Phytochemical Analysis, Evaluation of Antimicrobial, Antioxidant activities and Molecular Docking studies of Aerial Constituents of *Momordica foetida* (Yamora Misa/Yekura Hareg)

Betelhem Behailu Tefera

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 (Specialization in Medicinal Chemistry)

Office of Post Graduate Program

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Adama, Ethiopia

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Advisor: Aman Dekebo (Professor)

Co-advisor: Endale Mulugeta (PhD)

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DECLARATION

I hereby declare that this Thesis entitled "Phytochemical Analysis, Evaluation of Antimicrobial, Antioxidant activities and Molecular Docking studies of Aerial Constituents of *Momordica foetida***"** 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.

_____	_____	_____
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 Analysis, Evaluation of Antimicrobial, Antioxidant activities and Molecular Docking studies of Aerial Constituents of *Momordica foetida*" prepared under our guidance by Betelhem Behailu 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.

_____	_____	_____
Major Advisor	Signature	Date
_____	_____	_____
Co-advisor	Signature	Date

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LIST OF ABBREVIATIONS AND ACRONYMS

ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
CFU	Colony-forming Unit
CLSI	Clinical and Laboratory Standards Institute
$CDCl_3$	Deuterated chloroform
^{13}C NMR	Carbon 13-Nuclear Magnetic Resonance
DNA	Deoxy- ribose Nucleic Acid
DPPH	1, 1- diphenyl-2-picrylhydrazyl
EtOAc	Ethyl Acetate
GC-MS	Gas Chromatography/Mass Spectrometry
MAP-30	*Momordica* Anti-HIV Protein-30
MHA	Muller Hinton Agar
MIC	Minimum Inhibitory Concentration
NIST	National Institute of Standards and Technology
PDA	Potato Dextrose Agar
PDB	Protein Data Base
^{1}H NMR	Proton-Nuclear Magnetic Resonance
R_f	Retention Factor
RSA	Radical Scavenging Activity
SDB	Sauborated Dextrose Broth
TLC	Thin Layer Chromatography
TMS	Tetra Methyl Silane
UV	Ultra Violet-Visible

ABSTRACT

Medicinal plants are the nature's gift, serve as potential sources of bioactive compounds and lead compounds in drug development. Momordica foetida commonly known as "Yamora misa" or "Yekura Hareg", used to treat various diseases. The present study focused on isolation of chemical constituents, evaluation of biological activities and molecular docking studies of M. foetida. Here this, aerial of M. foetida (500g) were successively extracted using n-hexane, $CHCl_3$, $CHCl_3$/MeOH (1:1) and MeOH, yielded 2 g (0.4 %), 4.5 g (0.9 %), 12.47 g (2.49 %) and 32.5 g (6.5 %), respectively. Silica gel column chromatography of the $CHCl_3$/MeOH (1:1) and MeOH crude extract afforded five compounds, including colossolactone A derivatives ***(99)****, myristic acid* ***(100)****, pentan-1-ol* ***(101)****, bis (3-hydroxy-3-methylbutan-2-yl) phthalate* ***(102)*** *and heptane* ***(103)****. The structures of compounds were characterized using spectroscopic methods (^{1}H-NMR, ^{13}C-NMR and DEPT-135). Additionally, the GC-MS analysis of a fraction showed that Phenol, 2, 4-bis (1, 1-dimethylethyl) (11.18%) as the principal component. The in-vitro antibacterial activity was evaluated using disc diffusion method against S. aureus, S. pyogens, P. aeruginosa and E. coli. The antibacterial result revealed promising inhibition zone for MeOH extract (9.00±0.5, at 150 mg/mL) and pentan-1-ol* ***(101)*** *(11.5±00 mm, at 10mg/mL) against E. coli and S.pyogens compared to standard antibiotic ampicillin. In-vitro antifungal activity was evaluated using broth dilution method against C. albicans, penicillium. Species, T. mentagrophytes and A. niger. Compound* ***101*** *showed promising antifungal activity with MIC value of 1.25 and 5mg against T. mentagrophytes and P. species, respectively. The extracts and compounds were also assessed for their radical scavenging activity using DPPH assay. Promising percent RSA were observed with n-hexane (61.538%, IC_{50}:12.53), MeOH extract (61.538%, IC_{50}:7.98) and Compounds* ***99*** *and* ***100*** *were* ***(****71.123 and 72.784%, with IC_{50}: 0.46 and 0.85 respectively). Molecular docking study was done using Autodock vina 4.2 and it was found that compound* ***99*** *shows the best scoring pose (-9.6, -8.1, -11.1 and -8.0 Kcal/mole) against selected protein targets. Based on this study, it can be concluded that the aerial parts of M. foetida has bioactive compounds with the potential to treat bacterial and fungal infections and also used as an antioxidant.*

Key words: *M. foetida*, Antimicrobial Activity, Antioxidant Activity, Molecular Docking

1. INTRODUCTION

1.1. Background of Study

Infectious diseases caused by bacteria, fungi, viruses and parasites remain a major threat to public health, despite tremendous progress in human medicines. Their impact is particularly great in developing countries because of the relative unavailability of medicines and the emergence of wide spread drug resistance (Priyavardhini *et al.,* 2012). Traditional medicine appears to be the source of healthcare particularly in the rural majority communities of Africa due to its intrinsic qualities, unique and holistic approaches as well as its accessibility and affordability (Anza *et al*., 2021).

Medicinal plants are the nature's gift to people to make illness free healthy life (Vasantha *et al*., 2012). Medicinal plants have been the subject for very intense pharmacological studies and serve as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development (Anza *et al*., 2021). Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants growth in different parts of the country (Nirmala & Pandian, 2015).

Use of plant as a source of medicine has been inherited and is an important component of the health care system (Oduselu *et al.,* 2019). Historically, drug discovery relied solely on natural products as the primary source of medicines that have significantly influenced the advances in biology and inspired drug discovery and development. Around 50% of modern drugs are of natural products origin and as such these natural products play an important role in the drug development (Ayoola *et al.*, 2008; Nuru & Hepburn, 2001).

Plants have always been used in Ethiopia as a remedy for various ailments. Due to its long history traditional medicine has in fact become an integral part of the culture. Despite their significant contribution to society, traditional medicine has received very little attention (Giday, 2001).

The family Cucurbitaceae contains about 130 genera and 800 species (Olarewaju *et al*., 2021). The *Momordica* genera contain a special group of Cucurbitacins called momordicosides (Kaushik *et al.*, 2015), comprises of over 60 species, including *M. balsamina, M. charantia, M.*

cochinchinensis and *M. foetida,* which are distributed in the warm tropics, predominantly in Africa and in Southeast Asia *(*Ramalhete *et al.,* 2022).

M. foetida commonly known as "Yamora misa" or "Yekura Hareg", which is a perennial climbing plant; the flowers are often cream in color with a reddish center. The male and female flowers are on the same plant. The distinctive fruit is bright orange with prickles (Nabifwo M. K, 2015).

Traditionally the *M. foetida* species is used to treat a number of ailments including, hypertension, diabetes mellitus, fever, peptic ulcer purgative, malaria (Froelich *et al*., 2007; Mukaila *et al*., 2023; Samje *et al*., 2021; Zin & Oo, 2019), diarrhea, toothache, wound, bronchitis, liver disease and smallpox (Tesfaye, 2021). The plant also used symptoms of tuberculosis in Uganda, leaves are taken orally to stop vomit in Rwandan society (Olarewaju *et al.*, 2021).

Previously, reported data states about the isolated bioactive compounds and pharmacological activities of *M. foetida*. For instance, The isolation of unreported compounds cucurbitane glycosides, named momordisides (Soh *et al*., 2020) and flavanone glycosides (Froelich *et al*., 2007). *M. foetida* has shown a varieties of biological activities such as antimalarial, antioxidant, antibacterial, antiplasmodial and larvicidal activity (Akono & Aphrodite, 2018; Food & Unit, 2014; Froelich *et al.,* 2007; Odeleye & Oyedeji, 2008; P Berlyn, 2021; Talukdar & Hossain, 2014; Tesfaye, 2021)

However, in Ethiopia the plant *M .foetida* which is found in lesser studied medicinal plant and still needed scientific studies on phytochemical investigation. Thus, the plant was designated for this study on the basis of ethno medicinal information by which local communities use it and rich source of variety classes of secondary metabolites with different pharmacological properties. Hence, this study was intended at phytochemical analysis, biological evaluation of aerial extracts and docking study of isolated compounds of *M. foetida.*

1.2. **Statement of the Problem**

Nowadays medicinal plants are major source of drugs used for prevention and treatment of pathogenic carriers (microbes, virus and fungi) and various diseases of human beings. Emergency of antimicrobial resistance has become the major public health challenge concern worldwide. It is estimated that, infectious diseases cause more than 90% of all morbidities and mortalities occurring in the world with more than 90% of them occurring in developing countries. Oxidative stress is a major contributer to anxiety and the development of human diseases including cardiovascular, diabetes, atherosclerosis, cancer, neurodegenerative disease and infertility (Nantia *et al*., 2018).

Many drugs available for treatment of these diseases have developed and many are still developing resistance to the present drugs (Nyigo & Malebo, 2005). Hence, there is still a need for phytochemical constituents investigating and effective antimicrobial drugs. *M. foetida* possesses various biological activities such as antimicrobial, antioxidant, antiplasmodial, larvicidal and laxative activities (Acquaviva *et al*., 2013; Froelich *et al*., 2007; Odusote & Awaraka, 2008; P Berlyn, 2021; Tesfaye, 2021).

To the best of our knowledge, isolation of compounds and compressive biological assay of the aerial part of *M. foetida* is the less explored medicinal plant, despite its traditional use for the treatment of different disease. The genus *Momordica* is rich source of various phytochemicals as reported by different research groups, which possess beneficial effects against fungi and bacteria strains (Odeleye & Oyedeji, 2008). Therefore, the aim of this study was phytochemical analysis, evaluation of antimicrobial, antioxidant activities and molecular docking studies of compounds isolated from aerial extracts of *M. foetida*.

1.3. Objectives

1.3.1. General Objective

The major objective of this study was investigation of chemical constitutents from the aerial parts of *M. foetida* and to examine the *in-vitro* antimicrobial, and antioxidant activities with their *in silico* molecular docking studies on selected protein targets.

1.3.2. Specific Objectives

- To successively extract the aerial part of *M. foetida* using *n*-hexane, chloroform, chloroform/ methanol (1:1) mixture and methanol using maceration method.
- To assess the phytochemical screening of the crude extracts of *M. foetida*.
- To isolate compounds from chloroform/ methanol (1:1) and methanol aerial extracts of *M. foetida* using silica gel column chromatography.
- To characterize the structures of isolated compounds using spectroscopic techniques (^{1}H-NMR, ^{13}C-NMR and DEPT-135) and GC-MS analysis and comparison with literature data.
- To evaluate antibacterial activities of the extracts and isolated compounds against Gram negative (*P. aeruginosa, E. coli)* and Gram positive (*S.aureus* and *S. pyogenes*) bacteria strain by using agar disc diffusion method.
- To evaluate the antifungal activities of the extracts and isolated compounds using broth dilution method against *C. albicans, Penicillium species, A. niger* and *T. mentagrophytes.*
- To evaluate DPPH radical scavenging activity of the extracts and isolated compounds.
- To examine the *in silico* molecular docking study of the isolated compounds using Auto dock Vina 4.2 software program on selected protein targets including, *P. aureginosa Quinolone signals* (**PDB ID: 5OE3**), *S. aureus Pyruvate kinase* (**PDB ID: 3T07**), *T. mentagrophytes* Squalene epoxidase (**PDB ID: 2AIB**) and *Human meyleoperoxidase* (**PDB: ID 6NGJ**).

1.4. Significance of the Study

The ethnobotanical uses of Ethiopian medicinal plants need a scientific evaluation to provide basic understanding of the plants' efficacy and lend further support to the widespread use of the traditional medicine in health care systems. The general scientific evidences on the isolation of constituents, GC-MS analysis, evaluation of biological activities and conducting *in silico* computational studies of the promising bioactive compounds originated from medicinal plants, especially herein from *M. foetida* might help to arrive at finding lead compound for drug discovery. Therefore, the present studies in general having significance of validating the medicinal potency of the selected plant species; providing a scientific rationale to support the claimed ethno medicinal knowledge and laying a scientific base for both modern and traditional medicines by providing a bridge between the modern and traditional claims of the medicinal application of the plant species.

2. LITERATURE REVIEW

2.1. Cucurbitaceae family

The family Cucurbitaceae includes a large group of plants which are medicinally valuable. It is a family of about 130 genera and about 800 species (Olarewaju *et al*., 2021). Cucurbitacins are multiplex category of diverse compounds found in the plants of family Cucurbitaceae. Medicinal and toxic properties of these compounds have stimulated a continuing interest in them. The plants of genera *Momordica* contain a special group of Cucurbitacins called momordicosides. The diversity of cucurbitacins lies in variety of its side chain derivatives that contribute to their disparate pharmacological actions. The bitter taste of plant species like cucumber has been attributed to the presence of cucurbitacins (Kaushik *et al.,* 2015).

The first cucurbitacin was isolated as a crystalline substance in 1831 and was named α-elaterin. Certain plant species rich in cucurbitacins like *Momordica* hold coveted position in different system of traditional medicines for curative effects in metabolic disease like diabetes. *Momordica* species fall within the Cucurbitaceae family commonly known as the pumpkin, cucumber and gourd or melon family. The *Momordica* genus comprises 59 species, geographically distributed across the globe (47 in Africa, 12 in Australia and Asia) (Kaushik *et al.,* 2015). *Momordica* species are cultivated not only for their nutritional purposes but also for traditional use in the treatment of various ailments. These include diabetes-related conditions, cancer and inflammatory diseases. Other activities that have been reported for species within this family are anti-obesity, antioxidant and potential anti-HIV activities (Daniel *et al*., 2014), (Kodidela *et al.,* 2021). These activities can be attributed to the diverse phytochemical composition of these plants. *Momordica* species have been reported to contain various health-promoting compounds such as cucurbitane triterpenoids, saponin, glycosides, chlorogenic acids and flavonoids (Daniel *et al*., 2014). These health benefits and bioactivities of *Momordica* species point to a diverse and rich source of numerous bioactive metabolites (Ramabulana *et al.,* 2021).

2.2. The Genus *Momordica*

Plants with potential therapeutic values have been used from time immemorial to cure various ailments and infectious diseases. of late, scientific evidences have been provided on the potential

therapeutic agent exhibited by certain traditionally used vegetable extracts (Nagarani *et al.*, 2014). *Momordica* genus embraces over 60 species, including *M. balsamina, M. charantia, M. cochinchinensis* and *M. foetida*, which are distributed in the warm tropics, predominantly in Africa and in Southeast Asia. The name of the genus derives from the Latin word ‘‘mordeo’’ that means ‘‘to bite’’ either because of the bitten appearance of the indented margins of the seeds and leaves, or for the well-known bitter taste of the ripe fruits of some of its species *(Ramalhete et al.*, 2022).

All the *Momordica* species have been consumed as vegetable and traditionally used for various disorders. The whole plant parts are ascribed to possess the anti-diabetic effect in traditional medicinal system. The active constituents of *Momordica* plant parts were cucurbitane type triterpenoids, phenolic, glycosides, and several kinds of peptides including *Momordica* anti-HIV protein (MAP 30). Recent reports revealed the presence of several kinds of cucurbitane type triterpenoids in leaf, stem and fruits of *Momordica* plant having several pharmacological activities (Nagarani *et al*., 2014).

2.2.1. Ethnobotanical uses of Genus *Momordica*

M. foetida is used in the treatment of malaria, for cough and vomiting in some parts of Uganda, expelling worms and stomach ache, while the leaf decoction is used to treat tuberculosis. The leaf decoction is also taken orally to treat diabetes in the lower Eastern Province of Kenya. In Dek Island of Ethiopia, the leaf and stem of the plant are boiled in water and the steam is inhaled during bedtime to treat febrile illnesses. *M. foetida* young leaves are cooked and eaten while the endocarp is eaten raw in the Bullen district, north-western Ethiopia (Muronga *et al*., 2021).

Different species of the genus *Momordica* are used as a traditional medicine to cure many diseases worldwide. *Momordica chlarantia* extracts (primarily from the fruit) are currently being used include for diabetes, microbial infections, and as a cytotoxic agent for certain types of cancer. The seeds, fruit, leaves, and root of the plant have been used in traditional medicine for microbial infections, sluggish digestion, wound healing, inflammation, hypertension, and as a laxative and emetic. In Guyana traditional medicine, a leaf tea is used for diabetes, to expel intestinal gas, and as an antiviral for measles, hepatitis, and feverish conditions (Panchal *et al.*, 2020).

Momordica charantia fruit juice and a leaf tea are employed for diabetes, malaria, infections, measles and hepatitis. Leaves are used for treating constipation, diabetes, diarrhea, leprosy, rheumatism, breast cancer, snake bite, anemia, gonorrhea, and rheumatoid arthritis (Rakh & Banurekha, 2021).

The fruits, leaves, roots and seeds of *Momordica balsamina* have been used, either as decoction, infusion, and poultice or as herbal tea, for several medicinal purposes in different provinces of Asia and Africa. It is used to treat vomiting associated with bile and fever, as an ingredient in aphrodisiac preparations, to treat externally malignant ulcers Pulverization, stomach and intestinal complaints, as an anthelminthic, skin problem and to relieve intercostal pains. The leaves used to treat abdominal pain, aqueous extract to induce lactation and regenerate the loss of blood during labor (Nigeria) and to treat extreme uterine bleeding (Ramalhete *et al.,* 2022).

Fresh fruit juice and cooked fruit in small amount of oil are prescribed for hypertension and diabetes, respectively. Oral administration of 50 mL of root juice is advised once a day with empty stomach to beat diabetes. The juice of root is a domestic remedy for the inflammation caused by contact with the urine of the house lizard. Root juice has stimulant, antiseptic, antidiabetic, anti-inflammatory and antiulcer effect. The mucilaginous tubers act as anthelminthic, spermicidal and antifertility abortifacient agent. Oral administration of leaf paste for skin disease, tender fruits are rubbed on skin for pimples and acne and roasted seeds are used for eczema and other skin problems. Root powder is also applied for softening skin and reducing perspiration (Talukdar & Hossain, 2014).

According to (Rakh & Banurekha, 2021), the fruit of *Momordica dieoca* used for Cure asthma, leprosy, fever, tumors, urinary discharges, excessive salivation, troubles of the heart, inflammation. Head troubles, all kinds of poisoning like snake bite & scorpion-sting, inflammation of urine of house-lizard, sedative in high fever with delirium (as paste) Antiseptic, bleeding piles, bowel affections, expectorant, powder to skin soft, supple and lessens perspiration. Anti-allergic, bronchial, anti-malarial,Contraceptive, Spermicidal activity and anthelmintic activity.

2.2.2. Botanical and Ethnobotanical Description of *Momordica foetida*

The plant is a dioecious, perennial herb, trailing or climbing with simple tendrils; stem is long, with dark green flecks when young, woody when old, rooting at the nodes. Leaves are alternate,

simple; stipules absent; petiole 1.5–17 cm long. The fruit is a long-stalked, ellipsoid berry orange when ripe, densely and softly spiny, dehiscing with 3 valves and exposing the many seeds embedded in scarlet pulp.

Division: *Magnoliophyta*

Class: *Magnoliosida*

Order: *violes*

Family: *Cucurbitaceae*

Genus: *Momordica*

Species: *foetida* (Zin & Oo, 2019).

Figure 1: Pictures of *M. foetida* (taken by Betelhem Behailu Nov, 2021).

Traditional uses of *M, foetida* have been eaten as vegetables in countries like Gabon, Malawi, Sudan, Tanzania and Uganda. Confirming it's widespread in the tropics while the pulps of ripe fruit are eaten in Tanzania. There are also magical uses reported, for example, in the case of an epidemic, the roots and leaves are powdered and sprinkled on burning charcoal, the smoke is then inhaled to be immune to the disease. Other uses include insecticides and fattening rabbits (Mukaila *et al*., 2023). It is also used as antidotes for venomous stings such as bee stings and also as an analgesic (Samje *et al*., 2021; Talukdar & Hossain, 2014).

Phytochemical screening of the crude extract showed the presence of various secondary metabolities which include alkaloids, flavonoids, terpenoids, steroids, saponnins and tannins (Odeleye & Oyedeji, 2008; Zin & Oo, 2019). *Momordica foetida* comprised high to moderate

levels of triterpenoids, steroids, sugars, glycosides, saponins and low concentrations of alkaloids, flavonoids and phenols (P Berlyn, 2021).

M. foetida has shown a kinds of biological activities such as antimalarial activity (Talukdar & Hossain, 2014), antioxidant activity (Akono & Aphrodite, 2018), (Food & Unit, 2014), (Akono & Aphrodite, 2018), antibacterial activity (Bedore & Geinoro, 2018), (Tesfaye, 2021), (Odeleye & Oyedeji, 2008), antiplasmodal activity (Froelich *et al*., 2007) and larvicidal activity (P Berlyn, 2021).

