



WOLLEGA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

**PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL
ACTIVITY STUDIES ON THE ROOT EXTRACTS OF *CARISSA
SPINARUM***

M.Sc. Research Thesis

By
Feyisa Gobena sori

January, 2025
Nekemte, Ethiopia



WOLLEGA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

**PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY
STUDIES ON THE ROOT EXTRACTS OF *CARISSA SPINARUM***

**A Thesis Submitted to the College of Natural Science Department of Chemistry in
Partial Fulfillment of the Requirements for the Degree of Master of Science in
Organic Chemistry**

BY: Feyisa Gobena sori

Advisor: Desalegn Abebe (PhD)

**January, 2025
Nekemte, Ethiopia**



WOLLEGA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
P.O. Box: 395, Nekemte, Ethiopia

APPROVAL SHEET FOR SUBMITTING FINAL THESIS.

As members of the Board of Examining of the Final MSc. thesis open defense, we certify that we have read and evaluated the thesis prepared by **Feyisa Gobena sori** under the title “**PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY STUDIES ON THE ROOT EXTRACTS OF *CARISSA SPINARUM***” and recommend that the thesis be accepted as fulfilling the thesis requirement for the **Degree of Master of science** in Organic chemistry.

Zelalem Abdisa(Ph.D)

Chairperson

Tamirat Tesfaye (Ph.D)

Internal Examiner

External Examiner

Signature

Date

Signature

Date

Signature

Date

Final Approval and Acceptance

Thesis Approved by

Department PGC

Signature

Date

Dean of College

Signature

Date

Certification of the Final Thesis

I hereby certify that all the correction and recommendation suggested by the board of examiners are incorporated into the final thesis entitled on “**PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY STUDIES ON THE ROOT EXTRACTS OF *CARISSA SPINARUM***”.

Dean of SGS

Signature

Date

STATEMENT OF THE AUTHOR

I **Mr. Feyisa Gobena Sori** hereby declare and affirm that the thesis entitled "**PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY STUDIES ON THE ROOT EXTRACTS OF *CARISSA SPINARUM***" is my own work conducted under the supervision of **Desalegn Abebe(PhD)**. I have followed all the ethical principles of scholarship in the preparation, data collection, data analysis and completion of this thesis. All scholarly matter that is included in the thesis has been given recognition through citation. I have adequately cited and referenced all the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and I have not misrepresented, fabricated, or falsified any idea / data / fact / source in my submission. This thesis is submitted in partial fulfillment of the requirement for a degree from the Post Graduate Studies at Wollega University. I further declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

I understand that any violation of the above will be cause for disciplinary action by the University and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

Name:

Signature: _____

Date:

School/Department:

College:

ACKNOWLEDGEMENTS

All praises are being to 'Almighty God' who gave me the strength to accomplish this work. Next, I would like to express my deepest gratitude to my advisor Desalegn Abebe (PhD) for his excellent guidance and timely advice. Close follow up, supervision, continuous encouragement, reading the manuscript and giving me valuable comments throughout the preparation of this proposal.

I am very thankful to my brother Ayana Gobena and for both material and idea support. I want to thank Limu secondary school for material contribution and which invariably helped me. I am also grateful to the Department of chemistry, Wollega University for the assistance provided to me.

List of Abbreviations and Acronyms

MeOH:	Methanol
DMSO:	Dimethyl sulfoxide
EtOAc:	Ethyl acetate
WHO:	World health organization
NMR:	Nuclear Magnetic Resonance
HPLC:	High Performance Liquid Chromatography
GC-MS:	Gas chromatography- Mass spectroscopy

TABLE OF CONTENTS

Contents	Pages
ACKNOWLEDGEMENTS	III
List of Abbreviations and Acronyms	IV
TABLE OF CONTENTS	V
LIST OF TABLES	VIII
LIST OF FIGURES.....	VIII
ABSTRACT	IX
1. INTRODUCTION.....	1
1.1. Back ground of the study	1
1.2 Statement of the problem.....	3
1.3 Objectives of the study	3
1.3.1 General Objective the study	3
1.3.2 Specific Objectives of the Study	3
1.4 significance of the study	3
2 LITERATURE REVIEW	5
2.1 Description of <i>Carissa spinarum</i>	5
2.3. Pharmacological Activity of <i>Carissa spinarum</i>	7
2.3.1 Anthelmintic Activity	7
2.3.2 Anticonvulsant Activity	7
2.3.3 Anti-Arthritic Activity	8
2.3.4 Anti-Inflammatory Activity	8
2.3.5. Antimicrobial Activity	9
2.3.6. Antidiabetic Activity	10
2.3.7. Hepatoprotective Activity	10

2.3.8 Anticancer Activity	11
2.3.9. Antioxidant Activity	11
2.3.10. Antiviral Activity	12
2.4 Phytochemistry of <i>Carissa spinarum</i>	12
3 .MATERIALS AND METHODS	21
3.1 Plant material	21
3.2 Instruments and apparatuses	21
3.3 Chemicals and Reagents to be used.....	21
3.4 Extraction of plant sample	21
3.5 Phytochemical Screening.....	21
3.5.1 Test for Terpenoids	21
3.5.2 Test for Flavonoids	22
3.5.3 Test for Phenolic compounds.....	22
3.5.4 Test for Tannins	22
3.5.5 Test for Alkaloids.....	22
3.5.6 Test for Steroids	22
3.5.7 Tests for coumarins:.....	22
3.5.8 Test for Glycosides	22
3.5.9 Test for saponins	22
3.6 Anti-bacterial activity test.....	23
3.6.1 Bacterial strains.....	23
3.6.2 Preparation of inoculum.....	23
3.6.3 Preparation of test solutions	23
3.6.4. Test for antibacterial activity	23
4. RESULTS AND DISCUSSIONS	24
4.1 Extraction of plant sample	24

4.2 Phytochemical Tests	24
4.3 Antibacterial test.....	25
5. CONCLUSION AND RECOMMENDATION	26
5.1 Conclusion	26
5.2 Recommendations.....	26
REFERENCE	27
Appendix 1: During the extraction of plant sample	31
Appendix 2 Image of all positive tests for phytochemical tests	32

LIST OF TABLES

Table 4.1: phytochemical screening test results	24
Table 4.2: Antibacterial activity of chloroform/methanol extract of <i>Carissa spinarum</i>	25

LIST OF FIGURES

Figure 1: <i>Carissa spinarum</i> (photo by Feyisa Gobena, 2024)	6
--	---

ABSTRACT

Carissa spinarum, a medicinal plant widely used in traditional medicine, has been reported to possess various pharmacological properties. This study aimed to investigate the phytochemical constituents and antibacterial potential of the root extracts of *Carissa spinarum*. The roots were extracted using chloroform and methanol and subjected to phytochemical screening to identify the presence of various bioactive compounds. The phytochemical screening revealed the presence of terpenoids flavonoids; phenolic compound, tannins, alkaloids, coumarins and steroids were present in the chloroform and methanol crude extracts. The antibacterial activity of the extracts was evaluated against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* of pathogenic bacteria using the disc diffusion method. The crude extracts and their dilutions of concentrations by 100% 50%, 25%, 12.5% solvent extracts were tested for their antibacterial activity. Gentamycin was used as positive control. The chloroform/methanol extract exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria. Strong antibacterial activity (18mm) was observed for chloroform/methanol crude extract against *S. aureus*, compared to standard gentamicine(30mm).The antibacterial activity of crude extracts of more concentrated is stronger than the extracts of their dilutions; this indicated that the antibacterial activities were increased with increased concentration of crude extracts. These findings suggest that the root extracts of *Carissa spinarum* possess promising antibacterial properties, which may be attributed to the presence of various bioactive compounds. Further studies are warranted to isolate and characterize the active compounds responsible for the observed antibacterial activity and to explore their potential therapeutic applications.

Keywords: Medicinal plants, Anti-bacterial activity, Phytochemical screening, Extract.

1. INTRODUCTION

1.1. Back ground of the study

Plants and their secondary metabolic constituents have been used in traditional medicine and modern medicine and were source of many important drugs. Medicinal plants are the richest bio-resource of drugs in traditional and modern systems of medicine, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for the development of synthetic drugs (Fatima,*et al.*,2013). Medicinal plant research has potentiated humans with diverse constituents which can be used as natural preservatives for the inhibition of different pathogens (Yetgin *et al.*, 2008). The ever-growing interest in medicinal plant research area has been instrumental in increasing the research that is being conducted in different countries of the world to use them as a part of traditional medicine (Pankhurst, 2001). Most traditional medicine applications involve the use of plant materials and could be the source of treatment for many diseases of humans and livestock and also, most of the drugs are of plant origin (Fullaset *al.*, 2001). Medicinal herbs have huge potential as a therapeutic aid, as they are used to combat multiple diseases but also serve as potential material for maintaining appropriate health conditions. Increased use of traditional medicinal plants is mainly due to the acceptability, accessibility, inadequate coverage of the modern medical system, high cost of modern drugs, and other biomedical benefits (Dawit. *et al.*, 2001).Moreover, people's interest in herbal medicine has grown steadily over the years. This is understood by many aspects such as its diversity, accessibility, low cost, effectiveness, medicinal usefulness, and less side effects.

The use of traditional medicinal plants for primary health care has dramatically increased throughout the world (Samie *et al.*, 2010). Majority of rural areas depend on traditional medicinal plants as their only source of health care due to the lack of adequate clinics, hospitals, volunteer health care professionals, the absence of vehicular/road facilities, and the expensiveness of modern medicines.

Most traditional medicines are locally available and cheaper than the standard treatments available in developing countries. Due to the shortage of health facilities, traditional medicinal plants are mostly the option to treat many diseases in such countries (Tena, 2008).

Scientists are in search of new phytochemicals that could be used to develop useful antimicrobials for the treatment of infectious diseases (WHO, (2010).

Even though plants are rich in a wide variety of secondary metabolites that have anti-microbial properties, very few of them are being explored in the development and synthesis of novel antimicrobials (Ramor, and Ponnampulam, 2008). Medicinal plants have been used in the production of modern drugs. They are used as a source of direct therapeutic agents, as raw materials in the preparation of complex semi-synthetic compounds, and as taxonomic markers in the search of new compounds.

The escalating global health crisis posed by antibiotic-resistant bacteria has prompted an urgent need for novel antimicrobial agents. The occurrence and spread of microbes resistant to most antibiotics (multi-drug resistant), including the major last-resort drugs are showing an increasing trend as evidenced by the available literature (Rubaka *et al.*, 2014). Currently, there is an increasing demand for pharmaceutical industries to conduct research and develop new drugs, majorly owing to the continuous emergence of microbial resistance to conventional therapeutic agents. Natural products, particularly those derived from medicinal plants, have emerged as a promising source of potential therapeutic compounds. .

Phytochemical screening is a crucial step in the evaluation of medicinal plants, as it allows for the identification of bioactive compounds that may contribute to the plant's therapeutic properties. By employing various analytical techniques, researchers can detect the presence of different classes of compounds, such as alkaloids, flavonoids, tannins, and terpenoids. These compounds have been shown to possess a wide range of biological activities, including antimicrobial, antioxidant, and anti-inflammatory effects.

