Phytochemical Composition and Biological Activities of Extracts and Essential Oil of the Stem Bark of *Pentas schimperiana* (A. Rich.) Vatke, and *In Silico* Molecular Docking and ADMET Study of Selected Compounds from its Essential Oil.

Darwin Demissie Assefa

A Thesis Submitted to the Department of Applied Chemistry

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Applied Chemistry (Medicinal Chemistry)

Office of Graduate Studies

Adama Science and Technology University

June, 2024

Adama, Ethiopia

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Advisor: Professor Aman Dekebo
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DECLARATION

I hereby declare that this thesis entitled "**Phytochemical Composition and Biological Activities of Extracts and Essential Oil of the Stem Bark of *Pentas schimperiana* (A. Rich.) Vatke, and *In Silico* Molecular Docking and ADMET Study of Selected Compounds from its Essential Oil.**" is my original work. That is, it has not been submitted for the award of any academic degree, diploma, or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

Darwin Demissie Assefa

Name of student	Signature	Date

RECOMMENDATION

We, the advisors of this thesis, hereby certify that we have read the revised version of the thesis entitled **"Phytochemical Composition and Biological Activities of Extracts and Essential Oil of the Stem Bark of *Pentas schimperiana* (A. Rich.) Vatke, and *In Silico* Molecular Docking and ADMET Study of Selected Compounds from its Essential Oil."** prepared under my guidance by Darwin Demissie Assefa submitted in partial fulfillment of the requirements for the degree of Masters of Science in Applied Chemistry (Medicinal Chemistry). Therefore, we recommend the submission of a revised version of the thesis to the department following the applicable procedures.

Professor Aman Dekebo		
Major Advisor	Signature	Date
Dr. Leta Deressa		
Co-Advisor	Signature	Date

APPROVAL PAGE OF M.Sc. THESIS

We, the advisors of the thesis entitled "**Phytochemical Composition and Biological Activities of Extracts and Essential Oil of the Stem Bark of *Pentas schimperiana* (A. Rich.) Vatke, and *In Silico* Molecular Docking and ADMET Study of Selected Compounds from its Essential Oil.**" and developed by Darwin Demissie Assefa, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Professor Aman Dekebo		
Major Advisor	Signature	Date
Dr. Leta Deressa		
Co-Advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by Darwin Demissie Assefa have read and evaluated the thesis entitled "**Phytochemical Composition and Biological Activities of Extracts and Essential Oil of the Stem Bark of *Pentas schimperiana* (A. Rich.) Vatke, and *In Silico* Molecular Docking and ADMET Study of Selected Compounds from its Essential Oil.**" and examined the candidate during the open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied Chemistry (Medicinal Chemistry).

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

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

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

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LIST OF ABBREVIATIONS

ADMET ……Absorption, Distribution, Metabolism, Excretion and Toxicity

ATCC ……American Type Culture Collection

BBB……...…. Blood Brain Barrier

BGC……. Beijing Gastric Cancer

CC…….……...Column Chromatography

FTIR……. Fourier Transform Infrared Spectroscopy

CYP ……..……Cytochrome-P

DPPH…….…….2,2-diphenyl-1-picrylhydrazyl

GI…….…… Gastrointestinal

IC_{50} …… Half-maximal inhibitory concentration

LD_{50}…….…Median lethal dose

MHA…….……. Muller Hinton Agar

NMR……Nuclear Magnetic Resonance

PDB ……. Protein Data Base

PET…….……. Petroleum ether

P-gp…….…… P-glycoprotein

Rf……..…… Retention Factor

ROS…… Reactive Oxygen Species

TLC……..... Thin Layer Chromatography

TPSA ……. total polar surface area

UV-Vis ……..... Ultra Violet-Visible

WHO……...…….....World Health Organization

ABSTRACT

Medicinal plants play a key role in the healthcare system facilities of local communities as the major parts of medicine mainly in the rural population. Pentas schimperiana is a plant traditionally used in different areas of Africa, including Ethiopia, for the treatment of blackleg, epilepsy, bone fractures, hepatitis B liver infection, diabetes mellitus, livestock mental problems, and calf fattening. This study was aimed to identify antibacterial and antioxidant phytochemicals from the stem bark of Pentas schimperiana. In this study the stem bark of Pentas schimperiana was successively extracted by maceration with petroleum ether (PET), chloroform, and methanol to afford 1.74 g (0.22 %), 6.55 g (0.83 %), and 26.71 g (3.38 %) yields, respectively. Silica gel column chromatographic separation of the extracts afforded four compounds-based TLC profile and their structures were partially characterized by FTIR. The essential oil was extracted by hydro-distillation technique and analyzed by GC-MS. A total number of 29 components were identified. The antibacterial and antioxidant activities were evaluated using agar disc diffusion method against four strains of bacteria and DPPH methods, respectively. All crude extracts displayed promising growth inhibitory effect at the concentration of 200 mg/mL against the E. coli and their zone of inhibition are 20±0.82 mm for MeOH extract, 21.07±0.47 mm for $CHCl_3$ extract and 21.67±0.47mm for PET extract compared to ciprofloxacin (26.17±0.9 mm). The essential oil of Pentas schimperiana stem bark showed good zone of inhibition 15±0.00 mm against E. coli at concentration of 1 mg/mL, followed by inhibition against P. aeruginosa with the zone of inhibition values of 14±1.00 mm. The highest percent inhibition of the DPPH radical was observed with PET extract (IC_{50}: 2.81 µg/mL) compared with ascorbic acid (IC_{50}: 0.5 µg/mL) and also essential oil showed promising inhibitions IC_{50}: 6.63 µg/mL compared with ascorbic acid (IC_{50}: 2.89 µg/mL). Based on highest percentage composition five major compounds namely: butyl 9,12-octadecadienoate ***(14),*** *linoelaidic acid* ***(15),*** *9-octadecenoic acid (Z)-, oxiranylmethyl ester* ***(16),*** *oleic acid* ***(17),*** *and β-Monolinolein* ***(18)*** *were selected from essential oil for in silico molecular docking and ADMET study and exhibited good results. The current study revealed that the plant is promising source of antibacterial and antioxidant phytochemicals which support the traditional use of Pentas schimperiana for the treatment of various ailments.*

Keywords: *Pentas schimperiana*, Phytochemicals, Bioactivity, *in silico* molecular docking, ADMET

1. INTRODUCTION

1.1. Background of the study

Plants are regarded as having nutritional, medicinal, and even mystical (magical) properties according to local communities. They hold significant importance in rural healthcare systems, functioning as a fundamental element of traditional medicine. Medicinal plants are also recognized as a valuable resource for acquiring a wide range of medications (Beshah et al., 2020). The utilization and understanding of plants are closely tied to specific ethnic cultures. Ethnomedicinal healing systems differ across cultures, while the availability, taxonomic diversity, and abundance of medicinal plants are influenced by geographical and climatic factors (Gillani et al., 2024; Hussain et al., 2024).

Herbal medicine is receiving attention in both developed and developing countries because of its natural origin and minimal adverse effects (Lawal et al., 2014). According to a report from the World Health Organization (WHO), approximately 80% of the global population relies on herbal plants for treating various human ailments. The report highlights the growing interest in investigating medicinal plants as alternative therapies and supports for healthcare activities (Belayneh et al., 2022). Traditional medicine encompasses the medical knowledge and practices rooted in indigenous traditions that have been passed down through generations prior to the development of modern medicine. WHO defines traditional medicine as a comprehensive system that incorporates skills, knowledge, and practices based on cultural theories, beliefs, and experiences, utilized in healthcare for prevention, diagnosis, and treatment of mental and physical illnesses (World Health Organization, 2019).

Traditional medicine which is mainly based on plants has been frequently confirmed by phytochemical investigations, pharmacological studies and clinical tests initiating further studies on medicinal plants in different parts of the world (Bekele et al., 2012). However, use of traditional medicines sometimes can have adverse effects also; and hence more investigation is required to assure the efficiency and safety of traditional medicine and practices used by traditional medicine practitioners and consumers. For this purpose, WHO has instigated a nine-year strategy plan to support member states in developing the proactive strategies and executing the action plans that fortify the role of traditional medicine in keeping healthy populations (World Health Organization, 2013).

In Ethiopia, the diversified culture with various ways of using plants in their respective habitat brings a high expectation of being rich in traditional knowledge (Yirga, 2010). About 80% of the human population and 90% of live-stock in Ethiopia rely on traditional medicine, as most plant species have shown very effective medicinal value for some ailments of human beings and domestic animals (Fullas, 2007). As indicated on the national health policy of Ethiopia; relevant research should be there to explore possibilities to integrate traditional medicine into modern medicine. Considering the wealth of traditional knowledge in Ethiopia and elsewhere a lot is expected to develop and enhance the beneficial aspects of traditional medicine. Additionally, it is also important to look towards the relevant research activities to explore the possibilities to integrate traditional medicine into modern medicine (Kassaye et al., 2007). Medicinal plants have been used since ancient times in virtually all cultures as a source of medicines and are of great importance to the health of individuals and communities. Traditional medicine is used in all parts of the World and has a rapidly growing economic importance, mainly with medicinal plants, especially in developing countries.

The Rubiaceae family has thousands of species in tropical regions, including East Africa. They have medicinal compounds, fight malaria, bacteria, and offer pain relief. For instance, quinine from Rubiaceae family treats malaria and guides drug development. Rubiaceae is a potential source of antiprotozoal agents (Martins & Nunez, 2015). The plant *Pentas schimperiana*, which belongs to the Rubiaceae family, has been traditionally used across various regions of Africa, including Ethiopia. This plant has demonstrated effectiveness in the treatment of several medical conditions, such as blackleg, epilepsy, skeletal issues (including bone fractures), hepatitis B liver infection, and diabetes mellitus (DM) (Fisseha et al., 2021) and livestock mental problem, internal parasite, constipation, and as well as calf fattening (Wendimu et al., 2023a). Some secondary metabolites such as anthraquinones, their derivatives, and certain coumarins reported from *Pentas schimperiana*. Some experimental studies showed that parts of *Pentas schimperi*ana were used for anti-inflammatory (Dzoyem et al., 2016), cytotoxicity (Kuete et al., 2015; Rusel et al., 2014), anticonvulsant (Fisseha et al., 2021b), antioxidant and antidiabetic activities (Amare et al., 2022; Dinku et al., 2011).

This study was intended to investigate phytochemical composition of the stem bark of the *Pentas schimperiana*, evaluate their antibacterial and antioxidant activity along with *in silico* molecular docking and ADMET properties of the compounds.

1.2. Statement of the problem

Currently, medicinal plants play a crucial role in the development of drugs aimed at preventing and combating a diverse array of disease-causing agents, while also providing treatment for various human ailments. The rise of drug resistance has emerged as a significant global public health issue, posing a considerable challenge. Consequently, there remains an ongoing necessity to extract, isolate, and explore potent drugs to address the growing prevalence of emerging diseases and drug-resistant conditions. *Pentas schimperiana*, a medicinal plant of great promise, exhibits diverse traditional applications and possesses a range of beneficial biological properties, including anti-inflammatory, cytotoxic, antidiabetic, and anticonvulsant activities. However, as far as the researchers' knowledge extends, *Pentas schimperiana*, despite being traditionally employed for treating various ailments, has received relatively little attention as a medicinal plant. The research on the medicinal plant *Pentas schimperiana* has primarily focused on investigating its ethnomedicinal uses and some biological activities using crude extracts, such as antidiabetic, antioxidant, and anticonvulsant. To address this gap, there is a need to conduct further studies to isolate and identify the bioactive compounds present in the stem bark of *Pentas schimperiana* and evaluate their specific biological activities, particularly their antibacterial and antioxidant properties, to confirm their ethnomedicinal claims. Given the inherent potential of plants to serve as abundant sources of diverse phytochemicals and previous research indicating the beneficial effects of *Pentas schimperiana* against bacteria, there is a compelling interest and significance in investigating the phytochemistry, biological activity, and in silico molecular docking and ADMET of *Pentas schimperiana*.

1.3. Objectives

1.3.1. General objective

The general objective of this study was to identify antibacterial and antioxidant phytochemicals from the stem bark of *Pentas schimperiana*.

1.3.2. Specific objectives

The specific objectives of the study are as follows:

- To successively extract the stem bark of *Pentas schimperiana* using petroleum ether, chloroform and methanol by using maceration method.
- To extract essential oil through hydrodistillation and analyze its chemical composition by GC-MS.
- To conduct a qualitative phytochemical screening test on the petroleum ether, chloroform and methanol extracts of stem bark of *Pentas schimperiana*
- To isolate compounds from the stem bark extracts of *Pentas schimperiana* using silica gel column chromatography
- To characterize the structure of compounds isolated from the stem bark extracts of *Pentas schimperiana* using spectroscopic methods (FTIR)
- To evaluate antibacterial activities of the extracts and essential oil from the stem bark of *Pentas schimperiana* against Gram-negative bacteria *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), and Gram-positive bacteria *Staphylococcus aureus* (ATCC25923) and *Streptococcus pyogens* (ATCC19615) by using agar disc diffusion method.
- To assess the antioxidant potential of the extracts and essential oil through *in vitro* assays such as DPPH scavenging assay
- To conduct *in silico* molecular docking study of selected compounds from essential oil using Auto Dock vina 4.2 against selected protein targets (DNA gyrase B, pyruvate kinase, and human myeloperoxidase).
- To predict ADMET of selected compounds from essential oil using SwissADME and Pro Tox II online servers

1.4. Significance of the study

The significance of this study lies in its comprehensive investigation of the stem bark of *Pentas schimperiana*. By examining its phytochemical composition, evaluating its *in vitro* antibacterial and antioxidant activities, and conducting *in silico* molecular docking and ADMET studies, this research aims to provide valuable insights. Firstly, it may contribute to the identification of novel bioactive compounds with potential applications in combating pathogenic bacterial strains. Secondly, understanding the plant's antioxidant activity can shed light on its potential role in preventing oxidative stress-related conditions. Lastly, *the in silico* molecular docking studies can offer important information about the interactions between the plant's constituents and target proteins, while ADMET studies can offer insights into the drug-likeness and safety of the phytochemicals, potentially leading to the development of new therapeutic interventions. Overall, this research holds significance in advancing our understanding of the medicinal properties of *Pentas schimperiana* and its potential contributions to natural product chemistry, pharmacology, and drug discovery.

2. LITERATURE REVIEW

2.1. The Rubiaceae Family

The Rubiaceae family has around 13,000 species in approximately 630 genera, predominantly found in tropical and subtropical regions. In East Africa, including Ethiopia, there are about 100 genera and 600 species of Rubiaceae. This family is well-known for producing bioactive metabolites with significant pharmacological potential. These metabolites can serve as chemotaxonomic markers, even at the level of genera and subfamilies. Classical taxonomy focuses on plant morphology, while modern systems consider a combination of characteristics, including chemical composition. Phytochemical compounds are valuable for characterizing and classifying plant species. The distribution of secondary metabolites in Rubiaceae follows patterns that aid in characterizing the botanical group, such as subfamilies, tribes, or genera. These chemotaxonomic patterns are often used to determine the botanical origin of plants (Martins & Nunez, 2015).

Several plants from the Rubiaceae family have been studied for various biological activities, including antiplasmodial, antibacterial, analgesic, and antiperoxidative properties. The family is rich in phytochemicals such as terpenoids, iridoids, anthraquinones, and indole alkaloids. Quinine, derived from a Cinchona species in the Rubiaceae family, was the first effective treatment for malaria and has served as a basis for developing synthetic analogues like chloroquine. The Rubiaceae family shows potential as a source of antiprotozoal agents (Martins & Nunez, 2015).

2.2. The Genus *Pentas*

The genus *Pentas* belongs to the Rubiaceae family and consists of approximately 40 species. These plants are widely used by indigenous people in Africa for medicinal purposes. *Pentas* species can be found as herbs, shrubs, or subshrubs, with varying stem lengths and leaf and flower shapes. They are used in the treatment of various diseases such as malaria, tapeworms, skin conditions, gonorrhea, cough, dysmenorrhea, hepatitis B, mental illness, epilepsy, abdominal cramps, and veterinary diseases. Iridoids and highly oxygenated compounds are the most common secondary metabolites reported from this genus. While some biological activities

like antimalarial and antimicrobial properties have been reported, only limited studies have investigated their analgesic, wound-healing, and antitumor characteristics. The secondary metabolites identified in *Pentas* are shared with other plants in the Rubiaceae family, but some unique compounds have also been reported (Sweelam et al., 2018).