Table 1: Different Species of Genus *Momordica* and their Ethno medicinal uses

Name of plants	Ethno medicinal uses	Reference
Momordica foetida	To treat hypertension, diabetes mellitus, peptic ulcer, malaria, diarrhoea, liver disease.	(Froelich *et al*., 2007; Mukaila *et al*., 2023; Samje *et al.*, 2021; Tesfaye, 2021)
Momordica charantia	To treat diabetes, microbial infections, menstrual stimulation and wound healing.	(Panchal *et al.*, 2020; Rakh & Banurekha, 2021)
Momordia balsamina	To treat externally malignant ulcers Pulverization, as an anthelminthic.	(Ramalhete *et al.*, 2022; Talukdar & Hossain, 2014)
Momordica dioca	To treat Cure asthma, leprosy, fever, tumours, urinary discharges.	(Rakh & Banurekha, 2021).

2.2.3. Phytochemical and Pharmacological Study

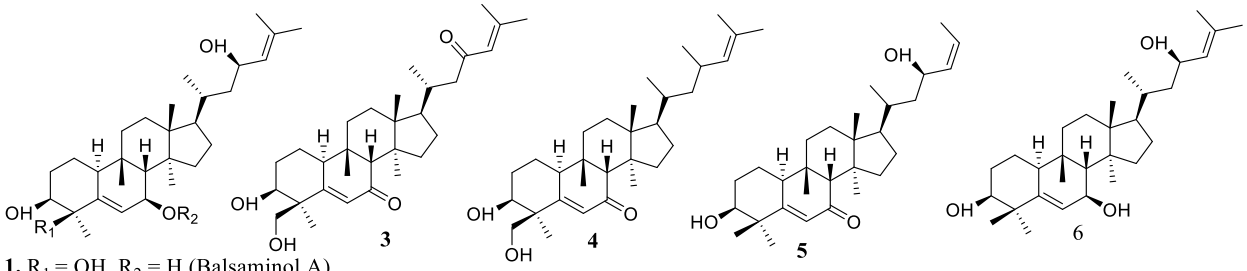
According to (Ramalhete *et al*., 2022), the phytochemical study of the methanol extract of *M. balsamina* aerial parts, led to the isolation of several cucurbitane-type triterpenoids, most of them reported for the first time. The compounds are divided in several groups, namely, the balsaminol set balsaminol A, B, C, D, E, F, triacetayl balsaminol F and balsaminapentaol (**1**–**9**) respectively, the balsaminoside A, B, C and kuguaglycosideA (**10**–**13**). Cucurbitacins with less common structures formation of cucurbitadienol (3S)-2, 3-oxidosqualene via chair-boat–chair conformation, balsaminagenin A, B and C(**14**–**16**). The karavilageninset (**17**–**36**) and the cucurbalsaminol set (**37**–**39**) some of the new triterpenes have unusual oxidation patterns, reported for the first time in cucurbitacins from plant sources. Balsaminols A-D,

balsaminagenins A, B and balsaminapentaol have an unusual oxidation pattern at C-29, while cucurbalsaminols. A-C (**37**–**39**) share the presence of an uncommon hydroxyl group at C-12 and cucurbalsaminane set (**40**–**42**). Cucurbitacins isolated from *M. balsamina* fruits (**43-45**). Nor-isoprenoid (**46**) and pimarane-type diterpenes (**47**–**51**) isolated from *M. balsamina* aerial parts and Flavonols identified in *M. balsamina* leaves, Cucurbitacins B (**62**), E (**63**) and I (**64**).

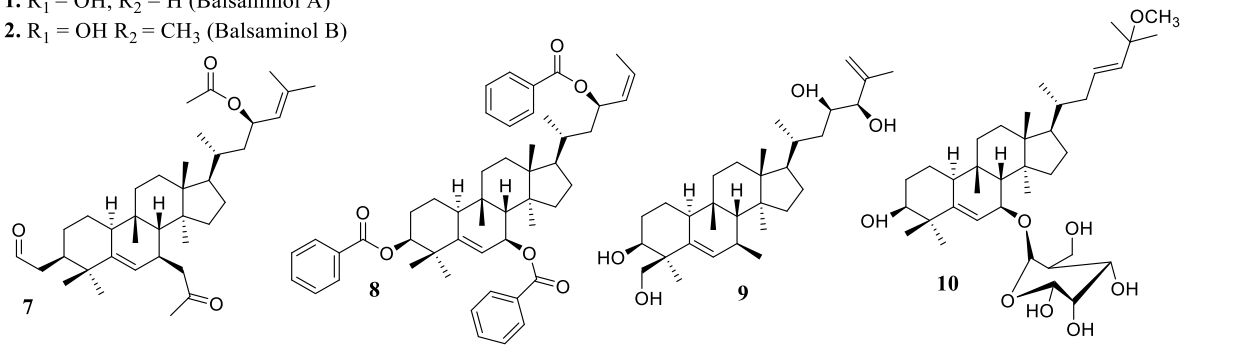
Momordica charantia is a good source of primary metabolites such as carbohydrates, fibers, proteins, minerals, and vitamins. The most important chemical constituents from *M. charantia* include heteropolysaccharides (arabinose, galactose, glucose, mannose and rhamnose); proteins and peptides (momordins, momorcharins); terpenoids and saponins (cucurbitanes and cucurbitacines); flavonoids and phenolic compounds. *M. charantia* also contains triterpenes, steroids, alkaloids and phenolic compounds some of compounds isolated from Momordica charantia from number (Momordicoside F2 (**65**), Goyaglycoside B (**66**), Karaviloside III (**67**), Charantoside VI (**68**), Charantagenin E (**69**), Charantoside II(**70**), Momordicoside G(**71**), Goyaglycoside D (**72**), Stigmasterol glucoside (**73**), β-sitosterol glucoside (**74**), (19R)-7β,19-epoxy-19-methoxycucurbita-5,24-dien-3β,23diol (**75**), Cucurbitane triterpenoid (**76** and **77**), Steroidal glycoside (**78**) (Muronga *et al.*, 2021).

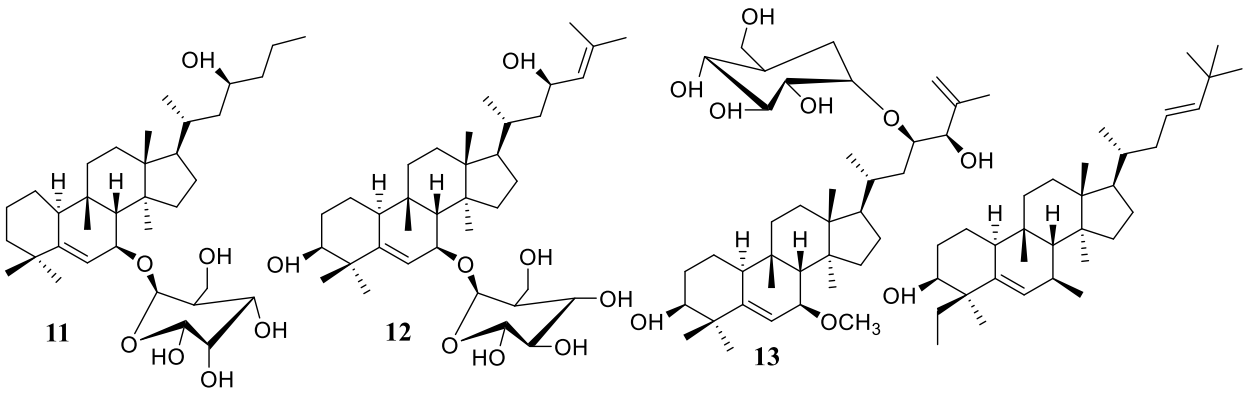
The chemotaxanomic and ethnopharmacological significance of the genus *Momordica* sparked the investigation of the secondary metabolites of *M. foetida*. According to (Soh et al., 2020). The isolation of two previously unreported compounds from *M. foetida* were cucurbitane glycosides, named momordisides A (**79**) and B (**80**) along with momordin (**81**), a previously undescribed pentanortriterpene. In addition, three known secondary metabolites namely β-sitosterol 3-O-β-D-glucopyranoside (**82**), β-sitosterol (**83**), kaempferol-7-O-ß-D-glucopyranoside (**84**), Quercetin malonlglycoside (**85**), Kaempferol malonyldiglycoside (**86**), Kaempferol Acetylglycoside (**87**), Quercetin acetylglycoside (**88**), Isorhamnetin acetylglycoside (**89**), Isorhamnetin glycoside(**90**), Quercetin glycoside (**91**), Kaempferol glycoside (**92**), Kaempferol diglycoside (**93**), Quercetin diglycoside were also isolated (**94**) (Khoza *et al.*, 2016; Muronga *et al*., 2021; Odusote & Awaraka, 2008). According to (Froelich *et al.*, 2007) isolated five flavanone glycosides from the leaves of *M. foetida* the name given as follows 5,7,4'-Trihydroxyflavanone-7-O-ß-D-glucopyranoside (Prunin) (**95**),5,7,3',4'-Tetrahydroxyflavanone-

7-O-ß-D-glucopyranoside (Eriodictyol-7-O-ß-D-glucopyranoside)(**96**), 5,7Dihydroxychromone-7-O-ß-D-glucopyranoside (**97**) and Kaempferol-7-O-ß-D-glucopyranoside (Populnin) (**98**).

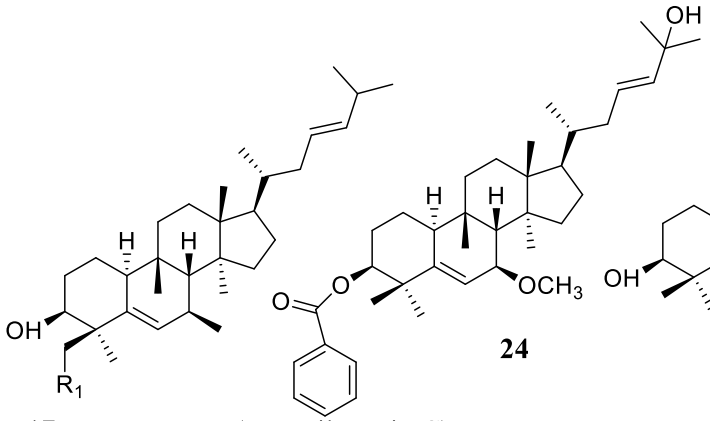


1. R_1 = OH, R_2 = H (Balsaminol A)
2. R_1 = OH R_2 = CH_3 (Balsaminol B)





14. R_1= OH, R= H(BalsaminageninA)
15. R_1= OH, R =CH_3 (Balsaminagenin B)
16, R_1= H, R_2 =H (Balsaminagenin C)



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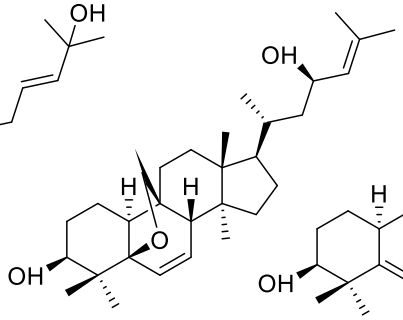

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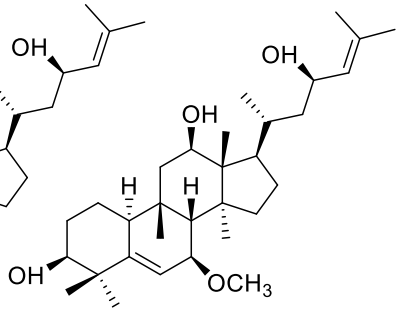
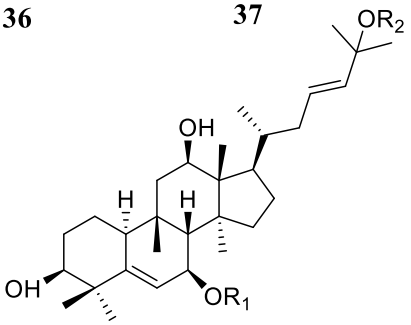
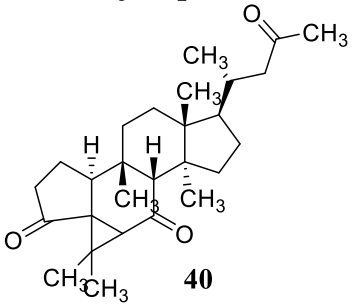


37

17. R_1 = H; R_2 = (Karavilagenin C)
18. R_1 = H; R_2 = $COCH_3$ (Karavoate A)
19. R_1 = R_2 = $COCH_3$ (Karavoate B)
20. R_1 = H; R_2 = $COCH_2CH_3$ (Karavoate C)
21. R_1 = R_2 = $COCH_2$ CH_3 (Karavoate D)
22. R_1 = H; R_2 = $COCH_2CH_2CH_3$ (Karavoate E)
23. R_1 = R_2 = $COCH_2CH_2CH_3$ (Karavoate F)
25. R_1 = R_2 = Bz (Karavoate H)
26. R_1 = H; R_2 = *p*-nitroBz (Karavoate I)
27. R_1 = R2 = *p*-nitroBz (Karavoate J)
28. R_1 = H; R_2 = *p*-chloroBz (Karavoate K)
29. R_1 = R_2 = *p*-chloroBz (Karavoate L)
30. R_1 = H; R_2 = *p*-methoxyBz (Karavoate M)
31. R_1 = R_2 = *p*-methoxyBz (Karavoate N)
32. R_1 = H; R_2 = Cin (Karavoate O)
33. R_1 = R_2 = Cin (Karavoate P)
34. R_1 = H; R_2 = $COCH_2CH_2COOH$ (Karavoate Q)
35. R_1 = R_2 = $COCH_2CH_2COOH$ (Karavoate R)



38. = CH_3, R_2 = CH_3 (Cucurbitasaminol B
39. = R_1 = R_2 = H (Cucurbitasaminol C)



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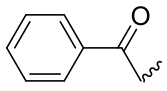
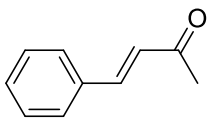
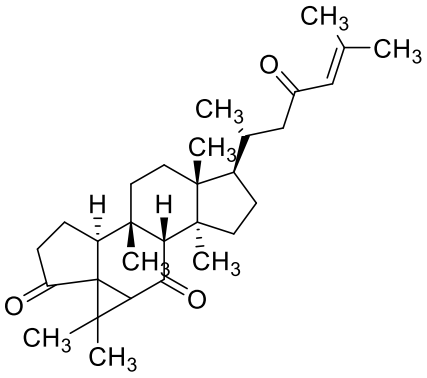
Bz =



Cin =





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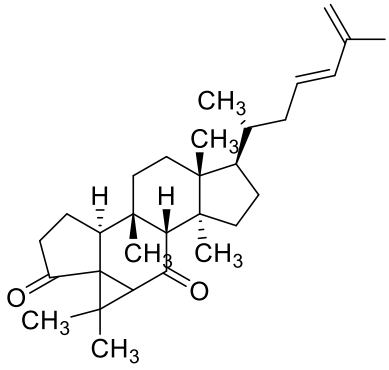

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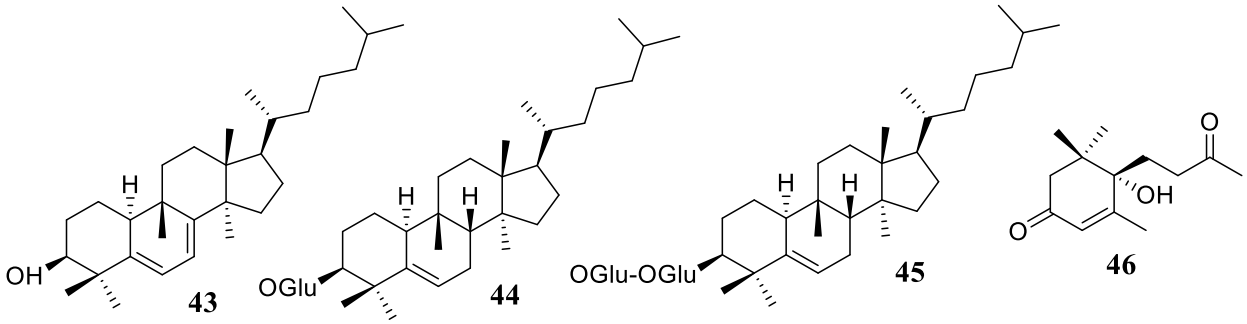
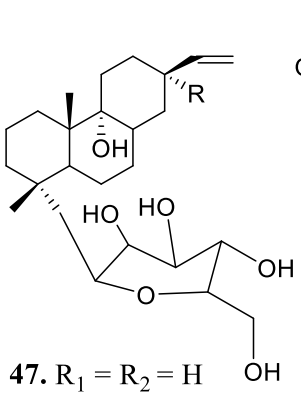
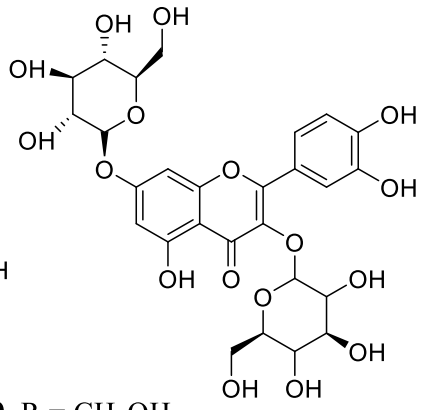
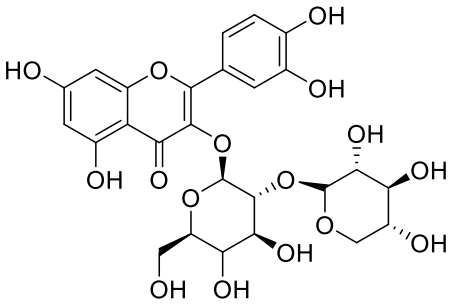




47. $R_1 = R_2 = H$
48. $R_1 = R_2 = Ac$
49. $R_1 = Ac$, $R_2 = H$



50. R = CH_2OH
51. R = COOH
52



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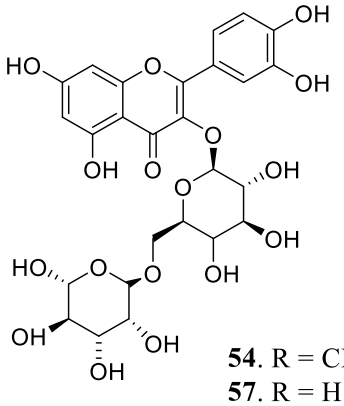
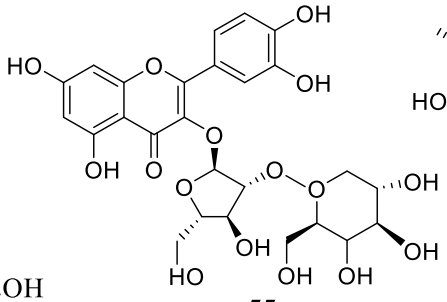


54. R = CH2OH
57. R = H



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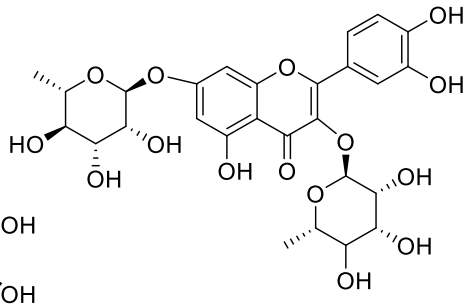

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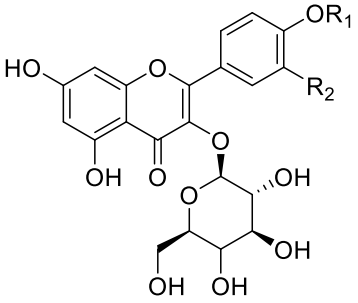
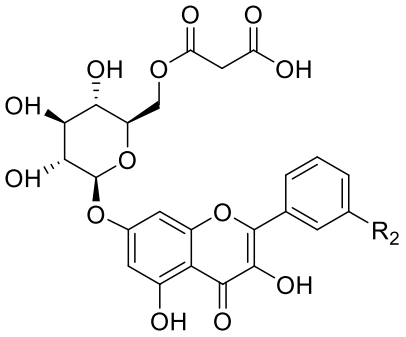


58. $R_1 = R_2 = H$

61. $R_1 = CH_3$; $R_2 = OH$



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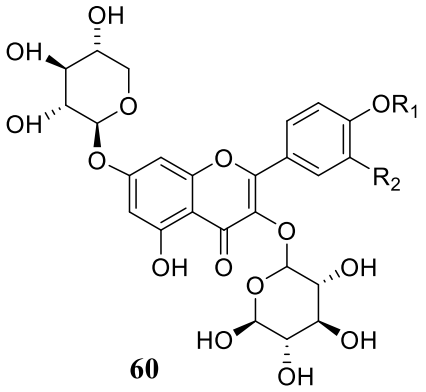

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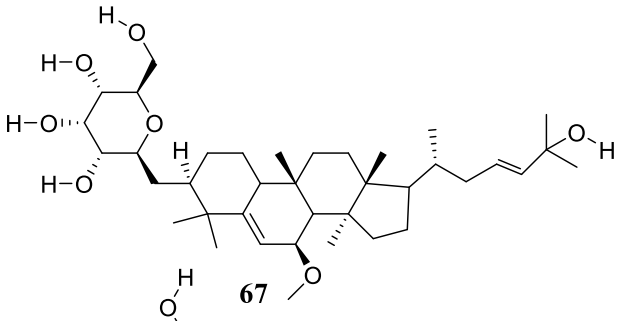

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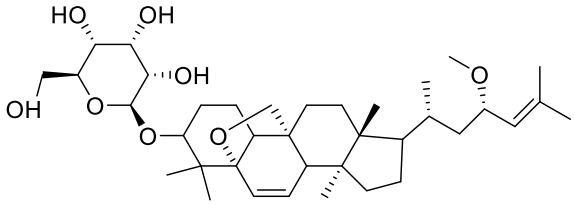

