not only has to bind to its target, it has to reach it in the first place. For an orally administered drug, that involves a long journey with many hazards to be overcome. The drug has to survive stomach acids then digestive enzymes in the intestine. It has to be absorbed from the gut into the blood supply and then it has to survive the liver where enzymes try to destroy it (drug metabolism). It has to be distributed round the body and not get mopped up by fat tissue. It should not be excreted too rapidly or else frequent doses will be required to maintain activity. However, it should not be excreted too slowly or its effects could linger on longer than required. The study of how a drug is absorbed, distributed, metabolized, and excreted (known as ADME in the pharmaceutical industry) is called pharmacokinetics. Thus, optimum ADME is one of the critical requirements for a lead to become a clinically useful drug. Thus, understanding the ADME of lead compounds from the on-set of drug discovery and development process prevents a lot of unnecessary wastage of money and time on leads that may not end-up becoming useful clinical drugs. Fortunately for the medicinal chemist, there are online resources such as the SwissADME online tool to estimate the physicochemical properties and drug-likeness (Lipinski's rule of five) of potential leads even before they are synthesized. In addition, there are online resources such as Pro Tox 3.0 online tool to estimate oral acute toxicity, organ toxicity and toxicity endpoints of potential leads without even conducting any animal toxicity studies.

As such, pharmacokinetic properties (ADME), physicochemical properties and drug-likeness of compounds **41** and **42** were determined using the Swiss ADME Web tool. Drug likeness is a prediction that screens whether a particular organic molecule has optimum properties for becoming an orally active drug. The drug-likeness of the isolated compounds was characterized according to "Lipinski's rule." According to Lipinski rule, the potential molecules should have the following physicochemical properties such as, (i) not more than 5 hydrogen bond donors; (ii) not more than 10 hydrogen bond acceptors; (iii) a molecular mass less than or equal to 500 Da; (iv) an octanol-water partition coefficient (log P) not greater than 5 (Lipinski *et al.*,1997). In the

present investigation, both compound **41** and **42** complied with Lipinski's rule of five and are likely to be orally active. Low molecular weight (MW) indicates that the molecules are light and can easily pass through the cell membrane and preferred for oral (Mathias & Hussain, 2010). The log P of the isolated compounds **41** and **42** were found to be less than zero. A negative log P value indicates a compound's higher affinity for the aqueous phase (Braeken *et al*., 2005). Therefore, both Compounds **41** and **42** have been shown to have good pharmacokinetics and bioavailability characteristics.

Table 12: Swiss ADME online drug-likeness predictions for isolated compounds and positive controls

Compound	Molecular Formula	MW/g/mol	NRB	NHA	NHD	TPSA (A^{o2})	Log P (MLOGP ≤ 4.15)	Lipinski rule of 5 violated
41	$C_{21}H_{30}O_9$	426.5	10	9	5	138.1	-1.47	0
42	$C_{20}H_{28}O_8$	396.4	8	8	6	139.8	-0.92	1
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	331.3	3	5	2	74.6	1.28	0
PKM2-IN-1	$C_{18}H_{19}NO_2S_2$	396.4	8	8	6	139.8	-0.92	1
Etoposide	$C_{29}H_{32}O_{13}$	345.5	4	2	-	94.8	2.05	0

Note. MW: molecular weight; NRB: number of rotatable bonds; NHA: number of hydrogen Acceptors; NHD: number of hydrogen donors; TPSA: total polar surface area.

The organ toxicity (hepatotoxicity) and toxicological endpoints (carcinogenicity, immune toxicity, mutagenicity and cytotoxicity) of the isolated compounds and their toxicity class and LD_{50} was predicted using Pro Tox II server (Table 13). Toxicological endpoints prediction analysis indicated median lethal dose (LD_{50}) values ranging from 100 mg/kg. Both compounds were predicted not to have mutagenicity and immunotoxicity. However, both compounds were predicted to show neurotoxicity and cardiotoxicity, which is not unexpected because no drug is totally safe and it is only the dose level of a drug that makes it safe or toxic. Therefore, all compounds have good profile of toxicity effect apart from that; they demonstrated neurotoxicity and cardiotoxicity effect if taken in higher doses. These data also confirmed by large value of LD_{50}, which refers to an estimate of the amount of poison that, under control conditions, will be a lethal dose to 50% of test animals of a particular species. Thus, for a healthy average adult of

70 kg, to die of toxicity of **41** or **42**, he must ingest 7000 mg (7 g) of any of the compounds, which is normally unlikely.

Table 13: Toxicity predictions of isolated compounds and standards calculated

Compound	LD_{50} (mg/Kg)	Toxicity class	Organ toxicity predicted	Toxicological endpoints predicted
41	100	3	Neurotoxicity and cardiotoxicity	-
42	100	3	Neurotoxicity and cardiotoxicity	-
Ciprofloxacin	2000	4	Neurotoxicity, nephrotoxicity, and respiratory toxicity	Mutagenicity and clinical toxicity
PKM2-IN-1	500	4	Respiratory toxicity	BBB-barrier
Etoposide	215	3	Respiratory toxicity and	Immunotoxicity

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

This research endeavor of discovering bioactive compounds from the endemic medicinal plant, *Lobelia rhynchopetalum* Hemsl stem bark has resulted in the successful identification of two pharmacologically important natural products, **41** and **42** .The extraction of *L .rhynchopetalum* dried and powdered stem bark with methanol resulted in a crude extracts with the yield of 20% w/w; whose preliminary phytochemical screening tests resulted in the identification of alkaloids, terpenoids, flavonoids, phenolics and tannins as phytoconstituents in this species. Silica gel column chromatographic analyses of the methanol extract furnished two compounds and the NMR structure elucidation of the isolated compound has led to the proposed structures, which are labeled as compound **41** and lobetyolin (**42**). Compound **41** has hitherto not been reported and is a novel structure and **42** is also discovered from this plant for the first time. Both the extracts and isolated compounds of the stem bark of *Lobelia rhynchopetalum* had promising antibacterial activity against Gram positive bacteria (*S. aureus*, *S. pyogenes*) and Gram negative (*E.coli*, *P. aeruginosa*). The compound **42** had better DPPH radical scavenging activity than both the methanol extract and compound **41** from the stem bark of *L.rhynchopetalum.* Compound **42** have a promising antibacterial activity against PqsA with binding affinities of (-7.3kcal/mol). Both compound **41** and **42** complied with Lipinski's rule of five and are likely to be orally active. Therefore, both Compounds **41** and **42** have been shown to have good pharmacokinetics and bioavailability characteristics.