2.2.1. *Pentas schimperiana*

*Pentas schimperi*ana (A. Rich) Vatke, commonly known as Schimper's *Pentas*, is a species of flowering plant belonging to the genus *Pentas* and the family Rubiaceae. It is native to East Africa and is found in countries such as Ethiopia, Kenya, Tanzania, Cameroon and Uganda. *Pentas schimperiana* is shrubby and semi-woody herb which reaches up to 2m high (Figure 1). The leaves are narrow at both ends, and the petiole is ranging up to nearly half inch. It has purplish black woody stems, and the corolla is tube funnel shaped and the fruits are 4–6mm long. It occurs in altitude range between 1710 and 2350 m. Its habitat is thickest at stream side, marshy edges, and open ground. *Pentas schimperiana* (A. Rich.) Vatke is subspecies of *Pentas schimperiana* found in Ethiopia, while subspecies occidental is occurring in Cameroon (Dinku et al., 2011. In Ethiopia, it is widely distributed in the area of Adaa district, Wonago woreda, Gedo dry evergreen mountain forest, Odo-Bulu, and Demaro in Bale Region. *Pentas schimperiana* (A. Rich.) Vatke is known by its vernacular name as Woinagrefet, also known as Dibexxo (Gideo), Moonyeer or Dasie (Afaan Oromo), Daanbursaa (Wolaita) (Megersa & Woldetsadik, 2022) and Dalbbantsa or Dawuridama (Dawuro) (Agize et al., 2013) with local name. Its dry root bark powder mixed with water is taken orally for epilepsy in Wonago woreda. In addition, root bark coarse powder is mixed with water given orally for treating livestock mental problem (Fisseha et al., 2021). The experimental studies showed that the decoctions of plants were used for anti-inflammatory (Dzoyem et al., 2016b), cytotoxicity (Kuete et al., 2015; Rusel et al., 2014), anticonvulsant (Fisseha et al., 2021b), antioxidant and antidiabetic activities (Amare et al., 2022; Dinku et al., 2011).

Figure 1: *Pentas schimperiana* (photo taken by Darwin Demissie from Wolaita Zone, Damot Sore Woreda, September 5, 2023).

2.2.2. Botanical Description of *Pentas schimperiana*

Botanical Name: *Pentas schimperiana* (A. Rich) Vatke

Class: Dicotyledons

Subclass: Asteridae

Order: Gentianales

Family: Rubiaceae

Genus: *Pentas*

Species: *Schimperiana* (Megersa & Woldetsadik, 2022; Wieringa, 2008).

2.2.3. Ethnobotanical uses of *Pentas schimperiana*

Ethnobotany is the scientific study of the relationships between plants and people, focusing on the traditional knowledge and practices of various cultures regarding the use of plants. It involves exploring the importance of plants used for food, medicines, and various economic applications, including their roles in providing shelter, clothing, fodder, fuel, furniture, and medicinal remedies. Ethnobotanical studies aim to identify, disseminate, and document indigenous knowledge related to plant diversity and its application in treating human and livestock ailments (Mekonnen et al., 2022). By understanding the interrelations between humans and plants, ethnobotany contributes to the preservation and utilization of traditional plant-based practices and the sustainable management of plant resources.

Pentas schimperiana (Rubiaceae) is among plants traditionally used in different areas of Africa including Ethiopia which is very effective in the treatment of epilepsy, skeletal problem (bone fracture), hepatitis B liver infection, diabetes mellitus (DM) and livestock mental problem, Fattening, internal parasite, constipation and bone broken (Table 1).

Table 1: Ethnobotanical uses of *Pentas schimperiana.*

Name of plant	Country	Ethnobotanical uses	References
Pentas schimperiana	Cameroon	Hepatitis B liver infection	(Rusel et al., 2014)
	Ethiopia	Diabetic Mellitus (DM)	(Amare et al., 2022)
	Wolaita and Dawuro (Ethiopia)	Blackleg, fattening of calf, mental problem, internal parasite, constipation for Livestock and bone fracture for Haman and livestock	(Agize et al., 2013; Megersa & Woldetsadik, 2022; Wendimu et al., 2023)
	Wonago (Ethiopia)	Epilepsy	(Fisseha et al., 2021)

2.3. Chemical composition of *Pentas Schimperiana*

Pentas schimperiana is recognized for its production of secondary metabolites, particularly anthraquinones and their derivatives, as well as certain coumarins. These compounds are considered the main chemical constituents of *Pentas schimperiana* (Dzoyem et al., 2016; Kuete et al., 2015; Rusel et al., 2014)

From the stem bark of *Pentas schimperi*, two new anthraquinone dimers named schimperiquinones A (**1**) and B (**2**) were isolated, along with several known compounds: 2-hydroxymethylanthraquinone (**3**), cleomiscosin A (**4**), oleanolic acid (**5**), and sitosterol 3-O-β-D-glucoside (**6**) **(Figure 2).** The structures of these compounds were determined through spectroscopic analysis and comparison with existing data or authentic samples (Rusel et al., 2014). A coumarin and nine anthraquinone derivatives, 3-hydroxy-1- methoxy-2-methylanthraquinone (**7**), 2-hydroxymethyl anthraquinone (**3**), schimperiquinone B (**2**), cleomiscosin A (**4**), damnacanthal (**8**), 1,2-dihydroxy anthraquinone (**9**), damnacanthol (**10**), 3-hydroxy-2-hydroxymethyl anthraquinone (**11**), 1-hydroxy-2-methoxyanthraquinone (**12**), and

2-hydroxymethyl-3-O-prenylanthraquinone (**13**) **(Figure 2)**, isolated from the roots of *Pentas schimperi* (Dzoyem et al., 2016). Anthraquinones from *Pentas schimperi*, namely damnacanthal (**7**), damnacanthol (**9**), 3-hydroxy-2-hydroxymethyl anthraquinone (**10**) and schimperiquinone B (**2**) (Figure 2) (Kuete et al., 2015.

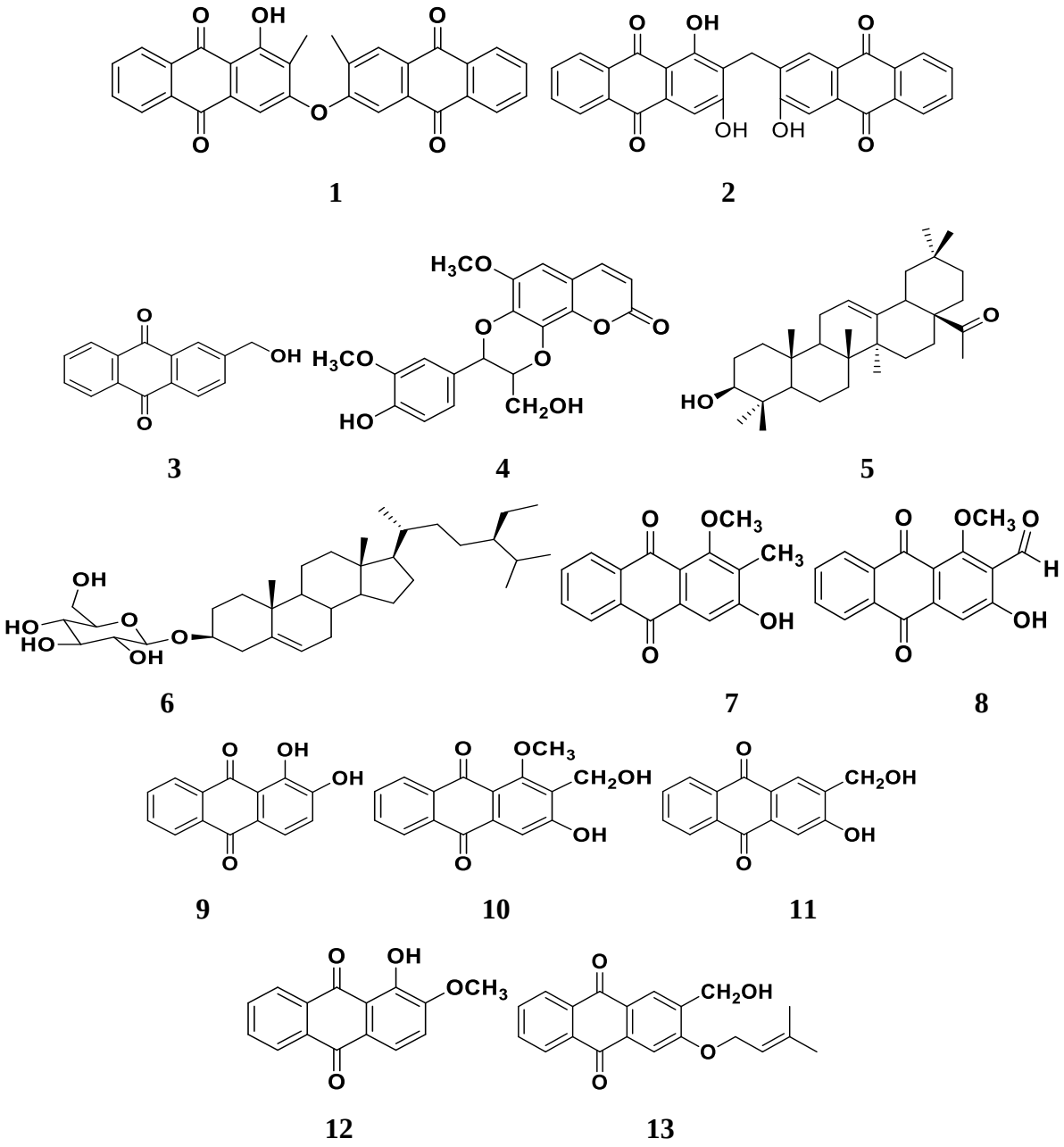


Figure 2 : Chemical structures of the compounds isolated and reported from *Pentas shimperiana*

2.4. Biological Activities of *Pentas schimperiana*

The remarkable traditional uses of *Pentas schimperiana* lead to the various research of biological activities, such as anti-inflammatory, anticonvulsant, cytotoxicity, acute toxicity, antidiabetic, and antioxidant activities. In this section, biological activities research of the extracts and some isolated compounds of the *Pentas schimperiana* are highlighted in below.

2.4.1. Anti-inflammatory Activity

A study investigated the anti-inflammatory activity of a coumarin and nine anthraquinone derivatives isolated from roots of *Pentas schimperiana*. The compounds showed significant concentration-dependent inhibition of nitric oxide production in lipopolysaccharide-stimulated macrophages. Several compounds displayed moderate inhibition of 15-lipoxygenase activity. Compounds:3-hydroxy-2-hydroxymethylanthraquinone **(11)** and 2-hydroxymethyl-3-O-prenylanthraquinone **(13)** were exhibited the most potent inhibition of nitric oxide production, while compounds: 2-hydroxy- methyl anthraquinone, damnacanthol **(9),** and 3- hydroxy-2-hydroxymethyl anthraquinone (**11**) showed good anti-15-lipoxygenase activity. These findings suggest that these compounds from *Pentas schimperiana* have potential anti-inflammatory properties (Dzoyem et al., 2016; Mohr et al., 2019)

2.4.2. Cytotoxicity

Multidrug resistance in cancer represents a major problem in chemotherapy. The study assessed the cytotoxicity of anthraquinones namely damnacanthal **(7)**, damnacanthol **(9),** 3-hydroxy-2-hydroxymethyl anthraquinone **(11)** and schimperiquinone B **(2)** from the roots of *Pentas schimperi* against drug-sensitive and multidrug-resistant cancer cell lines. Damnacanthal **(7)** and damnacanthol **(9)** displayed cytotoxic effects across all tested cell lines, while 3-hydroxy-2-hydroxymethyl anthraquinone **(11)** and schimperiquinone B **(2)** exhibited selective activities. Damnacanthal **(7)** and damnacanthol **(9),** induced apoptosis in leukemia cells by disrupting mitochondrial membrane potential and increasing reactive oxygen species (ROS) production. These findings suggested that anthraquinones from *Pentas schimperiana*, particularly compounds damnacanthal and damnacanthol, had potential as cytotoxic natural products for the development of novel drugs against multidrug-resistant cancers (Kuete et al., 2015).

Schimperiquinone B **(2),** an anthraquinone dimer isolated from the stem bark of *Pentas schimperi*, exhibits notable biological activities, such as cytotoxicity against human cancer cells. Specifically, it has been shown to possess in vitro cytotoxicity against the BGC-823 cell line, which represents human gastric cancer cells. With an IC_{50} value of 33.05 mM, Schimperiquinone B effectively inhibits the growth of BGC-823 cells, achieving a 50% inhibition rate at a concentration of 33.05 mM (Rusel et al., 2014).

2.4.3. Acute Toxicity

The acute toxicity study performed on Swiss albino mice indicated that the aqueous and hydroalcoholic extracts of *Pentas schimperiana* had a median lethal dose (LD_{50}) above 4000 mg/kg. This suggests a relatively low toxicity level for these extracts (Dinku et al., 2011).

2.4.4. Antioxidant Activity (Free Radical Scavenging Activity)

The hydroalcoholic extracts and methanol fraction of the dried leaves of *Pentas schimperiana* exhibited strong free radical scavenging activity, as measured by the DPPH assay. The IC_{50} values (concentration required to scavenge 50% of free radicals) for the hydroalcoholic extracts and methanol fraction were 7.83 µg/ml and 8.84 µg/ml, respectively. Ascorbic acid, used as a positive control, showed an IC_{50} value of 4.42 µg/ml (Dinku et al., 2011).

2.4.5. Antidiabetic Activity

The study tested the effects of different doses (500 mg/kg and 1000 mg/kg) of aqueous dried leaf extract, hydroalcoholic fresh leaf extract, and hydroalcoholic dried leaf extract of *P. schimperiana* on alloxan-induced diabetic mice. At the dose of 1000 mg/kg, all three extracts significantly lowered blood glucose levels, with reductions ranging from 21.90% to 26.97%. The extracts showed a better antidiabetic effect at the higher dose compared to the lower dose. The study also examined the antidiabetic activity of aqueous and methanol fractions prepared from the dried plant material. At a dose of 500 mg/kg, both the aqueous and methanol fractions significantly reduced blood glucose levels by 23% and 27.2%, respectively, compared to diabetic untreated mice. Comparison with Glibenclamide: Glibenclamide, a known antidiabetic drug, was used as a positive control. Treatment with glibenclamide (5 mg/kg) resulted in a 38% reduction in blood glucose levels (Dinku et al., 2011; Kifle et al., 2022)

In another study, three different doses (250 mg/kg, 500 mg/kg, and 1000 mg/kg) of methanolic leaf extracts of *Pentas schimperiana* were administered to three groups of mice.

The first group consisted of control mice with normal glucose levels, the second group had mice with glucose infusion, and the third group had alloxan-induced diabetic mice. Various parameters such as blood glucose levels, lipid levels, body weight, and physical diagnosis were assessed before and after the experimental treatment. After 14 days of treatment, all three doses of the methanolic leaf extract of *Pentas schimperiana* showed significant reductions in blood glucose levels in the diabetic mice compared to the normal mice (PS 250 mg/kg, $p < 0.01$; PS 500 mg/kg and 1000 mg/kg, $p < 0.001$). Additionally, the treatment for 14 days effectively corrected weight loss and dyslipidemic profiles in the diabetic mice. Based on these findings, the study concluded that the methanolic leaf extract of *Pentas schimperiana* could be a potential treatment for *Diabetes mellitus*, particularly in resource-limited settings. The study suggests the need for further follow-up studies to explore the full potential and mechanisms of action of this plant extract (Amare et al., 2022).

2.4.6. Anticonvulsant Activity

The anticonvulsant activity of an 80% methanolic root bark extract of *Pentas schimperiana* was evaluated using the pentylentetrazole (PTZ) and maximal electroshock-induced seizure tests. The results showed that the higher dose (400 mg/kg) of the methanolic extract (ME400) and butanol fraction (BF400) exhibited a significant anticonvulsant effect in both the PTZ and maximal electroshock-induced seizure tests compared to the control group. However, the chloroform fraction showed significant anticonvulsant activity only in the PTZ-induced seizure test, while the aqueous fraction had the least anticonvulsant activity in both seizure-induced tests (Fisseha et al., 2021). These findings suggest the potential of the methanolic extract and butanol fraction of *Pentas schimperiana* root bark as anticonvulsant agents, although further research is needed to understand their mechanisms of action and safety profiles.

3. MATERIALS AND METHODS

3.1. Plant Material Collection and Identification

The plant material was collected from Demba Zamine Kebele, Damot Sore Woreda, Wolaita Zone, Southern Ethiopia. The area is located at 398 km to the South West of Addis Ababa. The identification of the plant material was done by Botanist Melaku Wendaferash at the Department of Biology, National Herbarium of Ethiopia, Addis Ababa University (voucher code: DD001).