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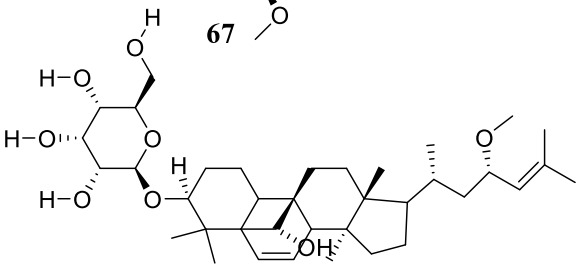

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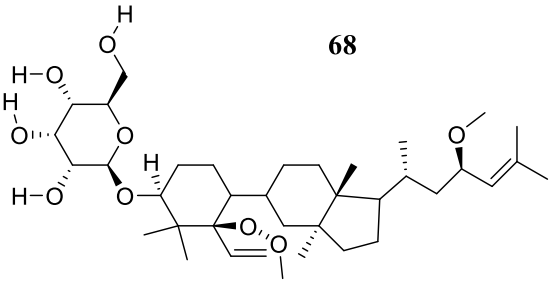

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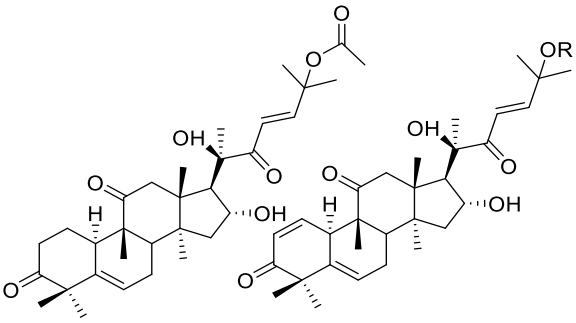
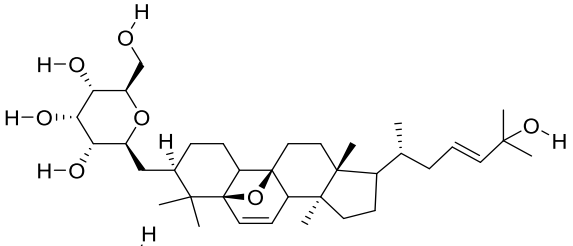


62

63. R = $COCH_3$

64. R = H



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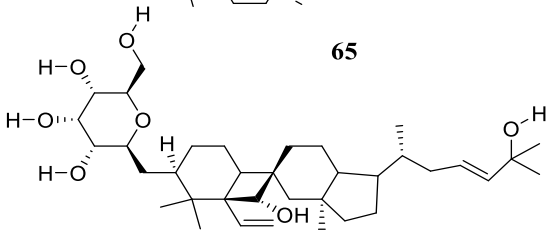

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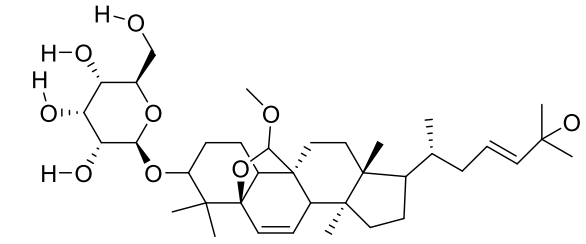

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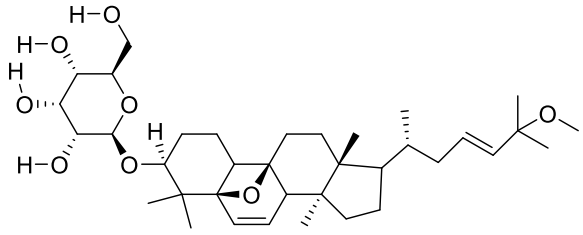

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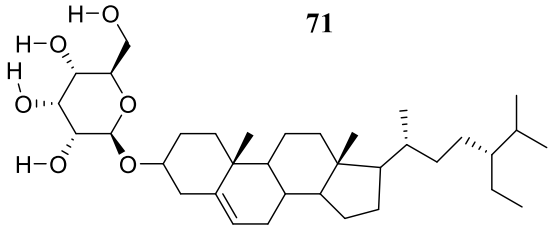

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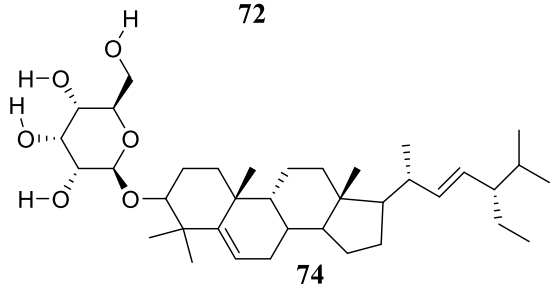

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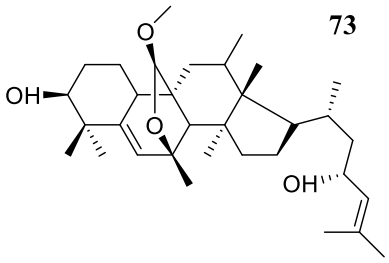

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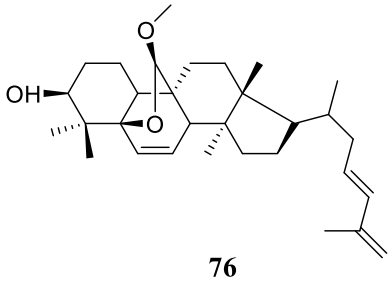

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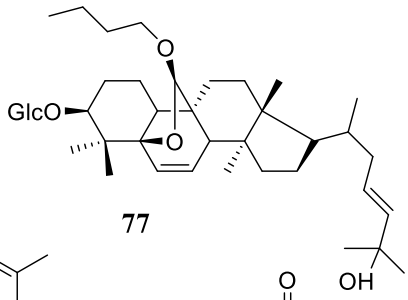

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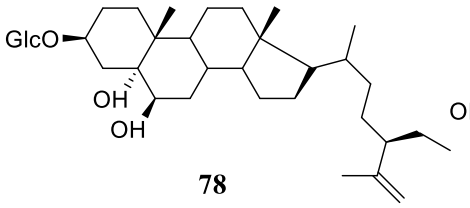

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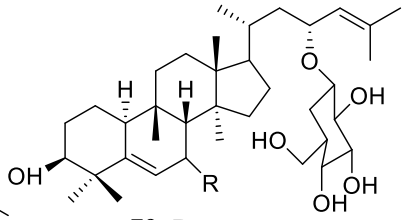
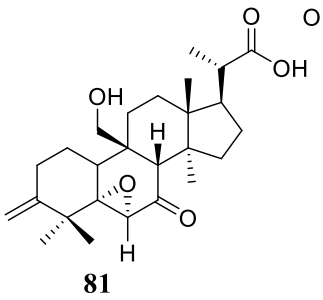


79. R = =O

80. R = -OCH_3



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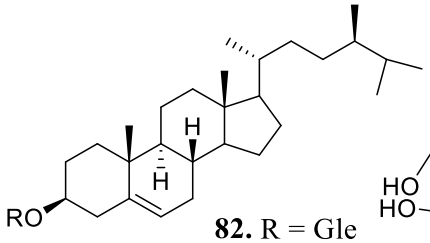
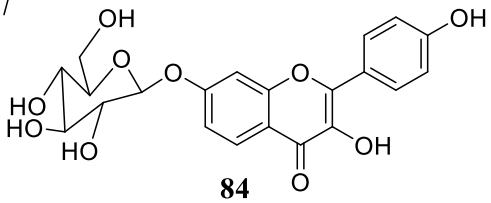


82. R = Gle

83. r = H



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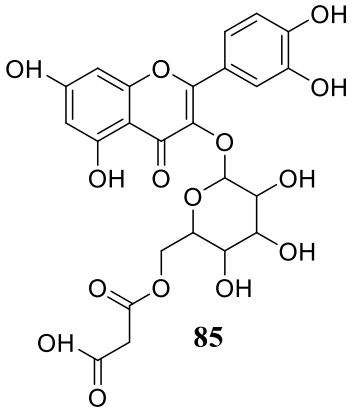

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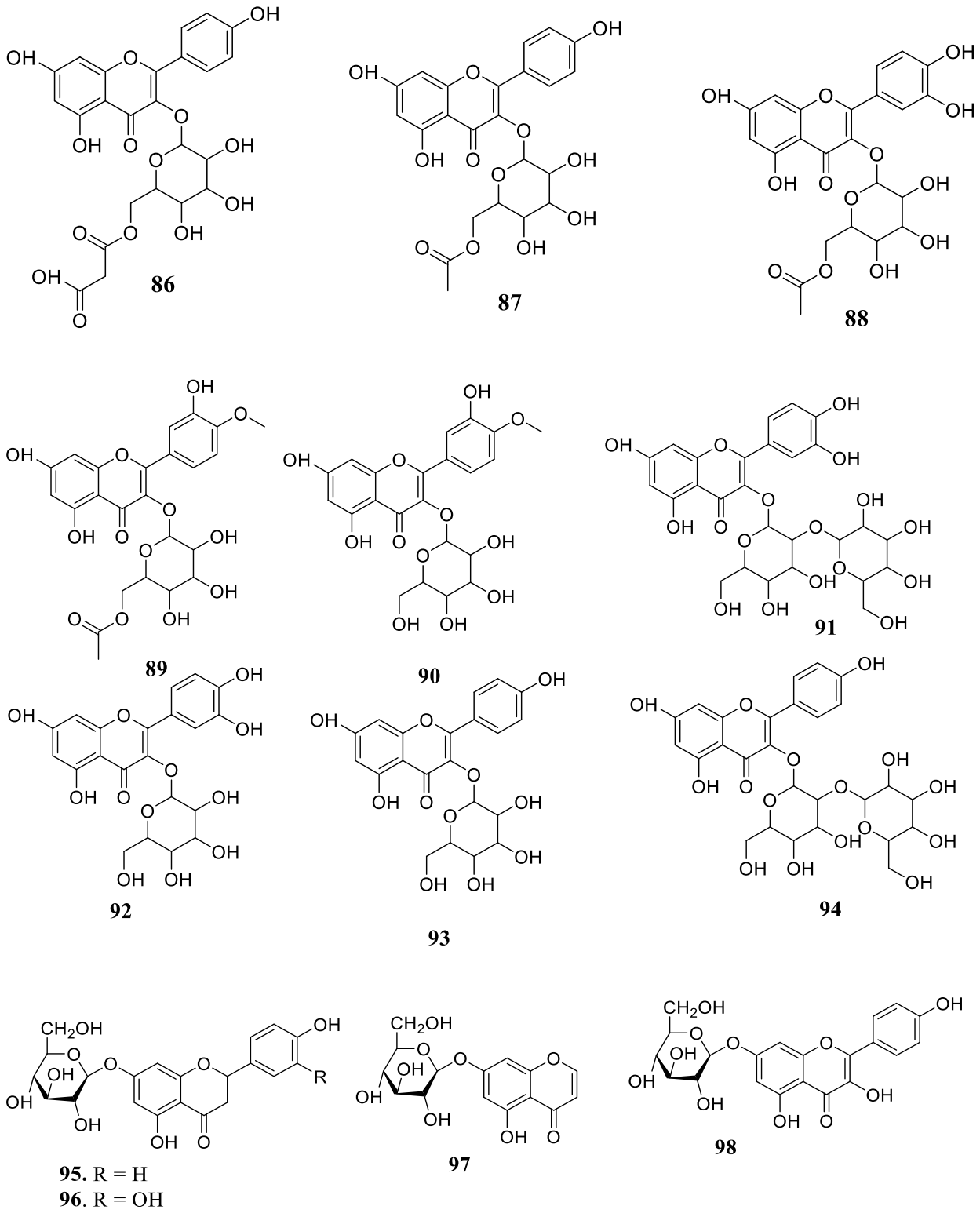


Figure 2: Structure of Compounds Isolated from the Genus *Momordica*

Table 2: Biological Activities of *M. foetida*

Momordica foetida	
Biological Activities	**Reference**
Antibacterial activity	(Bedore & Geinoro, 2018; Odeleye & Oyedeji, 2008; Tesfaye, 2021)
Antifungal activity	(Odhiambo *et al.,* 2011)
Antioxidant activity	(Acquaviva *et al.*, 2013), (Akono & Aphrodite, 2018), (Food & Unit, 2014)
Laxative activity	(Odusote & Awaraka, 2008)
Larvicidal activity	(P Berlyn, 2021)
Antiplasmodal activity	(Froelich *et al*., 2007).
Alleviates Parastar (Insecticide)-Induced Toxicity on Pancreatic and Duodenal α-amylase activity	(Akono *et al*., 2019)

3. MATERIALS AND METHODS

3.1. Plant Material Collection and Identification

The aerial of *M. foetida* were collected from surrounding Adama city located eastern part of Oromia region, Ethiopia. After collecting the plant authenticated by Mr. Melaku Wendafrash and its voucher specimen (BB001/2014 E.C) was deposited at the National Herbarium of Ethiopia, Addis Ababa University, Ethiopia.

3.2. Chemicals and Reagents

The chemicals and reagents which used in this investigation were solvents ($CHCl_3$, *n*-hexane, EtOAc and MeOH), anhydrous sodium sulphate, sulphuric acid, ascorbic acid, lead acetate solution, α-naphtol, Mg, HCl, acetic anhydride, KOH, NaOH, glacial acetic acid, Mayer's reagent, MHA, SDA, DPPH, and acetone and TLC visualizing reagents (vanillin reagent).

3.3. Instrumentation

TLC visualization was done using UV-light at λ 354 and 365 nm. Column chromatography was carried out using silica gel 100-200 mesh ASTM. GC (7890B, Agilent Technologies, USA) coupled with an MS (5977A Network, Agilent Technologies). The spectroscopic techniques 1D NMR were employed to elucidate the structure of isolated compounds. The NMR spectral data were obtained in $CDCl_3$ and Acetone-D_6 on Bruker Avance 400 MHz spectrometer $CDCl_3$ and Tetramethylsilane (TMS) was used as an internal standard.

3.4. Phytochemical Screening

The phytochemical tests were carried out for the aerial part of extracts using standard procedures to identify secondary metabolites (Kashyap & Hait, 2021; Mohamed *et al*., 2019).

Tests for alkaloids

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

Test for steroids

Libermann Burchard Reaction: 2 mL extract were mixed with $CHCl_3$. To this mixture, 2 mL acetic anhydride and 2 drops concentrated sulphuric acid were added from the side of test tube. Formation of red, blue and green color indicates the presence of steroid.

Test for Glycoside

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

Test for Terpenoids

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

Test for Flavonoids

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

Test for Tannins

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

Test for Saponins

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

3.5. Extraction and Isolation

3.5.1. Extraction

The powdered *M. foetida* (500 g) was extracted with 4L of *n*-hexane for 72 hrs with occasional shaking at room temperature. The solution was filtrated and concentrated by a vacuum rotary evaporator at 45 ^{o}C. The insoluble residue remaining after extraction of a solution (marc) left was further extracted with $CHCl_3$ (1.5 L), $CHCl_3$/ MeOH (1:1) (2 L) and MeOH (2 L) for 72 hrs with occasional shaking at room temperature. The solution was filtered and concentrated by a vacuum rotary evaporator. The TLC profiles of dried crude extracts were analyzed and finally the extracts were collected and placed under proper area until needed for further experiment.

3.5.2. Column Chromatography Technique

The crude $CHCl_3$/ MeOH (1:1) and MeOH extract (44.9 g) was adsorbed on 25 g of silica gel (mesh size 100-200), subjected to column packed with silica gel (120 g) using *n*-hexane. The column was eluted with *n*-hexane followed by increasing with increasing gradient of EtOAc in *n*-hexane and MeOH in EtOAc as eluent. A total of 319 fractions were collected (Table 3) and each fraction concentrated at 40 ^{0}C under reduced pressure. Fractions that showed similar R_f (retention factor) values and the same characteristic on TLC were combined. Fraction 19-26 afforded compound **99** (*n*-hexane/ EtOAc (85:15) *Rf* value of 0.63, 16 mg). Fraction 32-36 afforded compound **100**, TLC solvent system (*n*-hexane/ EtOAc) (70: 30), *Rf* value of 0.76, 20 mg). Fraction 37-46, TLC solvent system (80:20), *Rf* value of 0.65, 14 mg) compound **101** single spot on TLC**.** Fraction (188-192) was further purified by column chromatography using isocratic solvent elution (60:40) and collected 12 fractions, from which fraction B_8 obtained compound **102** with *Rf* value of 0.67, 12 mg) and 47-51, (80:20), *Rf* value of (0.58, 13 mg) afforded compound **103** with single spot on TLC, solvent system (*n*-hexane/ EtOAc) respectively.

Table 3: Summary of Fractions Collected from Column chromatography of $CHCl_3$/ MeOH (1:1) (1:1) and MeOH mixed Extract

Solvent system	**Ratio**	**Fractions**	**solvent system**	**Ratio**	**Fractions**
n-hexane :EtOAc	90:10	F_1-F_8	*n*-hexane: EtOAc	10:90	F_{166}-F_{180}
"	85:15	F_9-F_{29}	"	20:80	F_{181}-F_{192}
"	80:20	F_{30}-F_{42}	"	30:70	F_{193}-F_{212}
"	70:30	F_{43}-F_{59}	"	40:60	F_{213}-F_{235}
"	60:40	F_{60}-F_{80}	"	50:50	F_{236}-F_{247}
"	50:50	F_{81}-F_{93}	"	60:40	F_{247}-F_{256}
"	40:60	F_{93}-F_{100}	"	70:30	F_{257}-F_{269}
"	30:70	F_{101}-F_{117}	"	80:20	F_{270}-F_{281}

“	20:80	F_{118}-F_{138}	“	100%	F_{282}-F_{319}
“	10:90	F_{132}-F_{150}	“		
“	100%	F_{151}-F_{165}	“		

3.6. GC-MS Analysis of Fraction of *M. foetida*

Fraction 87 obtained from column chromatography analyzed by GC-MS. The GC-MS method is as follows. 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 100°C for 2 min and raised up from 100 to 200 °C at the speed of 10 ° C/min (inlet 250 ° C, split less injection/purge time 1 min), solvent delay 4min. Mass spectra was recorded in electron impact mode, with ionization energy of mode at 70 ev, scanning the 33-550 m/z range. The volatile compounds in the fraction were identified by comparing the mass spectra of the compounds in the fraction with those in the data base of NIST11 GC-MS libraries.

3.7. Evaluation of Biological Activities

The antibacterial and antifungal activities of crude extracts and isolated compounds were done in collaboration with the Department of Applied Biology, Adama Science and Technology University and Ethiopian Public Health Institute Addis Ababa Ethiopia.

3.7.1. Antibacterial Activity

In-vitro antibacterial activity of crude extracts and isolated compounds were done against Gram-negative *Escherichia coli (ATCC25922), Pseudomonas aeruginosa (ATCC27853),* and Gram-positive *Staphylococcus aureus (ATCC25923)* and *Streptococcus pyogens (ATCC19615)* bacterial species. The bacterial cultures were inoculated into the nutrient broth (inoculation medium) and incubated overnight at 37 ° C. Inoculated medium containing 24 hrs grown culture were added to the nutrient medium and mixed thoroughly to get a uniform distribution. The solution was poured approximately 20 mL of sterile MHA in sterile culture plates and allowed to

stay at room temperature. Sterile agar-disc diffusion previously soaked in a known concentration. Extract and isolated compounds of *M. foetida* and standard drugs prepared in using nutrient agar tubes and carefully placed at the center of the labeled seeded plate. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains ($1.5*10^8$ CFU/mL) and allowed to stay at 37 $^{\circ}$ C for 3 hrs. Control experiments were carried out under similar conditions using ampicillin for antibacterial activity as a standard drug. The zones of growth inhibition around the disks were measured after 24 hrs of incubation at 37 $^{\circ}$ C for bacteria. Diameter of zone of inhibition (mm) of plant extracts and isolated compounds was compared with inhibition exhibited by reference antibiotic (ampicillin) discs of the same concentration. DMSO used as negative control during the whole test on bacteria.

3.7.2. Antioxidant Activity

The DPPH assay is based on the measurement of the scavenging capacity of antioxidants. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine**.** The free radical scavenging activity of the extracts and isolated compounds was measured through the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) method by measuring the absorbance of DPPH at 517 nm. The crude extracts and isolated compounds were diluted in four vials as 100, 50, 25, and 12.5 µg/mL crude extracts and isolated compounds. To each 1 mL of the above solutions, 4 mL of 0.04% DPPH (prepared in methanol by dissolving 4 mg DPPH in 100 mL MeOH) were added to give 100, 50, 25 and 12.5µg/mL crude extract and isolated compounds. The resulting solution was placed for 30 min in the dark and then subjected to a UV-Vis spectrophotometer to record absorbance at 517 nm. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{DPPH scavenging activity (\%)} = \left(1 - \frac{A}{A0}\right) \text{ x } 100\% \qquad (1)$$

Where, A and A_0 are absorbance of DPPH containing tested samples including AA and negative control (DPPH solution, 0.004% w/v in MeOH), respectively.

3.7.3. Antifungal Activity

Antimicrobial MIC Determination of crude extract and isolated compounds from claimed medicinal plants were performed using 96-well micro titter plate broth dilution method.