According to medchemexpress website (https://www.medchemexpress.com/Lobetyolin.html), Compound **42**, lobetyolin, is a bioactive compound, which has proven anti-inflammatory, anti-oxidative and xanthine oxidase inhibiting activities. Lobetyolin also induces the apoptosis via the inhibition of ASCT2-mediated glutamine metabolism. Lobetyolin is a click_chemistry reagent; it contains an alkyne group and can undergo copper-catalyzed azide-alkyne cycloaddition (CuAAc) with molecules containing azide groups. It is most likely that compound **41** also share similar bioactivities as **42** since the two compounds are structural analogues of each other.

5.2. Recommendation

In this study only NMR spectrum (1D) and literature data was used for structure elucidation of isolated compounds, but the first compound is not reported before and its structure elucidation

requires more data, so it is recommend that 2D NMR and mass spectrometry for further confirmation of the structure of compound **41**.The results of these study supports the traditional use of the *Lobelia rhynchopetalum* stem bark for disease caused by pathogenic bacteria both Gram negative and Gram positive, since the result of invitro and insilico studies against bacteria are promisingly collaborated. But the traditional healer uses the plant for wide array of disease including rheumatism, muscle strains, heart failure and etc. this work recommend further activity test like anti-cancer, anti-viral and anti-inflammatory etc.

REFERENCES

Alamgir, A., & Alamgir, A. (2017). Herbal drugs: their collection, preservation, and preparation; evaluation, quality control, and standardization of herbal drugs. *Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1: Pharmacognosy*, 453-495.

Alamgir, A., & Alamgir, A. (2018). Secondary metabolites: Secondary metabolic products consisting of C and H; C, H, and O; N, S, and P elements; and O/N heterocycles. *Therapeutic Use of Medicinal Plants and their Extracts: Volume 2: Phytochemistry and Bioactive Compounds*, 165-309.

Aljeldah, M. M. (2022). Antimicrobial resistance and its spread is a global threat. *Antibiotics, 11*(8), 1082.

Assefa, T., Tesso, H., Ramachandran, V. P., Guta, L., Demissie, T. B., Ombito, J. O., . . . Melaku, Y. (2023). In Silico Molecular Docking Analysis, Cytotoxicity, and Antibacterial Activities of Constituents of Fruits of *Cucumis dipsaceus*. *ACS Omega, 9*(1), 1945-1955.

Atkin, N. D., Raimer, H. M., & Wang, Y.-H. (2019). Broken by the Cut: A Journey into the Role of Topoisomerase II in DNA Fragility. *Genes, 10*(10), 791.

Ayalew, H., Tewelde, E., Abebe, B., Alebachew, Y., & Tadesse, S. (2022). Endemic medicinal plants of Ethiopia: Ethnomedicinal uses, biological activities and chemical constituents. *Journal of Ethnopharmacology, 293*, 115307.

Barnes, P. (2012). Ornamentals *The Cultural History of Plants* (pp. 261-286): Routledge.

Bekoe, E. O., Kitcher, C., Gyima, N. A. M., Schwinger, G., & Frempong, M. (2018). Medicinal plants used as galactagogues. *Pharmacognosy-Medicinal Plants*.

Berhanu, S. (2017). Phytochemical Analysis and Evaluation for.

Bhat, V., & Chatterjee, J. (2021). The use of in silico tools for the toxicity prediction of potential inhibitors of SARS-CoV-2. *Alternatives to Laboratory Animals, 49*(1-2), 22-32.

Bittner, E. A., & Martyn, J. J. (2019). Neuromuscular physiology and pharmacology *Pharmacology and Physiology for Anesthesia* (pp. 412-427): Elsevier.

Borokini, T. I., & Omotayo, F. O. (2012). Phytochemical and ethnobotanical study of some selected medicinal plants from Nigeria. *Journal of medicinal plants research, 6*(7), 1106-1118.

Braeken, L., Ramaekers, R., Zhang, Y., Maes, G., Van der Bruggen, B., & Vandecasteele, C. (2005). Influence of hydrophobicity on retention in nanofiltration of aqueous solutions containing organic compounds. *Journal of Membrane Science, 252*(1-2), 195-203.

Brown, D. P., Rogers, D. T., Pomerleau, F., Siripurapu, K. B., Kulshrestha, M., Gerhardt, G. A., & Littleton, J. M. (2016). Novel multifunctional pharmacology of lobinaline, the major alkaloid from *Lobelia cardinalis*. *Fitoterapia, 111*, 109-123.

Bussmann, R. W., Paniagua-Zambrana, N. Y., & Njoroge, G. N. (2021). *Ethnobotany of Mountain Regions: Africa*: Springer.

Capson-Tojo, G., Moscoviz, R., Astals, S., Robles, Á., & Steyer, J.-P. (2020). Unraveling the literature chaos around free ammonia inhibition in anaerobic digestion. *Renewable and Sustainable Energy Reviews, 117*, 109487.

Chala, D., Brochmann, C., Psomas, A., Ehrich, D., Gizaw, A., Masao, C. A., ... & Zimmermann, N. E. (2016). Good-bye to tropical alpine plant giants under warmer climates? Loss of range and genetic diversity in *Lobelia rhynchopetalum*. *Ecology and evolution*, *6*(24), 8931-8941.

Christian Bailly, Nat Prod Bioprospect. 2021 Apr;11(2):143-153.

Clemants, S. E., & Gracie, C. (2006). *Wildflowers in the field and forest: a field guide to the northeastern United States*: Oxford University Press.

Crowl, A. A., Miles, N. W., Visger, C. J., Hansen, K., Ayers, T., Haberle, R., & Cellinese, N. (2016). A global perspective on *Campanulaceae*: Biogeographic, genomic, and floral evolution. *American journal of botany, 103*(2), 233-245.

Cseke, L. J., Kirakosyan, A., Kaufman, P. B., Warber, S., Duke, J. A., & Brielmann, H. L. (2016). *Natural products from plants*: CRC press.

David, B., Wolfender, J.-L., & Dias, D. A. (2015). The pharmaceutical industry and natural products: historical status and new trends. *Phytochemistry Reviews, 14*, 299-315.

Degfie, T., Endale, M., Tafese, T., Dekebo, A., & Shenkute, K. (2022). In vitro antibacterial, antioxidant activities, molecular docking, and ADMET analysis of phytochemicals from roots of Hydnora johannis. *Applied Biological Chemistry, 65*(1), 76.