3.2. Chemicals and Reagents

The chemicals and reagents used for extraction, isolation, phytochemical analysis, and other activities were silica gel (Loba Chemie PVT. LTD., 60-120 mesh), ethanol (Favor trading PLC, 99.9%), methanol (CDH (P) Ltd., 99.8%), chloroform (CDH (P) Ltd., 99.8%), n-hexane (Loba Chemie PVT.LTD., 99%), ethyl acetate (Loba Chemie PVT. LTD., 99.5%), dichloromethane (Loba Chemie PVT. LTD., 99.5%), Vanillin, ammonium solution (Avi chem, 25%), ferric chloride (Sigma Aldrich, 97%), sulfuric acid (Loba Chemie PVT. LTD., 98%), hydrochloric acid (Loba Chemie PVT. LTD., 35.4%), DPPH, Muller Hilton Agar (MHA) Ciprofloxacin, ascorbic acid, DMSO (Srlchem, 99.5%), Potassium Iodide (Samir Tech-Chem PVT. LTD., 99.8%), Iodine (Alpha Chemika, 98.5%), and distilled water.

3.3. Instruments

Column chromatography was carried out using silica gel (60-120 mesh) ASTM. Thin Layer Chromatography (TLC) was performed using pre-coated aluminum-backed supported silica gel $60F_{254}$ (0.2 mm thickness, 20*20). Uv cabinet or UV light (254 and 366 nm) for spots on TLC. The GC-MS analysis was performed by GC (7890B, Agilent Technologies, USA) coupled with an MS (5977A Network, Agilent Technologies) and Uv spectrophotometer (Shimadzu, Model 1800, Japan).

3.4. Preparation of Plant Material and Extractions

3.4.1. Preparation of Plant Material

The collected stem bark of *Pentas schimperiana* was cut into smaller pieces and dried under shade. Then it was pulverized into a fine powder using a mechanical grinder. The powder was stored in airtight containers at room temperature till further experimentation.

3.4.2. Extraction of the plant material by Maceration

The powdered stem bark of *Pentas schimperiana* (790 g) was soaked into petroleum ether (4L) for 72 hrs. and shaken with mechanical shaker at room temperature. The solution was filtrated and concentrated using a rotary evaporator at around 40ºC to afford petroleum ether extract. The marc was soaked in chloroform (4L) for 72 hrs. The mixture was filtered, and concentrated to afford chloroform crude extract. Finally, the marc left was further extracted in methanol (4L) for 72 hrs. followed by filtration and concentrated to afford methanol extract. The dried extracts were weighed and the percentage yield was calculated. The extracts' TLC profiles were checked to identify appropriate eluent for column chromatographic separation.

3.4.3. Extraction of Essential Oil by Hydrodistillation

The dried *Pentas schimperiana* (100 g) were subjected to Hydrodistillation using a Clevenger-type apparatus for 4h. The oil was extracted with $CHCl_3$ (100 ml) and then were dried over anhydrous Na_2SO_4 (1 g) filtered and concentrated on a rotary evaporator. The oil yield (w/w) was calculated by equation (1). The oil was stored in a vial at -4°C until GC-MS analysis and biological activity (Elyemni et al., 2019; Karakoti et al., 2022).

$$\textbf{Yield (\%)} = \frac{\textbf{Amount of extracted oil } (mg)}{\textbf{Amount of dry plant matter mass}(g)} * \mathbf{100} \quad \text{--------- (1)}$$

3.4.4. GC-MS Analysis of essential oil of *Pentas schimperiana* stem bark

The chemical composition of the stem bark of *Pentas schimperiana* essential oil extracted by hydro-distillation was analyzed by GC-MS using the following method. The analysis was performed by GC (7890B, Agilent Technologies, USA) coupled with an MS (5977A Network, Agilent Technologies). The GC had an HP5MS column (30x 250 µm internal diameter (i.d) and 0.25 µm). Helium was used as a carrier gas (flow rate 1mL/min). The initial oven temperature was 100ºC for 2 min and raised up from 100 to 200 ºC at the speed of 10 º C/min (inlet 250 º C, split less injection/purge time 1 min), solvent delay 4 min. Mass spectra was recorded in electron impact mode, with ionization energy of mode at 70 ev, scanning the 33-550 m/z range. Identification of essential oil components was done by comparing their relative retention index (RI) values with those compounds reported in the literature and, mass spectra NIST (NIST version 2.1) and WILEY (7th edition) libraries, and by matching the fragmentation pattern of the mass spectral data (Karakoti et al., 2022).

3.5. Phytochemical Screening

The preliminary qualitative phytochemical screening of the stem bark of *Pentas schimperiana* crude extracts were performed to identify the presence of secondary metabolites such as alkaloids, flavonoids, phenols, tannins, terpenoids, saponins and steroids present in the crude extracts. The following chemical tests were carried out on the extracts by following standard procedures reported in (Abdisa & Tarekegn, 2021; Dubale et al., 2023) with some modifications

Test for Alkaloids (Wagner's test): The extracts (30 mg) was separately stirred with 1% HCl (1 mL) in a water bath for 5 min and filtered into a test tube. 1 mL of Wagner's reagent (Iodine in Potassium Iodide) was added; the formation of a brown/reddish precipitate indicates the presence of alkaloids.

Test for flavonoids: The extracts (30 mg) was dissolved in 10 mL of ethyl acetate and heated for 3 min using a steam bath. The mixture was filtered, and to the filtrate 1 mL of dilute ammonia solution was added. The formation of intense yellow color ratifies the presence of flavonoids.

Test for Glycosides: The extracts (30mg) from each solvent was dissolved in 3 mL of glacial acetic acid containing 1 drop of ferric chloride solution. This was underlayed with 1 mL concentrated sulphuric acid. A brown ring at the interface was indicating the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

Test for Phenols: The extracts (30 mg) was dissolved in 5mL of distilled water. To this, a few drops of neutral 5% ferric chloride solution was added. A dark green color indicated the presence of phenolic compounds.

Test for Tannins: The extracts (30 mg) was dissolved in 5mL of distilled water, filtered and ferric chloride reagent was added to the filtrate. A blue-black, green, or blue-green precipitate was taken as evidence of the presence of tannins.

Test for Terpenoids (Salkowski's Test):

The extracts (30 mg) was dissolved in 2 mL of chloroform and concentrated H_2SO_4 (3 mL) was carefully added to form a layer. An appearance of reddish-brown color on the inner face indicated the presence of terpenoids.

Test for saponins: The extracts (30 mg) was dissolved in 5 mL of distilled water and shaken while heating to boil. Frothing showed the presence of saponins.

Test for Steroids: The extracts (30 mg) was dissolved in 10 mL of chloroform and an equal volume of concentrated H_2SO_4 was added by the sides of the test tube. A reddish-brown color at the interphase indicates the presence of a steroidal ring.

3.6. Isolation of Compounds

The crude extracts of chloroform and methanol were combined as they showed similar TLC profiles. The combined crude extracts (10 g) was adsorbed onto10 g of silica gel (mesh size 60-120), and subjected to silica gel column chromatography (150 g of silica gel). The column was packed with petroleum ether. Elution was carried out with increasing gradient of solvent polarity such as ethyl acetate in petroleum ether followed by increasing gradient of methanol in dichloromethane. A total of 130 fractions (100 mL each) were collected (Table 2). Fractions that showed identical Rf values and the same characteristic color on TLC were combined.

Fraction 7 (eluted with 15 % EtOAc in petroleum ether) was washed with n-hexane and afforded compound ***PS*-1** (30.4 mg) as white powder. Fraction 17 (eluted with 20% EtOAc in petroleum ether) afford compound ***PS*-2** (74.2 mg) as white powder. Fraction 41 (eluted with 30% EtOAc in petroleum ether) yielded compound ***PS*-3** (17.4 mg) as a pale-yellow compound and Fraction 119 (eluted with 20% MeOH in DCM) afforded compound ***PS*-4** (12.8 mg) as pale-yellow and their TLC profiles are shown in Figure 3.

Figure 3: TLC analysis of isolated compounds from stem bark of *Pentas schimperiana*

PS: Represent the name of investigated plant *Pentas schimperiana*

Solvent systems

***PS*-1.** 10% EtOAc to n-Hexane, ***PS*-2.** 30% EtOAc to n-Hexane,

***PS*-3.** 40% EtOAc to n-Hexane, ***PS*-4.** 10% MeOH to DCM.

Table 2: Summary of column chromatographic fractionation of chloroform-methanol crude extracts

Solvent System	**Ratio**	**Fractions**	**Solvent System**	**Ratio**	**Fractions**
Petroleum ether	100: 0	F_1-F_4	DCM	100:0	F_{107}
Petroleum ether: EtOAc	95:5	F_5		90:10	F_{108}-F_{115}
	90:10	F_6		80:20	F_{116}-F_{119}
	85:15	F_7-F_{16}		70:30	F_{120}-F_{122}
	80:20	F_{17}-F_{32}		60:40	F_{123}-F_{125}
	70:30	F_{33}-F_{39}		50:50	F_{126}-F_{128}
	60:40	F_{40}-F_{50}	MeOH	0:100	F_{129}-F_{130}
	50:50	F_{51}-F_{61}			
	40:60	F_{62}-F_{64}			
	30:70	F_{65}-F_{73}			
	20:80	F_{74}-F_{79}			
	10:90	F_{80}-F_{88}			
EtOAc	0:100	F_{89}-F_{106}			

3.7. Structural Elucidation

The FTIR spectroscopic technique were employed to partially elucidate the structure of isolated compounds. The FTIR were obtained at the Department of Chemistry, Addis Abeba Science and Technology University.

3.8. Biological Activities

The antibacterial and antioxidant activities of extracts and essential oil were done in the Department of Applied Biology laboratory, Adama Science and Technology University.

3.8.1. Antibacterial Activity (Disk Diffusion Assay)

In vitro antibacterial activity of the extracts and essential oil of *Pentas schimperiana* stem bark were studied against four pathogenic bacterial strains obtained from Adama Science and Technology University, Department of Applied Biology Laboratory. The strains used in the experiment were Gram-negative *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), and Gram-positive *Staphylococcus aureus* (ATCC25923) and *Streptococcus pyogen*s (ATCC19615) bacterial species. The bacterial strains were selected based on the availability and considering the likely bacterial strains that can cause bacterial infections.

The concentration of samples was prepared by dissolving required amounts in DMSO to give sets of four concentrations, 200, 100, 50, and 25 mg/mL for extracts and 1, 0.5, 0.25, and 0.125 mg/mL concentrations were used for essential oil.

Likewise, the reference antibiotics (Ciprofloxacin) were used at 0.5 mg/mL as positive control and DMSO as negative control (Blandino *et al.,* 2014).

In the experiment, Mueller-Hinton agar containing plates were inoculated with the standardized inoculum of the test micro-organisms (corresponding to 0.5 McFarland turbidity standard). Four sterile filter paper disks (Whatman No. 1 of approximately 6 mm in diameter) were impregnated with 20 µL of desired concentrations of either the crude, essential oil or controls, and placed on the inoculated agar surface. These plates were incubated under suitable conditions, at 37°C for 24 hrs (Girmay et al., 2014).The diameter of the growth inhibition zones around each disc was then measured in millimeters using a sliding caliper. The experiment was done in triplicate, and the results were expressed as mean ± standard deviation.

3.8.2. DPPH Radical Scavenging Assay

Antioxidant activity of the crude extracts and essential oil was measured based on their free radical scavenging activity, which was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical Scavenging Assay. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free

radical which has been used to simulate the antioxidant activity of chemical constituents of essential oils, extracts, and other substances from natural products (Do Nascimento et al., 2020). In this assay, DPPH solution (0.004%) was prepared in methanol by dissolving 0.004 g DPPH in 100 mL methanol. The solution was kept in darkness for 30 min to complete the reaction. The crude extracts and essential oil were serially diluted as 1000, 500, 250, 125, 62.5 µg/mL (crude extracts) and 400, 200, 100, 50, 25 µg/mL (essential oil). To each serially diluted solution of test samples, 1 mL of 0.004% DPPH was added. The resulting solutions were placed in the dark for 30 minutes and then subjected to a UV-Vis spectrophotometer to record absorbance at 517 nm. Ascorbic acid with a similar concentration of test samples was used as the positive control. The DPPH radical scavenging activity of each of the tested compounds was reported by percentage inhibition using the following formula (2) (Karakoti et al., 2022b).

$$\% \text{ of DPPH radical scavenging activity} = \frac{(Ac - As)}{Ac} * 100 \text{----------(2)}$$

Where A_c and A_s are the absorbance values of control (DPPH radical + methanol) and samples, respectively. Percent of DPPH radical scavenging activity was plotted against concentrations, and the equation for the line was used to obtain the IC_{50} (half-maximal inhibitory concentration) values.

3.9. Computational Study of selected compounds from essential oil

Based on highest percentage composition volatile compounds were selected from the essential oil of *Pentas schimperiana* stem bark and their structures were drawn using ChemDraw 22.0.0 for computational study.

3.9.1. *In silico* Molecular Docking Study

Virtual ligand screening is an *in-silico* method used to dock small molecules (ligand) to macromolecule (protein) to discover potent compounds that have the necessary biological effect (Hempel et al., 2015; Kitchen et al., 2004).

The molecular docking study of the selected compounds from essential oil *Pentas schimperiana* was carried out on *S. aureus* pyruvate kinase and *E. coli* DNA gyrase B receptors, as this protein has been reported as a molecular target for compounds with antibacterial activity (Nouioura et al., 2024) and the second protein taken was Human myeloperoxidase, which has a broader activity against reactive oxygen species (Alam et al., 2021). The three-dimensional (3D) structures of the DNA gyrase B (PDB ID: 6F86), pyruvate kinase (PDB ID: 3T07) and Human

myeloperoxidase (PDB ID: 1DNU) proteins were obtained from the (RCSB) https://www.rcsb.org/ Protein Data Bank. The 3D structures of the selected proteins converted into PDB formats by deleting the water molecules, HETATOMS, and adding polar hydrogens using Biovia Discovery Studio-2021 Client. The compounds from the essential oil for docking studies were selected based on their higher percentage contents and their concerned structures were obtained from the PUBCHEM database (https://pubchem.ncbi.nlm.nih.gov/, accessed on 12 August 2022) in the SDF (structure data file) format. Structures of the ligands in their SDF format were then imported into PyRx Software using an open babel tool embedded in PyRx software. Energy minimization (optimization) was performed by adding charges and optimizing the universal force field. Further, the ligands were converted into AutoDock Ligand format (PDBQT). To find out the binding affinity and to know the various ligand– receptor interactions responsible for the antibacterial and antioxidant activity, the molecular docking of the selected major constituents was performed using PyRx with Vina Wizard tool. The protein and multiple ligands to be docked were selected in the PyRx software using the Vina Wizard Control. The "Run Vina" control was selected to initiate the docking process. The results were observed by selecting the "Analyze Vina" tool and exported as CSV files. Biovia Discovery Studio-2021 Client was used for the visualization of 2D and 3D interactions of docking poses (Kim & Wyss, 2015).

3.9.2. *In Silico* ADMET Study

3.9.2.1. In silico Drug-Likeness

The selected compounds were evaluated for their pharmacokinetic profiles by *in silico* methods for predicting their molecular properties using Lipinski's Rule of Five, Egan and the Veber rule (Drwal et al., 2014). The structures of the selected compounds from the essential oil were drawn using ChemDraw 22.0.0 for the pharmacokinetics (absorption, distribution, metabolism, and excretion (ADME)) studies. The physicochemical and pharmacokinetics properties of the compounds were estimated using ADME descriptors by SwissADME (https://www.swissadme.ch/, accessed on 12 August 2022), online server as per the developed protocol (Daina et al., 2017). To estimate *in silico* and pharmacokinetic parameters and other molecular properties, the compound structures were submitted to SwissADME tools and converted to their canonical simplified molecular-input line-entry system "SMILES".

3.9.2.2. Toxicity Predictions

The organ toxicity profiles and toxicological endpoints of the selected compounds and toxicity classes were predicted using the Pro Tox II web server explore (https://toxnew.charite.de/, accessed on 12 August 2022). It calculates the prediction based on different parameters such as organ toxicity (hepatotoxicity), oral toxicity, and toxicological endpoints (cytotoxicity, mutagenicity, carcinotoxicity, and immunotoxicity) (Karakoti et al., 2022).