Antifungal experiment was performed in triplicate. 64 mg/mL extract, 20 mg/mL compounds test concentration was prepared by dissolving in 2 %Tween 80. *Trichophyton mentagrophytes* (ATCC 18748), Aspergillus *niger* (ATCC 10535) and two clinical isolates *Candida albicans*, and *Penicillium* species were tested. Using different sterilized vials all tested extracts and compounds were dissolved in 2 % Tween 80 in distilled water v/v dilution preparations, 64 mg/mL extract concentration, 20 mg/mL for compounds. 100 µL sterilized Sabouraud Dextrose Broth (SDB) was pipetted to each pre-labelled 96-microwell titter-plate and prepared in triplicate.

The first row (Row-A) of the 96-well plate from Well-1 to 8, [on (Well-1, Methanol Ext.), (Well-2, $CHCl_3$: MeOH (1:1) Ext.), (Well -3, $CHCl_3$ Ext.), (Well-4, *n*-hexane Ext.), (Well-5 Cpd-**101**), (Well-6, Cpd-**100**), Well-7 Cpd-**99**], (Well-8 Cpd-**102**)] were filled separately with 100 µL dissolved extract and compounds in 2 % Tween 80 from each vials containing (64 mg/mL for extract) and (20 mg/mL for compounds). Then after dilution in the first (Row-A) 96-well plate resulted with 32 mg/well and 10 mg/well concentration in each well. Similarly, (Well-9) were filled with dissolved 100 µg/mL Ketoconazole standard drug, which serves as a positive control whereas; (Well-10) filled with 100 µL 2% Tween 80 (negative controls). Micro (Well-11, left freely for Standardized fungal organisms (growth control), (Well-12 pipetted with 100 µL broth medium for sterility control finally all the (Row-A) wells having 200 µL volumes except well-11 which is left for fungal growth control to be added after standardization step.

Using multichannel pipette adjusted at 100 µL, two-fold serial dilutions were performed dawn ward across columns A to H micro well plates by sucking up and down 5-10 times, except well-11 the final 100 µL withdraw from row H and discarded. Active fungal test organism's (culture) were prepared from 4° C stored stock cultures and Sub-cultured at 25° C on Sabouraud Dextrose Agar (SDA) at 25° C for up to 7 days. Using sterile disposable inoculating loop, few fungal growth were transferred from sub-cultured plate to tubes containing sterile Sabouraud Dextrose Broth. For standardization of test organism, diluted inoculum was prepared in accordance with the CSLI recommendation. Using Spectrophotometer adjusted to 625 nm with OD (optical density) value range from (0.08 to 0.1) which is equivalent to 1.0×10^7 CFU/mL fungal species. From the above standardized inoculum 5×10^5 CFU/mL or 5×10^4 CFU/well test organism's suspensions were prepared by dilution in SDB. Within 15 min of standardization 100 µL, 5×10^5 CFU/mL suspension was applied to each well, except to the sterility control (column 12).

After 1-5 days incubation at 25 ° C, all the microtiter plate was examined for visible fungal growth. After incubation and growth, 40 µL of a 0.2 mg/mL solution of 2, 3, 5-triphenyltetrazolium chloride (TTC; Tetrazolium chloride) was added to each well as an indicator of microbial growth. After 30 min incubation, at 25 ° C the MIC values were determined visually by observing the presence or absence of pink color using magnifying instruments. The lowest concentration or dilution of each extract and compounds displaying no visible pink color is recorded as the MIC (Eloff, 1998).

3.8. Molecular Docking study of Isolated Compounds

Auto Dock Vina was used to dock the selected proteins and compounds into the active site of proteins. To investigate the mode of interaction between the *P. aeruginosa*, *Pseudomonas* quinolone signal PqsA (**PDB ID: 5OE3**), *S. aureus*, Pyruvate Kinase (**PDB ID: 3T07**), *T. mentagrophytes*, Squalene epoxidase (**PDB ID: 2AIB**) and Human myeloperoxidase (**PDB ID**: **6NGJ**) enzymes and isolated compounds in a 3D fashion; the compounds were docked within the binding site of the protein. The 2D chemical structures of the compounds were drawn using Chem Office tool (Chem Draw 15.0) assigned with proper orientation. The crystal structure of the receptor molecules complex with standard drug and DNA was downloaded from protein data bank. The preparation of protein and target protein file was carried out as per standard protocols. Then, the best docked conformation between the compounds and the protein were explored with the docking algorithm provided with Auto Dock Vina4.2.

3.9. Statistical Analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. The results were presented using tables, Microsoft Excel 2010, graph pad prism 9, ChemDraw 15.0, Auto dock vina4.2, Mendeley Desktop and Mnova Masterlab research chemistry software.

4. RESULT AND DISCUSSIONS

4.1. Extraction yields of the Aerial *M. foetida*

Maceration extraction of *M. foetida* with *n*-hexane, $CHCl_3$, $CHCl_3$: MeOH (1:1) and MeOH yielded of 2g (% of yield = 0.4), 4.5 g (% of yield =0.9) 12.47 g (% of yield =2.494) and 32.5 g (% of yield = 6.5) crude extract respectively. As shown below in Table 4, the yield of the $CHCl_3$: MeOH (1:1) and MeOH fraction was found to be highest as compared to that of the *n*-hexane and $CHCl_3$ fraction. This may be due to the presence of compounds with medium and high polarity in the plant.

Table 4: Yields of Extraction for crude extract of *M. foetida*

Crude extract	Yields/gram (g)	Percentage yield (%)
n-hexane	2	0.4
$CHCl_3$	4.5	0.9
$CHCl_3$: MeOH(1:1)	12.5	2.45
MeOH	32.5	6.5

4.2. TLC Analysis of the crude Extracts of the Aerial *M. foetida*

$CHCl_3$: MeOH (1:1) and MeOH were used for isolation of compounds due to their close TLC profile. The mobile phases used for $CHCl_3$: MeOH (1:1) and MeOH were *n*-hexane: ethyl acetate (95:5) and MeOH in EtOAc (30:70) were analyzed in order to confirm their close TLC profile. Spots were visualized using ultra violet lamp at 354 and 365 nm wave length and the components of crude extract was checked by using appropriate reagent.

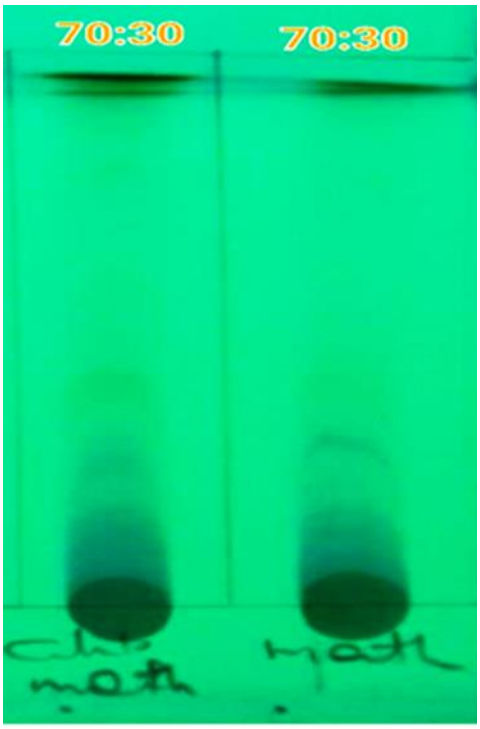


Figure 3: TLC Profile of the crude Extracts of $CHCl_3$: MeOH (1:1) and MeOH (*n*-hexane: ethyl acetate) (70:30)

4.3. Phytochemical Screening

Phytochemical screening was performed in order to reveal the constituents of the plants extract and the one that predominates over the others but also it is helpful in searching for bioactive agents those can be used in the synthesis of useful drugs. The detection of the bioactive principles presents in medicinal plants and subsequently may lead to drug discovery and development. The results suggested that alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, and glycosides are present within the crude aerial extract (Table 5). The results obtained in this study suggested that the identified phytochemical compounds may be the bioactive constituents of this plant and its substantial medicinal importance.

Table 5: Phytochemical Screening results of Aerial Extracts of *M. foetida*

Types of test	Types of crude extracts			
	***n*-hexane**	**$CHCl_3$**	**$CHCl_3$:MeOH (1:1)**	**MeOH**
Alkaloids	-	-	+	+
Flavonoids	-	+	-	+
Glycosides	+	+	+	+
Terpenes	+	+	+	+
Tannin	+	+	+	-
Steroids	+	+	+	+
Saponnin	-	-	-	+

+ = presence - = absence

As shown in Table 5 some of secondary metabolites are absence this is due to factors that affect secondary metabolite production. Such as, temperature, humidity, light intensity as well as environmental condition upset the growth of the plant and the assembly of secondary metabolites. The literature review mentioned below shows *M. foetida* has different kinds of phytochemicals. Based on the result the phytochemical screening tests are consistent from the previous investigation (Odeleye & Oyedeji, 2008; P Berlyn, 2021).

4.4. Characterization of Isolated Compounds

In this study, five compounds were isolated from the combined chloroform/methanol (1:1) and methanol crude extract of *M. foetida*, named as compound **99**, **100, 101**, **102** and **103**.

4.4.1 Characterization of Compound (99)

Compound **(99)** (16mg) was obtained as an orange solid from $CHCl_3$: MeOH (1:1) and MeOH extracts of *M. foetida*. TLC showed R_f value of 0.63 using 30% EtOAc in *n*-hexane as eluent.

The ^{1}H-NMR (400 MHz, Acetone-D_6) spectrum (Table-1, Appendix 1) of compound **99** showed signals at δ_H 3.61 (broad *s*, 1H) corresponding to the oxygenated methine proton of H-3. The signal at δ_H 3.91 (broad *s*, 1H) attributed to methylene protons adjacent to a hydroxyl group which attached to the cyclopentane ring (H-15). The spectrum displayed methylene signals at δ_H 1.71 (bro. *s*, 2H, H-12), 2.09 (*s*, 2H, H-11) and 2.31(*s*, 2H, H-23). The signal at δ_H 1.31 (*s*, 1H, H-15) and 1.61 (s, 1H, H-5) showed methine proton. The spectrum also showed signals at δ_H 0.89 (bro. *s*, 3H, H-21, H-27), 0.97 (*s*, 7H, H-19) and 1.11 (bro. *s*, 3H, H-28) corresponding to methyl proton. Two olefinic proton signals appeared downfield in the ^{1}H-NMR spectrum at δ_H 5.17 (*s*, 2H, H-2) and 5.37 (*s*, 2H, H-1, H-24).

The ^{13}C-NMR (400 MHz, Acetone-D_6) spectrum of compound **99** with the aid of DEPT-135 (Table 6, Appendix 2 and 3) displayed methyl carbons at δ_C 13.57, 15.34, 19.28, 22.94 and δ_C 24.98. The presence of methylene carbons exhibited at δ_C 22.2, 22.50, 26.41, 26.96, 27.9, 31.80, 33.78, 39.6, 33.56, and methine carbons at δ_C 41.9, 50.60, respectively. The signal observed at δ_C 39.26, 134.84 and 131.56 in the ^{13}C NMR spectrum were absent in DEPT-135 spectrum, suggesting the presence of quaternary carbon atoms that belong to (C-10), (C-8) and (C-25) respectively. The spectrum also resonated oxygenated methine carbon at δ_C 69.02 (C-3) and carbonyl of ester at δ_C 172.24 (C-1'). Olefinic methines appeared at δ_C 125.01 (C-1), 129.93 (C-2), 128.06 (C-7) and 124.24 (C-24). The signal δ_C 61.88 indicated the presence of methyl carbon adjacent to a hydroxyl group.

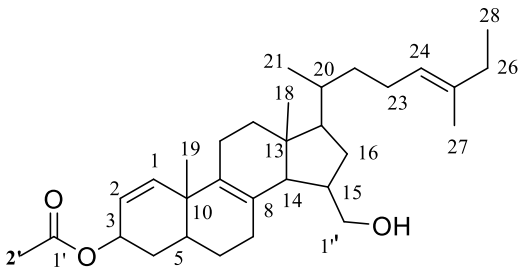
Based on the above NMR spectral data, compound **99** was found in nearby agreement with literatures data for collosolactoneA-type derivative dammarane triterpene, which was previously reported from aerial extracts of *Ganoderma* species (Anh *et al*., 2001; Xia *et al*., 2014) . The difference was the presence of extra olefinic carbon (C-1 and C-2) and the position of carbonyl ester (C-3) in the case of compound **99**. The proposed structure was depicted in Figure 4.

It was reported that various kinds of diseases such as liver, kidney disease, hyperlipidemia, cardiovascular, neuro-degenerative, diabetes mellitus, and cancer can be treated and prevented using dammarane-type triterpenoids (Noushahi *et al*., 2022).

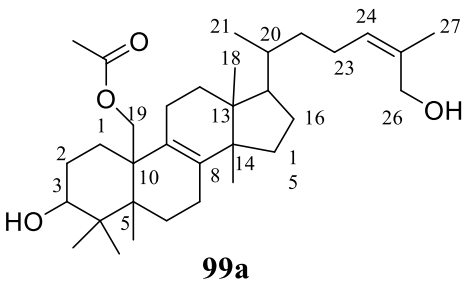
Table 6: ^{1}H, ^{13}C and DEPT-135 NMR Spectral data of Compound **99**

Position	NMR data of Compound 99		Reported data for damarrane triterpene (99a) (Anh *et al*., 2001)		
	^{1}H-NMR δ_H (Acetone-D_6)	^{13}C-NMR δ_C (Acetone-D_6)	Types of C	^{1}H- NMR	^{13}C-NMR
1	5.37 (*s*, 1H),	125.0	=CH		-
2	5.17 (*s*, 1H)	129.9	=CH		-
3	3.61 (*s*, 1H)	69.02	-C-O		-
4	-	39.63	CH_2		39.0
5	1.61 (*s*, 1H)	50.60	CH	1.69 (*m*, 1H)	50.1
6	-	22.50	CH_2	-	17.8
7	-	26.41	CH_2	-	26.2
8	-	134.8	=C	-	137.9
9	-	128.1	=C	-	130.4
10	-	39.26	C	-	42.2
11	2.09 (*s*, 2H)	22.20	CH_2	2.12 (2H, *m*)	22.16
12	1.71 (*s*, 2H)	31.80	CH_2	1.72 (1H, *m*)	31.0
13	-	-	C	-	44.4
14	-	-	CH	-	50.4
15	1.31(*s*, 1H)	29.5	CH		-
16	-	27.82	CH_2		27.5
17		41.90	CH		47.1
18	-	15.34	CH_3		16.6
19	0.97 (*s*, 3H)	19.28	CH_3		-
20	-	-	CH		41.6
21	-	13.57	CH_3	0.92 (3H, *d*, *J*=6.6Hz)	12.0
22	-	33.56	CH_2		-
23	2.31 (bro. *s*, 2H)	33.78	CH_2	2.47(1H, *m*)	34.0

24	5.37 (*s*, 1H)	124.04	=CH	5.49(1H, *t*, *J*=7.6Hz)	125.4
25	-	131.56	=C	-	137.9
26	-	26.96	CH_2	-	-
27	0.89 (bro.*s*, 3H)	13.57	CH_3		-
28	1.11 (bro.*s*, 3H)	24.90	CH_3		-
$CH_3\underline{CO}$	-	172.43	=CH		170.54
$\underline{CH_3}CO$	-	22.96	CH_3	-	21.1
$\underline{CH_2}OH$	3.91 (*s*, 2H)	61.88	-	-	-



99



99a

Figure 4: Structure of Compound **99**

4.4.2 Characterization of Compound 100

Compound **100** (20mg) was isolated as white crystal solid. TLC showed R_f value of (0.76) using (70:30) *n*-hexane/EtOAc solvent system as eluent. It provided a blue spot after spraying with vanillin.

The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum (Table7, Appendix4) of compound **100** ranged between δ_H 0.96-2.36. The ^{1}H-NMR spectrum showed the presence of one terminal methyl protons at δH 0.96-0.79 (*m*, 2H, H-14). The spectrum also displayed aliphatic methylene signals at δH 2.36(*t*, *J*= 7.6HZ, 1H, H-2), 1.36 (*s*, 1H, H-3), 1.29(*d*, *J*=17, 4HZ, 13H, H-4).

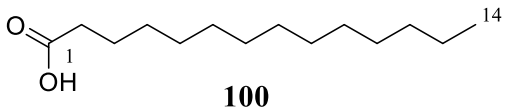
From the ^{13}C-NMR (400 MHz, $CDCl_3$), (Table 7, Appendix 5 and 6) with the aid of DEPT-135 data compound **100** revealed that 14 well resolved non-oxygenated aliphatic carbons signals. From observed carbon spectrum at δ_C 179.94 appeared as carboxyl carbonyl carbon. The signals at δ_C 34.07 (C-2), 31.93 (C-12), 29.7 (C-10), 29.66 (C-9), 29.59 (C-8), 29.44 (C-6) 29.36 (C-5),

29.25 (C-11), 29.07 (C-4), 24.69 (C-12), 22.69 (C-13) indicated that methylene carbons. The peaks at δ_C 14.11(C-14) corresponds to terminal methyl carbon. Thus, based on the above spectral data and comparison with literature value the structure of the compound is in good agreement with whose structure is depicted in Figure4 and the compound found to be myristic saturated fatty acid.

Fatty acids may interact with cellular membranes causing leakage of molecules from the cells, reduction of nutrient uptake or inhibition of cellular respiration. saturated fatty acids have been proven to be detrimental to health, in part associated with their effect on cholesterol metabolism as well as direct factor associated with disease (Zin & Oo, 2019).

Table 7: ^{1}H, ^{13}C and DEPT-135 NMR Spectral data of Compound **100**

Position	**^{1}H NMR** δ_H ($CDCl_3$)	**^{13}C NMR** δ_C ($CDCl_3$)	**Reported data** (Dida & Science, 2019)		**Type of C atom**
			^{1}H NMR	**^{13}C NMR**	
1	-	179.94	-	179.2	COOH
2	2.36 (*t, J*=7.6Hz, 1H)	34.07	2.36 (2H, *t, J* = 7.4)	34.0	CH_2
3	1.36 (*s*, 2H)	24.69	1.64 (2H, *m*)	24.7	CH_2
4	1.29(*d, J*=17.4Hz,2H)	29.07	1.27 (2H, *m*)	29.0	CH_2
5	1.29(*d, J*=17.4Hz,2H)	29.12	-	29.4	CH_2
6	1.29(*d, J*=17.4Hz,2H)	29.44	-	29.6	CH_2
7	1.29(*d, J*=17.4Hz,2H)	29.59	-	29.7	CH_2
8	1.29(*d, J*=17.4Hz,2H)	29.60	-	29.7	CH_2
9	1.29(*d, J*=17.4Hz,2H)	29.66	-	29.7	CH_2
10	1.29(*d, J*=17.4Hz,2H)	29.70	-	29.5	CH_2
11		29.25	-	29.3	CH_2
12		31.9	-	31.9	CH_2
13		22.69	-	22.7	CH_2
14	0.89 (t, *J* = 8.6, 7.1 Hz, 3H)	14.11	0.90(3H,*t,J*= 6.1Hz)	14.11	CH_3



100

Figure 5: Structure of Compound **100**

4.4.3. Characterization of Compound 101

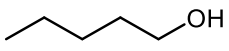
Compound **101** isolated as solid white crystal. TLC showed R_f value of (0.65) using (80:20) *n*-hexane/EtOAc solvent system as eluent. It provided purple spot after spraying with vanillin.

The ^{1}H-NMR (400 MHz, Acetone-D_6) spectral data (Table 8, Appendix7) of compound **101** showed signal at δ_H 5.05 (*s*, OH) the presence of hydroxyl methylene proton attached with (H-1). The peak at δ_H 3.89-3.92 (t, J = 6.0 Hz, 2H, H-1) and δ_H 1.59 (*s*, 2H) indicated that the existence of methylene protons at (H-2) and 1.30 (*s*, 4H) (H-3 and H-4) respectively. δ_H 0.89 (*s*, 3H) revealed terminal methyl proton (H-5).

The ^{13}C-NMR (400 MHz, Acetone-D_6) combination with DEPT-135 spectrum (Table 8, Appendix 8 and 9) obtained data the peak at δ_C 62.87 revealed that the presence of methylene hydroxyl carbon (C-1) position. The peak at δ_C 22.44, 31.75 and 33.41 indicated the methylene carbon at (C-4), (C-3) and (C-2) respectively, and there is terminal methyl carbon at δ_C 13.49. Based on these NMR data the compound that found to be is pentan-1-ol. In the present study the presence of alcohol compound recognized by the GC-MS analysis (1-heptadecanol) (Table 11) from the 26 identified compounds and it is consistent with the GC-MS results.

Table 8: ^{1}H, ^{13}C and DEPT-135 NMR spectral data of compound **101**

Position	**^{1}H-NMR** δ_H (Acetone-D_6)	**^{13}C-NMR** δ_C(Acetone-D_6)	**DEPT-135**	**Type of carbon atom**
1’	5.05 (*s*,-OH)	62.87	62.87	CH_2-OH
1	3.89-3.92 (t, J = 6.0 Hz, 2H)	-	-	CH_2-OH
2	1.59 (*s*, 2H)	33.41	33.41	CH_2
3	1.30 (*s*, 4H)	31.75	31.75	CH_2
4	1.30 (*s*, 4H)	22.44	22.44	CH_2
5	0.89 (*s*, 3H)	13.48	13.48	CH_3



101

Figure 6: Structure of Compound **101**

4.4.4. Characterization of Compound 102

Compound **102** (12 mg) was isolated as white amorphous. TLC showed R_f value of (0.67), 60:40 *n*-hexane/ EtOAc used as eluent. It gave a blue spot after spraying with vanillin.