Dhruv, P., Bavalatti, N., Rajagopala, M., & Kundal, P. (2019). A Review on Pharmacognostic and Physiochemical Study of *Lobelia Nicotianaefolia*-a folk Herb.

Dillard, C. J., & German, J. B. (2000). Phytochemicals: nutraceuticals and human health. *Journal of the Science of Food and Agriculture, 80*(12), 1744-1756.

Duguma, T., Melaku, Y., Rentsch, D., Ensermu, U., Endale, M., Dekebo, A., & Guta, L. (2023). In vitro Antibacterial Activities, DPPH Radical Scavenging, and Molecular Modeling of Isolated Compounds from the Bark of Grewia ferruginea.

Duke, J. (2012). *Handbook of legumes of world economic importance*: Springer Science & Business Media.

Dwoskin, L. P., & Crooks, P. A. (2002). A no0vel mechanism of action and potential use for lobeline as a treatment for psychostimulant abuse. *Biochemical pharmacology, 63*(2), 89-98.

Folquitto, D. G., Swiech, J. N., Pereira, C. B., Bobek, V. B., Possagno, G. C. H., Farago, P. V., . . . Miguel, O. G. (2019). Biological activity, phytochemistry and traditional uses of genus *Lobelia* (*Campanulaceae*): A systematic review. *Fitoterapia, 134*, 23-38.

Frick, W. F., Kingston, T., & Flanders, J. (2020). A review of the major threats and challenges to global bat conservation. *Annals of the new York Academy of Sciences, 1469*(1), 5-25.

Geleta, Mulatu; Thomas (2012). "Population genetic analysis of *Lobelia rhynchopetalum* Hemsl. (*Campanulaceae*) using DNA sequences from ITS and eight chloroplast DNA regions". The Scientific World Journal. 2012*:* 276451.

Henkel, A. (1904). *Weeds used in medicine*: US Government Printing Office.

Hosler, D. M. E. G. (1989). *Studies of medicinal plant use by residents of Catron County, New Mexico.* in partial fulfillment of the requirements for the degree, Master of Basic ….

Husen, A. (2023). *Exploring Poisonous Plants: Medicinal Values, Toxicity Responses, and Therapeutic Uses*: CRC Press.

Hussein, R. A., & El-Anssary, A. A. (2019). Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. *Herbal medicine, 1*(3), 11-30.

Johnson, D. (2018). *Ethnobotony, the leaves of life*: Lulu. com.

Joshi, S., Mishra, D., Bisht, G., & Khetwal, K. S. (2011). Essential oil composition and antimicrobial activity of *Lobelia pyramidalis* Wall. *EXCLI journal, 10*, 274.

Kebede, T., Gadisa, E., & Tufa, A. (2021). Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. *PloS one, 16*(3), e0249253.

Khan, M. S. A., & Ahmad, I. (2019). Herbal medicine: current trends and future prospects *New look to phytomedicine* (pp. 3-13): Elsevier.

Kichu, M., Malewska, T., Akter, K., Imchen, I., Harrington, D., Kohen, J., . . . Jamie, J. F. (2015). ScienceDirect database. *Journal of Ethnopharmacology, 166*, 5-17.

Kolap, R. M., Datkhile, K. D., & Zimare, S. B. (2021). Bioprospecting of *Lobelia nicotianifolia* Roth. plant parts for antioxidant and cytotoxic activity and its phytoconstituents. *Pharmacognosy Magazine, 17*(06).

Lammers, T. (2007). *Campanulaceae*: *Campanulaceae* Jussieu, Gen. Pl. 163 (1789), nom. cons *Flowering Plants· Eudicots: Asterales* (pp. 26-56): Springer.

Lans, C. (2007). Comparison of plants used for skin and stomach problems in Trinidad and Tobago with Asian ethnomedicine. *Journal of Ethnobiology and Ethnomedicine, 3*, 1-12.

Lattanzi, E., Del Vico, E., Tranquilli, R., Farris, E., Marignani, M., & Rosati, L. (2021). An unknown hotspot of plant diversity in the heart of the Central Apennine: flora and vegetation outline of Mt. Pozzoni-St. Rufo valley (Cittareale, Rieti). *PhytoKeys, 178*, 111.

Li, K.-C., Ho, Y.-L., Huang, G.-J., & Chang, Y.-S. (2015). Anti-oxidative and anti-inflammatory effects of *Lobelia chinensis* in vitro and in vivo. *The American journal of Chinese medicine, 43*(02), 269-287.

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced drug delivery reviews, 23*(1-3), 3-25.

Liveri, E., Crowl, A. A., & Cellinese, N. (2019). Past, present, and future of Campanula (*Campanulaceae)* systematics–a review. *Botanika Chronika, 22*, 209-222.

Lobo, M., Hounsome, N., & Hounsome, B. (2018). Biochemistry of vegetables: secondary metabolites in vegetables—terpenoids, phenolics, alkaloids, and sulfur-containing compounds. *Handbook of vegetables and vegetable processing*, 47-82.

Mahomoodally, M. F., Gurib-Fakim, A., & Subratty, A. H. (2005). Antimicrobial activities and phytochemical profiles of endemic medicinal plants of Mauritius. *Pharmaceutical biology, 43*(3), 237-242.

Máthé, Á. (2020). Indian Tobacco (*Lobelia inflata* L.). *Medicinal and Aromatic Plants of North America*, 159-186.

Mathias, N. R., & Hussain, M. A. (2010). Non-invasive systemic drug delivery: developability considerations for alternate routes of administration. *Journal of pharmaceutical sciences, 99*(1), 1-20.

Meesakul, P., Shea, T., Fenstemacher, R., Wong, S. X., Kuroki, Y., Wada, A., & Cao, S. (2023). Phytochemistry and Biological Studies of Endemic Hawaiian Plants. *International Journal of Molecular Sciences, 24*(22), 16323.

Mei-Wan, C., Wen-Rong, C., Zhang, J.-M., Xiao-Ying, L., & Yi-Tao, W. (2014). *Lobelia chinensis*: chemical constituents and anticancer activity perspective. *Chinese journal of natural medicines, 12*(2), 103-107.

Mokler, D. J., Rigdon, A., & Schildroth, S. (2016). The Herbal Cabinet.

Najmi, A., Javed, S. A., Al Bratty, M., & Alhazmi, H. A. (2022). Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. *Molecules, 27*(2), 349.