3.10. Statistical Data Analysis

The data of antibacterial and antioxidant activities were the averages of triplicate analyses. Data were recorded as mean ± standard deviation (SD). The results are expressed as 50% inhibition concentration (IC_{50}). The sample concentration that provides 50% activity (IC_{50}) was calculated from linear regression analysis of percent inhibitory activity versus sample concentration. All data were analyzed with the aid of Excel Microsoft office, and Origin pro 2022 software.

4. RESULTS AND DISCUSSIONS

4.1. Extraction Yields of *Pentas schimperiana*

The extraction yield measures the solvent efficiency to extract specific components from the original plant powder (Abdisa & Tarekegn, 2021). The plant material (stem bark) (790 g) of *Pentas schimperiana* was successively extracted by maceration with petroleum ether, chloroform, and methanol. The weight of extract as well as the percentage yield of the dry extracted sample from the three solvents were recorded in Table 3.

Table 3: Percentage yields of *Pentas schimperiana* crude extracts

Crude extracts	Yields (g)	Percentage yield (%)
PET	1.74	0.22
$CHCl_3$	6.55	0.83
MeOH	26.71	3.38

PET-Petroleum ether, **$CHCl_3$** -Chloroform, **MeOH-** Methanol

The results showed that the percentage yields increase with increasing the polarity of extractive solvents. The extraction yield obtained using methanol solvent was higher compared to petroleum ether and chloroform extracts suggesting the stem bark of the *Pentas schimperiana* contain polar secondary metabolites.

This observation is common in most of the extraction of bioactive compounds and is associated with the ability of methanol to dissolve polar compounds and to some extent non-polar groups. Methanol, having a polarity index of 5.1 next to water, is chosen as an extraction solvent to extract bioactive compounds (Ngo et al., 2017).

4.2. Phytochemical Analysis of *Pentas schimperiana* extracts

The preliminary phytochemical screening of extracts of the stem bark of *Pentas schimperiana* indicated the presence of various secondary metabolites and the result of phytochemical test has been summarized in Table 4. Alkaloids, phenolic compounds, flavonoids, saponins, tannins, steroids, glycosides and terpenoids were found in stem bark extracts. Methanol extract showed positive test for all of the secondary metabolites screened as compared to the remaining solvents utilized in this experiment.

Earlier phytochemical screening reports showed the presence of saponins, flavonoids, tannins, steroidal and phenolic compounds in leaf extracts of *Pentas schimperiana* (Dinku et al., 2011a).

The phytochemicals like phenolic, alkaloids, flavonoids and terpenoids are known to possess a variety of biological activities including antibacterial, antifungal, antioxidant, antiviral, anti-inflammatory, antitumor, anticancer, enzyme inhibition and antidiabetic (Akter et al., 2016a). The presence of these secondary metabolites supports the traditional medicinal use of different parts of *Pentas schimperiana* to treat various diseases caused by microorganisms.

Table 4 : Phytochemical screening tests on the stem bark extracts of *Pentas schimperiana*

Secondary Metabolites	Tests	Solvents Used for extraction		
		PET	$CHCl_3$	MeOH
Alkaloids	Wagner test	-	+	+
Flavonoids	Alkaline test	+	+	+
Glycosides	Salkowski's Test	-	-	+
Phenols	Ferric chloride test	+	+	+
Steroids	Salkowski's Test	+	+	+
Saponins	Foam Test	+	+	+
Tannins	Ferric chloride test	+	+	+
Terpenoids	Salkowski's Test	+	+	+

\+ shows Presence - shows Absence

4.3. Chemical composition of stem bark essential oil of *Pentas schimperiana*

Dried stem bark of *Pentas schimperiana* was subjected to Hydrodistillation for 3 h to isolate essential oil using a Clevenger-type apparatus. The oil yield (w/w) was 0.062 % (62 mg/100 g). A total of 29 components were detected. As summarized in Table 5. **14:** Butyl 9,12-octadecadienoate (14.26 %), **15:** linoelaidic acid (14.23 %), **16:** 9-octadecenoic acid (Z)-, oxiranylmethyl ester (13.56 %), **17:** oleic Acid (11.41 %), **18**: β-Monolinolein (8.44 %), **19**: glyceryl monooleate (6.86 %), **20:** methyl cinnamate (5.71 %), **21:** n-hexadecanoic acid (4.82 %), **22:** linalool (3.14 %), **23**: glycidyl palmitate (3.04 %), **24:** palmitic acid glycerol ester (1.54 %), **25:** hydroquinone (1.07 %) were the abundant compounds in essential oil of stem bark of *Pentas schimperiana*. Their structures and chromatogram were shown in figure 4 and Figure 5 respectively.

In terms of chemical class composition, essential oil of *Pentas schimperiana* was dominated by fatty acids and ester, oxygenated monoterpenes, aromatic compounds, sesquiterpenes, and phenolic compounds.

The compounds identified in the tested essential oil of *Pentas schimperiana* stem bark have potent biological applications and commercial uses. Linalool and linalool-rich essential oils have been reported to exhibit a wide range of biological activities, including antimicrobial, anti-inflammatory, anticancer, and antioxidant properties. Additionally, several in vivo studies have confirmed various effects of linalool on the central nervous system. The applications of linalool extend beyond its use as a fragrance in domestic products, as it plays an important role in plant pollination biology and is a key compound for the industrial production of various fragrance chemicals and vitamins. Furthermore, the repellent properties of linalool on crop-destroying insects have been well documented, highlighting its potential in eco-friendly pest management strategies (Kamatou & Viljoen, n.d.).

Eugenol has been reported to possess a wide range of beneficial biological properties, including antibacterial, antiviral, antifungal, anticancer, anti-inflammatory, and antioxidant activities. The compound has been extensively utilized in various areas such as cosmetology, medicine, and pharmacology for the development of diverse products and therapeutic applications (Ulanowska & Olas, 2021). Hydroquinone serves various applications as a reducing agent in photographic developers, an antioxidant, and a polymerization inhibitor in the production of monomers, polymers, dyes, pigments, rubber products, and various chemicals (Valenzuela et al., 2024).

Table 5 : Chemical composition of essential oil of stem bark of *Pentas schimperiana*

No	Compound Name	Molecular Formula	RI	% Composition
1.	Linalool	$C_{10}H_{18}O$	1025.17	3.14
2.	Phenylethyl Alcohol	$C_8H_{10}O$	1033.50	0.27
3.	Benzene acetaldehyde	C_8H_8O	1054.05	0.25
4.	α -Terpineol	$C_{10}H_{18}O$	1072.51	0.15
5.	Lemonol	$C_{10}H_{18}O$	1100.36	0.25
6.	Hydroquinone	$C_6H_6O_2$	1107.02	1.07
7.	Bromoacetic acid, 2-ethylcyclohexyl ester	$C_{10}H_{17}BrO_2$	1108.53	0.15
8.	Eugenol	$C_{10}H_{12}O_2$	1144.02	0.17
9.	Methyl cinnamate	$C_{10}H_{10}O_2$	1155.15	5.71
10.	β – elemene	$C_{15}H_{24}$	1158.86	0.23
11.	Germacrene D	$C_{15}H_{24}$	1194.10	0.23
12.	Bicylogermacrene	$C_{15}H_{24}$	1199.91	0.15
13.	Bisabolene	$C_{15}H_{24}$	1201.35	0.17
14.	γ-Cadinene	$C_{15}H_{24}$	1205.56	0.83
15.	τ-Cadinol	$C_{15}H_{26}O$	1253.51	0.59
16.	Tetradecanoic acid	$C_{14}H_{28}O_2$	1300.53	0.21
17.	Palmitoleic acid	$C_{16}H_{30}O_2$	1413.67	0.18
18.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	1431.76	4.82
19.	Linoelaidic acid	$C_{18}H_{32}O_2$	1590.24	14.23
20.	Oleic Acid	$C_{18}H_{34}O_2$	1596.03	11.41
21.	Octadecanoic acid	$C_{18}H_{36}O_2$	1614.01	0.93
22.	Glycidyl palmitate	$C_{19}H_{36}O_3$	1961.33	3.04
23.	Butyl 9,12-octadecadienoate	$C_{22}H_{40}O_2$	2065.26	14.26
24.	9-Octadecenoic acid (Z)-, oxiranylmethyl ester	$C_{21}H_{38}O_3$	2066.91	13.56
25.	Glycidyl (Z)-9-Heptadecenoate	$C_{20}H_{36}O_3$	2073.95	0.91
26.	Palmitic acid glycerol ester	$C_{19}H_{38}O_4$	2075.68	1.54

27.	Linoleic acid	$C_{18}H_{32}O_2$	2086.37	0.43
28.	β-Monolinolein	$C_{21}H_{38}O_4$	2181.80	8.44
29.	Glyceryl Monooleate	$C_{21}H_{40}O_4$	2185.08	6.87

RI value = Retention index value

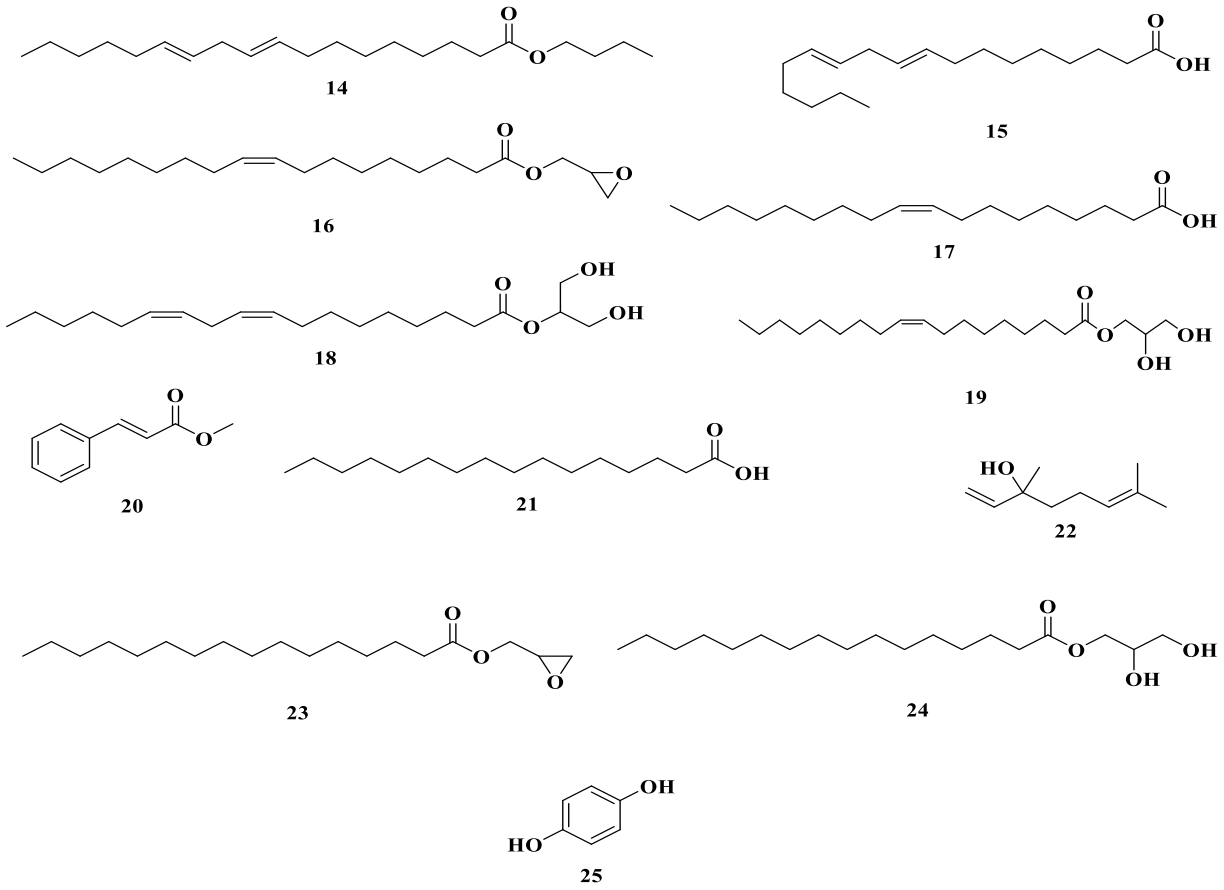


Figure 4 : The structures of major compounds from the essential oil of *Pentas schimperiana* stem bark

14: Butyl 9,12-octadecadienoate, **15:** Linoelaidic acid, **16:** 9-Octadecenoic acid (Z)-, oxiranylmethyl ester, **17**: Oleic Acid, **18:** β-Monolinolein, **19:** Glyceryl Monooleate, **20:** Methyl cinnamate, **21:** n-Hexadecanoic acid, **22:** Linalool, **23:** Glycidyl palmitate, **24:** Palmitic acid glycerol ester, **25**: Hydroquinone

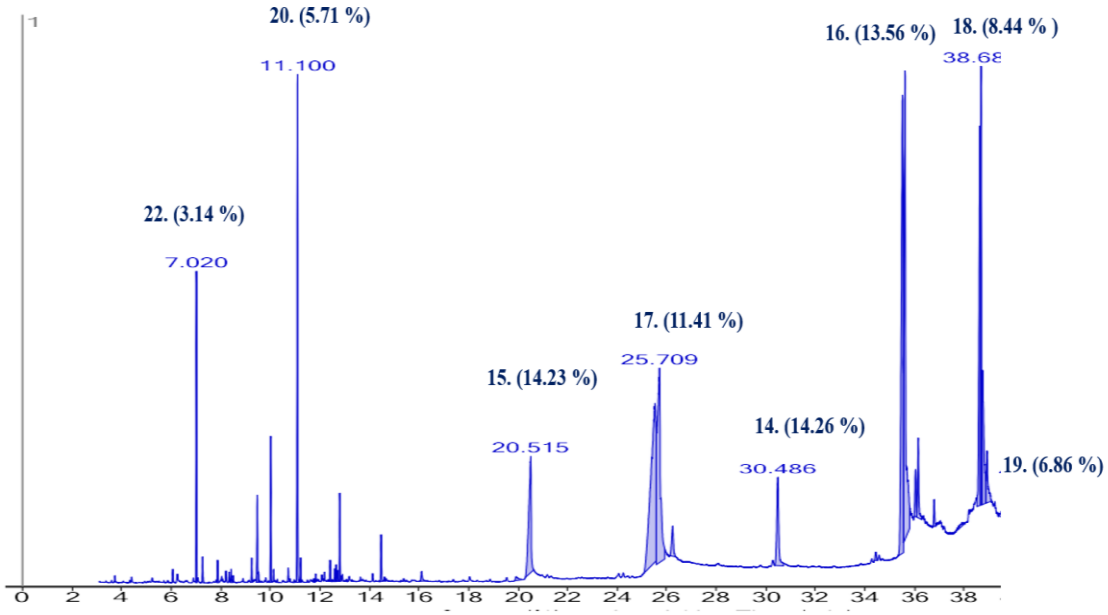


Figure 5: GC-MS chromatogram of essential oil extracted from *Pentas schimperiana* stem bark

4.4. Characterization of compounds isolated from the stem bark of *Pentas schimperiana*

Compound **PS-1** (30.4 mg) was obtained as a white powder from combined chloroform-methanol extracts of the stem bark of *Pentas schimperiana*. TLC showed a spot at Rf 0.6 using 10% EtOAc in n-hexane as mobile phase, which was visualized after spraying with vanillin/H_2SO_4 followed by heating on hot plate. The melting point was determined to be 135-140 ℃. The FTIR spectrum (Appendix 1) was analyzed, and the broad peak around 3400-3500 cm^{-1} was found to indicate the presence of hydroxyl (O-H) groups, which could be from alcohols, phenols, or water. The peaks in the 2800-3000 cm^{-1} range suggested the existence of C-H stretching vibrations, typically associated with alkyl groups. The sharp, intense peak around 1650-1750 cm^{-1} was likely due to C=O stretching vibrations, potentially from carbonyl groups in aldehydes, ketones, or carboxylic acids. The distinct features in the 1000-1300 cm^{-1} region were found to provide more detailed information about the specific molecular structures and functional groups present in the compound **PS-1**.

Compound **PS-2** (74.2 mg) was obtained as a white powder, and TLC showed a spot at Rf 0.7 using 30% EtOAc in n-hexane as mobile phase, which was visualized after spraying with vanillin/H_2SO_4 followed by heating on hot plate. The melting point was determined to be 175-180 ℃. The FTIR spectrum (Appendix 2) was analyzed, and the broad peak around 3400-3500 cm^{-1} was found to indicate the presence of hydroxyl (O-H) groups, which could be from alcohols and phenols. The peaks in the 2800-3000 cm^{-1} range suggested the existence of C-H stretching vibrations, typically associated with alkyl groups. The sharp, intense peak around 1650-1750 cm^{-1} was likely due to C=O stretching vibrations, potentially from carbonyl groups in aldehydes, ketones, or carboxylic acids. The distinct features in the 1000-1300 cm^{-1} region were found to provide more detailed information about the specific molecular structures and functional groups present in the compound **PS-2**.