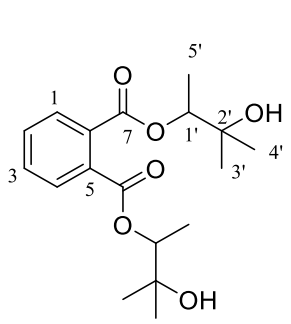
^{1}H-NMR (400 MHz, Acetone-D_6) (Table 9, Appendix10) spectrum of compound **102** showed two proton signals integrated to two protons each in the down field region at δ_H 7.67 (*dd*, *J* = 5.6, 3.4 Hz, 1H, H-2/3) and 7.77 (*dd*, *J* = 5.8, 3.3 Hz, 1H, H-1/4) were revealed that four symmetric aryl protons having the same coupling pattern. The signal at δ_H 3.94 (*h*, *J*= 6.1Hz, 2H) displayed methine proton (H-1'/1'). Besides, proton signal at δ_H 1.11 (*s*, 3H), (H-3'/3'), 2.31 (*s*, 3H, H-4'/4'), and 3.03 (*s*, 3H, H-5'/5') displayed the existence of equivalence methyl protons.

The ^{13}C NMR (400 MHz, Acetone-D_6) with the aid of DEPT-135 NMR spectra (Table 9, Appendix 11 and 12) 10 carbons were clearly exposed, which ascribed to 20 carbons, due to the presence of symmetry in compound **102**. The quaternary aryl ester carbon signal resonated at δ_C 167.9 (C- 7/8) methine carbon 62.85 (C-1'/1'), quaternary aromatic carbon at δ_C 132.56 (C-5/6), methine carbon attached with two equivalence methyl carbon and hydroxyl moiety δ_C 71.24 (C-2'/2') confirmed that the occurrence of symmetry respectively. Moreover, the presence of symmetry aromatic carbon was shown at δ_C 131.32, 128.75 (C-1/4), (C-2/3) respectively.

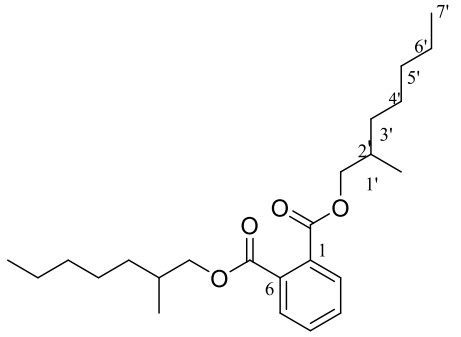
Based on the above NMR data the compound was found to be bis (3-hydroxy-3-methylbutan-2-yl) phthalate. These spectral features are in nearby agreement to those reported for di-(2-methylheptyl) phthalate. The aromatic carbon (C-1/4), (C-2/3), (C-5/6) and the carbonyl carbon (C-7/8) (Kiros *et al*., 2022). It had consistency by some level with literature compound **105** based on NMR data. From previously reported data (Zin & Oo, 2019) and present study of GC-MS analysis we recognized compound has phthalate skeleton. However, compound **102** (di-(2-methylheptyl) phthalate) reported for the first time from the species.

Table 9: ^{1}H, ^{13}C and DEPT-135 NMR Spectral data of Compound **102**

Position	**^{1}H-NMR** δ_H (Acetone-D_6)	**^{13}C-NMR** δ_H (Acetone-D_6)	**Type of C atom**
1/ 4	7.77 (*dd, J* = 5.8, 3.3 Hz, 1H)	131.32	CH
2/3	7.67 (*dd, J* = 5.6, 3.4 Hz, 1H)	128.75	CH
5/6	-	132.56	Q
7/8	-	167.49	Q(C=O)
1'/1'	3.90 (*h, J*= 6.1Hz, 2H)	62.85	CH
2'/2'	-	71.24	Q
3'/3'	1.11 (*s*, 3H)	18.49	CH_3
4'/4'	2.31 (*s*,3H)	24.86	CH_3
5'/5'	3.03 (*s*, 3H)	27.61	CH_3



102



105

Figure 7: Structure of Compound **102**

4.4.5. Characterization of Compound 103

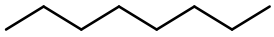
Compound **103** (13 mg) was isolated as light green amorphous. TLC showed R_f value of (0.58) using (80:20) *n*-hexane/EtOAc solvent system as eluent. It gave a blue spot after spraying with vanillin.

The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum (Table10, Appendix 13) ranged from δ_H 0.8-2.28. The signal at δ_H 1.27 (bro. *s*, 8H, H-4 – H-7), 1.62 (*m*, 2H, H-3) and δ_H 2.34 (*m*, 2H, H-2), resonated methylene proton respectively. The chemical shift δ_H 0.89 (t, *J* = 5.2 Hz, 3H, H-1 & H-8) indicated that the symmetry terminal methyl protons.

The ^{13}C-NMR (400 MHz, $CDCl_3$) spectrum (Table10, Appendix 14 and 15) of compound **103** analyzed with the aid of DEPT-135 revealed the presence of 7 carbon atoms. Six methylene carbon observed at δ_C 31.9 (C-2), 29.07 (C-3), 29.36 (C-4), 29.44 (C-5), 29.66 (C-6) and 22.7 (C-7) respectively. The symmetry terminal methyl carbon appeared at δ_C 14.1 (C-1 and C-8). Based on the spectral data compound **103** was found to be simple alkane octane. This compound is in good agreement with result of GC-MS analysis.

Table 10: ^{1}H, ^{13}C and DEPT-135 NMR Spectral data of Compound **103**

Position	**^{1}H-NMR** δ_H ($CDCl_3$)	**^{13}C-NMR** δ_C ($CDCl_3$)	**Type of C atom**
1	0.89 (t, *J* = 5.2 Hz, 3H)	14.1	CH_3
2	2.34 (*m*, 2H)	31.9	CH_2
3	1.62 (broad *s*, 2H)	29.1	CH_2
4	1.27 (*s*, 8H)	29.4	CH_2
5		29.4	CH_2
6		29.7	CH_2
7		22.7	CH_2
8	0.89 (t, *J* = 5.2 Hz, 3H)	14.1	CH_3



103

Figure 8: Structure of Compound **103**

4.5. GC-MS Analysis of Fraction of *M. foetida*

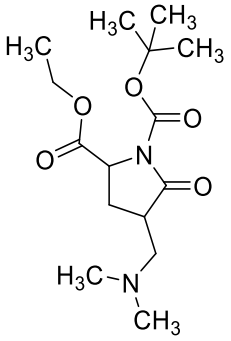
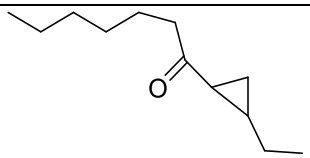
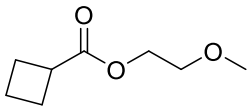
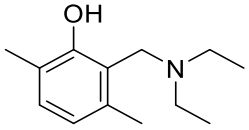
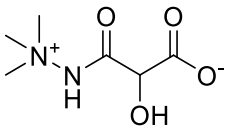
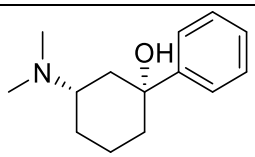
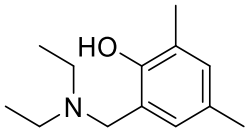
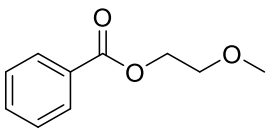
Identification of components was achieved based on their retention indices and interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NSIT) in order to support NMR data of isolated compounds. The database consists of more than 62,000 patterns of known compounds (Olivia et al., 2021). The spectra of the unknown components of *M. foetida* impurity fraction obtained were compared with the standard mass spectra of known components stored in NIST library.

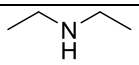
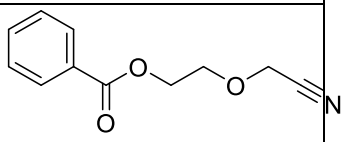
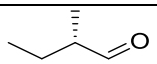
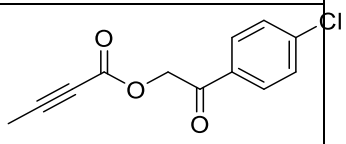
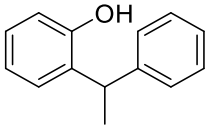
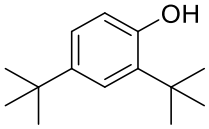
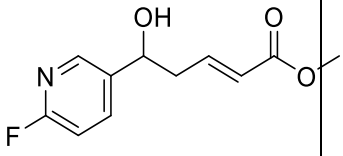
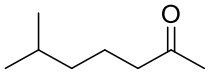
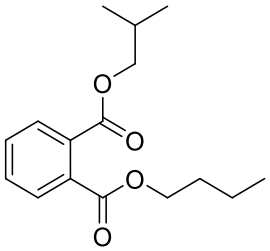
A total of 26 bioactive phtochemical compounds were identified from the GC-MS analysis of $CHCl_3$: MeOH (1:1) and MeOH impurity fraction 87 of the *M. foetida*. While the chemical constituents with their retention time (RT), molecular formula and concentration (%) are presented in Table 11. The following compounds were present in the GC-MS analysis carried on $CHCl_3$: MeOH (1:1) and MeOH fraction of *M. foetida*. Among the identified bioactive components Phenol, 2, 4-bis (1,1-dimethylethyl) (11.18 %), dibutylpthtalate (10.62 %) 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester (9.10 %), (E)-methyl5-(6fluoropyridin-3-yl)-5-hydroxypent-2-enoate (8.66 %) and 1-(2 methylcyclopropyl)-1 heptanone (7.40 %) are the principal component from identified compounds. The low content of 2-(N,N-Diethylaminomethyl)-4, 6-dimethylphenol (0.15 %).

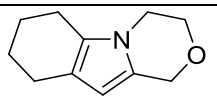
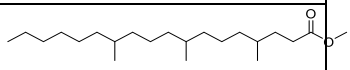
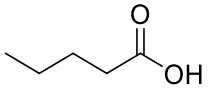
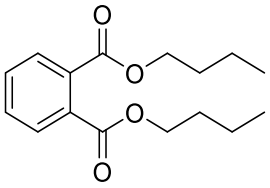
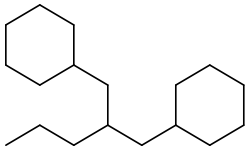
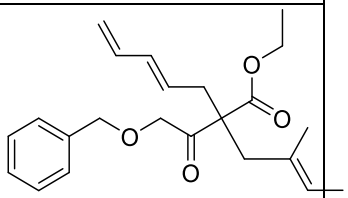
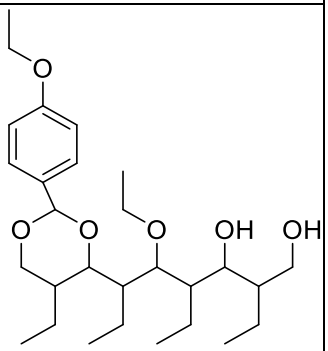
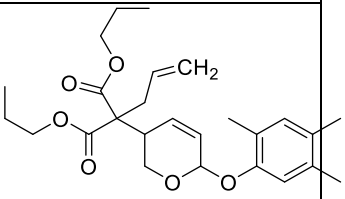
The major comound identified in this fraction Phenol, 2, 4-bis (1, 1-dimethylethyl) has several kinds biological activity. For instance, antioxidant, antibioflm, antimicrobial activities and against fungal phytopathogens (Devi *et al*., 2021). The compound dibutylpthtalate has antibacterial activity against *Staphylococcus epidermidis, Strephtococcus pneumoniae, Escherichia coli, Micrococcus luteus, Klebsiella pneumoniae, Shigella flexneri, Vibrio cholerae and Pseudomonas aeruginosa.* 1,2-Benzenedicarboxylic acid, butyl2-methylpropyl ester has antioxidant, hypocholestero-lemicactivity (Mini Shobi & Gowdu Viswanathan, 2018).

The results of qualitative identification of compounds by the GC-MS analysis recognized the presence of several kinds of compounds in *M. foetida* and support the result of phytochemical screening test in the present study. For instance, the compound 3,4,6,7,8,9-hexahydro-1H-[1,4]oxazino[4,3-a]indole demonstrates the existence of alkaloid compound and phenol compounds also found in this plant. Besides, this GC-MS analysis of the fraction *M. foetida* provisions the finding of fatty acid (myristic acid) (Figure3), alcohol compounds pentan-1-ol (Figure 4) phthalate (bis (3-hydroxy-3-methylbutan-2-yl) phthalate) (Figure 5) compounds in *M. foetida.*

Table 11: Compounds identified in fraction 87 of *M. foetida* by GC-MS

No	RT (min)	Name of the Compounds	Molecular formula	Peak area %	Structure of Compounds
1	5.649	Ethyl (2S,4S)-1-tert-Butoxycarbonyl-4-(dimethylaminomethyl)pyroglutamate	$C_{15}H_{26}N_2O_5$	0.152	
2	6.439	1-(2 methylcyclopropyl)-1-heptanone	$C_{11}H_{20}O$	7.40	
3	6.652	Cyclobutanecarboxylic acid, 2-methoxyethyl ester	$C_8H_{14}O_3$	0.375	
4	6.948	2-[(diethylamino)methyl]-3,6-dimethylphenol	$C_{13}H_{21}NO$	0.323	
5	7.876	N-trimethylammonio-2-hydroxypropanamidate	$C_6H_4N_2O_2$	1.18	
6	10.398	(trans)-3-dimethylamino-1-phenyl-1-hydroxycyclohexane	$C_{15}H_{23}NO$	0.18	
7	15.063	2-(N,N-Diethylaminomethyl)-4,6-dimethylphenol	$C_{13}H_{21}NO$	0.149	
8	15.473	Methoxyethyl benzoate	$C_{10}H_{12}O_3$	0.24	

9	17.060	Diethylamine	$C_4H_{11}N$	0.135	
10	17.128	Acetonitrile, [2 - (benzoyloxy)ethoxy]	$C_{11}H_{11}NO_3$	0.31	
11	18.145	(S)-2-Methylbutanal	$C_5H_{10}O$	0.49	
12	18.965	2-(4-Chlorophenyl)-2-oxoethyl but-2-ynoate	$C_{12}H9ClO_3$	0.21	
13	22.586	1-Heptadecanol	$C_{17}H_{36}O$	0.567	
14	22.752	Phenol, 2-(1-phenylethyl)	$C_{14}H_{14}O$	0.85	
15	25.129	Phenol, 2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	11.18	
16	26.826	(E)-methyl 5-(6-fluoropyridin-3-yl)-5-hydroxypent-2-enoate	$C_{11}H_{12}FNO_3$	8.66	
17	31.303	2-Heptanone, 6-methyl-	$C_8H_{16}O$	3.72	
18	31.70	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	$C_{16}H_{22}O_4$	9.1	

19	31.869	3,4,6,7,8,9-hexahydro-1H-[1,4]oxazino[4,3-a]indole	$C_{11}H_{15}NO$	1.50	
20	32.642	Octadecanoic acid, 4,8,12-trimethyl-, methyl ester	$C_{22}H_{44}O_2$	0.89	
21	33.156	Pentanoic acid	$C_5H_{10}O_2$	0.726	
22	33.270 - 33.353	Dibutyl phthalate	$C_{16}H_{22}O_4$	10.618	
23	35.122	Cyclohexane, 1,1'-(2-propyl-1,3-propanediyl)bis-	$C_{18}H_{34}$	0.92	
24	36.202	Ethyl 4-Benzyloxy-2-[2-methyl-2(E)-butenyl]-2-[2(E),4-pentadienyl]acetoacetate	$C_{23}H_{30}O_4$	0.72	
25	36.321	7,9-(p-Methoxyphenylidenedioxy)-5-methoxy-2,4,6,8-tetraamethylnonan-1,3-diol	$C_{22}H_{36}O_6$	0.682	
26	36.528	Phenyl 4-[bis(ethoxycarbonyl)but-3-ynyl]-2,3,4-trideoxy-.alpha.,L-glcero-pent-2-enopyranoside	$C_{21}H_{26}O_6$	0.589	

4.6. Biological Activity of crude Extract and Isolated Compounds of *M. foetida*

4.6.1. Antibacterial Activity

The *in-vitro* antibacterial activities of crude extract and isolated compounds from the aerial *M. foetida* were tested against the Gram-negative bacteria *E. coli* and *P. aeruginosa, the* Gram-positive bacteria *S. aureus, and S. pyogens* pathogens at concentrations of 150, 75, and 10, 5mg/mL respectively. The obtained mean inhibition zone diameters (in mm) are presented in Table 12.

The MeOH crude extract showed promising antibacterial activity in all bacterial strains against Gram-negative bacteria *E. coli, P .aeruginos* with an inhibition zone of 9.00±0.5, 8.16±0.28 mm and the Gram-positive bacteria *S. aureus and S. pyogens* 9.66±0.28 and 10.00±0.5mm at 150 mg/mL compared to ampicillin (13.00±0.00) and (14.00±0.00) used as a standard antibiotic (300 µg/mL) respectively. *n*-hexane extracts of *M. foetida* showed remarkable antibacterial activity against Gram-positive bacteria *S. aureus and S. pyogens* with an inhibition zone of (8.166±0.57) and (9.00±0.5 mm) at 150 µg/mL respectively. The $CHCl_3$ crude extract had no showed activity against the two *E.coli* and, *P .aeruginosa.* Whereas, the $CHCl_3$/MeOH (1:1) crude extract showed that less antibacterial activity against *S. aureus* (7.403± 0.35 mm), *P .aeruginosa (*7.00±0.5mm) *and S, pyogens* (7.00±0.5 mm) at 150 mg/mL. The MeOH extract demonstrated remarkable activity against *S. pyogens* (8.10±0.4 mm) compared with other extracts at 75 mg/mL. However, all crude extracts were not showed pronounceable antibacterial activity against the given strains at 75 mg/mL. Moreover, the inhibition zone of all the extracts and isolated compounds activity increased with increasing concentration in a dose-dependent manner.

Compound **101** exhibited a promising activity against the Gram-negative bacteria *E. coli* (10.00±0.5 mm), *P. aeruginosa* (10.00±0.5 mm) and Gram-positive bacteria *S. aureus* (11.16±0.577 mm) and *S. pyogens* with inhibition zone of (11.5±0.00 mm) at 10mg/mL relative to the standard drug ampicillin. Compound **101** showed less activity against *S. aureus* and *S. pyogens* (8.33±0.28 mm) and (8.97±0.26 mm) respectively. Compound **99** has showed a promising activity against *S. pyogens* (10.83±0.5mm) and *S. aureus* (10.00±0.55mm) at 10 mg/mL. Compound **102** has showed a promising and remarkable activity against *S. pyogens* (10.00±0.5 and 8.47±0.44 mm) at 10 mg/mL and 5 mg/mL respectively, it had no activity against

the Gram-negative bacteria *E.coli*. Compound **100** showed remarkable inhibition zone against *S, pyogens* **(**9.00±0.55mm) but, it had no activity against the gram negative bacteria *E.coli*. All compounds have less activity at 5mg/mL compared to 10mg/mL concentration. In general, the result of the antibacterial activity verified MeOH aerial extract of *M. foetida* had showed promising antibacterial activity against the Gram-negative and Gram-positive bacteria comparable to the other extract at 150mg/mL. All compounds had a promising antibacterial activity against the given strain at 10mg/mL. The negative control DMSO did not show any inhibition effect against the tested bacterial species.

In this study the phytochemical screening supports the antibacterial activity of *Momordica foetida*. The presence of constituents like terpenoids, steroids and flavonoids in the extract are likely to be responsible for the anti-bacterial activity. Accordingly, antibacterial activity of crude extract and isolated compounds might be due to the presence of active secondary metabolite in the plant seeds. This study revealed that some crude extracts and compounds had pronounced inhibitory activity against some bacteria. Thus, the crude extracts and isolated compounds of *M. foetida* are useful in management of such infections in which these bacteria are implicated.

The susceptibility of the Gram-positive bacteria *S. aureus* and Gram-negative bacteria *P. aeruginosa* to crude extracts and the Gram-positive bacteria *S. aureus* and S. pyogens for isolated compounds demonstrates promising antibacterial activities in terms of growth inhibition of the tested organisms. This makes methanol crude extract and isolated compounds could be a good candidate for investigation of in control of new multi-drug resistant on the tested bacteria. The result was consistent to some extent with the previous reported data for bacterial strain *S. aureus* and *P. aeruginosa* (Odeleye & Oyedeji, 2008).