Nigussie, G., & Ashenef, S. (2020). Isolation, characterization, structural elucidation and anti-bacterial activities of roots extracts of Cucumis ficifolius.

Oliveira, N. J. C., dos Santos Júnior, V. S., Pierotte, I. C., Leocádio, V. A. T., Santana, L. F. d. A., Marques, G. V. d. L., . . . Freitas, T. R. (2023). Discovery of Lead 2-Thiazolylhydrazones with Broad-Spectrum and Potent Antifungal Activity. *Journal of Medicinal Chemistry, 66*(24), 16628-16645.

Oluwole, O., Fernando, W. B., Lumanlan, J., Ademuyiwa, O., & Jayasena, V. (2022). Role of phenolic acid, tannins, stilbenes, lignans and flavonoids in human health–a review. *International Journal of Food Science & Technology, 57*(10), 6326-6335.

Organization, W. H. (2019). *WHO global report on traditional and complementary medicine 2019*: World Health Organization.

Paschoal, B. S., Carvalho, G. C., Cauzzo, G., Campo, V. L., Zoccal, K. F., & Tefé-Silva, C. (2020). The anti-inflammatory and antitumor effects of medicinal plants: Arctium lappa, Solanum torvum and *Lobelia inflata*. *Revista Saúde & Diversidade, 4*(2), 84-91.

Pattanayak, S. (2018). Alternative to antibiotics from herbal origin–outline of a comprehensive research project. *Current Pharmacogenomics and Personalized Medicine (Formerly Current Pharmacogenomics), 16*(1), 9-62.

Poddar, S., Sarkar, T., Choudhury, S., Chatterjee, S., & Ghosh, P. (2020). Indian traditional medicinal plants: A concise review. *International Journal of Botany Studies, 5*(5), 174-190.

Riyaphan, J., Pham, D.-C., Leong, M. K., & Weng, C.-F. (2021). In silico approaches to identify polyphenol compounds as α-glucosidase and α-amylase inhibitors against type-II diabetes. *Biomolecules, 11*(12), 1877.

Rizvi, S. M. D., Shaikh, S., Naaz, D., Shakil, S., Ahmad, A., Haneef, M., & Abuzenadah, A. M. (2016). Kinetics and molecular docking study of an anti-diabetic drug glimepiride as acetylcholinesterase inhibitor: implication for Alzheimer's disease-diabetes dual therapy. *Neurochemical research, 41*, 1475-1482.

Seaton, K., Bettin, A., & Grüneberg, H. (2014). New ornamental plants for horticulture. *Horticulture: Plants for People and Places, Volume 1: Production Horticulture*, 435-463.

Shaheen, S., Ahmad, M., Haroon, N., Shaheen, S., Ahmad, M., & Haroon, N. (2017). Status of edible wild plants in Pakistan: Case studies. *Edible Wild Plants: An alternative approach to food security*, 65-125.

Shaki, N. B. W. V., & Areas, A. W. P. (2022). DISCOVERY.

Sindhu, R. K., Sofat, M., Kaur, H., Taneja, A., Babu, M. A., Singh, V., . . . Kumar, A. (2022). Nanodeliovery based Chinese medicine's bioactive compounds for treatment of respiratory disorders. *Pharmacological Research-Modern Chinese Medicine, 5*, 100180.

Singh, R., Upadhyay, S. K., Rani, A., Kumar, P., & Kumar, A. (2020). Ethanobotanical study of Subhartipuram, Meerut, Uttar Pradesh, India. II. Diversity and pharmacological significance of shrubs and climbers. *International Journal of Pharmaceutical Research, 12*(2), 383-393.

Stansbury, J., Saunders, P. R., & Zampieron, E. R. (2013). The use of *lobelia* in the treatment of asthma and respiratory illness. *Journal of Restorative Medicine, 2*(1), 94.

Stolom, S., Oyemitan, I. A., Matewu, R., Oyedeji, O. O., Oluwafemi, S. O., Nkeh-Chungag, B. N., . . . Oyedeji, A. O. (2016). Chemical and biological studies of *Lobelia flaccida* (C. Presl) A. DC leaf: a medicinal plant used by traditional healers in Eastern Cape, South Africa. *Tropical Journal of Pharmaceutical Research, 15*(8), 1715-1721.

Subarnas, A., Oshima, Y., & Ohizumi, Y. (1992). An antidepressant principle of *Lobelia inflata* L.(Campanulaceae). *Journal of pharmaceutical sciences, 81*(7), 620-621.

Sun, W., Yan, S., Li, J., Wu, L., Xiang, L., Ma, W., & Chen, S. (2017). Study of commercially available *Lobelia chinensis* products using Bar-HRM technology. *Frontiers in plant science, 8*, 234615.

Tadege, G., Alebachew, Y., Hymete, A., & Tadesse, S. (2022). Identification of lobetyolin as a major antimalarial constituent of the roots of *Lobelia giberroa* Hemsl. *International Journal for Parasitology: Drugs and Drug Resistance, 18*, 43-51.

Tamboli, A., Khan, I., Bhutkar, K., & Rub, R. (2011). Systematic review on phytochemical and pharmacological profile of *Lobelia nicotianaefolia* Roth E & S. *Pharmacologyonline, 3*, 7-22.

Van Wyk, B.-E., & Wink, M. (2018). *Medicinal plants of the world*: Cabi.

Wubetu, M., Sintayehu, M., & Aeta, M. A. (2018). Ethnobotany of medicinal plants used to treat various mental illnesses in Ethiopia: a systematic review Muluken Wubetu1, Mezinew Sintayehu2, Mohammedbrhan Abdelwuhab Aeta2, Haimanot Reta2 and Dagninet Derebe2. *Asian Journal of Plant Science and Research, 8*(1), 9-33.

Xu, Z., Chang, L., Xu, Z., & Chang, L. (2017). Campanulaceae. *Identification and Control of Common Weeds: Volume 3*, 433-439.

Zhang, L., Khoo, C., Koyyalamudi, S. R., Pedro, N. d., & Reddy, N. (2017). Antioxidant, anti-inflammatory and anticancer activities of ethanol soluble organics from water extracts of selected medicinal herbs and their relation with flavonoid and phenolic contents. *Pharmacologia*, 59-72.