4.5. Antibacterial Activity

The *in-vitro* antibacterial activity of extracts and essential oil from the stem bark of *Pentas schimperiana* were tested against the Gram-negative bacteria *E. coli* and *P. aeruginosa, the Gram-positive bacteria S. aureus, and S. pyogens* pathogens. The extracts tested were methanol (MeOH), chloroform ($CHCl_3$), and petroleum ether (PET) extracts, as well as the essential oil. The concentrations tested ranged from 25 mg/mL to 200 mg/mL for the extracts, and 0.125 mg/mL to 1 mg/mL for the essential oil. Ciprofloxacin (CPFX) was used as a positive control at a concentration of 0.5 mg/mL and DMSO as negative control. The obtained mean inhibition zone diameters (in mm) are presented in Table 6.

The results showed that all the extracts and the essential oil exhibited antibacterial activity against the tested bacterial strains, except the MeOH extract at a concentration of 25 mg/mL, which showed no effect on *S. pyogens*. The inhibition diameters were observed to increase with increasing concentrations.

All extracts displayed promising growth inhibitory effect at the concentration of 200 mg/mL against the *E. coli* and their zone of inhibition are 20±0.82 mm for MeOH extract, 21.07±0.47 mm for $CHCl_3$ extract and 21.67±0.47mm for PET extract.

The finding is promising in comparison to the standard drug and the drug showed inhibition zones of 26.17±0.9 mm against *E. coli*. In comparatively, the PET extract showed the highest

inhibition among the extracts, with inhibition diameters ranging from 16.67 mm to 21.67 mm at the different concentrations. Ciprofloxacin, the positive control, showed the highest inhibition against all the bacterial strains, with inhibition diameters ranging from 22.83 mm to 26.5 mm.

The essential oil of *Pentas schimperiana* stem bark showed highest zone of inhibition 15±0.00 mm against *E. coli* at concentration of 1 mg/mL, followed by inhibition against *P. aeruginosa* with the zone of inhibition values of 14±1.00 mm. Generally, the results of the *in vitro* antibacterial activity showed that all extracts and essential oil exhibited growth inhibition against all selected bacterial strains in dose-dependent manner.

The antibacterial activities of the extracts and essential oil were likely due to the ability of their secondary metabolites to interact with the proteins and cell walls of the microorganisms. The higher inhibition effect observed may have been because the crude extracts and essential oil contained a wider range of antimicrobial compounds, which were more soluble and therefore more effective (Akter et al., 2016b). Therefore, phytochemical compounds found in various plant parts are abundant and serve as natural protection against microbial illnesses.

The phytochemical examination of the crude extract of *Pentas schimperiana* revealed the presence of several classes of secondary metabolites, including alkaloids, flavonoids, terpenoids, tannins, saponins, phenolic compounds, glycosides, and steroids, as shown in Table 5. These diverse phytochemical compounds present in the stem bark extract and essential oil of *Pentas schimperiana* are likely responsible for the observed antibacterial activities (Dubale et al., 2023b).

Table 6 : Antibacterial activity of the crude extracts and essential oil of the stem bark of *Pentas schimperiana* against selected bacterial strains.

Tested samples	Concentration (mg/mL)	Inhibition Diameter mm (mean±SD)			
		E. oil	*S. aureus*	*P. aeruginosa*	*S. pyogens*
MeOH Extract	200	20±0.82	14±0.82	16±0.82	11±0.82
	100	17.67±0.47	11.33±0.47	13±0.82	8.67±0.47
	50	15.33±1.70	9±0.82	8.67±0.47	6.67±0.47
	25	10±0.82	6.67±0.47	6.67±0.47	NE
$CHCl_3$ Extract	200	21.07±0.47	15.33±0.47	16.33±0.47	13.67±0.47
	100	20.67±0.47	13±0.82	15±0.00	12±0.82
	50	19.67±0.47	8.33±0.47	11.33±0.47	8.67±0.47
	25	15±0.82	6.67±0.47	7.67±0.94	6.33±0.47
PET Extract	200	21.67±0.47	17±0.82	18±0.82	18.33±0.47
	100	20.33±0.47	14.33±0.47	15±0.82	14±0.82
	50	19±0.82	9.67±0.47	12.33±0.47	10.33±0.47
	25	16.67±0.47	7±0.82	10.33±0.47	7.33±0.47
Essential oil	1	15±0.00	12.67±1.15	14±1.00	10.67±1.15
	0.5	14±2.00	12.33±1.15	13.67±0.58	10±1.73
	0.25	11.33±0.58	12±.00	12±1.00	8.33±2.52
	0.125	11±0.00	9.33±2.08	10.67±0.58	7.67±2.89
CPFX	0.5	26.17±0.9	22.83±1.77	26.5±1.38	24.83±0.9

NE= No Effect**, CPFX=**ciprofloxacin, **SD=**Standard deviation**, *S. aureus*** = *Staphylococcus aureus*, ***S. Pyogenes*** = *Streptococcus pyogenes*, ***P. aeruginosa*** = *Pseudomonas aeruginosa*, ***E. coli*** = *Escherichia coli*

4.6. DPPH Radical Scavenging Activity

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The antioxidant compounds present in the essential oil and crude extracts react with the DPPH, thus converting it to 1-diphenyl-2-picryl hydrazine (Sadeer et al., 2020).

The reaction mechanism of DPPH radical with a phenolic antioxidant is shown in Figure 6.

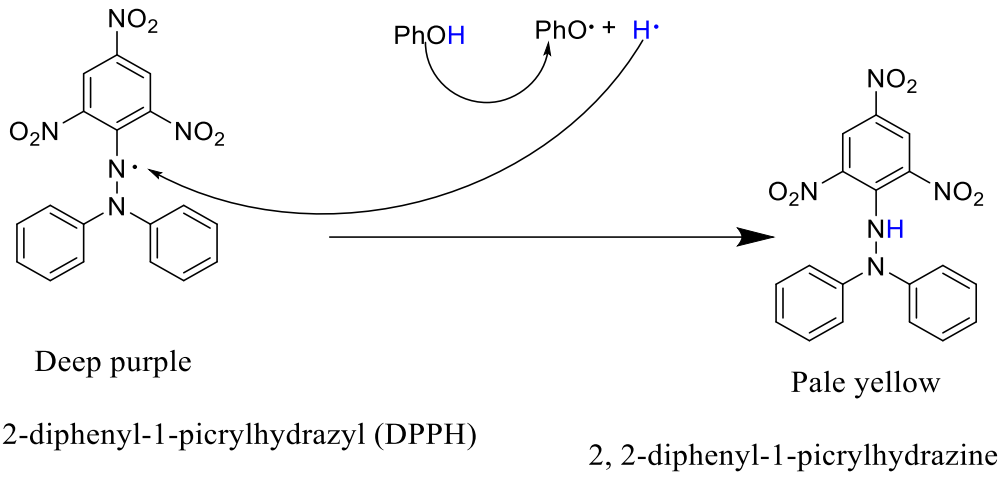


Figure 6: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) reaction mechanism

The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants and visually noticeable by the color change from purple to yellow. Therefore, DPPH is commonly used as a substrate to evaluate the antioxidant activity (Abdisa & Tarekegn, 2021b).

The antioxidant activity assay using the DPPH free radical scavenging method was conducted on three different crude extracts (PET, $CHCl_3$, and MeOH) as well as a positive control, ascorbic acid. The results were presented in Table 7 and Figure 7.

The results were found that the antioxidant activity (% RSA) increased with increasing concentration for all the extracts and the positive control. The PET extract showed the highest antioxidant activity, with % RSA values ranging from 74.73% at 62.5 µg/mL to 87.61 % at 1000 µg/mL. The $CHCl_3$ extract had the lowest antioxidant activity among the three extracts, with % RSA values ranging from 22.70 % at 62.5 µg/mL to 65.21% at 1000 µg/mL. The MeOH extract had moderate antioxidant activity, with % RSA values ranging from 48.36 % at 62.5 µg/mL to 86.9 % at 1000 µg/mL. The positive control, ascorbic acid, showed the highest antioxidant activity, with % RSA values ranging from 90.98 % at 62.5 µg/mL to 94.35 % at 1000 µg/mL.

The IC_{50} values (the concentration required to inhibit 50% of the DPPH radicals) is a parameter widely used to measure the antioxidant activity of test samples. It is calculated as the

concentration of antioxidants needed to decrease the initial DPPH concentration by 50 %. Thus, lower IC_{50} values correspond to a higher antioxidant capacity (Karakoti et al., 2022a).

The IC_{50} values were calculated, and it was found that the PET extract had the lowest IC_{50} value of 2.81 µg/mL, followed by the MeOH extract (5.3 µg/mL) and the $CHCl_3$ extract (12.83 µg/mL). Ascorbic acid had an IC_{50} of 0.5 µg/mL.

Compared to the standard ascorbic acid, the DPPH radical scavenging activities of the extracts were found to be lower, except for the PET extract, which showed comparable activity with a lower IC_{50} value (2.81 µg/mL), indicating its strong antioxidant activity.

Based on these results, the PET extract was determined to have the highest antioxidant activity among the three extracts tested, with activity comparable to the positive control, ascorbic acid. This suggests that the PET extract may be a promising source of natural antioxidants. The superior radical scavenging activity displayed by the petroleum ether (PET) crude extract can be attributed to the polarity and solubility of the antioxidant compounds, as well as the specific phytochemical composition of the extract. Petroleum ether, being a relatively non-polar solvent, is more effective at extracting non-polar or lipophilic antioxidant compounds from the plant material. The bioactive antioxidant compounds present in this plant, such as terpenoids, carotenoids, or fat-soluble vitamins, are likely more soluble in the non-polar petroleum ether, allowing for a higher extraction yield of these potent antioxidants (Shen et al., 2023). As a result, the PET extract may contain a higher concentration or a different composition of antioxidant compounds that are more effective at neutralizing the free radicals in the assay compared to the other solvent extracts, leading to its superior radical scavenging activity.

In earlier study, both the hydroalcoholic extracts and the methanol fraction of the dried leaves of *P. schimperiana* displayed a very good DPPH scavenging activity with IC_{50} values of 7.83 and 8.84 µg/ml, respectively, while ascorbic acid showed an IC_{50} value of 4.42 µg/ml (Dinku et al., 2011a). Therefore, *Pentas shimperiana* contain antioxidant phytochemicals in leaf as well as stem bark.

Table 7 : DPPH radical scavenging activities (%) of *Pentas schimperiana* stem bark extracts.

Concentration (µg/ml)	Control	Crude Extracts						Positive control	
		PET		$CHCl_3$		MeOH		Ascorbic Acid	
		A	%RSA	A	%RSA	A	%RSA	A	%RSA
1000	1.009	0.125	87.61	0.351	65.21	0.14	86.9	0.057	94.35
500	1.009	0.147	85.43	0.359	64.42	0.151	85.03	0.066	93.46
250	1.009	0.186	81.56	0.494	51.04	0.252	75.02	0.075	92.57
125	1.009	0.212	78.99	0.648	35.78	0.342	66.10	0.083	91.77
62.5	1.009	0.255	74.73	0.78	22.70	0.521	48.36	0.091	90.98
IC_{50}		2.81		12.83		5.3		0.5	

Each value is a mean of three biological replicates.

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

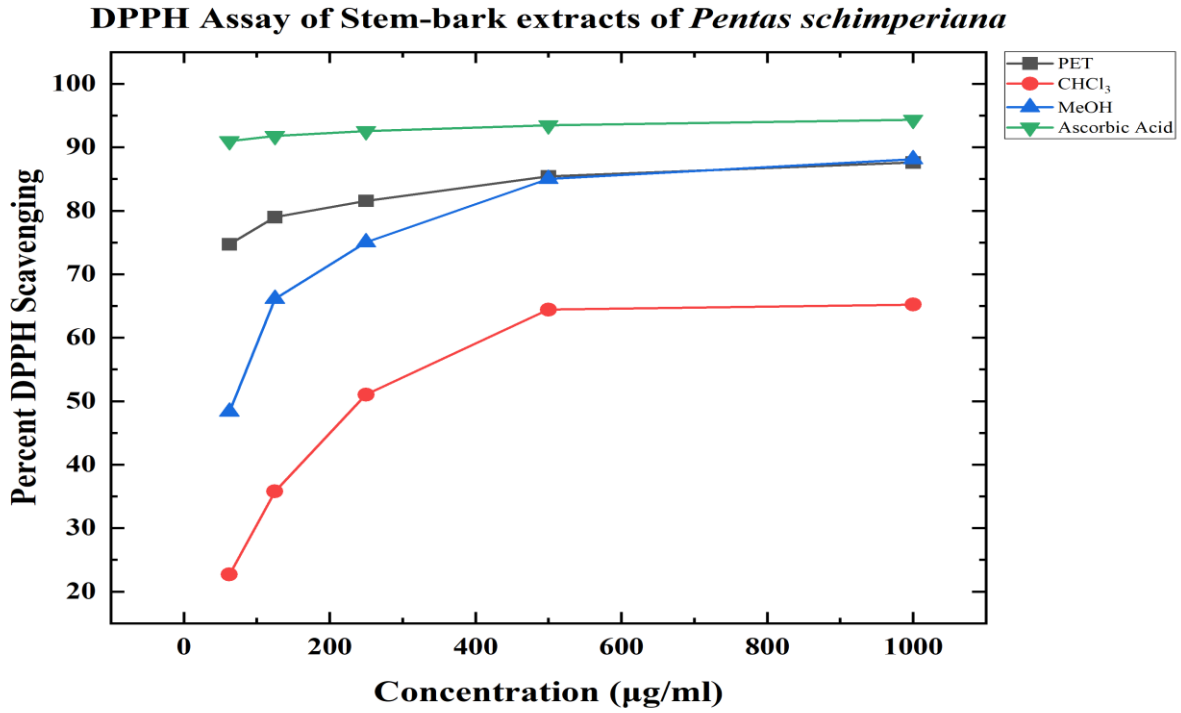


Figure 7: DPPH radical scavenging activities (%) of *Pentas schimperiana* stem bark extracts.

The antioxidant activity assay using the DPPH free radical scavenging method was conducted on an essential oil and a positive control (ascorbic acid) The results were presented in Table 8 and Figure 8, and it was found that the antioxidant activity (% RSA) increased with increasing concentration for both the essential oil and the positive control. The positive control, ascorbic acid, showed higher antioxidant activity compared to the sample (essential oil).

The essential oil exhibited good antioxidant activity, with % RSA values ranging from 55.59% at 25 µg/mL to 72.08 % at 400 µg/mL. In comparison, the % RSA values for ascorbic acid, used as a positive control, were slightly higher, ranging from 59.10 % at 25 µg/mL to 77.88 % at 400 µg/mL. While the essential oil's antioxidant activity, as measured by % RSA, was moderately lower than that of ascorbic acid at the corresponding concentrations, the essential oil still demonstrated strong antioxidant potential, with % RSA values above 55 % even at the lowest concentration tested. Overall, the essential oil showed good antioxidant activity, though it was moderately lower than the antioxidant activity of the ascorbic acid positive control across the tested concentration range.

The IC_{50} values (the concentration required to inhibit 50 % of the DPPH radicals) were calculated as 6.63 µg/mL for the essential oil and 2.89 µg/mL for the positive control (ascorbic acid). Based on these results, the positive control (ascorbic acid) was determined to have higher antioxidant activity compared to the essential oil. However, the essential oil was also found to exhibit good antioxidant activity, suggesting its potential as a source of natural antioxidants.

The high antioxidant activity of the *Pentas schimperiana* stem bark essential oil for the DPPH radical scavenging is likely due to the presence of a substantial amount of hydroquinone, as well as other constituents of the *Pentas schimperiana* stem bark essential oil, such as eugenol, oleic acid, and linoleic acid, which already possess antioxidant potential through various parameters (Deshmukh & Gaikwad, 2024). Since essential oils are complex mixtures of numerous compounds, their overall biological activity is challenging to fully explain. Therefore, research on the antioxidant and antibacterial activity of essential oils typically indicates that other minor chemical constituents, which may interact synergistically or antagonistically, can also contribute

to the overall antioxidant and antibacterial activity by producing an additive and effective system against free radicals and microorganisms, respectively (Asdadi et al., 2015; Ciftci et al., 2011).