Table 12: Inhibition zone (mm) of the crude Extracts and Isolated Compounds of the Aerial *M. foetida* against selected bacterial strain

Tested samples	**Concentration (mg/mL)**	**Inhibition Diameter mm (mean±SD)**			
		E.coli	***S. aureus***	***P. aeruginosa***	***S. pyogens***
$CHCl_3$	75	NI	NI	NI	NI
	150	NI	7.00±0.5	NI	7.00±0.5
$CHCl_3$/MeOH	75	NI	6.5±0.5	NI	NI

(1:1)	150	NI	7.40± 0.35	7.00±0.5	7.00±0.5
n-hexane	75	NI	7.00±0.5	NI	6.65±0.85
	150	7.5.00±0.5	8.16±0.57	7.16±0.28	9.00±0.5
MeOH	75	6.97±0.55	7.33±0.76	NI	8.1±0.4
	150	9.00±0.5	9.66±0.28	8.16±0.28	10.00±0.5
100	5	NI	6.9±0.35	6.53±0.2	8.1±0.36
	10	8.00±0.5	8.16±0.5	8.00±0.5	9.00±0.55
102	5	NI	7.5±0.30	6.77±0.25	8.47±0.44
	10	8.16±0.28	9.00±0.55	8.16±0.28	10.00±0.5
99	5	6.8±0.25	7.33±0.28	6.67±0.76	8.60±0.24
	10	9.00±0.00	10.00±0.5	9.00±0.5	10.83±0.5
101	5	7.83±0.28	8.33±0.28	7.50±0.50	8.97±0.26
	10	10.00±0.5	11.16±0.5	10.00±0.5	11.5±0.00
Ampicillin	300µg/mL	13.00±0.5	13.50±0.5	13.00±0.00	14.00±0.5
DMSO	5	NI	NI	NI	NI
	10	NI	NI	NI	NI

NI: No Inhibition**;** **$CHCl_3$/ MeOH (1:1):** chloroform/ methanol (1:1); **DMSO**: Dimethylsulfoxide; Results were presented as mean ±SD (Standard deviation)

4.6.2. Antioxidant Activity

In the DPPH scavenging assay, crude extracts and isolated compounds of *M. foetida* were examined for their free radical scavenging activity and their reaction with the stable DPPH radicals, and presented in Table13.

Table 13: Radical scavenging activity (%) of crude Extracts and Isolated Compounds from Aerial of *M. foetida*

Tested samples	**Control**	**Concentration µg/mL**								**IC_{50}**
		100		**50**		**25**		**12.5**		
		A	**% RSA**	**A**	**%RSA**	**A**	**% RSA**	**A**	**% RSA**	

n-hexane	0.026	0.01	61.538	0.011	57.692	0.012	53.846	0.018	30.769	12.53
$CHCl_3$	0.026	0.01	61.538	0.014	46.153	0.018	30.769	0.024	7.692	67.56
$CHCl_3$/MeOH (1:1)	0.026	0.012	53.846	0.018	30.769	0.023	11.538	0.025	3.846	681.4
MeOH	0.026	0.01	61.538	0.011	57.692	0.014	46.153	0.015	42.307	7.986
Cpd 100	1.264	0.344	72.784	0.388	69.303	0.415	67.167	0.453	64.161	0.85
Cpd 101	1.264	0.379	70.015	0.389	69.224	0.394	68.829	0.425	66.376	0.26
Cpd 102	1.264	0.322	74.525	0.328	74.050	0.358	71.677	0.422	66.613	1.58
Cpd 99	1.264	0.365	71.123	0.421	66.693	0.447	64.636	0.453	64.161	0.46
A. acid	1.264	0.057	94.60	0.057	94.60	0.062	94.10	0.053	93.05	0.06

A: Absorbance; **$CHCl_3$/MeOH**: Chloroform:Methanol; **% RSA**: Radical Scavenging Activity

DPPH radical scavenging activity of crude extracts and isolated compounds showed good to moderate activity at different concentration i.e. *n*-hexane, $CHCl_3$, $CHCl_3$/MeOH (1:1) and MeOH. The DPPH radical scavenging activities (%) of extract were found to be 61.53, 61.53, 53.84 and 61.5% for *n*-hexane, $CHCl_3$, $CHC5l_3$/MeOH (1:1) and MeOH respectively, at the same concentration 100 µg/mL. The IC_{50} values were *n*-hexane (12.53 µg/mL), $CHCl_3$ (67.56 µg/mL) $CHCl_3$/MeOH (1:1) (681.4 µg/mL) and MeOH (7.986 µg/mL). As clearly shown from (Table 13) *n*-hexane and MeOH extract displayed comparable radical scavenging activities relative with other extracts. However, the percentage inhibitions of the crude extracts had shown moderate activity comparable with the positive control ascorbic acid. Ascorbic acid showed maximum scavenging effect at very high and low concentration (94.6, 94.6, 94.1 and 93.05% at 100, 50, 25and 12.5 µg/mL) respectively, and IC_{50} value of 0.06 µg/mL.

Among the tested compounds the maximum antioxidant effect was demonstrated by compounds **99** 71.123 % with IC_{50} value of 0.46 µg/mL relative to positive control. Compounds **100**, **101**, and **102** showed promising activity which is 72.784, 70.015and 74.252 % respectively, at higher dose 100 µg/mL (Table 13). The IC_{50} values were 0.85, 0.26 and 1.85 µg/mL compounds **100**, **101** and **102** respectively. It was observed that the scavenging activity increase with increasing concentration of samples. Therefore, the results showed that the isolated compounds can serve as

a source of radical scavenging and hence can be employed as an antioxidant. In general, the extracts and isolated compounds of *M. foetida* showed promising antioxidant scavenging activity. In the previous study, the whole plant and fruit extracts of *M. foetida* showed different richness in antioxidants phenolic compounds, flavonoids and vitamin C, this finding support ability of *M. foetida* might be a useful tool in preventing metabolic syndrome. However, there is a method difference of reported phytochemical information with the present study (Nantia *et al*., 2018).

Figure 9: DPPH Radical Scavenging Activity (%) Vs Concentration graph of Extracts

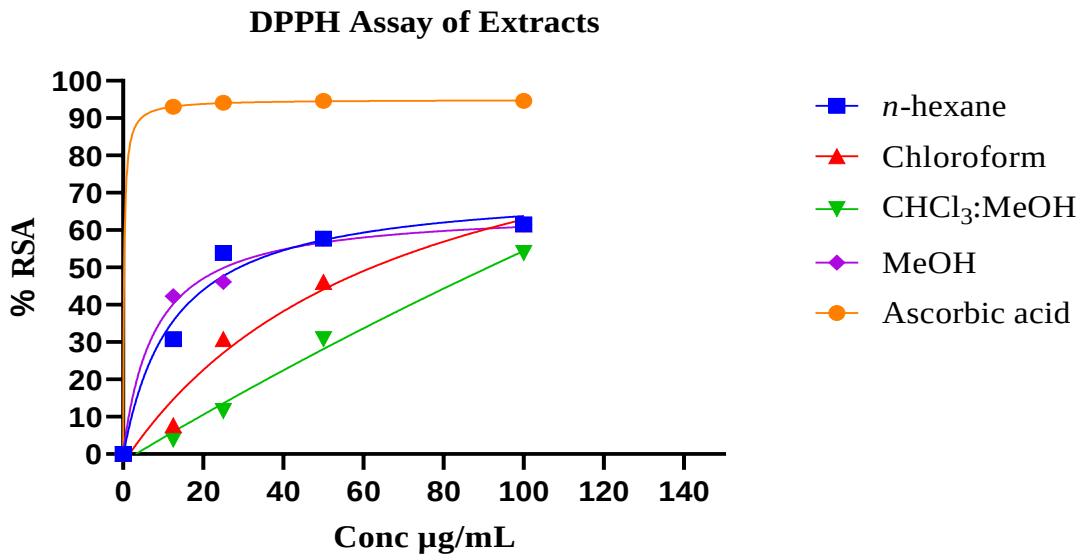
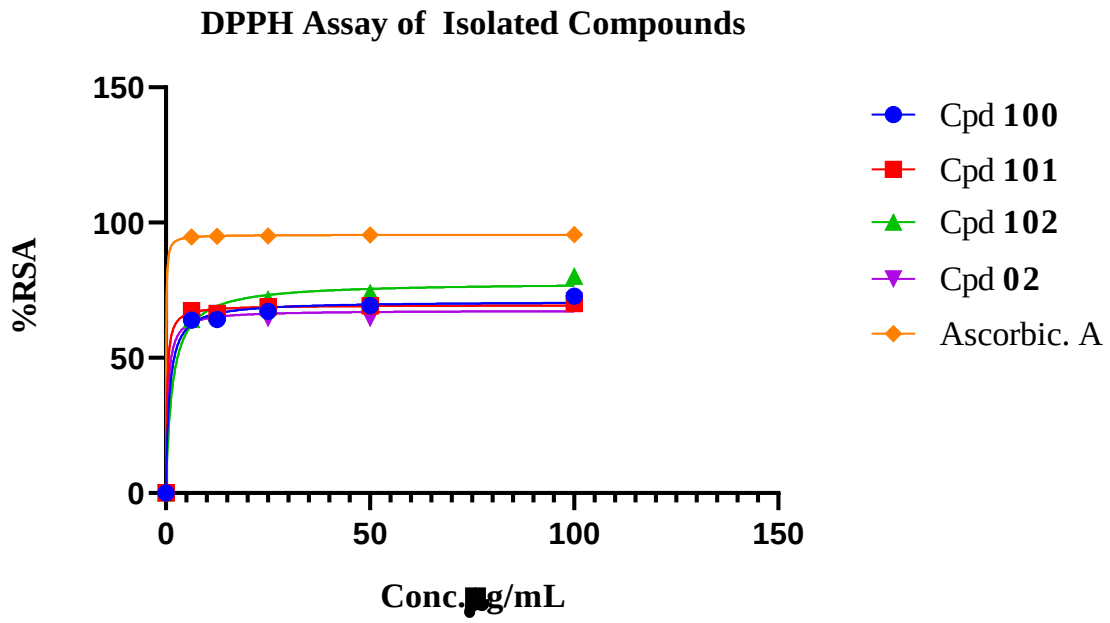


Figure 10: DPPH Radical Scavenging Activity (%) Vs Concentration graph of Isolated Compounds



4.6.3. Antifungal Activity

The *n*-hexane, $CHCl_3$, $CHCl_3$/MeOH (1:1) and MeOH crude extracts and isolated compounds of *M. foetida* were tested *in-vitro* MIC antifungal activity against *C. albicans*, *Pencillium species*, *T. mentagrophytes* and *A. niger*. A standard drug (antibiotic) ketoconazole was also used to act as a positive control and 2 % Tween 80 (100 µL) was used as a negative control. This was done to test the viability of the experiment (Table14).

$CHCl_3$ and $CHCl_3$/ MeOH (1:1) aerial extract of *M. foetida* showed a promising antifungal activity with MIC of (3.33 mg) and (6.67 mg) against *T. mentagrophytes* and *C. albicans* respectively. Whereas, the $CHCl_3$/ MeOH (1:1) and *n*-hexane crude extract of of *momordica foetida* showed a promising activity with MIC (10.67 mg) and (16 mg) against *C. albicans and P. species* respectively. Therefore, $CHCl_3$ extract of this plant exhibited better activity compared to $CHCl_3$/ MeOH (1:1) and *n*- hexane. Besides, the MeOH crude extract was showed remarkable activity against *T. mentagrophytes* (16mg) and less MIC value against *C. albicans* and *A. niger* (32 mg) respectively.

Compound **101** showed a promising antifungal activity with MIC of (1.25 mg) and (5 mg) against *T. mentagrophytes* and *P. species* respectively. Likewise, Compound **101** was showed less antifungal activity against *C. albicans* and *A. niger* (MIC >10 mg) respectively. Compound

99, **100** and **102** had remarkable MIC of (>10mg) against *C. albicans, T. mentagrophytes, P. species* and *A. niger.*

The result suggests that $CHCl_3$ and $CHCl_3$/ MeOH (1:1) from crude extract and Compound **101** were promising inhibitory to the tested microbes *T. mentagrophytes* and merit further investigation. A relatively the less MIC result showed in MeOH compared with other tested samples.

Table14: Minimum Inhibitory Concentration (MIC) of crude Extracts and Isolated Compounds of the Aerial *M. foetida* against the selected fungal strains

Tested samples	***C. albicans* (mg)**	***Penicillium species* (mg)**	***T. mentagrophytes* (mg)**	***A. niger* (mg)**
MeOH	32	32	16	> 32
$CHCl_3$/MeOH (1:1)	10.67	16	6.67	>32
$CHCl_3$	16	>32	3.33	>32
n-hexane	10.67	16	16	>32
Compound 101	>10	5	1.25	>10
Compound 100	> 10	> 10	> 10	> 10
Compound 99	> 10	> 10	> 10	> 10
Compound 102	> 10	> 10	> 10	> 10
Ketoconazole (100 µg/mL)	50µg	50µg	25 µg	100µg

4.7. Molecular Docking Study

4.7.1. Molecular Docking Study for Antibacterial Activity

Molecular docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex (Mishra *et al.*, 2019). Molecular docking studies are generally employed to investigate the binding energy and to further validate the molecular mechanisms for ligands at the active site of a protein.

The molecular docking study was carried out to assess the binding affinity and binding interaction of isolated compounds **99, 100** and **102** toward target proteins *P. aeruginosa*, *Pseudomonas* quinolone signal (PqsA) (**PDB ID: 5OE3**), and *S. aureus* Pyruvate Kinase (PK) (**PDB ID: 3T07**).

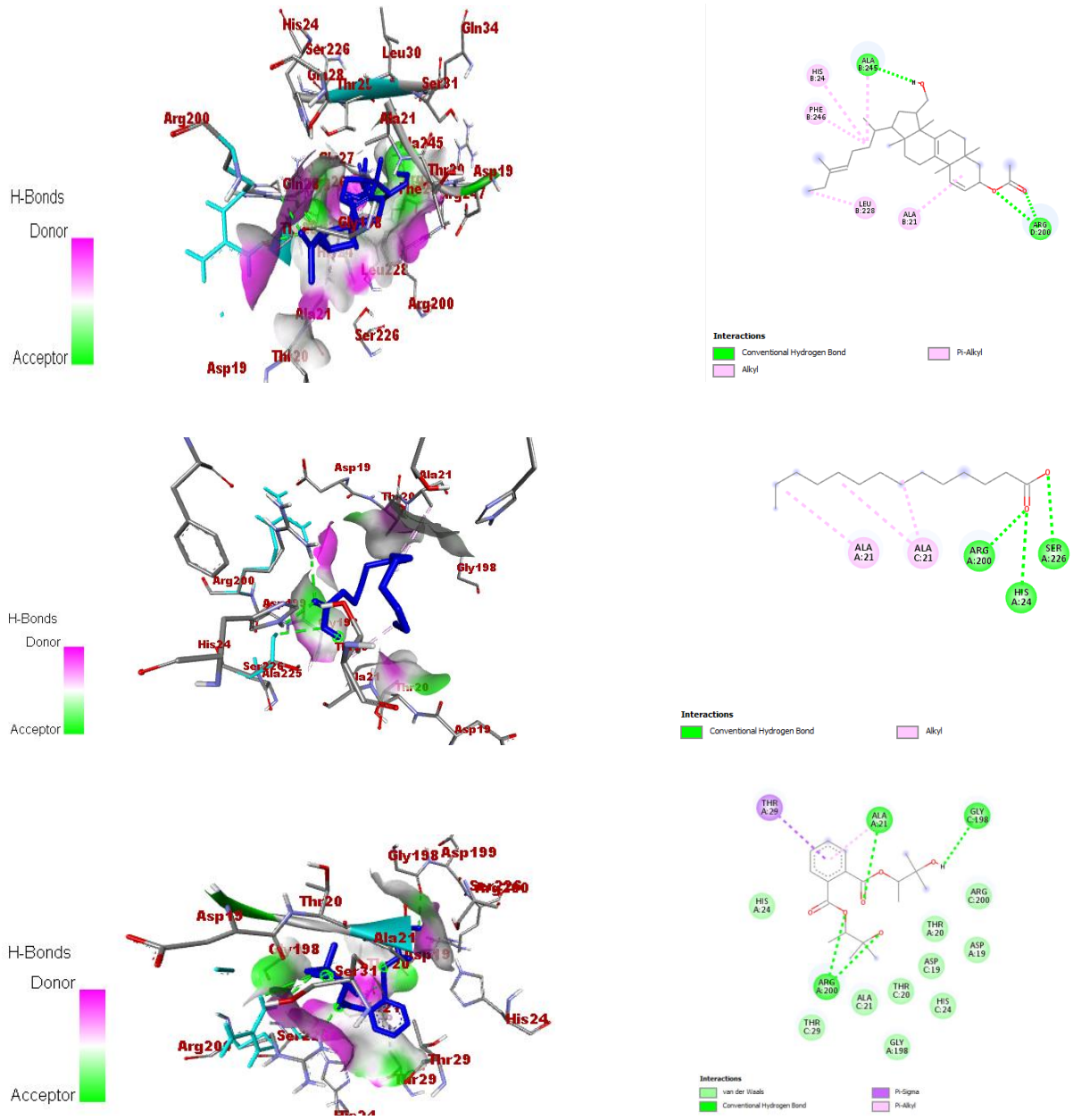
P. aeruginosa, a prevalent pathogen in nosocomial infections and a major burden in cystic fibrosis, uses three interconnected quorum-sensing systems to coordinate virulence processes. At variance with other Gram-negative bacteria, one of these systems relies on 2- alkyl-4(1*H*)-quinolones (*Pseudomonas* quinolone signal, PQS) and might hence be an attractive target for new anti-infective agents (Witzgall et al., 2017). In this study *P. aeruginosa* PQS *(***PDB ID: 5OE3**) was used as a target protein. The result showed a binding pocket of *Pseudomonas* quinolone signal (**PDB ID: 5OE3**) of isolated compounds **99**, **100** and **102** were found to have minimum binding energy ranging from (-4.6 to -9.6 kcal/mole) (Table 15).

The binding affinity, H-bond and residual interaction with different amino acids of compound **99, 100** and **102** along the standard drug ampicillin were descried in (Table15). In comparison to ampicillin, all compounds have shown no similar hydrogen bond amino acid interaction. However, all compounds have showed similar amino acid interaction with **Ar-200** (hydrogen bond). Similarly, compound **99** and **100** showed similar residual amino acid interaction with **Ala-21** (Hydrophobic interaction). Among all tested compound **99** showed the remarkable binding affinity score (-9.6kcal/mol). This data is in agreement with *in-vitro* activity of this compound against *P. aeruginosa.* 3D and 2D binding interaction is depicted in below (Figure 10).

Table 15: Molecular Docking results of Compounds Isolated from the Aerial *M. foetida* and ampicillin against *P. aeruginosa*, PqsA **(PDB ID: 5OE3).**

Cpds	Ligands	Binding Affinity (kcal/mol)	Hydrogen Bond	Residual interactions	
				Hydrophobic/ Electrostatic	Van der Waals
99	$C_{33}H_{52}O_3$	-9.6	Arg-200: HE, Arg-200: HH21, Arg-200: HH11, Ala-245	Ala-21, Ala-245, Leu-228, His-24 Phe-246	-
100	$C_{14}H_{28}O_2$	-4.3	His-24(2.54876), Arg 200(2.41678), Ser-226(2.73739)	A:Ala-21(3.69647), C:Ala-21(3.88612) ,C:Ala-21 (4.75127)	-
102	$C_{18}H_{26}O_6$	-6.0	Ala-21(2.32204), Arg-200: HH21(2.4645), Arg-200: HH21: B (2.85846), Arg-200:HH22:B(2.80796), Gly-198(2.56652)	Pi-SigmaThr 29(3.99715), Ala-21(4.31058)	Arg200,Thr20, Asp-19,Asp-19,Thr-20,His-24,Thr-20,Ala-21,Gly-198,Thr-29,His-24
Ampicillin	$C_{16}H_{19}N_3O_4S$	-7.6	Arg-354(2.34291), Gly- (2.50094), Arg-333(2.26757), **Ser-359(2.76843), Gly-329(2.80288), Gly-329(3.01454)**	Electrostatic Pi-Cation Arg-333(4.82219), Hydrophobic Pi-Alkyl Arg-333(4.77674)	

Figure 11: Binding Interactions (3D and 2D) of Compound **99**, **100** and **102** against *P. aeruginosa* **(PDB ID: 5EO3)**



Staphylococcus aureus (*S. aureus*) is a very common human pathogenic microorganism that can trigger a variety of infectious diseases (Guo *et al*., 2020). In this study *S. aureus* Pyruvate Kinase (**PDB ID: 5OE3**) was selected for docking as target protein. The result showed a binding pocket N-terminal domain of PK (**PDB ID: 3T07**) of isolated compounds **99, 100** and **102** were found to have minimum binding energy ranging from (-3.2 to -8.1 kcal/mole) (Table 16). The binding

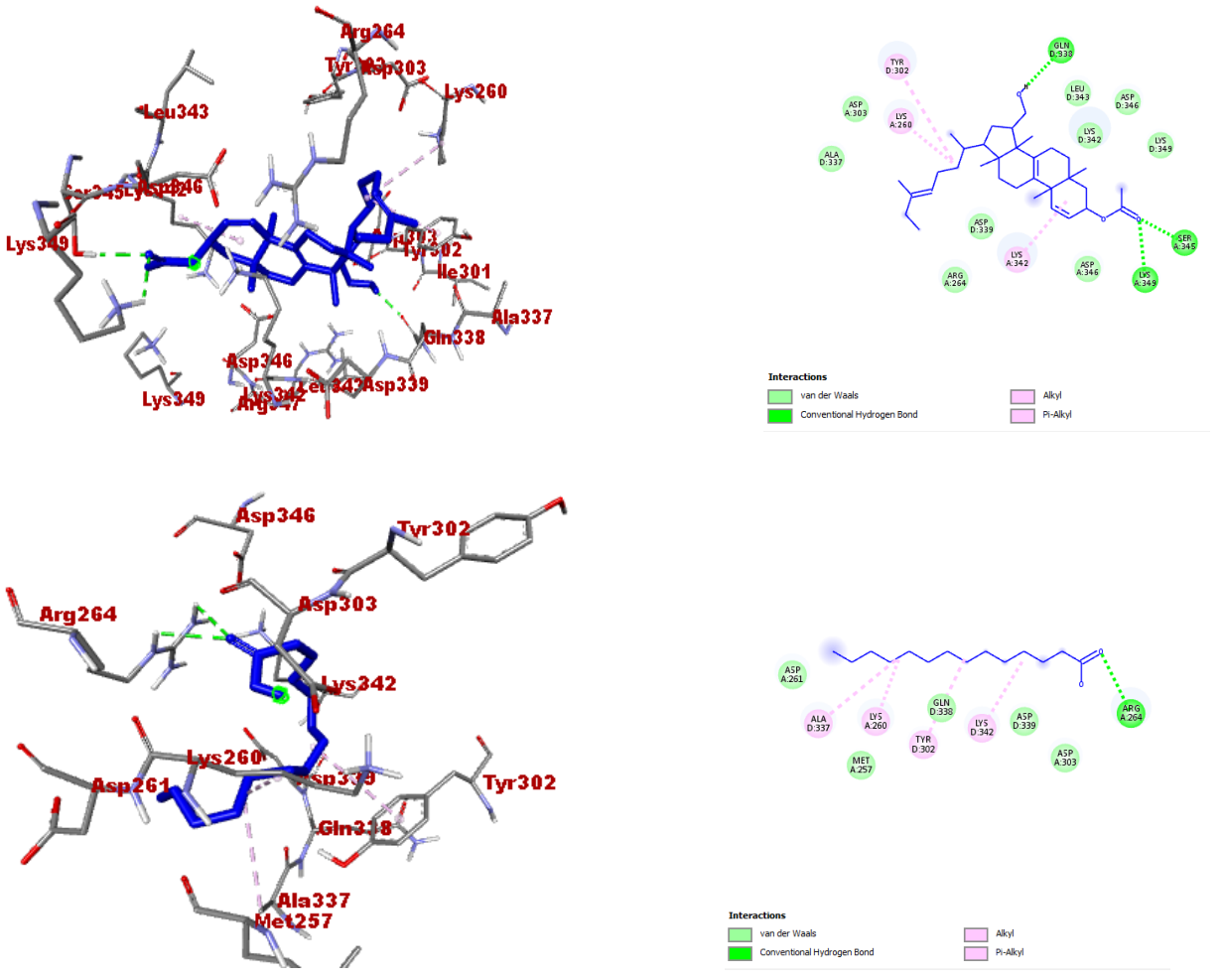
affinity, H-bond and residual interaction with different amino acids of compound **99, 100** and **102** along the standard drug ampicillin were used (Table 16). Among all tested compound **99** showed the remarkable binding affinity score (-8.1 kcal/mol). In comparison to ampicillin, all compounds have shown no similar hydrogen bond interaction of amino acid. However, compound **99**, **100** and **102** were displayed similar residual interaction with similar **Lys-342** amino acid and compound **99** and **100** showed similar residual interaction with **Tyr-302** amino acid.