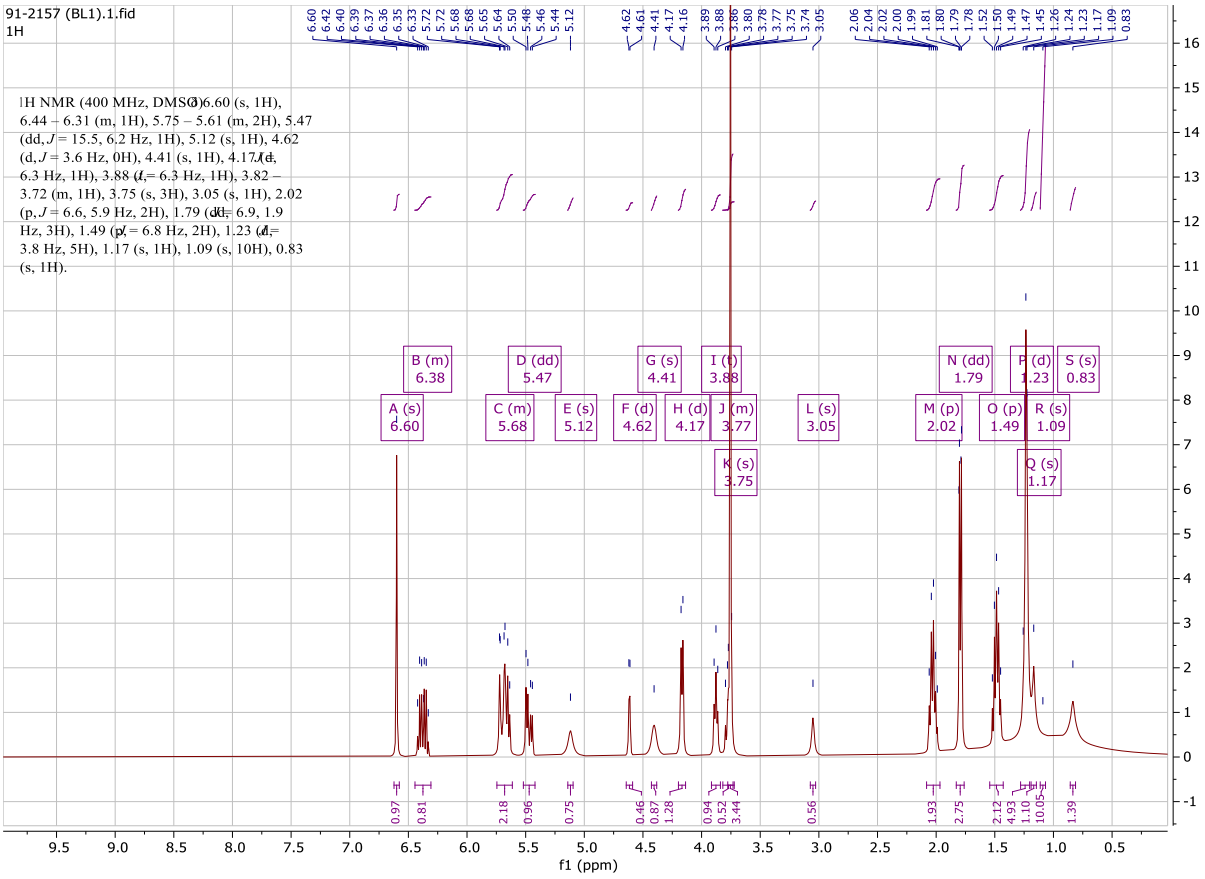
Zheng, Q., Wang, Y., & Zhang, S. (2021). Beyond alkaloids: novel bioactive natural products from *Lobelia* species. *Frontiers in Pharmacology, 12*, 638210.

ZIMARE, S. B., & KAKADE, P. S. (2023). Comprehensive Overview of the Phytochemistry and Pharmacological Studies of the Genus *Lobelia*. *Phytochemical Composition and Pharmacy of Medicinal Plants: 2-volume set*, 385.