Table 8 : DPPH radical scavenging activity (%) of Essential oil.

Concentration (µg/ml)	Control	Sample (Essential oil)		Positive Control (Ascorbic Acid)	
		A	%RSA	A	%RSA
400	1.225	0.342	72.08	0.271	77.88
200	1.225	0.351	71.35	0.32	73.88
100	1.225	0.401	67.26	0.341	72.16
50	1.225	0.472	61.47	0.461	62.37
25	1.225	0.544	55.59	0.501	59.10
IC_{50}		6.63		2.89	

Each value is a mean of three biological replicates.

A- Absorbance, **RSA**-Radical Scavenging Activity, **IC_{50}** -half-maximal inhibitory concentration

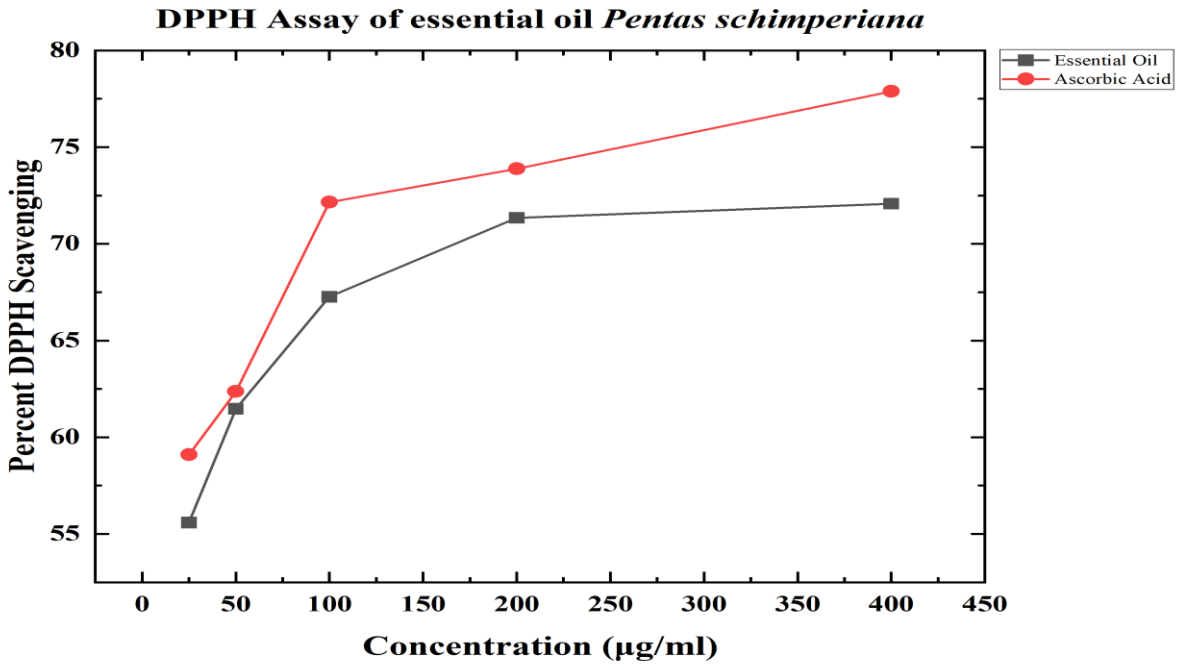


Figure 8 : DPPH radical scavenging activity (%) of essential oil *Pentas schimperiana* stem bark

4.7. Computational Analysis

The five major compounds were selected from the essential oil of *Pentas schimperiana* stem bark based on the highest percentage composition. They were arranged in order and assigned by rank for computational analysis. The compounds were named as follows: Butyl 9,12-octadecadienoate **(14),** linoelaidic acid **(15),** 9-octadecenoic acid (Z)-, oxiranylmethyl ester **(16),** oleic acid **(17),** and β-Monolinolein **(18).** The percentages for each compound were: Butyl 9,12-octadecadienoate (14.26 %), linoelaidic acid (14.23 %), 9-octadecenoic acid (Z)-, oxiranylmethyl ester (13.56 %), oleic acid (11.41 %), and β-Monolinolein (8.44 %). The structures of these compounds were summarized above in Figure 4.

4.7.1. *In silico* Molecular Docking Study

From in vitro studies, it was found that the essential oil from *Pentas schimperiana* stem bark had exhibited potent antibacterial and antioxidant activities. To support these *in vitro* findings, the major compounds from the essential oil were further examined for their potential to physically bind with key antibacterial and antioxidant proteins.

The essential oil was observed to have displayed good inhibition against the bacterial strains *Escherichia coli and Staphylococcus aureus*. To investigate the potential antibacterial mechanisms, the enzymes DNA gyrase subunit B (PDB ID: 6F86) and pyruvate kinase (PDB ID: 3T07) were selected as target proteins for *E. coli* and *S. aureus,* respectively, as these enzymes were known to play crucial roles in bacterial metabolism and physiology (Aliye et al., 2021).

Furthermore, the essential oil also had demonstrated good free radical scavenging activity. To elucidate the antioxidant mechanisms, the enzyme human myeloperoxidase (PDB ID: 1DNU) was selected as a target protein, as it was known to have a capable of producing reactive oxidants through its peroxidase which leads to several inflammation problems and was mostly involved in the cellular stress (Aouadi et al., 2021).

The subsequent protein-ligand interaction studies were expected to provide insights into the specific binding interactions between the major essential oil compounds and the selected antibacterial and antioxidant target proteins, thereby helping to understand the underlying molecular mechanisms behind the observed *in vitro* biological activities (Agu et al., 2023).

4.7.1.1. Molecular Docking *with E. coli* DNA gyrase

In this study, the *E. coli* DNA gyrase subunit B (PDB ID: 6F86) was used as the target protein to investigate the antibacterial mechanisms of selected compounds from the essential oil of *Pentas schimperiana* stem bark. For comparison, ciprofloxacin (CPFX) was also used as a reference control compound and docked against the same DNA gyrase B target protein.

The binding affinity and specific amino acid interactions between the selected compounds and the DNA gyrase B target were presented in Table 9. Additionally, the 3D and 2D binding interactions between the selected compounds and the DNA gyrase B target were depicted in Figure 9.

By examining the binding affinity and binding interactions of the selected compounds with the DNA gyrase B target, the potential antibacterial mechanisms of the selected compounds at the molecular level were analyzed and it was compared to the known antibacterial drug **CPFX.**

Compound **17** showed the highest binding affinity of -5.6 kcal/mol among the test compounds. Compound **16** had the next highest binding affinity of -5.5 kcal/mol, followed by compound **18** at -5.3 kcal/mol, compound **15** at -5.1 kcal/mol, and compound **14** with the lowest affinity of -4.8 kcal/mol. Compared to the standard **CPFX**, which had a binding affinity of -7.6 kcal/mol, the binding affinities of the test compounds were lower, with compound **17** showing the highest affinity among the test compounds.

CPFX has the most H-bond interactions with Asn-46, Arg-76, and Asp-73. Compound **17** and compound **15** both have H-bond interactions with Val-167, but compound **17** also interacts with Asp-73. Compound **3** has H-bond interactions with Asn-46, Val-120, and two with Gly-119. β-compound **18** has H-bond interactions with Ser-121, Leu-98, and Ile-94. Compound **14** has the fewest H-bond interactions, with only Gly-77. **CPFX** has the most diverse residual interactions, including hydrophobic/electrostatic interactions with Glu-50, Arg-76, Gly-77, Ile-94, and Ile-78, as well as van der Waals interactions with Pro-79, Thr-165, and Ala-47. Compound **15, 17,** and **18** have more extensive hydrophobic/electrostatic and van der Waals interactions compared to the other compounds. Compound **14** and **16** have fewer residual interactions compared to the other compounds.

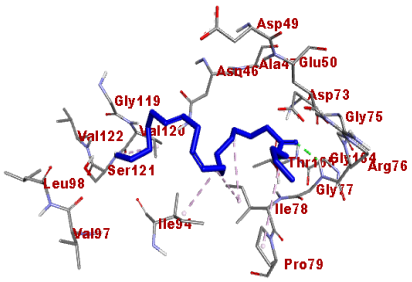
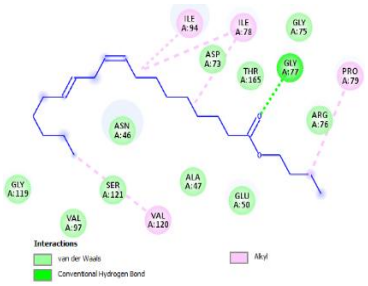
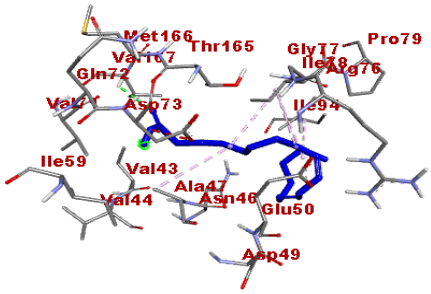
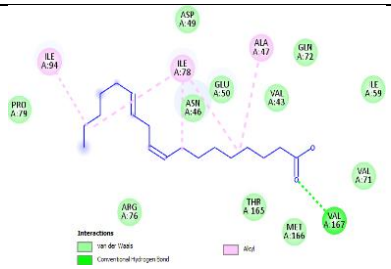
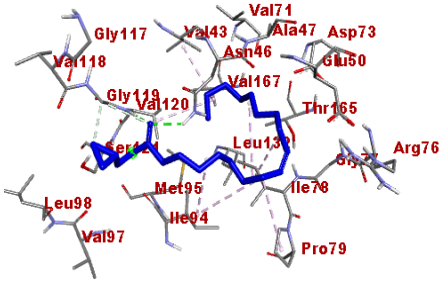
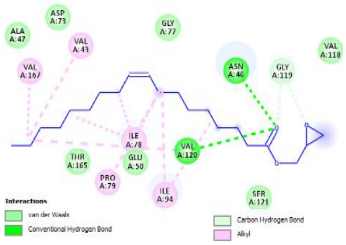
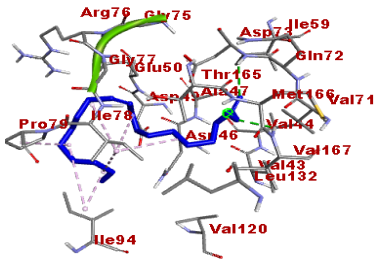
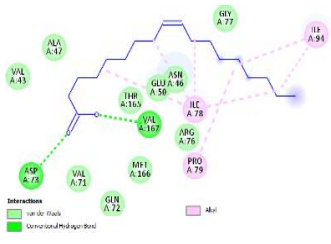
Although compound **17** exhibited a lower binding affinity (-5.6 kcal/mol) compared to the standard **CPFX** (-7.6 kcal/mol), it was identified as the most promising lead compound among the selected set based on the comprehensive binding affinity and interactions with amino acids.

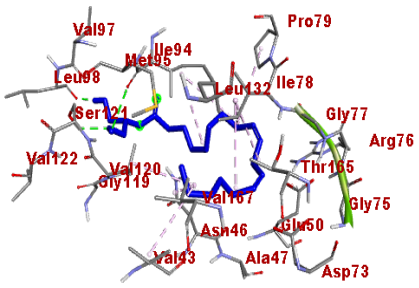
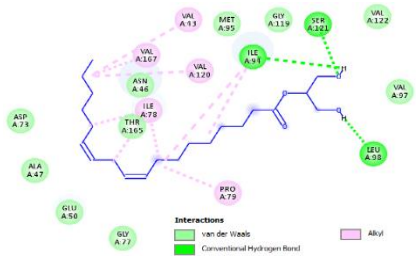
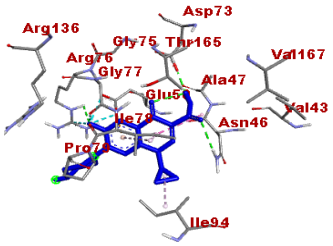
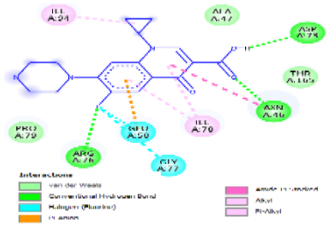
Table 9: Molecular docking binding affinity and interactions of selected compounds and standard with the target *E. coli* DNA gyrase B (PDB ID: 6F86)

Cpds	Affinity (kcal/mol)	H-bond	Residual interactions	
			Hydrophobic/Electrostatic	Van der Waals
14	-4.8	Gly-77	Ile-78, Pro-79, Ile-94, Val-120	Asp-73, Thr-165, Gly-75, Arg-76, Glu-50, Ala-47, Asn-46, Ser-121, Val-97, Gly-119
15	-5.1	Val-167	Ala-47, Ile-78, Ile-94	Pro-79, Asp-49, Asn-46, Glu-50, Val-43, Gln-72, Ile-59, Val-71, Met-166, Thr-165, Arg-76
16	-5.5	Asn-46, Val-120, Gly-119, Gly-119	Val-43, Pro-79, Ile-94, Val-120, Val-167, Ile-78	Ala-47, Asp-73, Gly-77, Val-118, Ser-121, Glu-50, Thr-165
17	-5.6	Asp-73, Val-167	Ile-78, Pro-79, Ile-94	Val-43, Ala-47, Gly-77, Asn-46, Glu-50, Arg-76, Thr-165, Met-166, Gln-72, Val-71
18	-5.3 (pose 7)	Ser-121 Leu-98, Ile-94	Val-43, Pro-79, Val-120, Val-167, Ile-94 Ile-78	Gly-77, Glu-50, Ala-47, Asp-73, Thr-165, Asn-46, Met-95, Gly-119, Val-122, Val-97
CPFX	-7.6	Asn-46, Arg-76, Asp-73	Glu-50, Arg-76, Gly-77, Glu-50, Asn-46, Ile-94, Ile-78	Pro-79, Thr-165, Ala-47

Cpds= Selected Compounds, **CPFX**=Ciprofloxacin

Figure 9: Molecular docking binding interactions (3D & 2D) of selected compounds and standard with the target *E. coli* DNA gyrase B (PDB ID: 6F86)

Cpds	2D	3D
14		
15		
16		
17		

18		
CPFX		

Cpds= Selected Compounds, **CPFX** =Ciprofloxacin, **2D**= Two Dimension, **3D**=Three Dimension

4.7.1.2. Molecular Docking with *S. aureus* Pyruvate kinase

For the docking studies, the *S. aureus* pyruvate kinase (PK) enzyme (PDB ID: 3T07) was used as the target protein. Ciprofloxacin (CPFX) was used as the reference control compound and docked against the same *S. aureus* PK target protein.

The binding affinity and specific amino acid interactions between the selected compounds and the *S. aureus* PK target were presented in Table 10. Furthermore, the 3D and 2D binding interactions between the selected compounds and the *S. aureus* PK target were depicted in Figure 10. The binding affinity and interactions of the selected compounds with the *S. aureus* pyruvate kinase (PK) enzyme were examined. This was done to better understand the potential antibacterial mechanisms of these compounds, focusing on their effects on the PK enzyme. The results were then compared to the known antibiotic **CPFX.**

Among the selected compounds, compound **18** was found to have the highest binding affinity at -5.8 kcal/mol, whereas standard **CPFX** exhibited -7.9 kcal/mol. CPFX formed hydrogen bond interactions with Asn-369 and Ser-362, and hydrophobic/pi-sigma interactions were observed with Ile-361, as well as hydrophobic/pi-pi stacked interactions with His-365. Various van der Waals interactions were also exhibited with Thr-353, Thr-348, Asn-369, Ile-361, His-365, Ser-362, Met-467, Ala-358, and Thr-366.

Hydrogen bonds interactions of compound **18** were formed with Arg-264, Arg-347, and Asp-346. Hydrophobic/alkyl interactions were observed with Lys-342 and Leu-343, as well as hydrophobic/pi-alkyl interactions with Tyr-302. Van der Waals interactions were exhibited with Asp-339, Gln-338, Ala-337, Lys-260, Leu-350, Asn-267, Asp-303, Gly-304, Leu-343, and Tyr-302.

Both compound **17** and **16** had a binding affinity of -5.4 kcal/mol. Compound **17** formed hydrogen bonds with Arg-264, Arg-347, and Asp-346, and displayed hydrophobic/alkyl interactions with Lys-260 and Arg-264, as well as hydrophobic/pi-alkyl interactions with Tyr-302. Van der Waals interactions were observed with Asp-261, Asn-299, Asp-303, Asn-267, and Gly-304. Compound **16** formed a hydrogen bond with Asp-339, exhibited hydrophobic/alkyl interactions with Lys-260 and Leu-343, as well as hydrophobic/pi-alkyl interactions with Tyr-302. Van der Waals interactions were observed with Gln-338, Lys-342, Asp-346, Leu-343, Asp-339, Gln-338, Asp-303, Asn-299, and Lys-260.