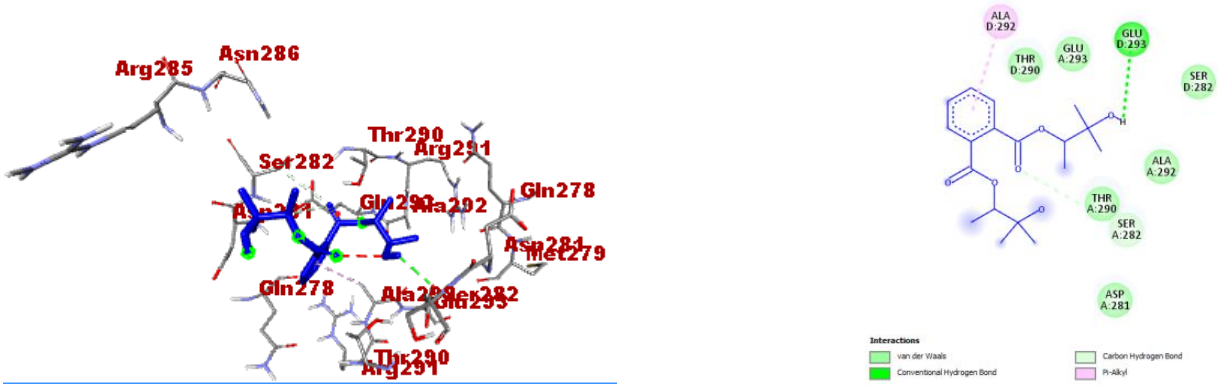
Table 16: Molecular Docking results of Compound **99**, **100** and **102** Isolated from the Aerial *M. foetida* and Ampicillin against *S. aureus* Pyruvate Kinase **(PDB ID: 3T07).**

Cpds	**Ligands**	**Binding Affinity (kcal/mol)**	**H-Bond**	**Residual interaction**	
				Hydrophobic/ Electrostatic	**Van der Waals**
99	$C_{33}H_{52}O_3$	-8.1	Ser-345(2.21594), Lys-349(2.50823), Gln-338(2.16336)	Lys-260 (4.91801), Lys-342 (4.94393), Pi-AlkylTyr-302 (5.43881)	Leu-343, Asp-346, Lys,342, Lys 349, Asp-346, Asp-339, Arg-264,Ala 337,Asp-303
100	$C_{14}H_{28}O_2$	-3.2	Arg-264(2.74117), Arg-264(2.77408)	Lys260(3.9559),Ala337(4.86444),Lys342(5.40412),Tyr302(5.39793)	Asp-261, Met-267, Gln-338, Asp-339, Asp-303
102	$C_{18}H_{26}O_6$	-5.5	Glu-293(2.57429), Ser-282(3.78896), UNK1:O (3.49767)	Lys-342, Ala-292 (4.40636)	Thr-290,Glu-293,Ser-282,Ala-292,Thr-290,Ser-282,Asp-281
Ampicillin	$C_{16}H_{19}N_3O_4S$	-6.7	Arg-204(3.07006),	Pi-Cation Lys-390 (4.78505)	Glu-234, Gly-483, Arg-

			Arg-204(2.3253), Lys-271(2.925), Arg-386(2.74309), Arg-386(2.41526), Leu-269 (2.26807), Gly-270(2.84417), Glu-234(3.78248)		484,Thr-387,val-356,Asp-237,Ser-236,Val-235

Figure 12: Binding Interactions (3D and 2D) of Compound **99**, **100** and **102** against *S. aureus* Pyruvate Kinase **(PDB ID: 3T07)**





4.7.2. Molecular Docking Study for Antifungal Activity

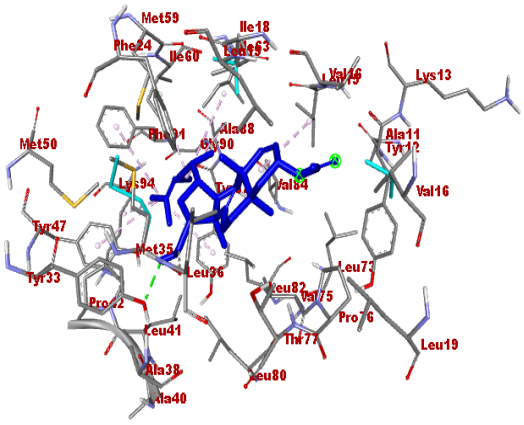
Currently, the main targets of the antifungal agents are the cytochrome P450 sterol 14a-demethylase (CYP51), squalene epoxidase (SE) and β-1, 3-glucansynthase. (Teng et al., 2018).The mechanism of molecular docking was targeted at the positive drug, Ketoconazole. Docking experiments select SE (**PDB ID**: **2AIB**) to perform molecular docking since allylamine antifungal agents mainly acts on SE. The docking score of compounds is shown in Table 17. The results show compounds **99** had remarkable binding affinity than ketoconazole and compound **100** and **102** demonstrate better score. The 2D and 3D interactions of compounds **99, 100** and **102** with **2AIB** are shown in Figure 12. The binding affinity, H-bond and residual interaction with different amino acids of compound **99, 100** and **102** along the standard drug ketoconazole were used (Table 17). Among all tested compound **99** showed the remarkable binding affinity score (-11.1 kcal/mol) compared with ketoconazole (-10.2 kcal/mol). However, it has less hydrophobic interaction. Compound **99** and **102** formed the hydrogen bonding interactions with amino acid **Tyr-33,** compound **99** showed residual hydrophobic interaction with **Lys94, Tyr47 and Phy91** and compound **100** with **Val84.** Besides, compound **99, 100** and **102** showed hydrophobic interaction with amino acid **Ile63.** Thus, all these docked compounds exhibited less hydrophobic interaction than the standard drug ampicillin of binding affinity (**-10.**2 kcal/mol**)** except compound **99** which have comparable hydrophobic interaction with standard drug ketoconazole.

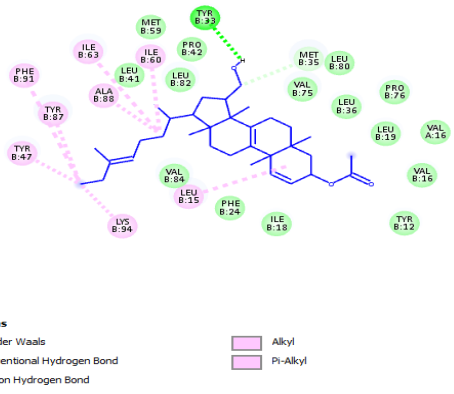
Table 17: Molecular Docking results of Compounds **99, 100** and **102** Isolated from the Aerial *M.foetida* and Ketoconazole against *T. mentagrophytes* squalene epoxidase **(PDB ID: 2AIB)**

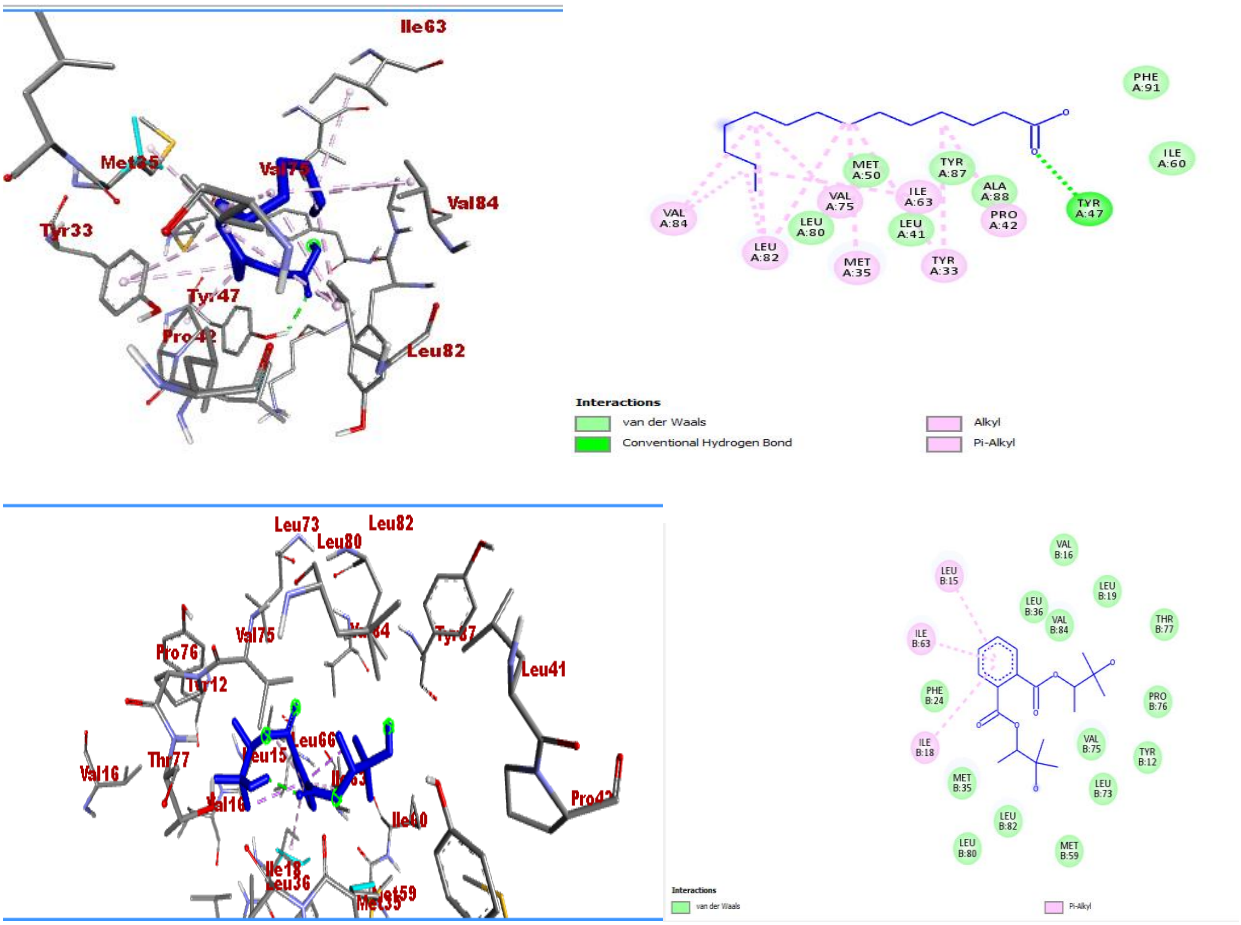
Cpds	**Ligands**	**Binding Affinity (kcal/mol)**	**H-Bond**	**Residual interaction**	
				Hydrophobic/ Electrostatic	**Van der Waals**
99	$C_{33}H_{52}O_3$	-11.1	Tyr-33 (Dist. 2.32782), Met-35 (Dist..71777)	Leu-15(Dist. 5.05483). Ile-60 (*Dist.* 5.2268), Ile-63(*Dist.* 5.43237), Ala-88(Dist. 4.82686), Lys-94(*Dist.* 4.1239), Tyr-47(*Dist.* 5.16405), Tyr-87(*Dist.* 5.25018) -Phe-91(*Dist.* 5.45619)	Leu-80, Val-75, Leu-36,Pro-76, Leu-19,Val-16, Tyr-12,Ile-18, Phe-24,Val-84, Leu-41,Leu-82, Pro-42,Met-59,
100	$C_{14}H_{28}O_2$	-5.8	Tyr-47 (2.17737)	Pro-42(4.10216), Val-75(4.96631), Val-84 (5.0205), Val-84(4.05905), Met-35(4.96777),Leu-82 (5.25956) Leu-82(4.5844), Ile-63(5.13999), Leu-82(5.11539), Tyr-33 (5.00368), Tyr-33(5.49868)	Phe-91, Ile-60, Ala-88,Tyr-87, Met-50,Leu-80, Leu-41
102	$C_{18}H_{26}O_6$	-8.2	UNK1:H (Dist. 2.14674)	Pi-Sigma-UNK1:C(Dist.3.9034), Pi-Sigma-UNK1:C(Dist.3.92318),Leu-15(Dist. 4.86398), Ile-18(Dist. 5.02736), Ile-63(Dist. 4.75678)	Val16,Leu36,Val-84,Leu-19, Thr-77,Pro 76,Tyr-12,Val-75,Leu-73,Met-59,Leu-82,Leu-80,Met-35,Phe-24
Keto conaz	$C_{26}H_{28}Cl_2N_4O_4$	-10.2	Tyr-33(Dist.3.74058)	Pi-Sigma-Ile-63(Dist.3.29363), Pi-	Tyr-12,Leu-19,Val-16,Leu-

ole				Sigma-Val-75(Dist.3.5133),Pi-Sigma- Val-75 (Dist.3.70273), Other Pi-Sulfur-Met-35 (Dist.5.17605), Pi-Pi Stacked-UNK1(Dist.4.33682), Pro-76(Dist.5.15754), Lys-94(4.47504), Tyr-47(Dist.4.70011),Phe-91(Dist.4.53685), Val-84(Dist.5.0834)	15,Lue-36,Leu-80,Tyr-33,Leu-82,Met-50,Pro-42,Ile-60,Tyr-87,Ala-88,Met-59,Phe-24,Thr-77

Figure 13: Binding Interactions (3D and 2D) of Compound **99**, **100** and **102** against *T. mentagrophytes* Squalene Epoxidase **(PDB ID: 2AIB)**







4.7.3. Molecular Docking Study for Antioxidant Activity

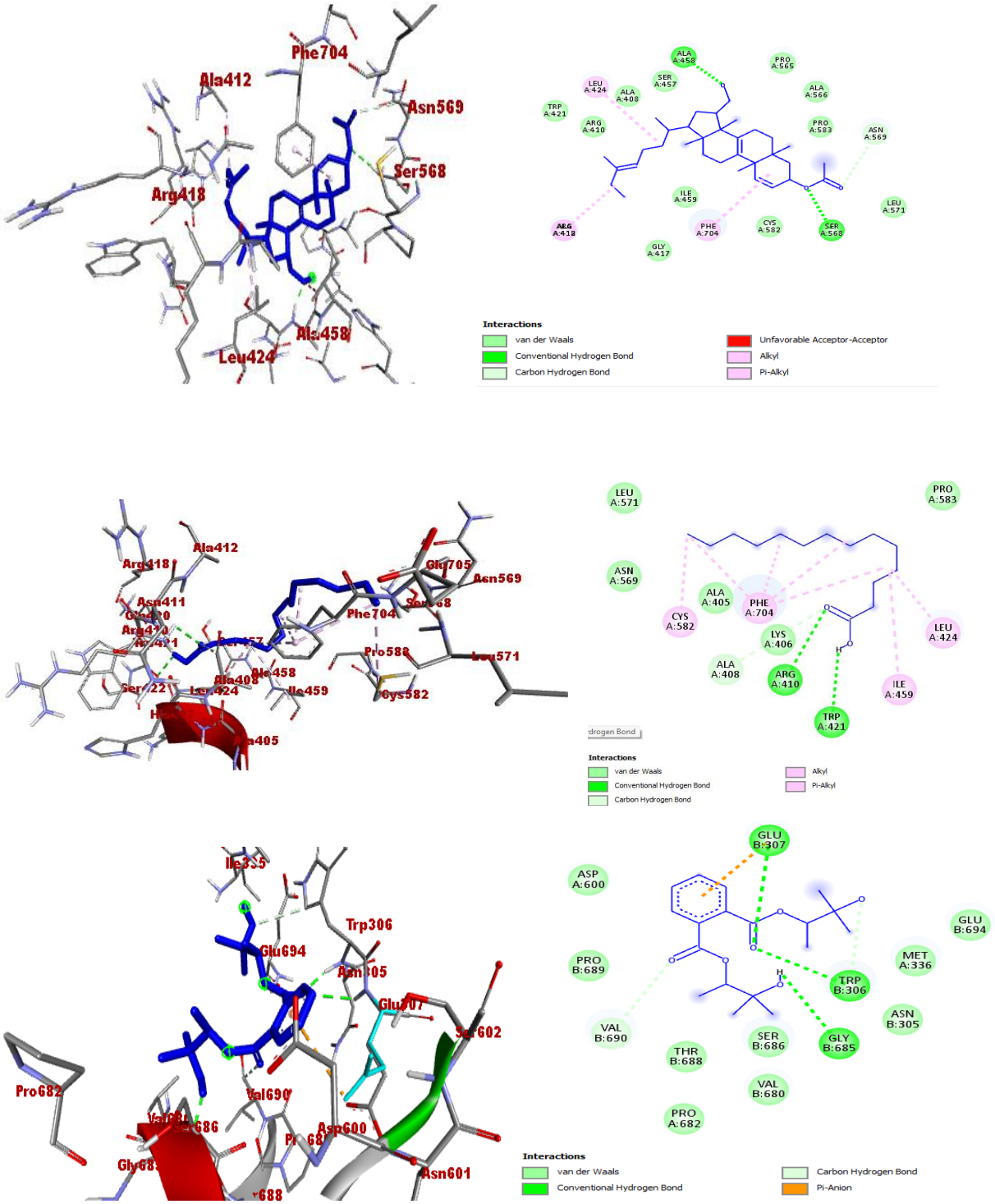
The binding mode of the ligands, some the isolated compounds were subjected to molecular docking studied against selected proteins viz. Human myeloperoxidase (**PDB ID 6NGJ**) and the results were compared with standard and antioxidant ascorbic acid. The tested compounds **99** and **102** were found to have minimum binding energy ranging from – 5.7 to – 8.0 kcal/mol (Table18). Comparing with ascorbic acid (-5.5 kcal/mol) the isolated compounds **99** and **100** have shown comparable binding affinity and similar residual interaction profile with many amino acid residues. The compound **100** (-4.3 kcal/mol), have shown comparable binding affinity and similar residual amino acid binding within the binding pockets of Human myeloperoxidase (**6NGJ)** as compared to ascorbic acid (Figure 13). *In-silico* interaction results have shown that the compounds **99** and **102** have comparable binding affinity, among them Compound **99** has shown the best binding score (–8.0 kcal/mol). Based on the molecular docking analysis results, all the tested compounds have shown comparable residual interactions, as well as all the docking

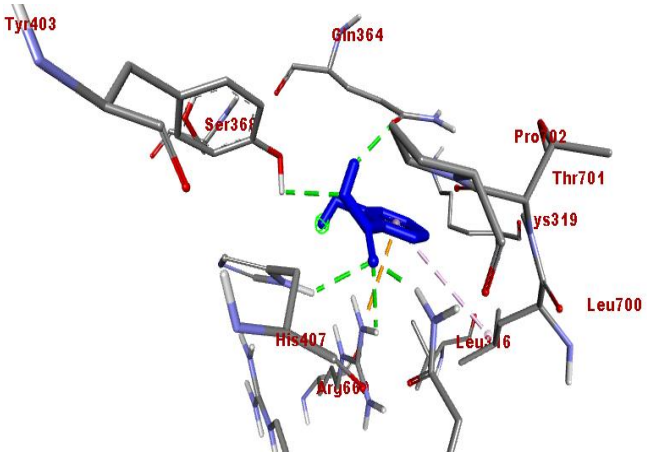
results are in good agreement with *in-vitro* analysis. Hence, these compounds might prove to be comparable antioxidant agents compared to standard ascorbic acid. The binding affinity, H-bond and residual interaction of all the isolated compounds are summarized in (Table 18).