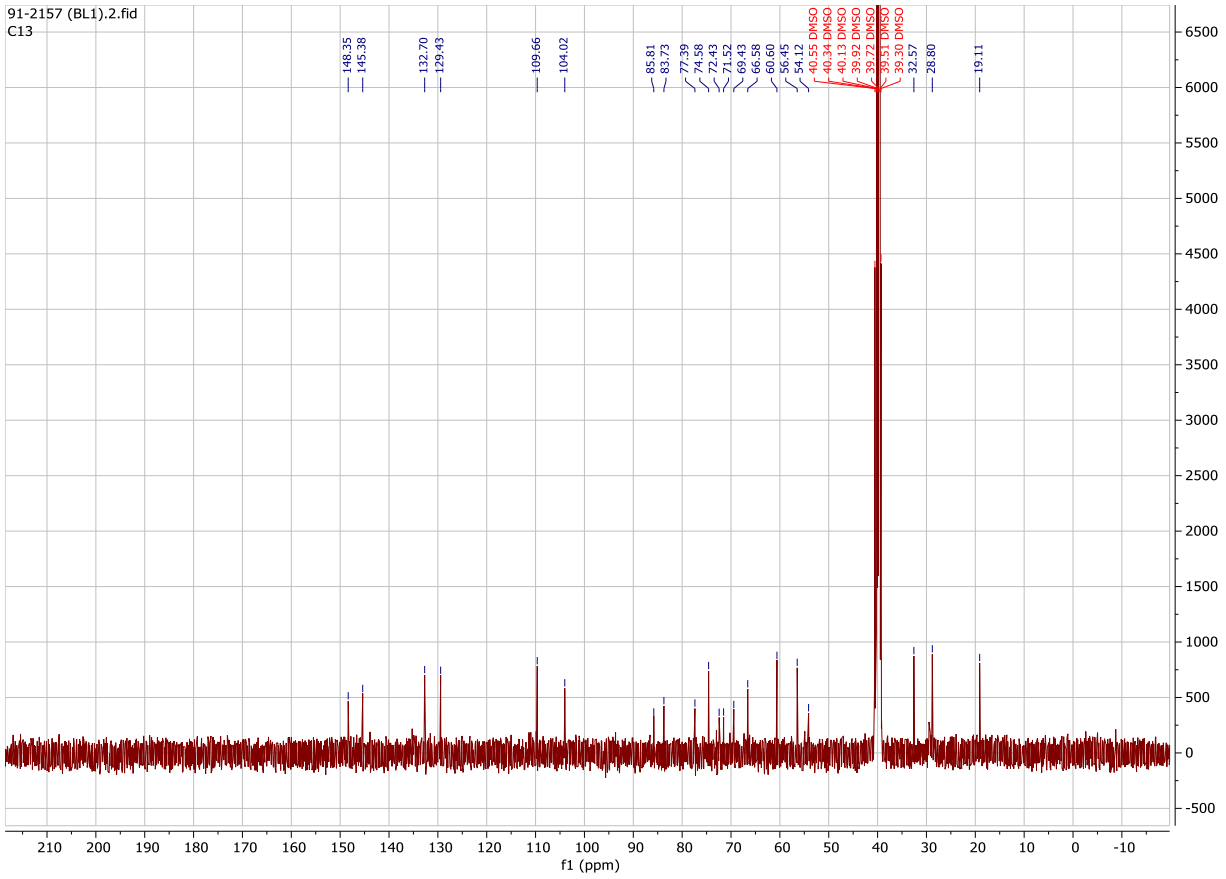
LISTS OF APPENDIX

1.1.Phytochemical analysis of *L.rhynchopetalum* extract

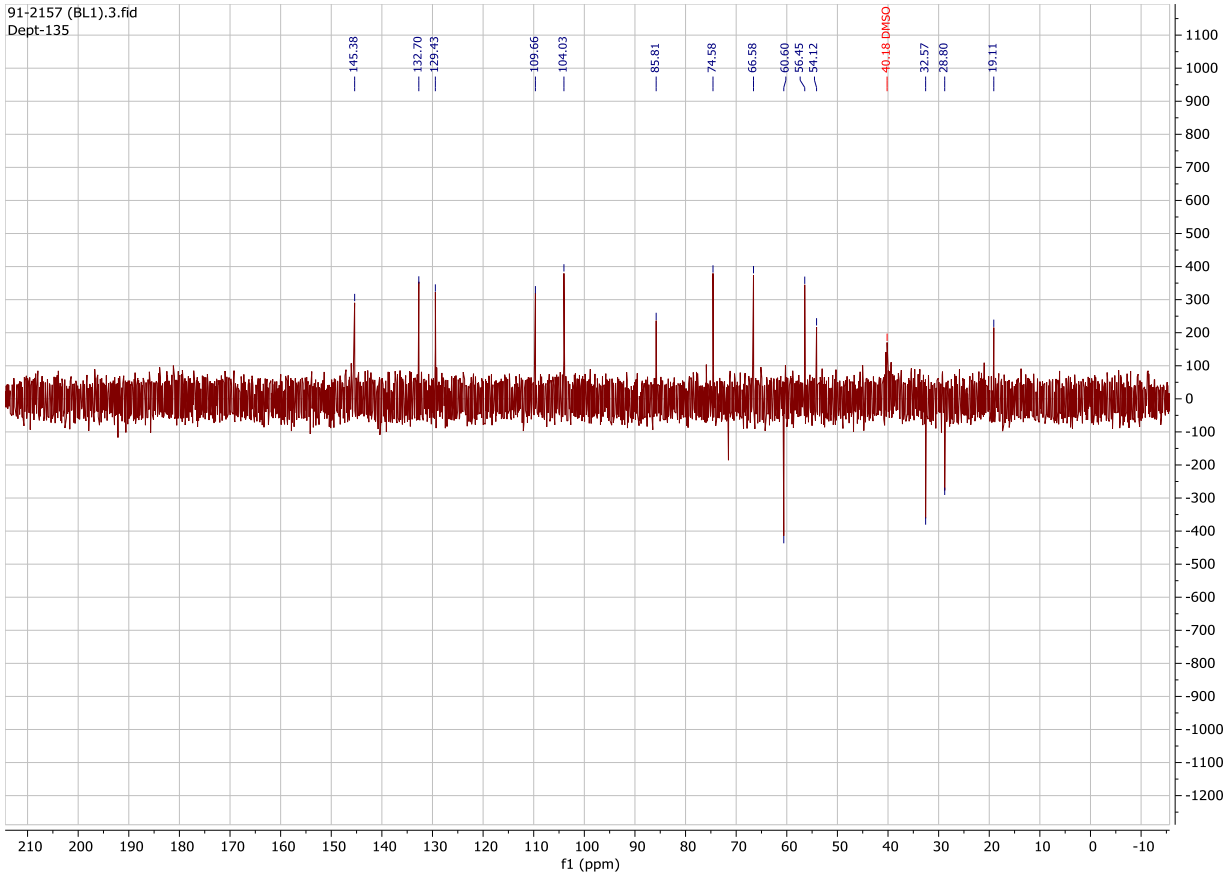
1. 2.[1] H (NMR 400 MHz, DMSO) spectrum of Compound 41(BL1)



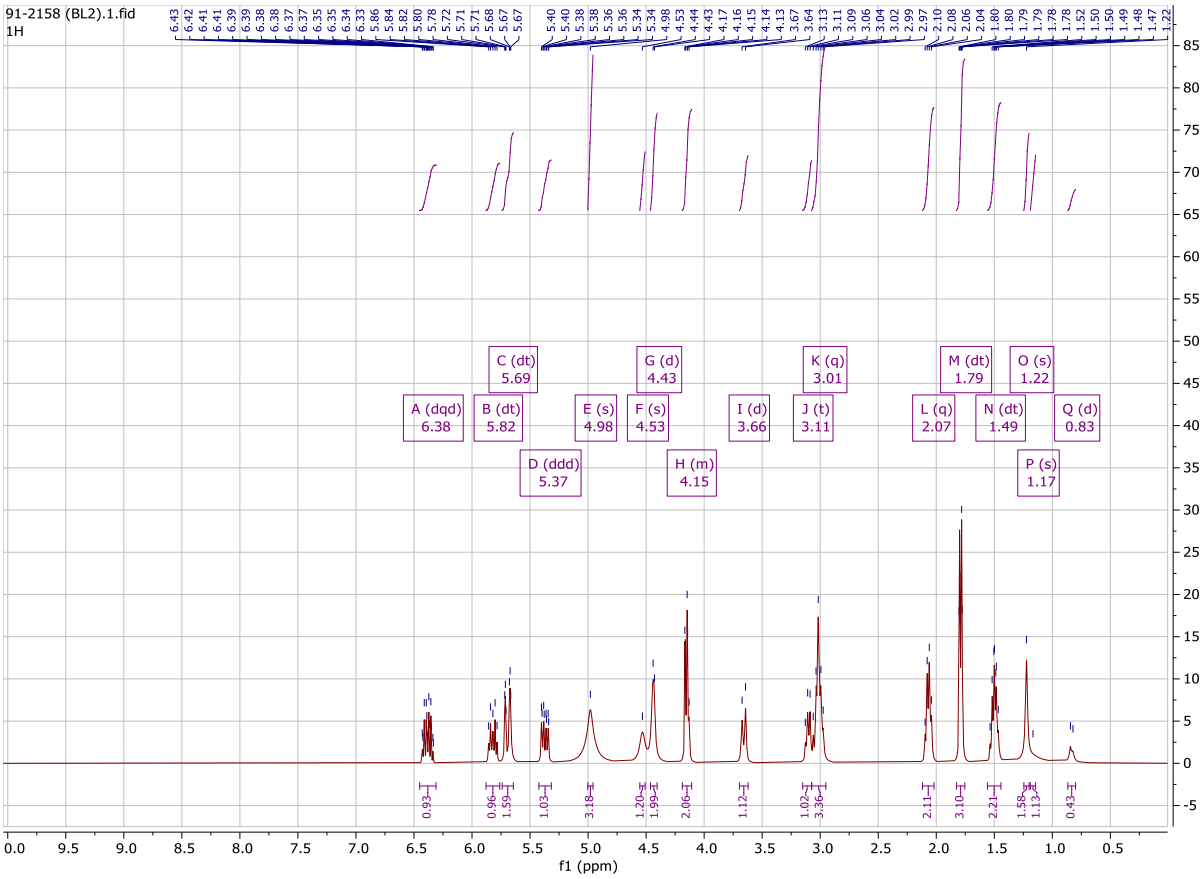
1. 3.^{13}C NMR (101 MHz, DMSO) spectrum of Compound 41(BL1)