Carissa spinarum is widely used traditionally as incense plant in South Wollo and for the treatment of numerous diseases (Fatima, *et al.*, 2013) though the plant is widely used in traditional medicine in Limu Woreda, Eastern Wollega, Western Ethiopia. Antibacterial activity testing is essential to assess the potential of plant extracts to inhibit the growth of pathogenic bacteria. By evaluating the susceptibility of various bacterial strains to the extracts, researchers can determine their efficacy and identify potential lead compounds for drug development. Therefore, this study aims to scientifically investigate the phytochemical profile and antibacterial activity of the root extracts of *Carissa spinarum*.

1.2 Statement of the problem

Despite the widespread use of *Carissa spinarum* in traditional medicine, there is a lack of scientific evidence to support its therapeutic claims, particularly regarding its antimicrobial properties.

While some studies have explored the phytochemical composition and biological activities of different parts of the plant, there is limited research specifically focused on the root extracts. Therefore, this study aims to address the following research questions:

- What are the major phytochemical constituents present in the root extracts of *Carissa spinarum*?
- Does the root extract of *Carissa spinarum* exhibit antibacterial activity against a panel of pathogenic bacteria?

By answering these questions, this study will contribute to the scientific validation of the traditional use of *Carissa spinarum* and provide insights into its potential as a source of natural antimicrobial agents.

1.3 Objectives of the study

1.3.1 General Objective the study

To study phytochemical screening and antibacterial activity of the root extracts of *Carissa spinarum*.

1.3.2 Specific Objectives of the Study

- To identify the major phytochemical constituents present in the root extracts of *Carissa spinarum* using standard phytochemical screening methods.
- To evaluate the antibacterial activity of the root extracts against a panel of pathogenic bacteria using the disc diffusion method.

1.4 significance of the study

This study will contribute to the scientific understanding of *Carissa spinarum* and its potential as a source of natural antimicrobial agents. By exploring the phytochemical composition and antibacterial activity of the root extracts, we aim to validate the traditional use of this plant and provide a scientific basis for its future applications in medicine. This study can also provide basic information about the phytochemical components of *Carissa spinarum* root extract.

In addition, the study can provide baseline information for researchers who are interested to conduct further studies on this area. Furthermore, the antibacterial study might result in data that support the traditional use of the plant to treat different diseases.

2 LITERATURE REVIEW

2.1 Description of *Carissa spinarum*

Carissa spinarum L. belongs to the Genus *Carissa*. The plant is known as ‘wild Karanda’, or ‘bush plum’ and is an underutilized wild fruit closely related to *C. carandas*. (Mundaragi, and Thangadurai, 2017). *Carissa spinarum* is also called as “Magic Shrub” in some of the African countries, as it is considered as a source of treatment for various diseases and ailments (Ansari and Patil, 2017). *Carissa spinarum* is a small spinous evergreen erect thorny shrub. Morphologically it is woody, extremely hard with forked branches, and grows up to a height of 2-3 meters. It is light brown to green in appearance, brown to greenish at the base, and deep brown towards the tip. It can be stripped off longitudinally, exposing the white to light-green wood beneath with 3.2 cm long thorns, (Rubaka *et al.*, 2014). The shrubs of this genus are found in the tropical and subtropical regions of Africa, Asia, and Australia (Mundaragi and Thangadurai, 2017). They are grown as decorative plants in America ranging from Florida to California. The green leaves of the plant are glossy, waxy, and tough oval-shaped, while the white flowers are fragrant. The plant also develops oval green berries that emerge from white star-shaped flowers. The berries turn black or dark in color when ripe. Ethnobotanically, the plant has been used as a remedy against numerous diseases, and its fruits are taken as a rich source of vitamin C and iron (Ansari and Patil, 2017).

Carissa spinarum (Synonyms: *Carissa edulis*, *Carissa tomentosa*, *Carissa richardiana*, *Carissa candolleana* and *Carissa cornifolia*) Family: Apocynaceae, *Carissa spinarum* is locally called Agamsa (Afan Oromo) is a thorny, much-branched shrub or climber growing to 5 m high. It is widely distributed in mountain bushland, at forest margins, along roadsides, riverbanks and rocky slopes at altitudes from 550 – 2500 m (Inga *et al.*, 2003).



Figure 1: *Carissa spinarum* (photo by Feyisa Gobena, 2024)

2.2. Traditional uses of *Carissa spinarum*

The use of *Carissa spinarum* has a very long history. Traditionally, the whole plant is used in India, Ethiopia, and other african countries for the treatment of venereal, respiratory, and gastrointestinal infections(Dhatwalia *et al.*,2021), fever, jaundice, hepatitis, cardiac diseases, diabetes, malaria, pneumonia , chronic joint pain , asthma, rheumatism, stomachache, cough, chest complaints, sickle-cell anemia, hypertension, kidney complications, diarrhea, worms, eye cataracts, gastric ulcers, and cancer(Sharma *et al.*,2017).

It has also been used for treating microbial infections such as herpes, gonorrhea, syphilis, rabies, typhoid fever, jaundice, and polio, and as an antivenin for snake bites (Kelemu *et al.*,2018). It has been also conventionally used to treat infertility and sexual problems such as asthenia and premature ejaculation in males (Bhadane *et al.*,(2018). It is also used as an ethnoveterinary medicine for the treatment of anaplasmosis. The leaves of cs are also used as a mosquito repellent (Dhatwalia *et al.*, 2021).

Carissa spinarum has been reportedly used to treat ulcers, muscle cramps, to stop bleeding after delivery, and to treat worm infestations in wounded animal's (Berhanu *et al.*, 2020).

2.3. Pharmacological Activity of *Carissa spinarum*

Several pharmacological activities have been reported from different parts of *Carissa spinarum* some of these properties have been discussed as follows:

2.3.1 Anthelmintic Activity

Several studies were conducted to investigate the anthelmintic properties of *Carissa spinarum*. Methanolic extract (100 mg/ml) and chloroform extract (50 mg/ml and 100 mg/ml) had a potency almost equivalent to that of the standard drug piperazine citrate (10 mg/ml), and both solvent extracts showed similar paralysis time as well as death of the *P. Posthuma*, indicating their efficacy against human intestinal helminthes. In another in vitro experiment, the anthelmintic effect of crude aqueous extracts of *Carissa spinarum* leaves on *H. Contortus* was reported (Harwansh *et al.*, 2010). Other aqueous extract of *Carissa spinarum* leaves (0.5 mg/ml) and the standard drug albendazole (0.25 µg/ml) were found to induce 100% egg hatch inhibition. In adult *H. Contortus*, aqueous extracts of *Carissa spinarum* leaves (4 mg/ml) induced significant mortality of parasites (96.8%), as compared to albendazole (0.5 mg/kg), which showed 100% parasite mortality (Mohammed *et al.*, (2013). On condensed tannins derived from *Carissa spinarum* leaves showed a protective role of *Carissa spinarum* against the pathogenic gastrointestinal nematode *H. Contortus* in goats (Jan *et al.*, 2015).

The animals were fed with 1.96% condensed tannins (CT)-rich leaf meal mixture for 90 days; a group of animals with 0% CT in their feed were taken as controls. Significant improvements in hematological parameters were observed in terms of increased levels of hemoglobin, total protein and globulin, hematocrit, calcium, and glucose in nematode-infected animals.

There were also no detrimental health effects of the CT-rich diet on the goats. However, there is also a need to explore the anthelmintic properties of other parts of *Carissa spinarum*. Future studies could be conducted using different drug doses, model systems, and to explore the mechanism of action of *Carissa spinarum* against pathogenic helminthes.

2.3.2 Anticonvulsant Activity

Carissa spinarum also exhibits anticonvulsant activity, as reported by different scientists. (Hedge *et al.*, 2011) reported an anticonvulsant effect of ethanolic extracts of *Carissa spinarum* roots.

Three differently induced murine models—i.e., maximal electroshock-induced convulsion, picrotoxin-induced seizures, and pentylenetetrazole (PTZ)-induced convulsion—were used to study the anticonvulsant

effects of ethanolic extracts of *Carissa spinarum* roots at three different dose levels: 100 mg/kg, 200 mg/kg, and 400 mg/kg. Significant reductions in the latency of tonic seizures were observed with all three doses. It was found that the ethanolic extract of *Carissa spinarum* roots protected 50% and 62.5% of the total mice from PTZ-induced convulsions and 25% and 62.5% of the total mice from maximal electroshock-induced convulsions at *Carissa spinarum* root extract doses of 200 mg/kg and 400 mg/kg, respectively. Further studies need to be conducted to explore the anticonvulsant activity of this plant using different model systems. Moreover, studies could also be carried out to determine the optimal drug dose without any toxic effects, as well as the mechanism of action of *Carissa spinarum*.

2.3.3 Anti-Arthritic Activity

The anti-arthritic properties of ethanolic extracts of *Carissa spinarum* roots were studied by (Hegde *et al.*, 2011) for the treatment of Freund's-adjuvant-induced polyarthritis in rats, using phenylbutazone (100 mg/kg) as a standard drug.

Arthritic mice were administrated with ethanolic extracts of *Carissa spinarum* roots at different doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg. The results in non-established arthritis were 36.23%, 49.27%, and 66.66%, while in established arthritis they were 30.43%, 44.92%, and 57.97% reduction in edema of affected joints at the doses of 100, 200, and 400 mg/kg, respectively. The *Carissa spinarum* root extract was found to reduce the pain and swelling of the affected joints, while also reducing the development of secondary lesions. Further studies need to be conducted to explore the anti-arthritic activity of this plant using different model systems. More research could also be carried out to determine the optimal drug dose without any toxic effects, as well as the mechanism of action of *Carissa spinarum*.

2.3.4 Anti-Inflammatory Activity

Different parts of *Carissa spinarum* have been traditionally used to treat pain and inflammation.(Beck and Namdeo,2016) observed significant ($p < 0.01$) anti-inflammatory activity of different extracts of *Carissa spinarum* leaves at a dose of 200 mg/kg, which was comparable to that of analgin (30 mg/kg), in the treatment of formalin-induced edema in the hind paws of rats. The aqueous extracts of *Carissa spinarum* leaves showed the greatest inhibition percentage (17.04%) after the standard drug (33.87%), followed by chloroform extracts (6.93%), ethanolic extracts (6.93%), and petroleum ether extracts (4.76%).

The anti-inflammatory potential of the crude root bark, dichloromethane (DCM) fraction, petroleum ether (PE) fraction, ethyl acetate (EA) fraction, and n-butanol (BUT) fraction was determined via COX-2 inhibition assay, using indomethacin (INM) as a positive control.

Among the crude extract and its fractions, the EA fraction ($0.2 \pm 0.0 \mu\text{g/ml}$) exhibited the lowest IC_{50} value, followed by the DCM ($0.5 \pm 0.0 \mu\text{g/ml}$), BUT ($0.2 \pm 0.0 \mu\text{g/ml}$), crude extract ($0.2 \pm 0.0 \mu\text{g/ml}$), and PE ($0.2 \pm 0.0 \mu\text{g/ml}$) fractions, whereas INM showed an IC_{50} of $0.4 \pm 0.1 \mu\text{g/m}$ (Liu *et al.*, (2021).