Both compound **14** and **15** had a binding affinity of -4.8 kcal/mol. Compound **15** formed a hydrogen bond with Asp-303, displayed hydrophobic/alkyl interactions with Lys-260 and Arg-264, as well as hydrophobic/pi-alkyl interactions with Tyr-302. Van der Waals interactions were exhibited with Lys-342, Tyr-302, Asn-299, and Lys-260. Compound **14** did not form any hydrogen bonds, but showed hydrophobic/alkyl interactions with Lys-342, Lys-260, and Arg-264, as well as hydrophobic/pi-alkyl interactions with Tyr-302. Van der Waals interactions were observed with Asp-261, Asp-303, Asn-299, Tyr-302, Leu-343, Gln-338, and Asp-339.

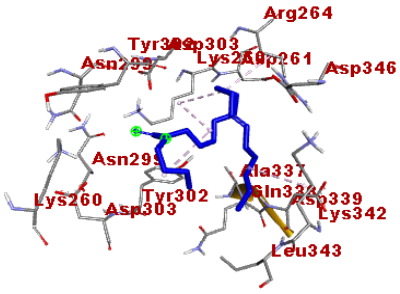
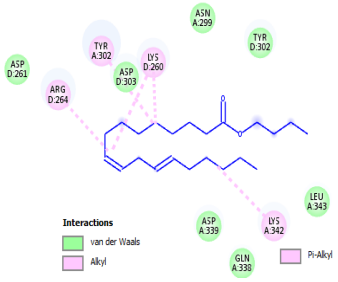
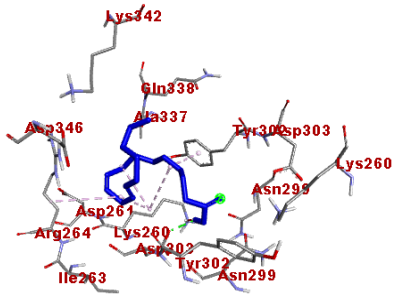
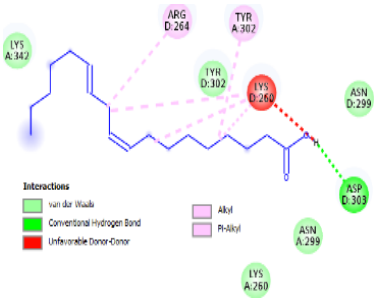
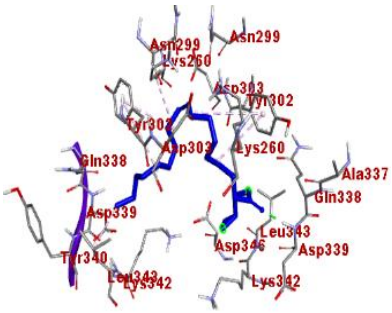
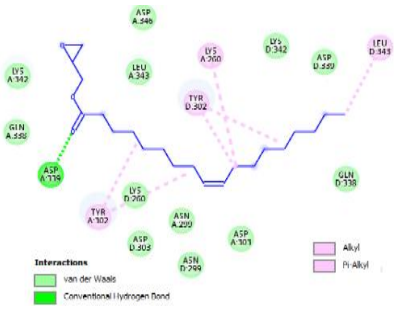
Therefore, based on the binding affinity and the specific interactions formed, compound **18** was identified as the most promising lead compound among the selected compounds, even though it was still slightly weaker than the standard **CPFX**.

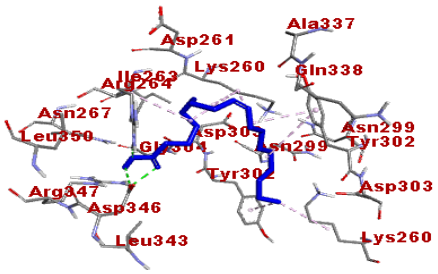
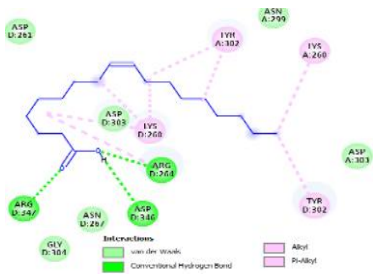
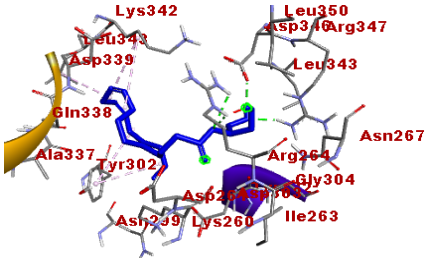
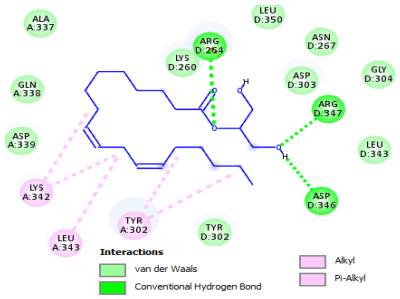
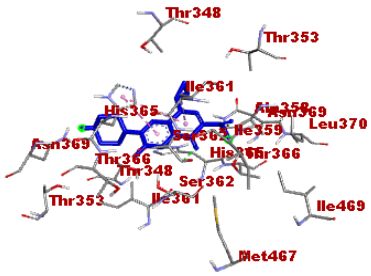
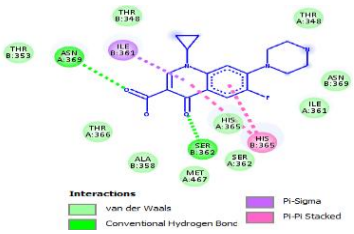
Table 10 : Molecular docking binding affinity and interactions of selected compounds and standard with the target *S. aureus* PK (PDB ID: 3T07)

Cpds	Affinity (kcal/mol)	H-bond	Residual interactions	
			Hydrophobic/ Electrostatic	Van der Waals
14	-4.8	___	Lys-342, Lys-260 Arg-264, Tyr-302	Asp-261, Asp-303, Asn-299, Tyr-302, Leu-343, Gln-338, Asp-339
15	-4.8	Asp-303	Lys-260, Arg-264 Tyr-302	Lys-342, Tyr-302, Asn-299, Lys-260
16	-5.4	Asp-339	Lys-260, Leu-343 Tyr-302	Gln-338, Lys-342, Asp-346, Leu-343, Asp-339, Gln-338, Asp-303, Asn-299, Lys-260
17	-5.4	Arg-264, Arg-347, Asp-346	Lys-260, Arg-264, Tyr302	Asp-261, Asn-299, Asp-303, Asn-267, Gly-304
18	-5.8	Arg-264, Arg-347, Asp-346	Lys-342, Leu-343 Tyr-302	Asp-339, Gln-338, Ala-337, Lys-260, Leu-350, Asn-267, Asp-303, Gly-304, Leu-343, Tyr-302
CPFX	-7.9	Asn-369, Ser-362	Ile-361, His-365	Thr-353, Thr-348, Asn-369, Ile-361, His-365, Ser-362, Met-467, Ala-358, Thr-366

Cpds= Selected Compounds**, CPFX**=Ciprofloxacin

Figure 10: Molecular docking binding interactions (3D & 2D) of selected compounds and standard with the target *S. aureus PK* (PDB ID: 3T07)

Cpds	2D	3D
14		
15		
16		

17		
18		
CPFX		

Cpds= Selected Compounds, **CPFX** =Ciprofloxacin, **2D**= Two Dimension, **3D**=Three Dimension

4.7.1.3. Molecular docking with human myeloperoxidase

In this study, the human myeloperoxidase (PDB ID: 1DNU) enzyme was used as the target. Ascorbic acid (AA) was utilized as a reference control and docked with the myeloperoxidase enzyme.

The binding affinity and interactions with different amino acids were shown in Table 11. The 3D and 2D binding interactions were also depicted in Figure 11.

By examining the binding affinity and interactions with the myeloperoxidase enzyme, the potential mechanisms of the selected compounds were aimed to be further understood and compared to the reference compound ascorbic acid.

All compounds except **14** (-5.0 kcal/mol), and **15** (-5.1 kcal/mol), were shown to have higher binding affinity compared to the standard, **AA** (-5.1 kcal/mol). Among all, compound **17** and **18** were shown to have the highest binding affinity (-5.3 kcal/mol), followed by **3** (-5.2 kcal/mol).

AA was found to have H-bonding interactions with Arg-31, Ala-35, Arg-323, Ile-160, Arg-161. All selected compounds were shown to have H-bonding interactions with one or two of these amino acids except **17** (Asn-162). In addition to this, **16** and **18** were shown to have H-bonding interactions with Asn-162 and Phe-29 respectively. Regarding the residual interactions, all the selected compounds were exhibited to have hydrophobic-alkyl interactions with various amino acid residues, such as Ala-28, Arg-31, Ala-152, And Ile-160. Additionally, a range of Van der Waals interactions with different amino acids were had by the compounds. The specific residual interaction patterns were found to differ among the compounds. In contrast, Ascorbic acid was not reported to have any hydrophobic-alkyl interactions and instead was relied more on the hydrogen bonding network for its binding interactions.

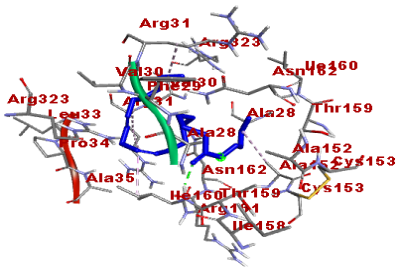
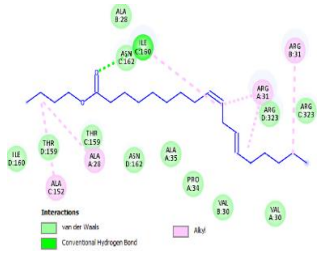
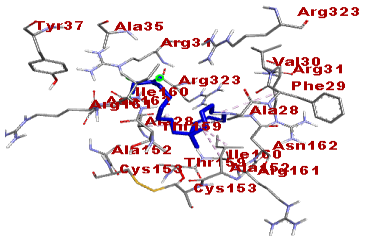
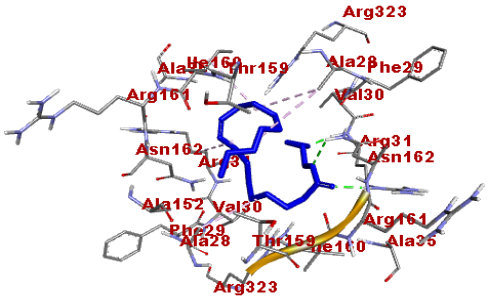
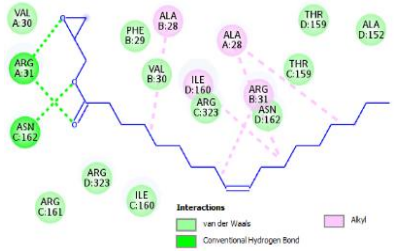
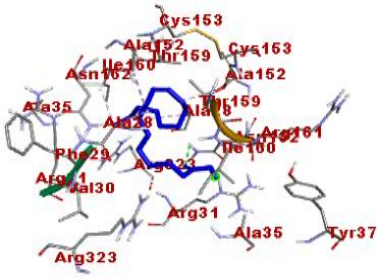
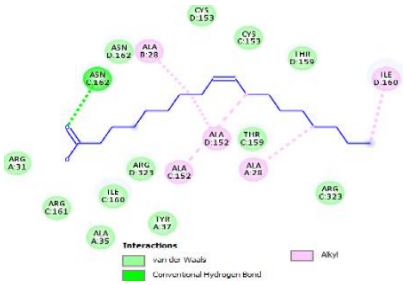
Therefore, based on the higher binding affinity and the specific binding interactions observed, compound **17** and compound **18** were identified as the most promising lead compounds among the selected compounds, as they were shown to have exhibited the highest binding affinity at -5.3 kcal/mol, exceeded even the standard ascorbic acid (-5.1 kcal/mol). Additionally, these two compounds were shown to have promising H-bonding interactions with key amino acid residues, as well as a range of hydrophobic-alkyl and Van der Waals interactions, suggesting they may have improved binding compared to the **AA**.

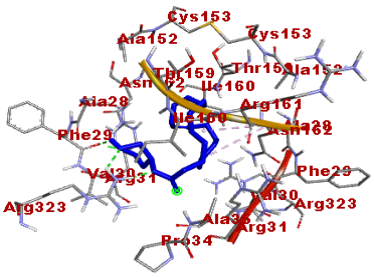
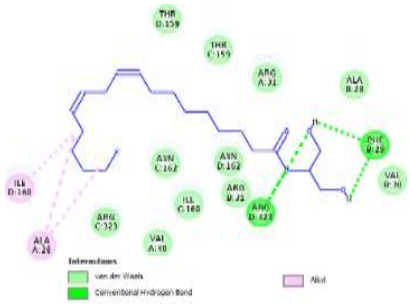
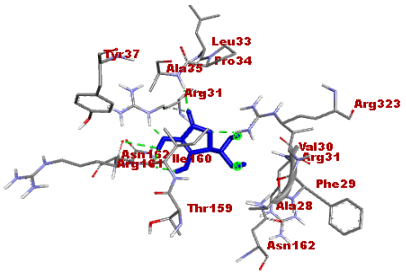
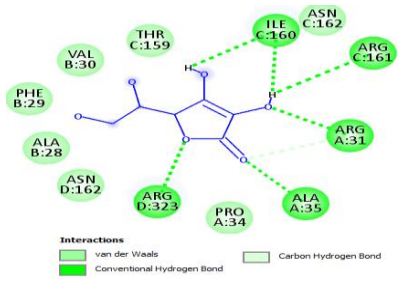
Table 11: Molecular docking binding affinity and interactions of selected compounds and standard with the target human myeloperoxidase (PDB ID: 1DNU).

Cpds	Affinity (kcal/mol)	H-bond	Residual interactions	
			Hydrophobic/ Electrostatic	Van der Waals
14	-5.0	Ile-160	Ala-28, Arg-31 Ala-152, Ile-160	Ala-28, Asn-162, Arg-323, Val-30, Pro-34, Ala-35, Thr-159, Ile-160
15	-5.1	Arg-31, Ile-160	Ala-28, Arg-31 Ala-152, Ile-160	Ala-152, Arg-323, Asn-162, Cys-153, Thr-159, Ala-35, Arg-161, Asn-162
16	-5.2	Arg-31, Asn-162	Ala-28, Arg-31, Ile-160	Val-30, Phe-29, Arg-323, Asn-162, Thr-159, Ala-152, Ile-160, Arg-161
17	-5.3	Asn-162	Ala-28, Ala-152, Ile-160	Arg-31, Asn-162, Cys-153, Thr-159, Arg-323, Tyr-37, Ile-160, Ala-35, Arg-161
18	-5.3	Arg-323, Phe-29	Ala-28, Ile-160	Thr-159, Arg-31, Ala-28, Val-30, Asn-162, Ile-160, Arg-323
AA	-5.1	Arg-31, Ala-35, Arg-323, Ile-160, Arg-161	-	Ala-28, Phe-29, Val-30, Thr-159, Asn-162, Pro-34

Cpds = Selected Compounds, **AA**= Ascorbic Acid

Figure 11: Binding interactions (3D & 2D) of selected compounds and standard compounds with human myeloperoxidase (PDB ID: 1DNU).

Cpds	2D	3D
14		
15		
16		
17		

18		
AA		

Cpds = Selected compounds**, AA=** Ascorbic Acid, **2D**= Two Dimension, **3D**=Three Dimension

4.7.2. *In silico* ADME Analysis

The forecasting of ADME (absorption, distribution, metabolism, and excretion) properties of the selected compounds, including their pharmacokinetic and drug-likeness properties, have been estimated using the SwissADME online server (http://www.swissadme.ch/, accessed on 12 August 2022). The collective rules of Lipinski's, Veber's and Egan's, which determine the properties of a drug, were followed. According to the rule, the bioavailability of a compound is filtered using the following: Lipinski's rule of 5 considers the values of four parameters that indicate good bioavailability: the molecular weight (MW) ≤ 500; H-bonds donors (NHD) ≤ 5; H-bonds acceptors (NHA) ≤ 10; water partition coefficient (cLogP) ≤ 5. One of these parameters is accepted to be out of range; if two parameters are out of the ranges, only a poor absorption or permeability is possible (Lipinski Pfizer, n.d.). The Veber rule considers a good bioavailability for compounds with number of rotatable bonds (NRB) ≤10 and topological surface area (TPSA) ≤ 140 $Å^2$ (Veber et al., 2002). The Egan rule considers good bioavailability for compounds with 0 ≥ TPSA ≤ 132 $Å^2$ and-1≥ cLogP ≤ 6 (Egan et al., 2000).