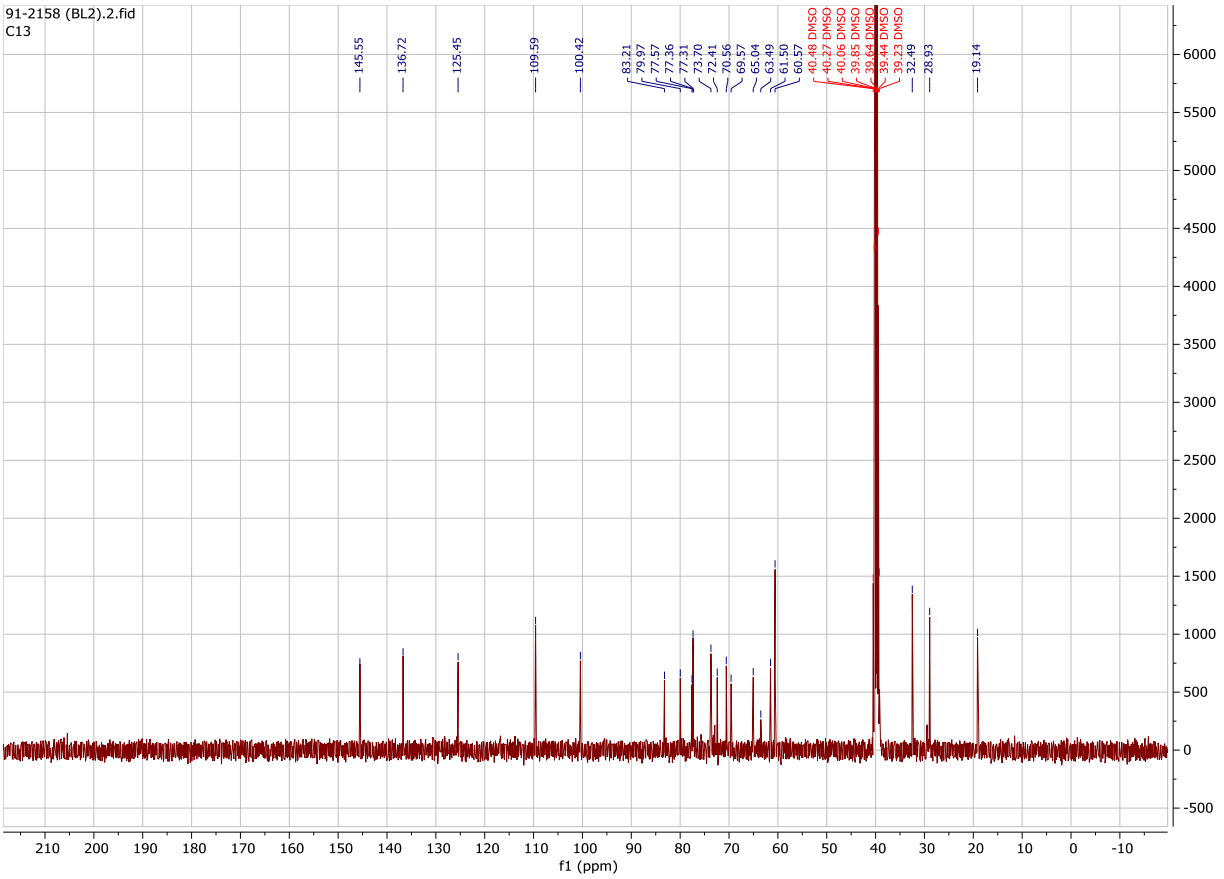
1. 4. Dept 135 spectrum of Compound 41(BL1)