2.3.5. Antimicrobial Activity

The methanolic extract of *Carissa spinarum* roots was evaluated for its antimicrobial activity against five pathogenic bacteria and fungi, with MIC values of $125 \pm 10 \mu\text{g/ml}$ (*Escherichia Coli*), $512 \pm 43 \mu\text{g/ml}$ (*Bacillus subtilis*), $110 \pm 28 \mu\text{g/ml}$ (*Staphylococcus aureus*), $165 \pm 20 \mu\text{g/ml}$ (*Streptococcus sp.*), and $256 \pm 30 \mu\text{g/ml}$ (*Aspergillus niger*) (Sanwal and Chaudhary, 2011). (Rubaka *et al.*, 2014) reported strong antibacterial activity of ethanolic and methanolic root extracts of *Carissa spinarum* against *S. Aureus* and *E. coli*, with relative inhibition zone diameters of 66.97–70.13% and 54.66–57.24%, respectively.

The PE extract of *Carissa spinarum* leaves displayed the lowest MIC value against *S. Aureus* ($312 \mu\text{g/ml}$), while the ethanolic and methanolic root extracts of *Carissa spinarum* showed the lowest MIC ($312 \mu\text{g/ml}$) against *E. coli*. (Kumar *et al.*, 2017) reported strong inhibition of the EA extract (25 mm) and PE extract of *Carissa spinarum* roots against *E. Coli* (23 mm), of the EA extract (20 mm) and chloroform extract (CE) of *Carissa spinarum* roots (20 mm) against *Proteus*, PE (20 mm), and of the CE (20 mm) against MRSA. The PE and hexane extract of *Carissa spinarum* leaves showed strong inhibition against *E.coli*, *Proteus*, and MRSA, with zones of inhibition ranging between 16 and 30 mm.

The modest antibacterial activity of the ethyl acetate extract of *Carissa spinarum* leaves against *S. Aureus* was reported by (Feyissa and Melaku, 2016) with a zone of inhibition of 15 mm at a 1.5 mg/ml concentration, which was due to the presence of ursolic and betulinic acids in the leaves of *Carissa spinarum*. Doshi *et al.*, 2017 showed the strong antimicrobial activity of a nanoemulsion prepared from *Carissa spinarum* fruits against *S. Aureus*, *B. Subtilis*, *S. Typhi*, and *E. Coli*, with MIC values ranging between 30 and 50 $\mu\text{g/ml}$. Tiruneh *et al.*, 2022 reported a relative antibacterial effect with varying zones of inhibition. However, the ME showed superior antibacterial activity compared with the DMSO, EA, and CE, possibly due to the different phytoconstituents in the different solvents. Saka *et al.*, 2022 reported the antibacterial activity of Nano particles from fresh leaves of *Carissa spinarum* against *Staphylococcus aureus* and *Bacillus*, while

nanoparticles prepared from the leaves of *Carissa spinarum* were found to have strong antifungal activity against *Sporisorium scitamineum*.

Extensive studies are required to explore the antimicrobial nature of different parts of *Carissa spinarum* and to determine the phytoconstituents responsible for their antimicrobial activity. Scientists can also work on the preparation of nanoparticles with antimicrobial properties from different parts of *Carissa spinarum*.

2.3.6. Antidiabetic Activity

Plant-based drugs have always been attractive to humans for the treatment of diseases such as diabetes. (Fatima *et al.*, 2013) investigated the antidiabetic effect of acetone extract of *Carissa spinarum* leaves on alloxan-induced diabetic rats. In overnight-fasted rats, alloxan (150 mg/kg body weight) was injected intraperitoneally to induce diabetes mellitus. The oral administration of acetone extract of *Carissa spinarum* leaves in diabetic rats at doses of 200, 400, and 600 mg/kg was found to significantly decrease fasting blood glucose and urine sugar levels. The treatment also reduced cholesterol, triglyceride, low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) levels and increased high-density lipoprotein (HDL) levels significantly in the treated diabetic rats, thereby showing both anti-hyperglycemic and anti-hyperlipidemic activity. More research could be carried out to explore the antidiabetic potential of *Carissa spinarum* against various model systems.

2.3.7. Hepatoprotective Activity

The ethanolic extract of *Carissa spinarum* roots (100–400 mg/kg dose) also showed hepatoprotective activity against chloroform- and paracetamol-induced hepatotoxicity by decreasing the activities of bilirubin and lipid peroxidation, while significantly increasing the levels of uric acid, glutathione, superoxide dismutase, catalase, and protein in a dose-dependent manner. Recently, (Liu *et al.*, 2021) reported the hepatoprotective effects of (+)-isolariciresinol 3 α -O- β -D-glucopyranoside and protocatechuic acid from root bark extract of *C. spinarum* using a H₂O₂-induced oxidative stress model in L02 cells. There were improvements in the viability of L02 cells in the H₂O₂ model group from 68.2% to 79.4% and 80.9% by (+)-isolariciresinol 3 α -O- β -D-glucopyranoside and protocatechuic acid at concentrations of 5 μ m, respectively, whereas the positive control (vitamin C) improved the cell viability from 65.2% to 82.7% at 5 μ m.

2.3.8 Anticancer Activity

The anticancer activity of *Carissa spinarum* has been reported using different cell lines. (Sehar *et al.*, 2011) showed significant anticancer activity of *Carissa spinarum* root extract against HL-60 leukemia cell lines, with $IC_{50} = 34.58 \mu\text{g/ml}$ and $GI_{50} = 18.1 \mu\text{g/ml}$.

Later, cytotoxic effects of hexane, CE, and methanolic extracts of *Carissa spinarum* were reported on A375 melanoma cells using a sulforhodamine B staining assay, with IC_{50} values of $40 \mu\text{g/ml}$, $47 \mu\text{g/ml}$, and $>100 \mu\text{g/ml}$, respectively. Cell-cycle analysis also validated the cytotoxic activity of hexane extract of *Carissa spinarum* through induction of cell-cycle arrest at the S phase, along with the induction of caspase-3/7 activities (Alqathama *et al.*, (2015]).

2.3.9. Antioxidant Activity

Chloroform extract of *Carissa spinarum* stems (IC_{50} — $47.03 \mu\text{g}$), along with (-)-carissanol (IC_{50} — $47.87 \mu\text{m}$) and (-)-carinol (IC_{50} — $37.12 \mu\text{m}$), showed strong DPPH scavenging activity as compared to that of Trolox (IC_{50} — $32.19 \mu\text{m}$) (Chanda *et al.*, 2014).

However, a study by (Feyissa and Melaku, 2016) reported low antioxidant activity of EA extract of *Carissa spinarum* leaves (5% at 2.5 mg/ml). Moderate anti-DPPH activity was also reported by (Wangteeraprasert *et al.*, 2012) for eight lignans isolated from *Carissa spinarum* stem extract. (Afanyibo *et al.*, 2019) also reported strong radical scavenging of aqueous extract of *Carissa spinarum* roots, with an IC_{50} value of $75.65 \pm 5.02 \mu\text{g/ml}$, as compared to that of hydroalcoholic extract (IC_{50} — $96.10 \pm 1.11 \mu\text{g/ml}$). The antioxidant activity of crude *Carissa spinarum* root bark extract and its different fractions (i.e., PE, DCM, EA, and BUT) was also reported by Liu *et al.* (Liu *et al.*, 2021) using a DPPH assay. The ethyl acetate fraction was found to have the highest DPPH scavenging activity (IC_{50} — $31.8 \pm 1.3 \mu\text{g/ml}$), followed by the BUT ($92.4 \pm 8.6 \mu\text{g/ml}$) and DCM ($136.0 \pm 7.6 \mu\text{g/ml}$) fractions.

Similarly, the EA fraction ($14.9 \pm 2.4 \text{ mmol Fe}^{2+}/\text{g}$) also exhibited the best FRAP activity, followed by the BUT ($4.1 \pm 0.0 \text{ mmol Fe}^{2+}/\text{g}$) and DCM ($3.0 \pm 0.7 \text{ mmol Fe}^{2+}/\text{g}$) fractions. Protocatechuic acid from the root bark extract of *Carissa spinarum* showed strong DPPH activity, with an IC_{50} value of $16.5 \pm 1.2 \mu\text{m}$, which was lower than that of the positive control (vitamin C, $25.5 \pm 0.3 \mu\text{m}$). However, compounds such as (+)-isolariciresinol 3α -O- β -D-glucopyranoside, erythro-1-(3-methoxy-4-hydroxy-phenyl)-propan-1,2-diol, threo-1-(3-methoxy-4-hydroxy-phenyl)-propan-1,2-diol, and protocatechuic acid showed better FRAP activity as compared to that of vitamin C (Liu *et al.*, 2021).

The antioxidant activity of neochlorogenic acid, 3, 4-dicaffeoylquinic acid, 3, 5-dicaffeoylquinic acid, 4, 5-dicaffeoylquinic acid, methyl 3, 5-dicaffeoylquinic acid, methyl 4,5-dicaffeoylquinic acid, and 4-O-caffeoyl-3-O-syringoylquinic acid from the root bark of *Carissa spinarum* was reported by Liu et al. (Liu *et al.*, 2021). Recently, Nazareth et al. (Tiruneh *et al.*, 2022) also reported the strong antioxidant activity of ripe *Carissa spinarum* fruits based on a DPPH assay (IC_{50} —4.69 mg/ml).

2.3.10. Antiviral Activity

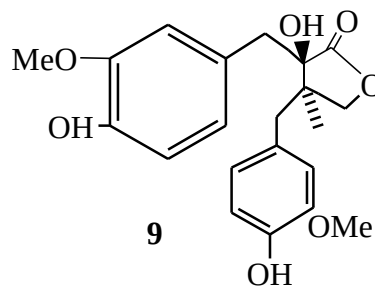
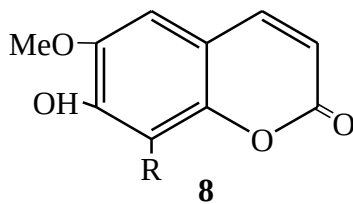
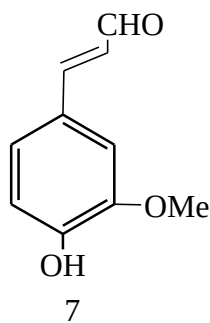
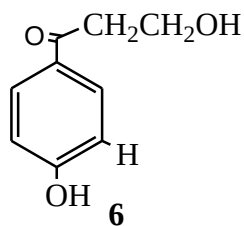
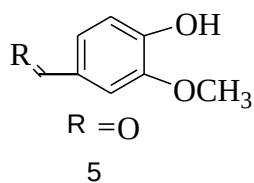
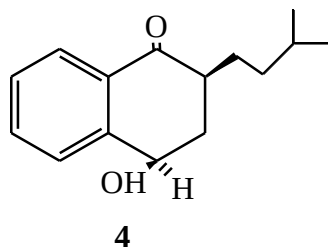
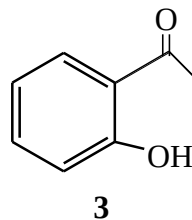
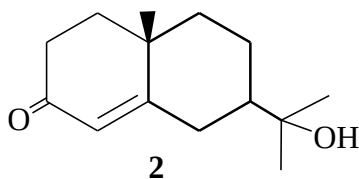
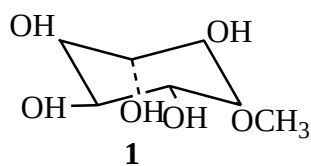
The antiviral activity of aqueous extracts of the root bark of *Carissa spinarum* against herpes simplex virus (HSV) was reported by (Tolo *et al.*, 2006) based on in vitro and in vivo anti-HSV activity, using tests such as plaque inhibition assay, cell cytotoxicity assay, and virus yield reduction assay, as well as against BALB/C mice cutaneously infected with HSV. The root bark of *Carissa spinarum* significantly inhibited the formation of plaques in Vero E6 cells infected with 100 PFU of wild-type strains of HSV (7401H HSV-1 and Ito-1262 HSV-2) or resistant strains of HSV (TK-7401H HSV-1 and apr 7401H HSV-1) by 100% at 50 μ g/ml in vitro, with minimal cell cytotoxicity (CC_{50} = 480 μ g/ml). When the extract was examined for in vivo efficacy in a murine model using BALB/C mice cutaneously infected with wild-type or resistant strains of HSV, the extract at an oral dose of 250 mg/kg significantly delayed the onset of HSV infections by over 50%. It also increased the mean survival time of treated infected mice by between 28 and 35% relative to the infected untreated mice ($p < 0.05$ versus control, according to Student's t-test).