Based on the current findings in Table 12, the Lipinski's Rule of 5 was considered for the selected compounds. It was found that compounds **14, 18**, and **17** had one violation of Lipinski's rule, while compounds **16, 18** followed the rule as **CPFX**. Compounds can have one violation and still be considered drug-like

All the selected compounds were found to have 1 violation of Veber's rule, which considers the number of rotatable bonds (NRB) and the topological polar surface area (TPSA). However, all the selected compounds were found to follow Egan's rule, which looks at TPSA and cLogP. Finally, the bioavailability score (BAS) was calculated and found to range from 0.55 to 0.85 for the selected compounds, indicating good potential for oral bioavailability.

Table 12: Calculated parameters of *in silico* bioavailability (drug-likeness) prediction & physicochemical properties of the selected compounds and ciprofloxacin

Cpds	Formula	MW (g/mol)	NHD	NHA	LogP (cLogP)	Lipinski's rule violation	NRB	TPSA (Å ^{2})	Veber's rule violation	BAS
14	$C_{22}H_{40}O_2$	336.55	0	2	7.14	1	18	26.30	1	0.55
15	$C_{18}H_{32}O_2$	280.45	1	2	5.88	1	14	37.30	1	0.85
16	$C_{21}H_{38}O_3$	338.52	0	3	5.97	0	18	38.83	1	0.55
17	$C_{18}H_{34}O_2$	282.46	1	2	6.11	1	15	37.30	1	0.85
18	$C_{21}H_{38}O_4$	354.52	2	4	4.7	0	18	66.76	1	0.55
CPFX	$C_{17}H_{18}FN_3O_3$	331.34	2	5	1.98	0	3	74.57	0	0.55

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

The selected compounds were evaluated for their P-glycoprotein (P-gp) substrate properties, and it was found that they were not P-gp substrates, suggesting good intestinal absorption. Except for compound **14**, all the selected compounds were found to have high gastrointestinal absorption and were predicted to cross the blood-brain barrier (BBB). The interactions of the selected compounds with the isoenzymes of the cytochrome (CYP) system were also assessed.

It was found that all the selected compounds interacted mainly with the CYP2C9 isoenzyme, suggesting their efficiency while having minimal toxicity (Table 13).

The rate at which a substance penetrates the stratum corneum was measured by skin permeability (Kp). This value was commonly used to quantify the transport of molecules in the epidermal skin's outermost layer and to highlight the importance of skin absorption. The more negative the log Kp, the lower the cutaneous permeability of the molecule, with a permissible range of LogKp from -8.0 to -1.0 cm/s (Théry et al., 2018).

In this study, compared to **CPFX**, all the selected compounds showed a lesser LogKp value ranging from -3.80 to -2.57 (Table 13). This suggested that these selected compounds might have better skin permeation than CPFX (-9.09 cm/s).

Table 13: Pharmacokinetic (ADME) properties prediction of the selected compounds

Cpds	GI ABS	BBB per	P-gp Sub	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor	log Kp (cm/s)
14	Low	No	No	No	No	Yes	No	No	-2.57
15	High	Yes	No	No	No	Yes	No	No	-3.05
16	High	Yes	No	No	No	Yes	No	No	-3.19
17	High	Yes	No	No	No	Yes	No	No	-2.60
18	High	Yes	No	No	No	Yes	No	No	-3.80
CPFX	High	No	Yes	No	No	No	Yes	No	-9.09

Cpds= Compounds, **GI** = Gastro-Intestinal, **ABS** = Absorption, **BBB** = Blood Brain Barrier, **P-gp** = P-glycoprotein, **CYP** = Cytochrome-P, **Sub**=Substrate

Drug-like properties and GI absorption of selected compounds from essential oil of *Pentas schimperiana* stem bark were also represented by the boiled-egg prediction (Figure 12) and bioavailability radar graph (Figure 13). The compounds present in the yellow zone in the boiled-egg graph can permeate through the blood–brain barrier (BBB), and the pink area of the

bioavailability radar graphs shows the drug-likeness of the compounds. Except compound **14**, all selected compounds were presented in the yellow zone in the boil- egg model.

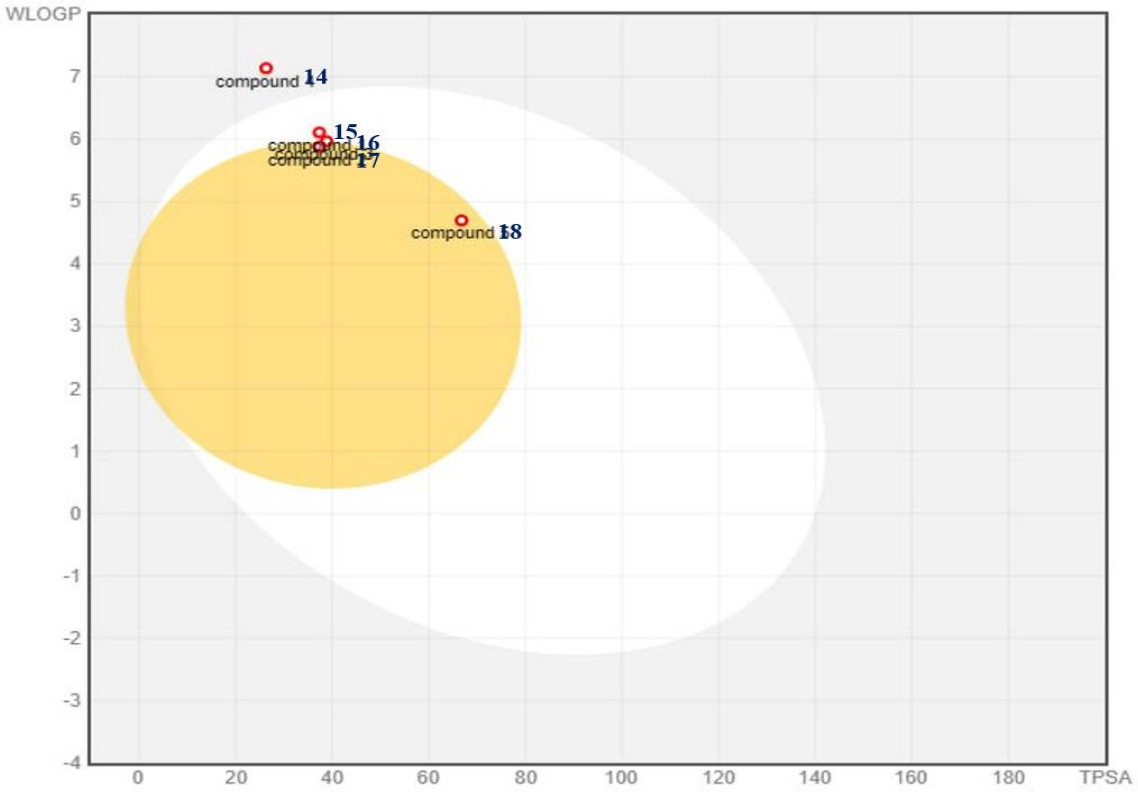
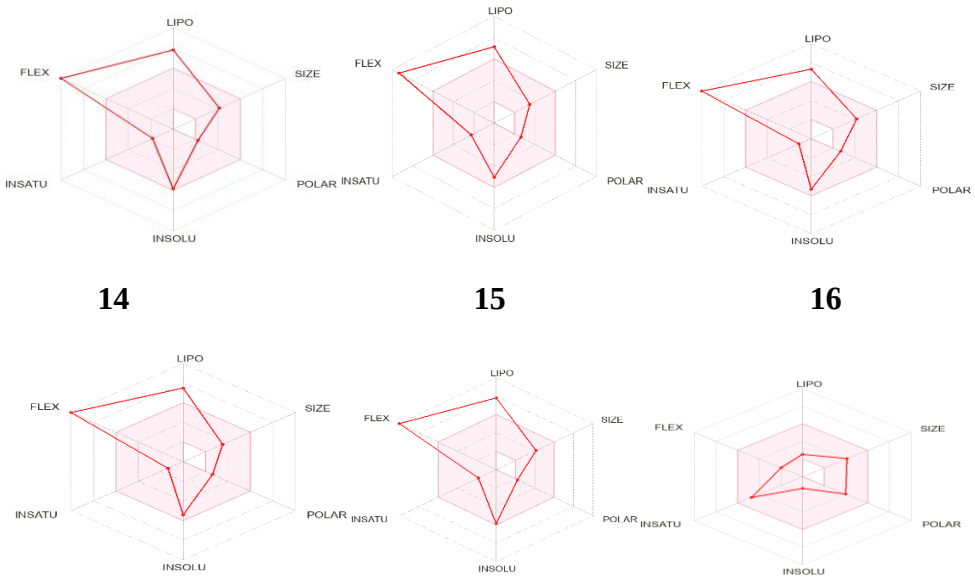


Figure 12: Boiled-egg model of the selected compounds for predicting gastrointestinal absorption and brain access



14 **15** **16**

17 **18** **CPFX**

Figure 13 : Bioavailability radar of selected phytoconstituents (pink area showed the drug likeness properties of selected compounds)

4.7.3. Toxicity Predictions

The toxicity parameters of selected phytocompounds from essential oil of *Pentas schimperiana* stem bark were predicted using web server ProTox II (https://toxnew.charite.de/) (Table 14). All the selected compounds, including the reference compound CPFX, were found to be inactive for hepatotoxicity, cytotoxicity, immunotoxicity, and mutagenicity, suggesting they were unlikely to cause liver damage, cell-killing effects, immune system-related toxicity, or genetic mutations. However, compounds **14** and **16** were classified as active for carcinogenicity, indicating a potential risk of cancer development. Additionally, compound **16** was found to be active for mutagenicity, similar to the standard drug **CPFX,** raising concerns about its ability to induce heritable genetic changes. The remaining compounds (**15, 16**, and **18**) did not show any major toxicological concerns. The LD_{50} values were also calculated to ensure the safety of the selected compounds as shown in Table 14.

The level of toxicity is expressed on a scale from 1 to 6, with a greater number indicating a lower level of toxicity. It is determined based on the LD_{50} (mg/kg) value, which denotes a dose

that will kill 50% of the test animal. Toxicity class: (Class 1: fatal if swallowed (LD50 ≤ 5), Class 2: fatal if swallowed ($5 < LD_{50} \leq 50$), Class 3: toxic if swallowed ($50 < LD_{50} \leq 300$), Class 4: harmful if swallowed ($300 < LD_{50} \leq 2000$), Class 5: may be harmful if swallowed ($2000 < LD_{50} \leq 5000$), Class 6: non-toxic ($LD_{50} > 5000$)).

The predicted LD_{50} (mg/kg) for the tested compounds range from 36 to 20000, and their toxicity class as classified by Pro Tox-II ranges from grade 2 to 6. The compounds with $LD_{50} > 2000$ mg/kg, suggesting their safety for biological administration and as potential drugs (Drwal et al., 2014). Based on this standard,

Compound **14, 15**, and **18** were classified as toxicity class 6, indicating they were found to be non-toxic with LD_{50} values greater than 5000 mg/kg. This suggested these compounds may be considered safe for biological administration and have potential as drug candidates.

In contrast, compound **16** and **17** were classified as toxicity class 2, meaning they were determined to be fatal if swallowed with LD_{50} values between 5 and 50 mg/kg. These compounds would be considered highly toxic and unsuitable for biological applications without further modification

The reference compound **(CPFX),** was classified as toxicity class 4, indicating it was found to be harmful if swallowed with an LD_{50} value between 300 and 2000 mg/kg. This placed it in a lower toxicity category compared to compounds **16** and **17**.

Table 14 : Toxicological properties of selected compounds and standard, computed by Pro-Tox II

Compounds	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	LD_{50} (mg/kg)	Toxicity Class
14	Inactive	Active	Inactive	Inactive	Inactive	20000	6
15	Inactive	Inactive	Inactive	Inactive	Inactive	10000	6
16	Inactive	Active	Inactive	Active	Inactive	36	2
17	Inactive	Inactive	Inactive	Inactive	Inactive	48	2
18	Inactive	Inactive	Inactive	Inactive	Inactive	20000	6
CPFX	Inactive	Inactive	Inactive	Active	Inactive	2000	4

CPFX- Ciprofloxacin, **LD_{50}**-Median Lethal Dose

4. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Natural products represent an important source of new lead compounds in drug discovery and development. The phytochemical screening tests showed that the presence of alkaloids, tannins, glycosides, steroids, flavonoids and terpenoids in the crude extract of the *Pentas schimperiana* stem bark. The essential oil of *Pentas schimperiana* was also found to be a rich source of potentially bioactive compounds, including high levels of fatty acid derivatives and other interesting components like methyl cinnamate, linalool, and hydroquinone. Column chromatographic separation of the combined crude extracts of chloroform and methanol furnished 4 compounds and their structures were partially characterized by FTIR.

All the tested crude extracts and essential oil showed moderate to good *in vitro* antibacterial and antioxidant potentials as assessed with different assays. In addition to *in vitro* findings, the *in silico* molecular docking study suggested that the selected major compounds from the essential oil can be good antibacterial and antioxidant agents by the analysis of ligand interaction with the proteins. The ADMET analysis revealed the drug likeness and safety of most of the major compounds in the essential oil. Overall, this study identified some interesting biological activities of crude extracts and essential oil, especially as natural antioxidants and antibacterial agents, which justifies the use of the Pentas schimperiana in traditional medicine.

5.2. Recommendation

Further biological activity evaluations, including antifungal, anticancer, and cytotoxicity studies, on the crude extracts, essential oil, and isolated compounds from Pentas schimperiana are required to validate the traditional use of this plant. Future research efforts should focus on isolating and characterizing the active compounds responsible for the pharmacological activities of *Pentas schimperiana.* This will enable the identification of potential drug candidates and provide a more comprehensive understanding of the underlying mechanisms of its therapeutic effects. Additionally, in vivo studies are necessary to investigate and assess the potency and safety of the essential oil and its active components.

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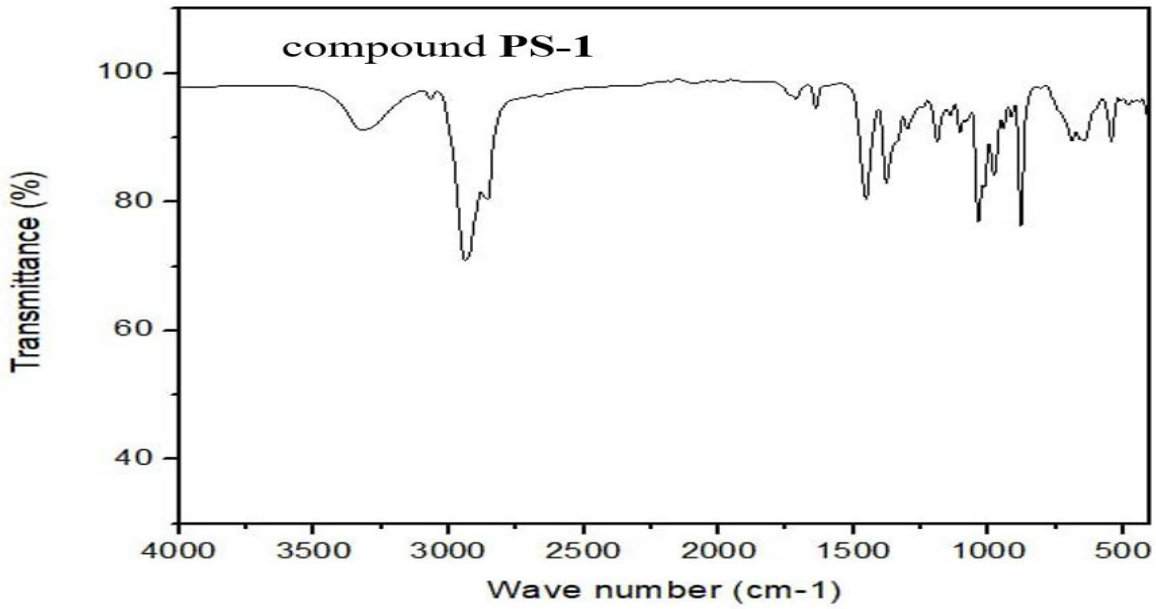
APPENDIX – 1: FTIR spectrum of compound **PS-1**



APPENDIX – 2: FTIR spectrum of compound **PS-2**