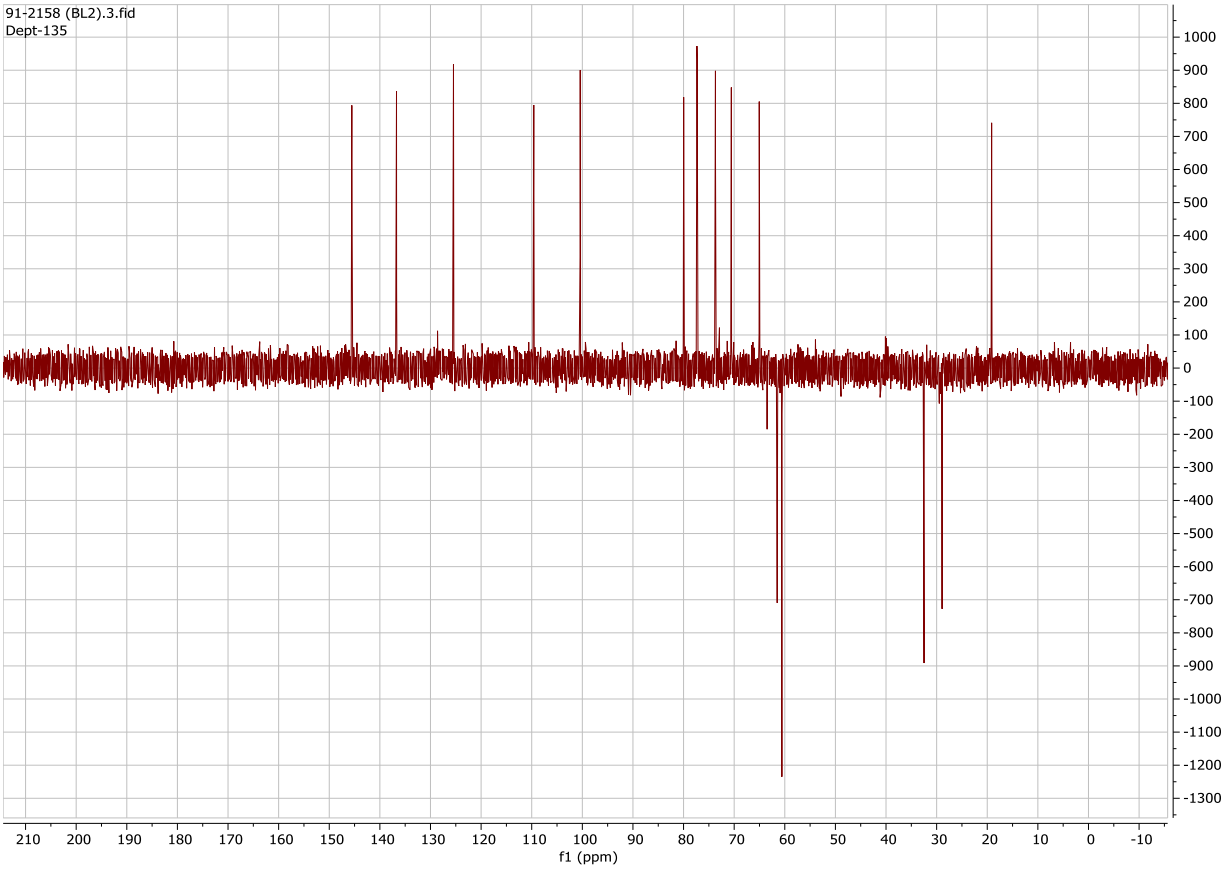
1. 5.[1] H (NMR 400 MHz, DMSO) spectrum of Compound 42(BL2)



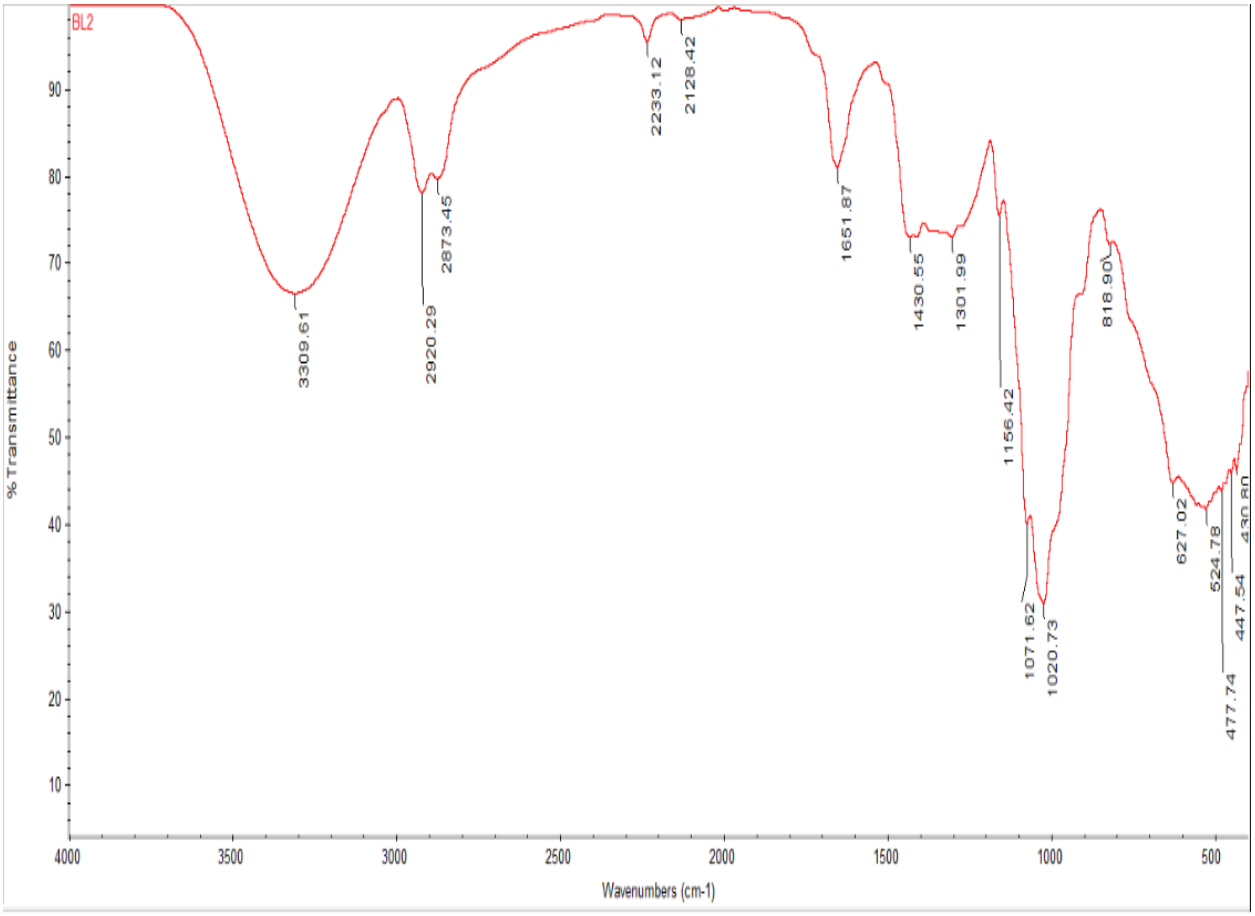
1. 6.^{13}C NMR (101 MHz, DMSO) spectrum of Compound 42(BL2)



1. 3. Dept 135 spectrum of Compound 42(BL2)



1. 4. IR spectrum of Compound 42(BL2)



Research Fund Acknowledgement

This thesis work was funded by Adama science and Technology University under the grant ASTU/SM-R/682/23