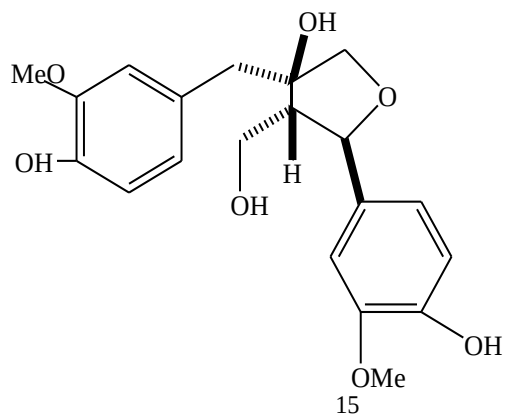
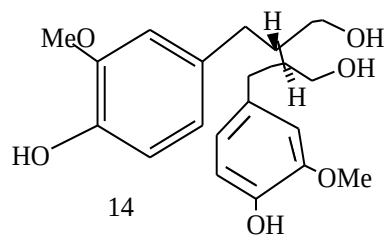
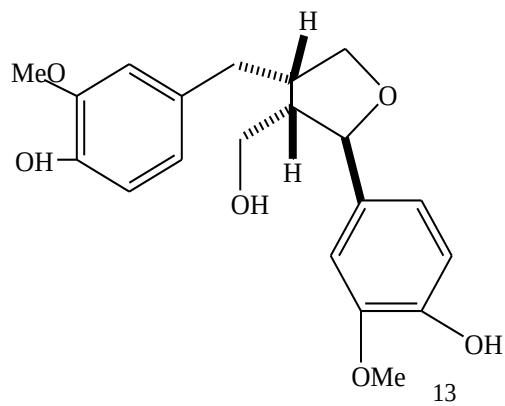
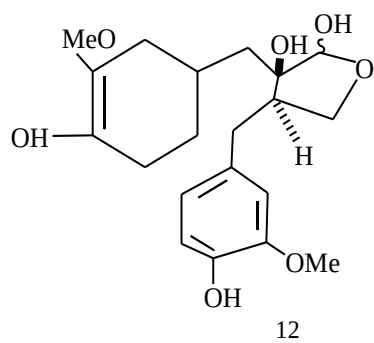
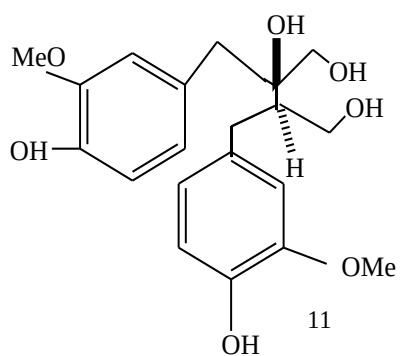
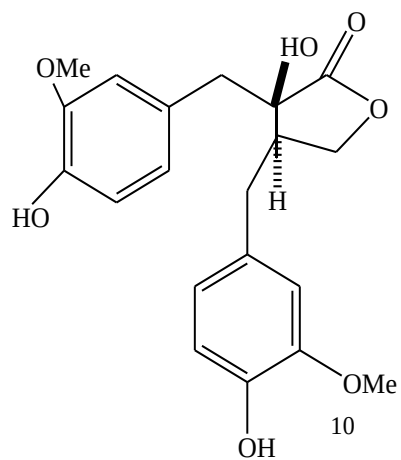
The mortality rate for mice treated with the extract was also significantly reduced by between 70 and 90% as compared with the infected untreated mice, which exhibited 100% mortality. No acute toxicity was observed in mice at the oral therapeutic dose of 250 mg/kg.

2.4 Phytochemistry of *Carissa spinarum*

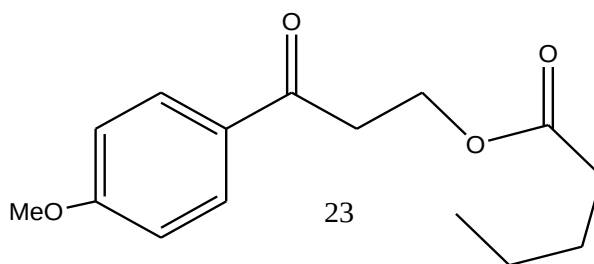
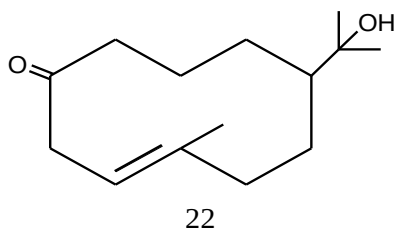
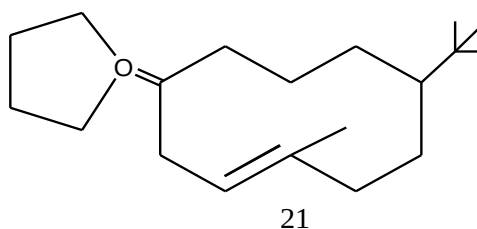
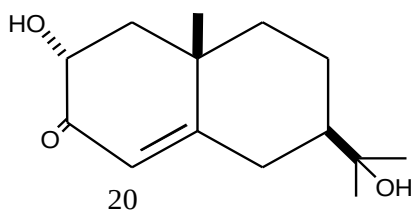
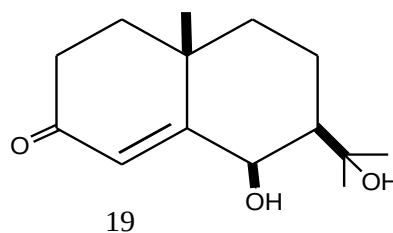
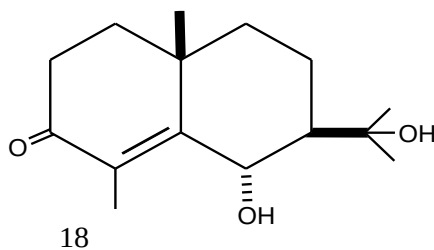
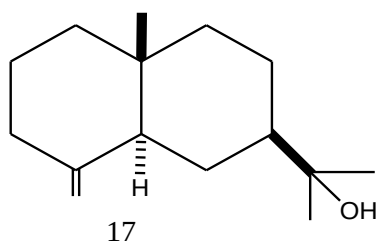
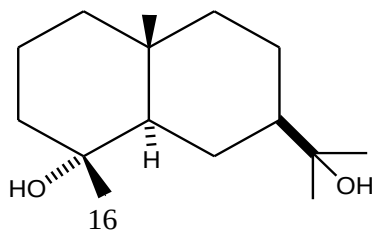
The genus *Carissa* is a rich source of different natural classes of compounds. A literature search for the phytoconstituents of *Carissa spinarum* indicated the isolation of lignans, phenolic compounds, sesquiterpenes, chlorogenic acid derivatives, flavonoids and their glycosides, cumarins, triterpenes and cardiac glycosides. The inosit derivative quebrachitol (1) was isolated from the twigs of *Carissa spinarum*. Chemical studies on the MeOH extract of the roots of *Carissa spinarum* resulted in the isolation of a sesquiterpene, carissone (2), seven aromatic compound (2) *O*-hydroxyacetophenone(3),catalponol (4), vanillin (5), 4-hydroxy-(3-hydroxypropionyl)benzene (6), coniferaldehyde (7), scopoletin (8), and isofraxidin and six lignans,

(-)- nortrachegenin (9), carinol (10), carissanol (11), (+)-lariciresinol (12), (-)- secoisolariciresinol (13), and (-)-olivil (14). (Bentley *et al.*,1984).

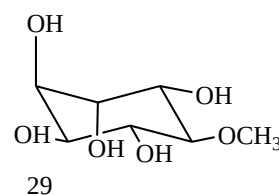
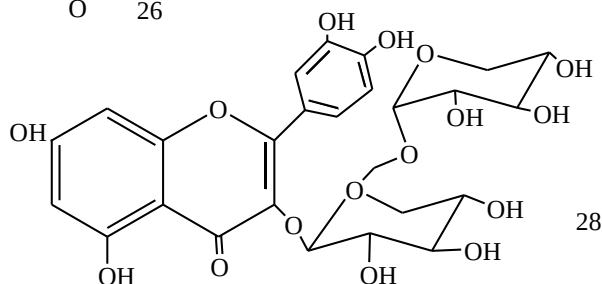
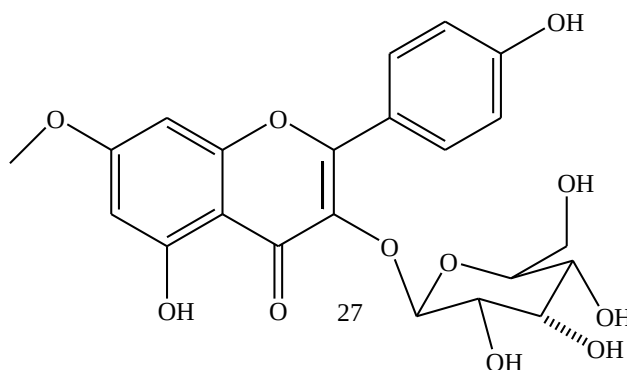
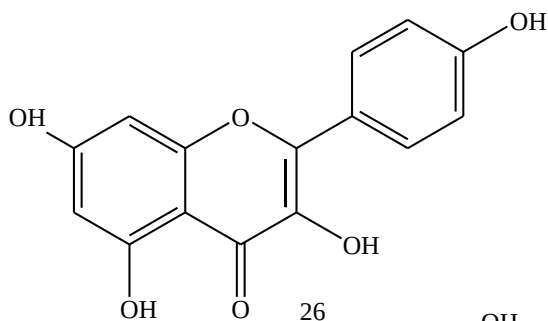
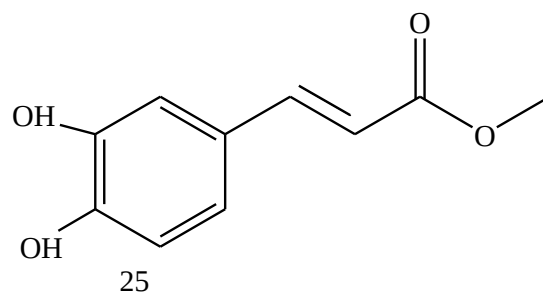
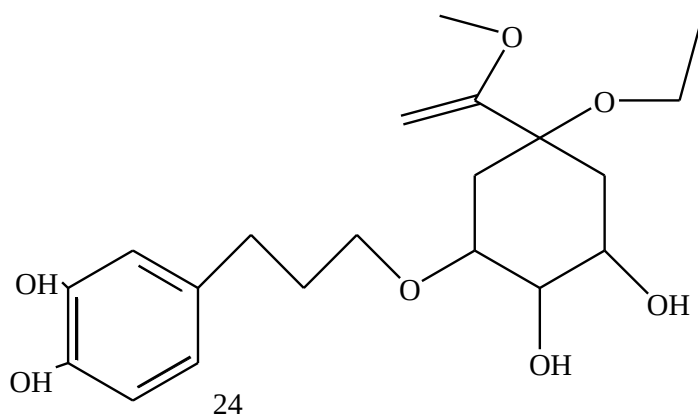


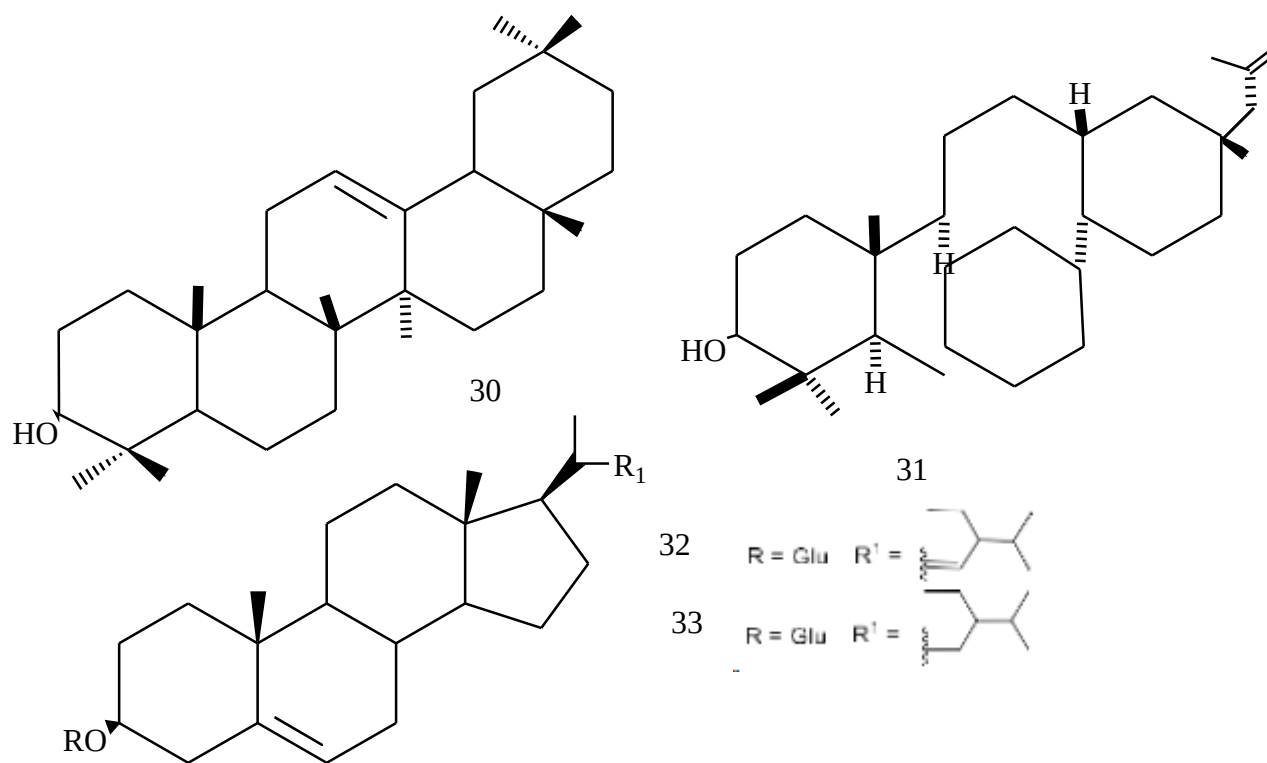


Phytochemical investigation of the petrol- and tetrachloromethane-soluble fractions of the MeOH extract of the roots of *Carissa spinarum* resulted in the isolation of seven sesquiterpenes, namely, carissone (15), cryptomeridiol (16), β -eudesmol (17), 6 α -carissano(18)6 β -carissanol (19), α -carissanoland(20)germacrenone and from the stems of *Carissa spinarum*, the germacrane derivative,(21)carenone together with (22)3'-(4'-methoxyphenyl)-3'-oxopropionyl hexadecanoate were also isolated((Rao *et al.*,2005).

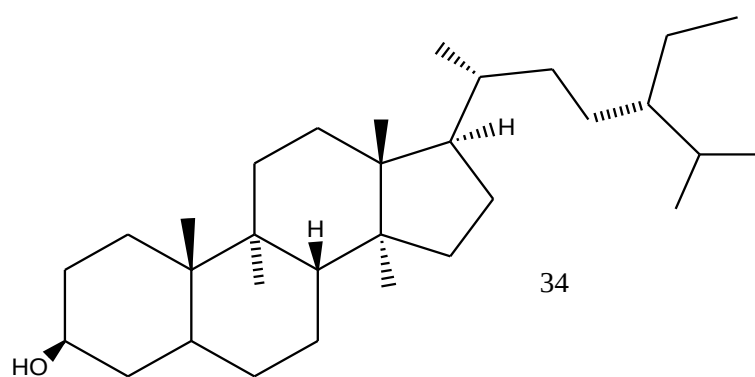


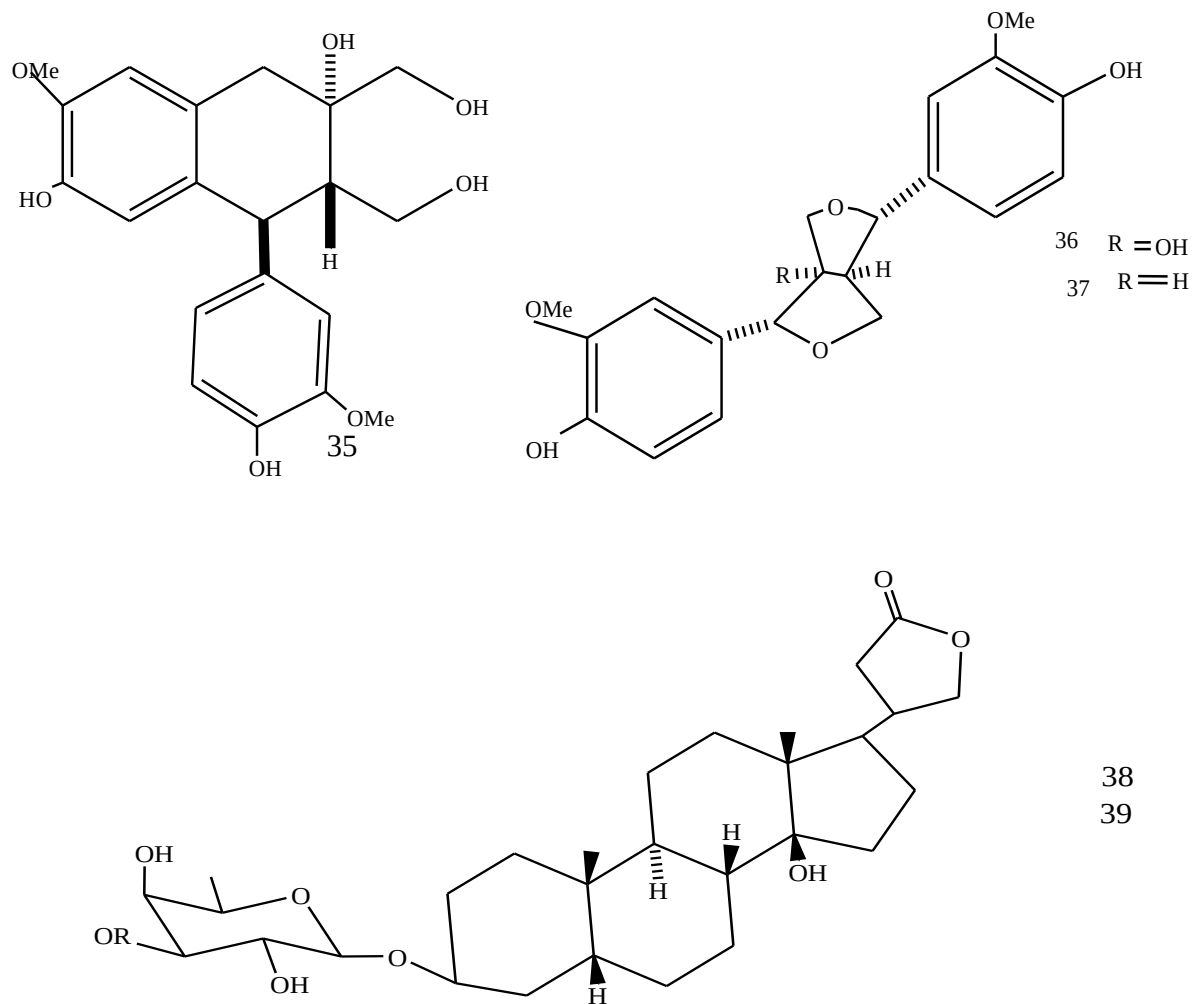
Eleven compounds, namely, (23) chlorogenic acid-1-ethyl ether-1-methyl ester (24) caffeic acid methyl ester (25), kaempferol (26), quercetin-3-O-D-glucoside-7,3',4'-trimethyl ether (27), rutin (28) pinitol (29) β -amyrin (30), lupeol (31), stigmasterol glucoside (32), β -sitosterol (33) and β -sitosterol glucoside were isolated from the ethanol extract of the aerial parts of *Carissa spinarum* (Al-yuossef *et al.*, 2012).



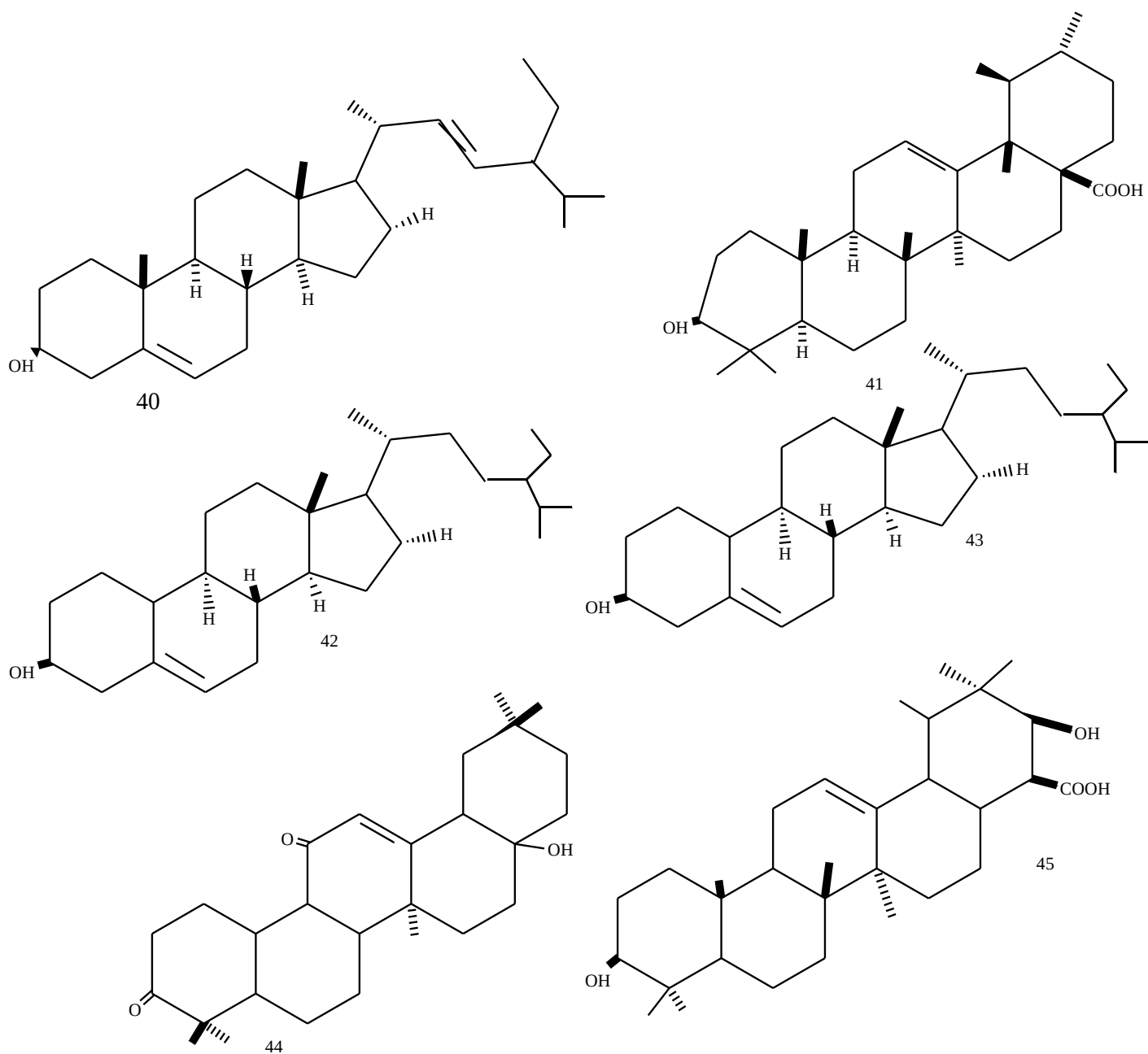


Three lignans(34)(+)-cycloolivil(35)(+)-8-hydroxypinoresinol(36)(+)-pinoresinol and (37) two cardiac glycosides,digitoxigenin O- β digitalopyranoside and(38) evomonoside stem *Carissa spinarum* (Wangteeraprasertetal.,2012).

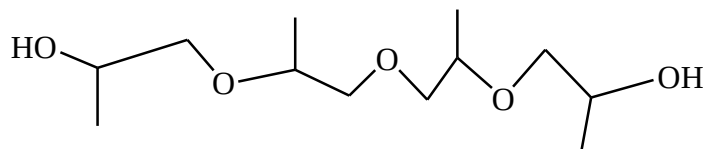
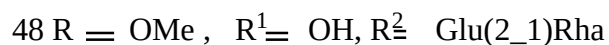
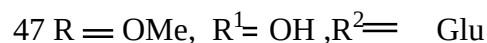
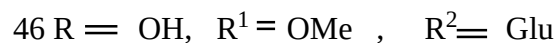
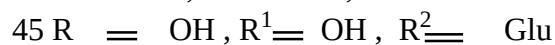
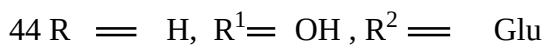
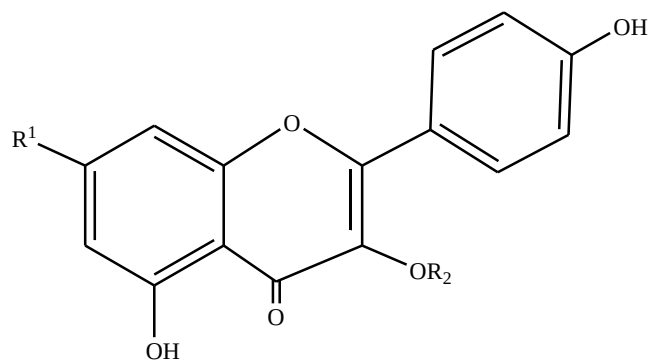




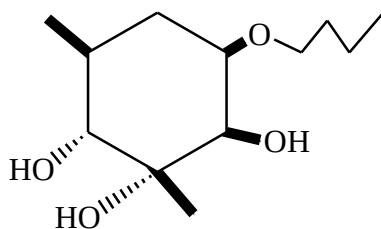
Phytochemical studies on the pet ether extract of the roots of *Carissa spinarum* resulted in the isolation of six compounds, namely, stigmasterol (39), ursolic acid (40), lupeol (41), campesterol (42), 17-hydroxy-11-oxo-nor- β -amyrone (43) and urs-12-ene-3 β ,22 β -diol-17- carboxylic acid (44)(Karunakar *et al.*,2012)



Investigation of the EtOAc and butanol fractions from the ethanol extract of the aerial parts of *Carissa spinarum* afforded 3-O-acetyl chlorogenic acid(46), along with five flavonol glucosides, namely, kaempferol 3-O-β-D-glucopyranoside (45), quercetin-3-O-β-D- glucopyranoside (46), rhamnetin-3-O-β-D-glucopyranoside (47), isorhamnetin-3-O-β-D-glucopyranoside (48) and isorhamnetin-3-O-β-D-glucopyranoside-(2''□1''')- rhamnopyranoside (49), together with caredulis and (+) butyl-O-α-L-rhamnoside (50) (Al-Youssef *et al.*,2017).



49



50

3 .MATERIALS AND METHODS

3.1 Plant material

The roots of *Carissa spinarum* were collected from East Wallaga Zone, Limu Woreda, Mukarba Dimma Village 485 km West from Addis Ababa. The collected plant sample was washed with tap water, cut into small bits and shade dried at room temperature. After dried, it was grinded into powder by using a mortar and pestle before being subjected to solvent extraction.

3.2 Instruments and apparatuses

The instruments and apparatuses utilized to conduct the experiments were What man No,1 filter paper, separatory funnel, rotary evaporator, beakers (different sizes), mortar and pestle, flasks (different sizes), Petri dish, measuring cylinder, ruler, pencil, micropipettes (different sizes), electronic balance.

3.3 Chemicals and Reagents to be used

The chemicals and reagents that were used in this study includes analytical grade solvents (methanol, chloroform), hydrochloric acid, sulphuric acid, Ammonia, Ferric Chloride, DMSO, standard antibiotic (gentamicin), Wagner's reagent.

3.4 Extraction of plant sample

About 250g of the powdered root of *Carissa spinarum* was extracted with 1:1 ratio of chloroform and methanol (1 Lx3) for 24 hrs each with random shaking by maceration method. The extracts were filtered first using cotton plug followed by Whatman No. 1 filter paper.

Solvents were removed by rotary evaporator under reduced pressure. The semisolid extract was made to dry at about room temperature and the dried masses were weighed and the percentage yield was calculated. The dried crude extracts were kept in closed container for phytochemical screening and antibacterial tests.

3.5 Phytochemical Screening

The qualitative phytochemical analysis was done to identify the types of secondary metabolites present, which assumed to contain the bioactive component present in the crude extract of *Carissa spinarum*.

3.5.1 Test for Terpenoids

Plant extract sample was mixed with 2ml of chloroform in a test tube. Then 3ml of concentrated sulphuric acid (H₂SO₄) was carefully added to the mixture to form a layer (Yadav *et al.*, 2014).

3.5.2 Test for Flavonoids

Alkaline reagent test: An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. (Sahira and Cathrine, 2015).

3.5.3 Test for Phenolic compounds

Ferric Chloride test: The extract (0.5g) was dissolved in 3mL of distilled water. To this few drops of neutral 15% ferric chloride solution were added (Sahira and Cathrine, 2015).

3.5.4 Test for Tannins

0.5g of the extract was boiled in 10mL of water in a test tube and filtered. To the filtrate, few drops of 0.1% ferric chloride were added (Babe *et al.* 2018).

3.5.5 Test for Alkaloids

0.5gm of dried powdered sample free extract were measured, agitated with 3mL diluted hydrochloric acid and filtered separately. Then the filtrates were tested for alkaloids using Wagner's reagent: To 2mL of filtrate and 2mL Wagner's reagent was added (Harborne, 2005).

3.5.6 Test for Steroids

The plant material was extracted in 10mL chloroform. First 2mL of acetic anhydride was added, and then 2mL of conc. H_2SO_4 (Yadav *et al.*, 2014).

3.5.7 Tests for coumarins:

On to filter papers, a few drops of ammonia were added and then a drop of *extracts* was added (Sangeetha *et al.*, 2014).

3.5.8 Test for Glycosides

Borntrager's test: A 2mL of filtrate were mixed with 3mL of chloroform and 10% ammonia is added to the sample (Gusthinnadura *et al.*, 2017).

3.5.9 Test for saponins

To 0.5mg of crude extract, 5mL of distilled water was added and shaken while heating to boil (Babe *et al.* 2001).

3.6 Anti-bacterial activity test

3.6.1 Bacterial strains

The two Gram negative bacteria, *E. coli* and *P. aeruginosa* and Gram-positive *S. aureus*, bacteria were used in this test depending on availability at regional laboratory.

3.6.2 Preparation of inoculum

The test bacterial strains were transferred from the stock cultures and streaked on Mueller Hinton plates and incubated for 24hrs. Well separated bacterial colonies were then used as inoculum. Bacteria was transferred using bacteriological loop to autoclaved Mueller Hinton that were cooled to about 45°C in a water bath and mixed by gently swirling the flasks. The medium was then poured to sterile Petriplates, allowed to solidify and used for the anti-bacterial activity.

3.6.3 Preparation of test solutions

The test solution was prepared by dissolving 10mg of the crude extract in 1mL of DMSO to achieve final stock concentration. Then five serial dilutions (100%, 50%, 12.5% and 6.25%) concentrations were prepared and used to test antibacterial activities.

3.6.4. Test for antibacterial activity

The crude extract and each of the isolates were then pipetted to the sterile paper discs. Sterilized paper discs were transferred to Mueller Hinton agar plates seeded with bacteria and incubated at 37°C for 24hrs performed. The antibacterial activity was evaluated by measuring the zone of inhibition against the tested organism.

4. RESULTS AND DISCUSSIONS

4.1 Extraction of plant sample

The extraction of roots of *Carissa spinarum* followed by removal of solvent using rotary evaporator to afford 1.819 g (0.73 %) dark brown crude extract.

4.2 Phytochemical Tests

Table 4.1: phytochemical screening test results

Sr.no	Secondary metabolites	Color observed	Methanol and chloroform (1:1) Crude extracts of the root of <i>Carissa spinarum</i>
1	Terpenoids	An interface with red dish brown	+
2	Alkaloids	reddish brown precipitate	+
3	Tannins	blue-black	+
4	Saponins	No frothing	-
5	Flavonoids	Yellow fluorescence	+
6	Glycosides	No pink color	-
7	Phenols	A dark green	+
8	Coumarins	Fluorescence	+
9	Steroids	Color changes from violet to blue	+

+ indicates presence; – indicates absence

These tests reveal the presence of various bioactive secondary metabolites which might be responsible for medicinal attributes.

4.3 Antibacterial test

Table 4.2: Antibacterial activity of chloroform/methanol extract of *Carissa spinarum*

Bacteria used		Concentrations and zone of inhibition (mm)					Standard disc (mm)
Concentrations		100	50	25	12.5	6.25	Gentamicin 10µg
Gram positive	<i>S. aureus</i>	18.00± 0.1	12.00±0.2	0	0	0	30.00± 0.1
Gram negative	<i>E. coli</i>	15.30±0.2	9.00±0.3	0	0	0	28.00±0.1
	<i>P. aeruginosa</i>	0	0	0	0	0	26.00±0.1

The information obtained from (Table 4.2) indicated that chloroform methanol (1:1) extract of the root of *Carissa spinarum* was effective against *Staphylococcus aureus* (18mm) and *Escherichia coli* (15mm) intermediate or medium with *Pseudomonas aeruginosa*.

. The effectiveness of chloroform methanol (1:1) extract of root *Carissa spinarum* was due to the presence of various secondary metabolites like alkaloids, flavonoids, terpenoids, tannins and etc. Those confirmed in photochemical screening tests (table 4.1).

Geo *et al.*, 1998 reported that *Carissa spinarum* root extract have highest antibacterial activity against urinary tract infection causing pathogens like *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, External Ear infection, Infection of blood, infection of bone and joints which occurs commonly under ten years old causing bacteria *Staphylococcus aureus*.

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The phytochemical screening and antibacterial activity studies on the root extracts of *Carissa spinarum* have revealed the presence of several bioactive compounds, including alkaloids, flavonoids, phenols, tannins, terpenoids, steroid and coumarins. These compounds are known for their potential antimicrobial properties. The antibacterial activity tests demonstrated that the extracts, particularly the methanol/chloroform extract, exhibited significant inhibitory effects against *S. aureus*, *E. coli* and *P. aeruginosa*. Strong antibacterial activity (18 mm) was observed against *Staphylococcus aureus* compared to standard gentamicine (30mm). These findings support the traditional use of *Carissa spinarum* in treating infections. The presence of diverse phytochemicals suggests that the plant's antimicrobial activity is likely due to a synergistic effect of multiple compounds.

5.2 Recommendations

Based on the findings of the study the following recommendations are forwarded:

1. Isolation and Characterization:

- Conduct studies to isolate and characterize the specific compounds responsible for the observed antibacterial activity.
- Utilize advanced analytical techniques like HPLC, GC-MS, and NMR spectroscopy for compound identification and structural elucidation.

2. Mechanism of Action:

- Investigate the precise mechanism of action of the bioactive compounds to understand their mode of interaction with bacterial cells.

3. Sustainability and Conservation:

- Promote sustainable harvesting practices to ensure the long-term availability of *C. spinarum* and its medicinal properties.
- Implement conservation strategies to protect the plant's natural habitats and biodiversity.

REFERENCE

- Afanyibo, Y. (2019); Esseh, K.; Idoh, K.; Koudouvo, K.; Agbonon, A.; Gbeassor, M. Toxicity and antioxidant activity of *Syzygium aromaticum*, *Mondia whitei*, *Carissa spinarum* and *Caesalpinia bonduc*. *J. Phytopharm.* 8, 124–128.
- Agbor, G.A., Kuate, D. and Oben, J.E. (2007). Medicinal plants can be good source of antioxidants: case study in Cameroon. *Pakistan Journal of Biological Sciences*, 10(4): 537-544.
- Alqathama, A. (2015); Bader, A.; Khondkar, P.; Gibbons, S.; Prieto, J. Bioguided isolation of cytotoxic compounds Against melanoma cells from *Carissa spinarum* L. *Planta Med.* 81, 51.
- Al-youssef, H. M.; Hassan, W. H. B. *Biomed.& Pharmacol. J.* 2012, 5, 09-18.
- Al-Youssef, H. M.; Hassan, W. H. B. *Arabian J. Chem.* 2017, 10, 109-113.
- Ansari, I., and Patil, D.T. (2017). A brief review on phytochemical and pharmacological profile of *carissa spinarum*. *Asian Journal of Pharmaceutical and Clinical Research*, 11(9)12- 18.
- Babe.G,Yadessa.M, andMilkyas.E(2018).AntibacterialActivityof TwoFlavansfromthe Stem Bark of *Embelias chimperi*.*Advances in Pharmacological Sciences*.Volume2018, Article ID 5870161,6
- Beck, N.(2016) R.; Namdeo, K.P. Evaluation of in vivo anti-inflammatory activity of leaves extract of *Carissa spinarum* on formalin induced paw edema in albino rats. *Pharma. Biosci. J.*4, 36–40.
- Bentley, M. D.; Brackett, S. R.; Chapya, A. J. *Nat. Prod.* 1984, 47, 1056-1057.
- Berhanu, G (2020); Atalel, D.; Kandi, V. A review of the medicinal and antimicrobial properties of *Carissa spinarum* L. *Am. J. Biomed. Res.* 8, 54–58.
- Bhadane.B(2018) S.; Patil, M.P.; Maheshwari, V.L.; Patil, R.H. Ethnopharmacology, phytochemistry, and biotechnological advances of family Apocynaceae: A review. *Phytother. Res.* , 32, 1181–1210.
- Chanda, J.(2014); Mukherjee, P.K.; Harwansh, R.; Bhadra, S.; Chaudhary, S.K.; Choudhury, S. RP-HPLC simultaneous estimation of betulinic acid and ursolic acid in *Carissa spinarum*. *Nat. Prod. Res.* 28, 1926–1928.
- Dawit.A. (2001). The role of medicinal plants in Health care Coverage of Ethiopia, the possible benefits of integration. *Conservation and Sustainable Use of Medicinal plants in Ethiopia. Proceeding of the National workshop on Biodiversity Conservation and Sustainable use of medicinal plants in Ethiopia*, 28 April- 01 May 1998, IBCR, Addis Ababa. Pp. 107-118.
- Dhatwalia. J.(2021). Kumari, A.; Verma, R.; Upadhyay, N.; Guleria, I.; Lal, S.; Thakur, S.; Gudeta, K.; Kumar, V.; Chao, J.C.; et al. Phytochemistry, pharmacology, and nutraceutical profile of *Carissa* species:An updated review. *Molecules*, 26, 7010.

- Doshi, G. (2017); Chaskar, P.K.; View, M. Antiinflammatory and antibacterial potential of *Carissa carandas* and *Carissa spinarum* nanoemulsions. *Int. J. Pharma Sci. Res.* 8, 5137–5145.
- Fatima, A., Singh, P. P., Agarwal, P., Irchhaiya, R., Alok, S., and Verma, A. (2013). Treatment of various Diseases by *Carissa spinarum* L.- a Promising Shrub. *International Journal of Pharmaceutical Sciences and Research*, 4(7): 2489.
- Feyissa, D.; Melaku, Y (2016). Phytochemical, antibacterial and antioxidant studies of the leaves of *Carissa spinarum*. *Int. J. Chem. Pharma. Sci.* 7, 25–30.
- Gemechu, B., Dagmawit, A., Venkataramana, K. (2020). A Review of the Medicinal and Antimicrobial Properties of *Carissa spinarum* L. *American Journal of Biomedical Research*, 8(2), 54-58.
- Geo. F. (1998) Brooks, Janet S. Butel, Stephen A. Morse. Jawetz, Malnick and Adelberg's *Medical Microbiology*. 21st edition. Appleton & Lange. 3
- Gusthinnadura, O., Achala, T., and Malamige M. (2017). Extraction methods, qualitative and Quantitative techniques for screening of phytochemicals from plants. *American Journal of Essential Oils and Natural Products*. 5 (2), 29-32.
- Harwansh. R (2010) K.; Garabadu, D.; Rahman, M.A.; Garabadu, P.S. In vitro anthelmintic activity of different extracts of root of *Carissa spinarum*. *Int. J. Pharma. Sci. Res.* 1, 84.
- Hegde, K (2011).; Satyanarayana, D.; Joshi, A.B. Evaluation of anticonvulsant activity of *Carissa spinarum* root extract. *Rajiv Gandhi Univ. Health Sci. J. Pharm. Sci.* 1, 64–68.
- Inga, H.; Sue, E.; Sileshi, N., *Carissa spinarum*. In *Flora of Ethiopia and Eritrea*, The National Herbarium, Biology Department, Addis Ababa University and The Department of Systematic Botany, Uppsala University: Addis Ababa, Ethiopia, 2003; Vol. 4, Part 1, p 90 .
- Jan, O. (2015). Q.; Kamili, N.; Ashraf, A.; Iqbal, A.; Sharma, R.K.; Rastogi, A. Haematobiochemical parameters of goats fed tannin rich *Psidium guajava* and *Carissa spinarum* against *Haemonchus contortus* infection in India. *J. Parasitic. Dis.* 39, 41–48.
- Karunakar, H.; Satyanarayana, D.; Joshi, A. B. *J. Pharm. Sci.* 2012, 2, 55-59.
- Kelemu, (2018); Wolde, W. Ethnobotanical study of indigenous knowledge on medicinal plants used to treat diseases in selected districts of Amhara regional state, Ethiopia. *J. Med. Plants Res.* **2018**, 12, 528–536.
- Kumar, V., Joshi, V., and Singh, R. (2017). Antibacterial activity of Root and Leaf Extracts of *Carissa spinarum* against Methicillin-resistant *Staphylococcus aureus* (MRSA) and other Clinically Pathogenic bacterial strains. *Octa Journal of Biosciences*, 5(2): 61- 64.

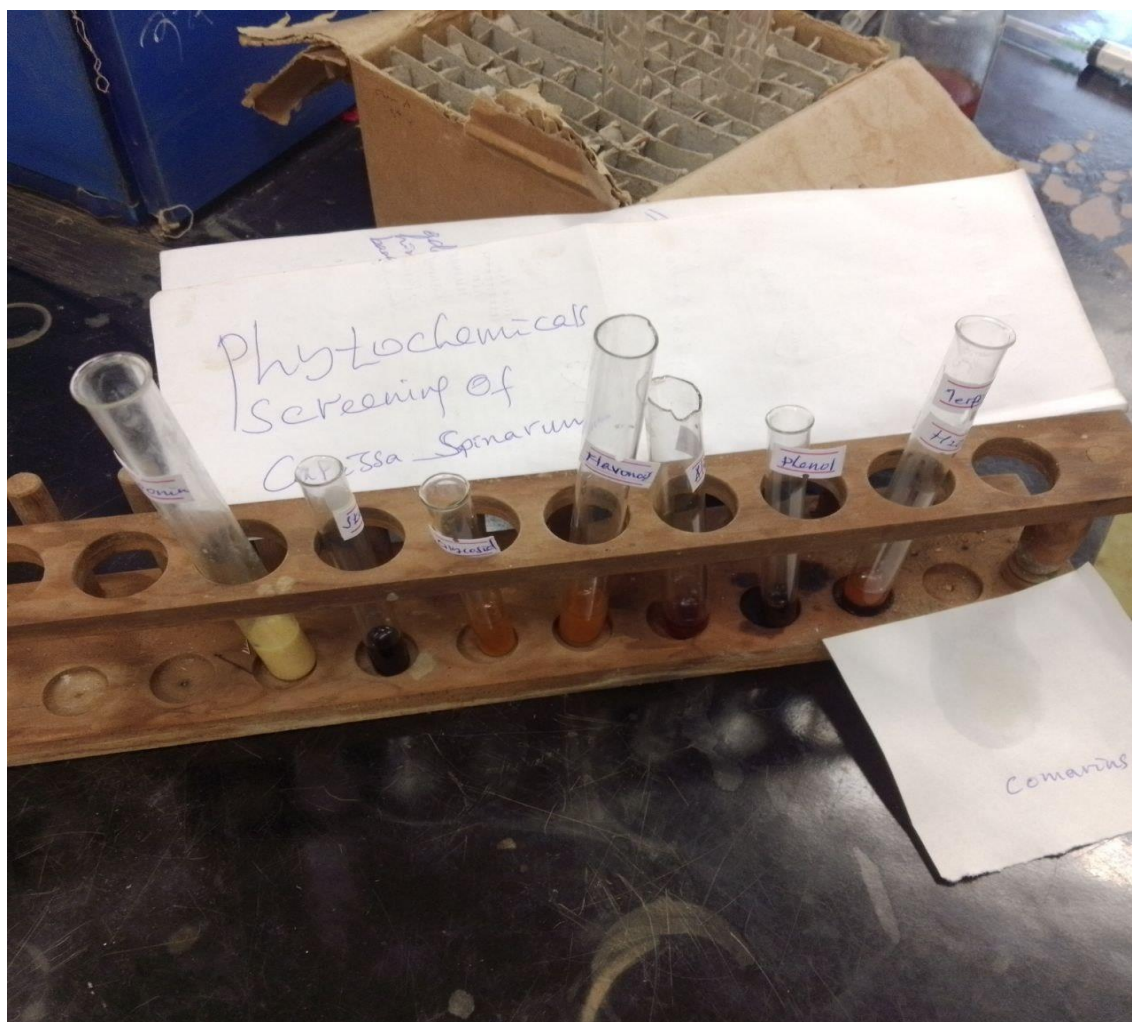
- Liu, Y.(2021); Zhang, Y.; Muema, F.W.; Kimutai, F.; Chen, G.; Guo, M. Phenolic Compounds from *Carissa spinarum* are characterized by their antioxidant, anti-inflammatory and hepatoprotective activities. *Antioxidants*, 10, 652.
- Mohammed, A.(2013); Wossene, A.; Giday, M.; Tilahun, G.; Kebede, N. In vitro anthelmintic activities of four medicinal plants against *Haemonchus contortus*. *Afr. J. Plant Sci.* 7, 369–373.
- Mundaragi, A. and Thangadurai, D. (2017). Process optimization, physicochemical characterization and antioxidant potential of novel wine from an underutilized fruit *Carissa spinarum* L. (Apocynaceae). *Journal of Food Science and Technology*, 38(3): 428-433.
- NegussieB.(1988).Traditional wisdom and modern development: A case study of traditional peri-natal knowledge among women in southern Shewa, Ethiopia. Doctoral dissertation, University of Stockholm, December. *Ethiop. J. Health Dev.* 20(2), 127-134
- Omino, E. A.; Kokwaro, J. O. J. *Ethnopharmacol.* 1993, 40, 167-180.
- Pankhurst, R. (2001). The status and Availability of oral and written knowledge on traditional health care in Ethiopia. In *Proceedings of the National Workshop on Biodiversity Conservation and Sustainable Use of Medicinal Plants in Ethiopia Addis Ababa: IBCR*. Pp 92- 106.
- Parekh, J. and Chanda, S. (2008). Antibacterial activities of aqueous and alcoholic extracts of 34 Indian medicinal plants against some *Staphylococcus* species. *Turkish Journal Of Biology*,
- Rao, R. J.; Kumar, U. S.; Reddy, S. V.; Tiwari, A. K.; Rao, J. M. *Nat. Prod. Res.*
- Rubaka, C., Ndakidemi, P., H. M., and Shahada, and F. (2014). Analysis of Phytochemical and Antibacterial Activity of *Carissa spinarum* Linn Crude Extracts. *European Journal of Medicinal Plants*, 4(8), 937-945.
- Sahira.KandCathrine.L,2015GeneralTechniquesInvolvedinPhytochemicalAnalysis. *International Journal of Advanced Research in Chemical Science (IJARCS)*.2(4),25-32.
- Saka, A.(2022); Tesfaye, J.L.; Gudata, L.; Shanmugam, R.; Dwarampudi, L.P.; Nagaprasad, N.; Rajeshkumar, S. Synthesis, characterization, and antibacterial activity of ZnO nanoparticles from fresh leaf extracts of *Apocynaceae, Carissa spinarum* L. (Hagamsa). *J. Nanomater.* 6230298.
- Samie, A., Tambani, E., Harahfield, E., Green, E., Ramalivhana, J.N. and Bessong, P.O. (2010). Antifungal activities of selected Venda medicinal plants against *Candida albicans*, *Candida krusei*
- Sangeetha,V.S.,Michael,B.and Beena,L.(2014).Phytochemical Analysis of *Annona Reticulata*L.Leaf Extracts.*Int.ResJPharm.AppSci.*,4(5),4-8.

- Sanwal, R., and Chaudhary, A. K. (2011). Wound healing and antimicrobial potential of *Carissa spinarum* Linn. in albino mice. *Journal of Ethnopharmacology*, 135(3): 792-796. [34].
- Sehar, I.(2011).; Pal, H.C.; Shukla, S.; Bhushan, S.; Hamid, A.; Gupta, B.D.; Saxena, A.K. Cytotoxic evaluation and induction of mitochondria-mediated apoptosis in human leukaemia HL-60 cells by *Carissa spinarum* stem isolate. *J. Pharma. Pharmacol.* 63, 1078–1090.
- Sharma, I. (2017)P.; Kanta, C.; Semwal, S.C.; Goswami, N. Wild fruits of Uttarakhand (India): Ethnobotanical and medicinal uses. *Inter. J. Complement. Alter. Med.* **2017**, 8, 1–8.
- Tena, R. (2008). Ethno Botanical Study on Traditional Medicinal Plants of Limu Woreda, Eastern Welega, Western Ethiopia, M.Sc. Thesis. Addis Ababa, Ethiopia.
- Tiruneh, A.(2022)T.; Ayalew Tiruneh, G.; Chekol Abebe, E.; Mengie Ayele, T. Phytochemical Investigation and Determination of Antibacterial Activity of Solvent Leave Extracts of *Carissa spinarum*. *Infect. Drug. Res.* 15, 807–819.
- Tolo, F.(2006)M.; Rukunga, G.M.; Muli, F.W.; Njagi, E.N.; Njue, W.; Kumon, K.; Kofi-Tsekpo, M.W. Anti-viral activity of the extracts of a Kenyan medicinal plant *Carissa edulis* against herpes simplex virus. *J. ethnopharmacol.* 104, 92–99.
- Wangteeraprasert R, Lipipun V, Gunaratnam M, Neidle S, Gibbons S, Likhitwitayawuid K. Bioactive compounds from *Carissa spinarum*. *Phytother Res.* 2012; 26(10): 1496- 1499.
- WHO, (2010).Report of Traditional Medicine.World Health Organization, Geneva, WHO. [14]
- Yadav, M., Chatterji, S., Gupta, S.K.,&Watal,G.(2014).Preliminary phytochemical Screening of six medicinal plants used in traditional medicine.*International Journal of Pharmacy and Pharmacological Science*,6(5),539-542.
- Yetgin, A., KeremCanl, and Altuner, E.M., (2018). Comparison of Antimicrobial Activity of *Allium sativum* Cloves from China and Taskopru, Turkey.*Advances in pharmacological and Pharmaceutical Sciences*, 1-5.

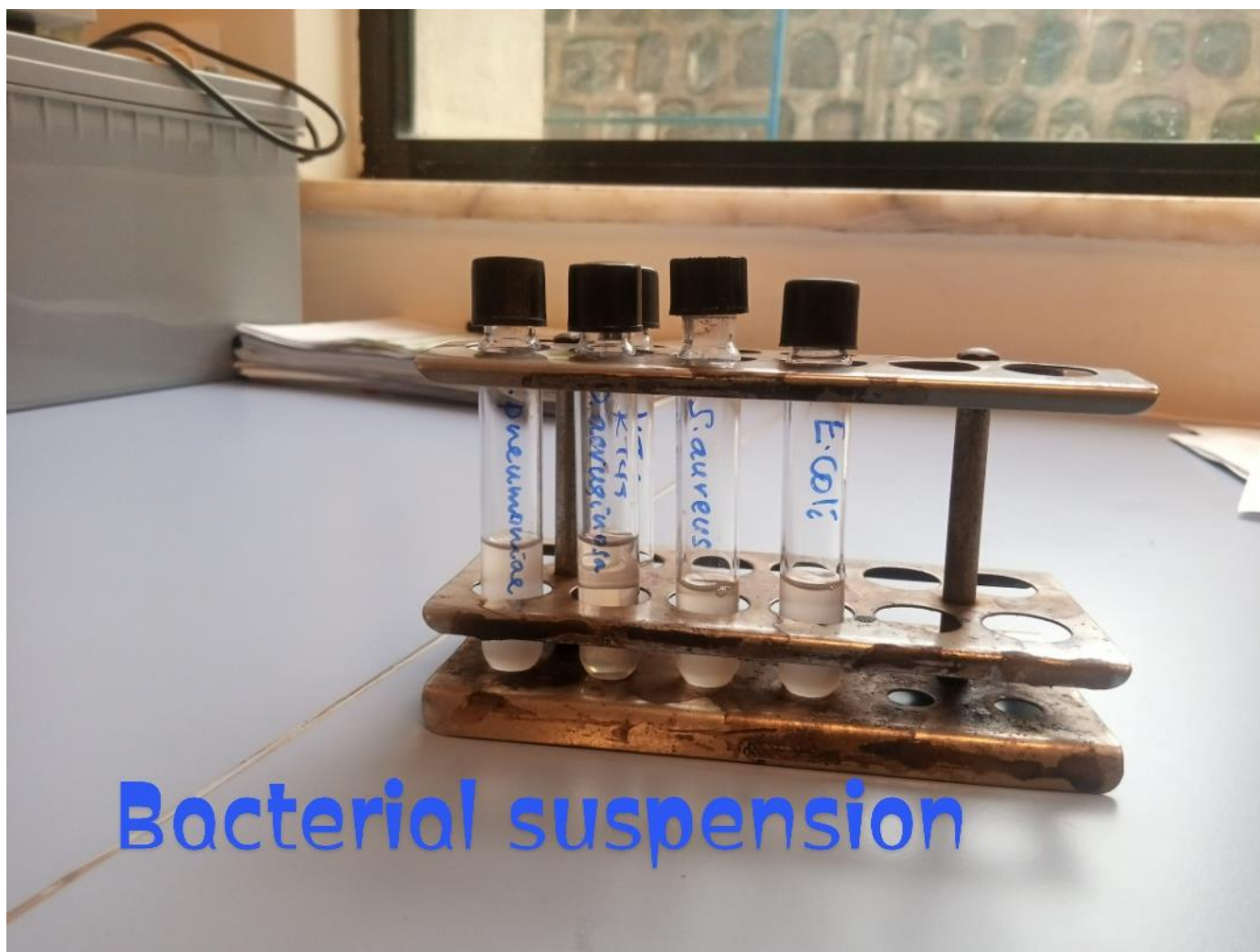
Appendix 1: During the extraction of plant sample



Appendix 2 Image of all positive tests for phytochemical tests



Antibacterial test for chloroform and methanol original extract root of *Carissa spinarum* against the three bacteria's *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.







usceptibility Test Result