Table 18: Molecular Docking results of Compounds 99, 100 and 102 Isolated from the Aerial *M. foetida* and against selected proteins viz. Human Myeloperoxidase (**PDB ID 6NGJ**)

Cpds	**Ligands**	**Binding Affinity (kcal/mol)**	**H-Bond**	**Residual interaction**	
				Hydrophobic/ Electrostatic	**Van der Waals**
99	$C_{33}H_{52}O_3$	-8.0	Ala-458(1.98663), Ser-568(2.60145), Asn- (3.48063)	Ala-412(4.2832), Arg-418 (4.26261), Leu-424(4.7748), Phe-704 (4.98053)	Pro-565, Ala-566, Pro-583, Leu-571, Cys-582,Ile-459, Gly-417,Arg410, Trp-421,Ala-408, Ser-457
100	$C_{14}H_{28}O_2$	-4.3	Arg-410(2.67999), Trp-421(2.47853), Ala-408 (3.55816)	Leu-424(4.32505),Ile-459(4.74762), Cys-582(5.26082), Phe-704 (5.14058), Phe-704 (5.34008), Phe-704 (3.89085), Phe-704 (4.71754)	Pro-583, Lys-406, Ala-405, Asn-569,Leu-571
102	$C_{18}H_{26}O_6$	-5.7	Trp-306(2.00244), Glu-307(3.0837), Gly-685(2.44891), Trp-306(3.38566), Val-690(3.49506)	Pi-Anion Glu-307(4.09671)	Glu-694, Met-336, Asn-305,Ser-686,Val-680,Pro-682,Thr-688,Asp-600,Pro-689
AA	$C_6H_8O_6$	-5.5	Tyr-403 (2.58164), His-407 (2.3732), Asn-411(1.90927), Arg-669 (2.30124), Gln-364 (2.03715)	Pi-cation-Arg-669 (3.85017),Leu-700 (5.26239)	Leu-316, Thr-701, Pro-702

Figure 14: The3D and 2D Binding Interactions of Compound **99, 100** and **102** and Ascorbic acid against Human myeloperoxidase **(PDB ID: 6NGJ**). Hydrogen bond between Compounds and Amino acids are shown as green dash lines, π-anion/ π-cation/ π-alkyl interactions

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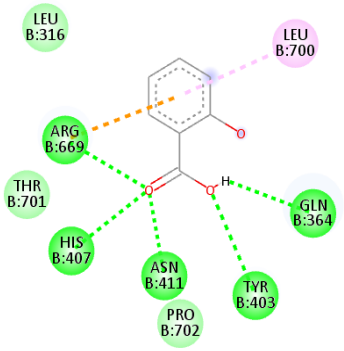
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LEU
B:700
ARG
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THR
B:701
GLN
B:364
HIS
B:407
ASN
B:411
TYR
B:403
PRO
B:702

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The phytochemical screening tests showed that the presence of alkaloids, tannins, glycosides, steroids, flavonoids and terpenoids in the crude extract of the plant. Column chromatographic separation of the crude extracts chloroform: methanol (1:1) and methanol furnished five compounds namely, collosolactone A derivatives **99**, myristic acid **100**, pentan-1-ol **101**, 3-bis (3-hydroxy-3-methylbutan-2-yl) phthalate **102** and heptane **103**, from which 3-bis (3-hydroxy-3-methylbutan-2-yl) phthalate **102**. GC-MS analysis revealed the principal component to be Phenol, 2, 4-bis (1, 1-dimethylethyl), i.e this component might be the bioactive compound of this *M. foetida*. The *in-vitro* antibacterial activities of extract and compounds exhibited promising inhibition zone for MeOH extract and Compound **101** against *E. coli* and *S. aureus,* compared to the standard antibiotic ampicillin. Moreover, Compound **101** showed promising antifungal activity *against T. mentagrophytes and P. species* with pronounced MIC value compared to other samples. The DPPH radical scavenging activities (%) of extracts and compounds were displayed remarkable IC_{50} value with MeOH extract, compound **99** and compound **100**. Molecular docking study was done using Autodock vina 4.2 and it was found compound **99** shows the best scoring pose (lowest energy) against *P. aureginosa* quinolone signal PqsA (**PDB ID: 5OE3**), *S. aureus* Pyruvate kinase PK **(PDB ID: 3T07**), *T. mentagrophytes* Squalene Epoxidase SE (**PDB ID: 2AIB**) and Human meyleoperoxidase (**PDB: ID 6NGJ**) respectively. Furthermore, this study provided the scientific basis the use of the *M. foetida* in future research and brings the attention experts to look more on the medicinal importance of the plant. Based on results shown in this study, it can be concluded that the aerial parts of *M. foetida* has bioactive compounds with the potential to treat bacterial and fungal infections and also used as an antioxidant. This observation supports the traditional uses of the plant for skin infections (small pox) and other reported activities.

5.2. Recommendation

Based on the present study we recommend further research works such as isolation of more compounds from polar part of the plant, evaluate biological activities (anticancer, antimalarial and cytotoxicity) on crude extracts as well as isolated compounds. Additional research works are also recommended such as, 2D NMR and MS analysis to fully elucidate structures of the isolated compounds.

6. REFERENCES

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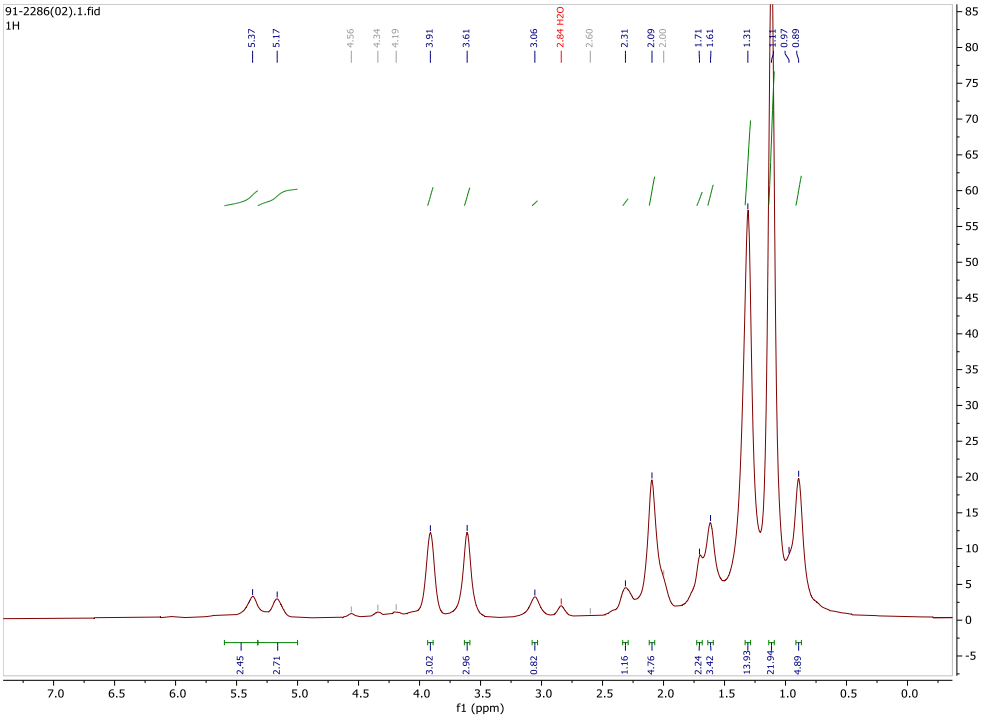
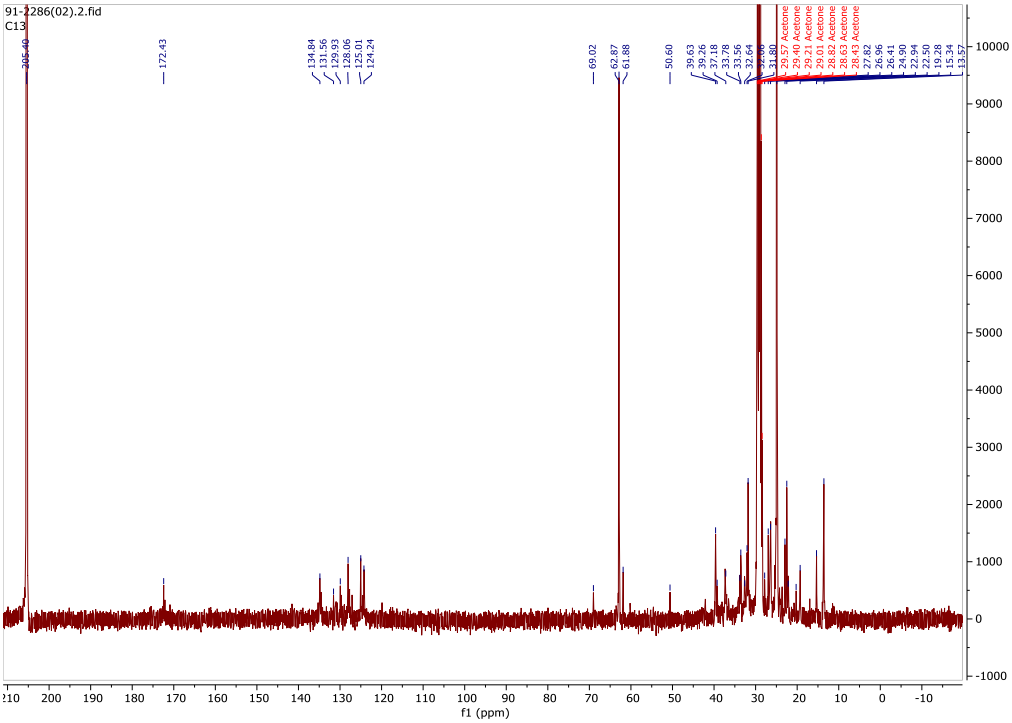
APPENDIXES

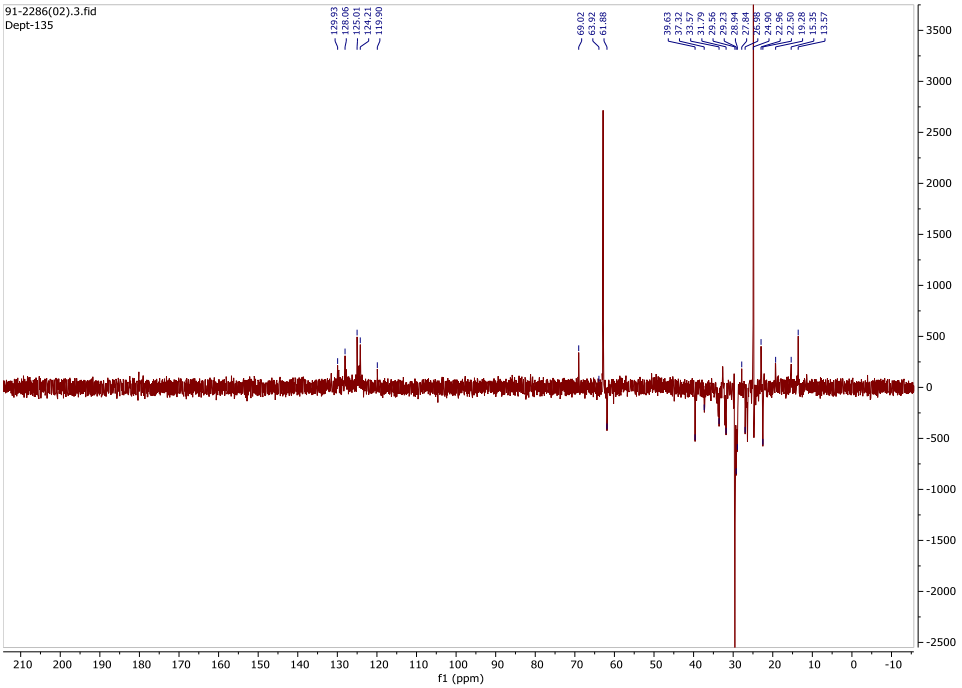
Appendix 1: ^{1}H-NMR Spectrum (400 MHz, Acetone-D_6) of Compound **99**



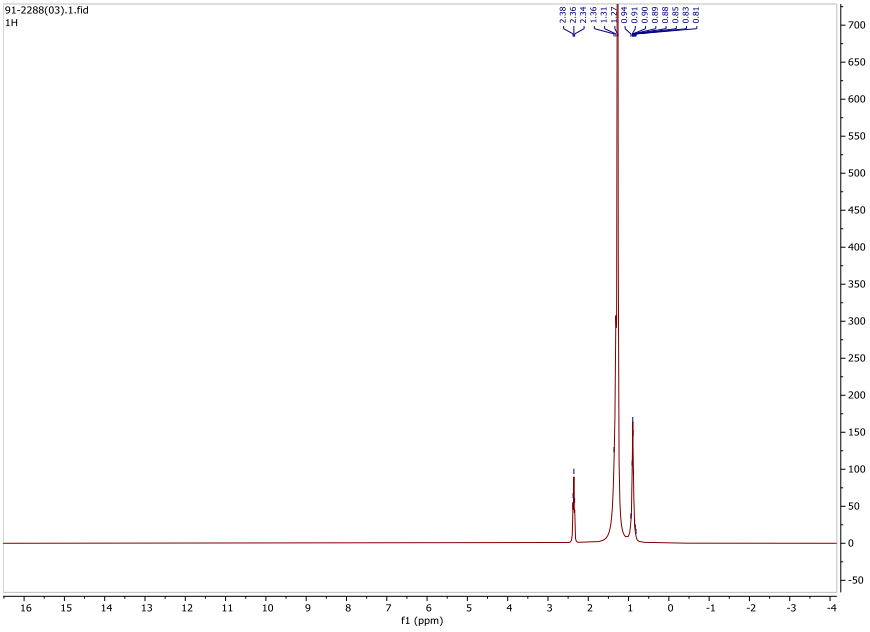
Appendix 2: ^{13}C-NMR Spectrum (400 MHz, Acetone-D_6) of Compound **99**



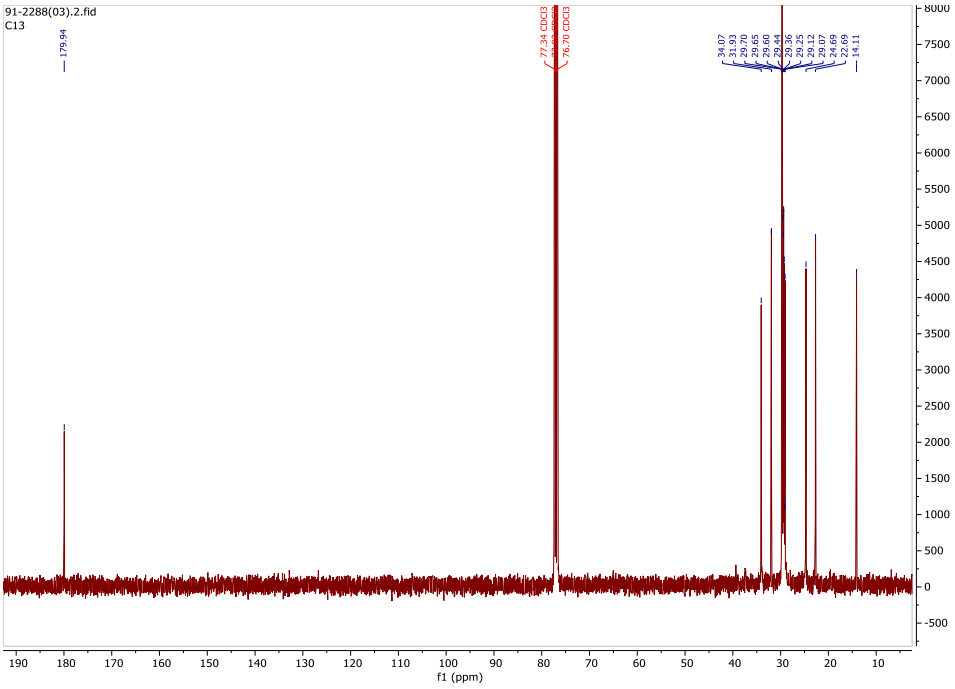
Appendix 3: DEPT-135 Spectrum (400 MHz, Acetone-D_6) of Compound **99**



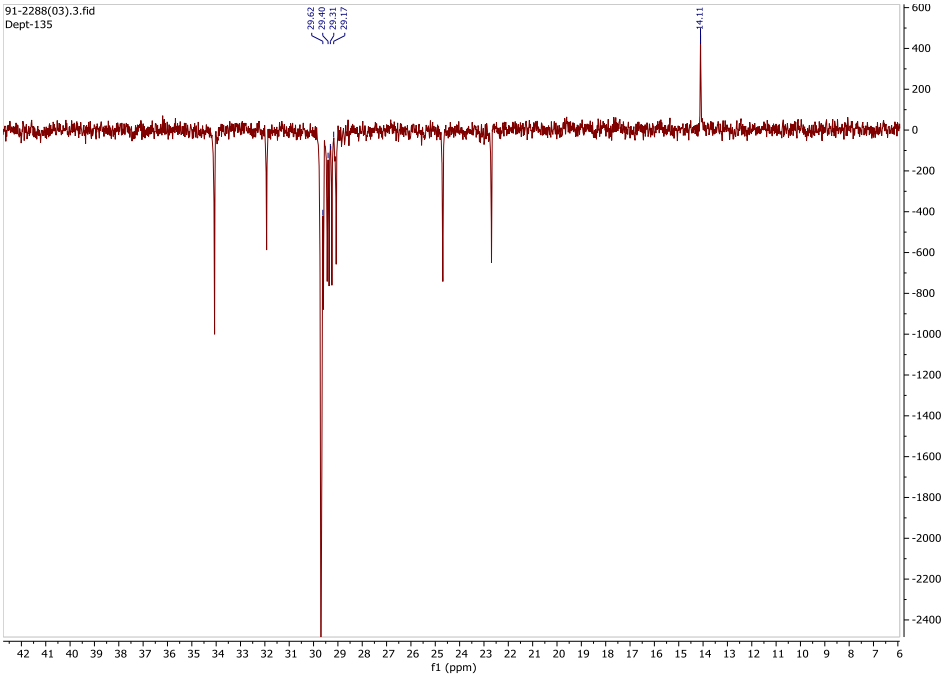
Appendix 4: ^{1}H-NMR Spectrum (400 MHz, $CDCl_3$) of Compound **100**



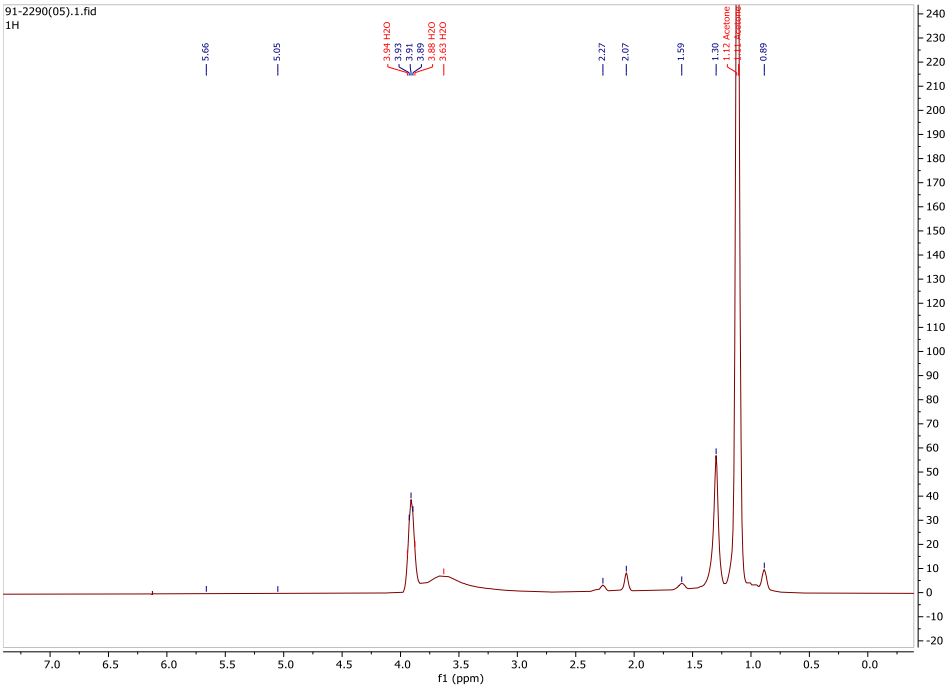
Appendix 5: ^{13}C-NMR Spectrum (400 MHz, $CDCl_3$) of Compound **100**



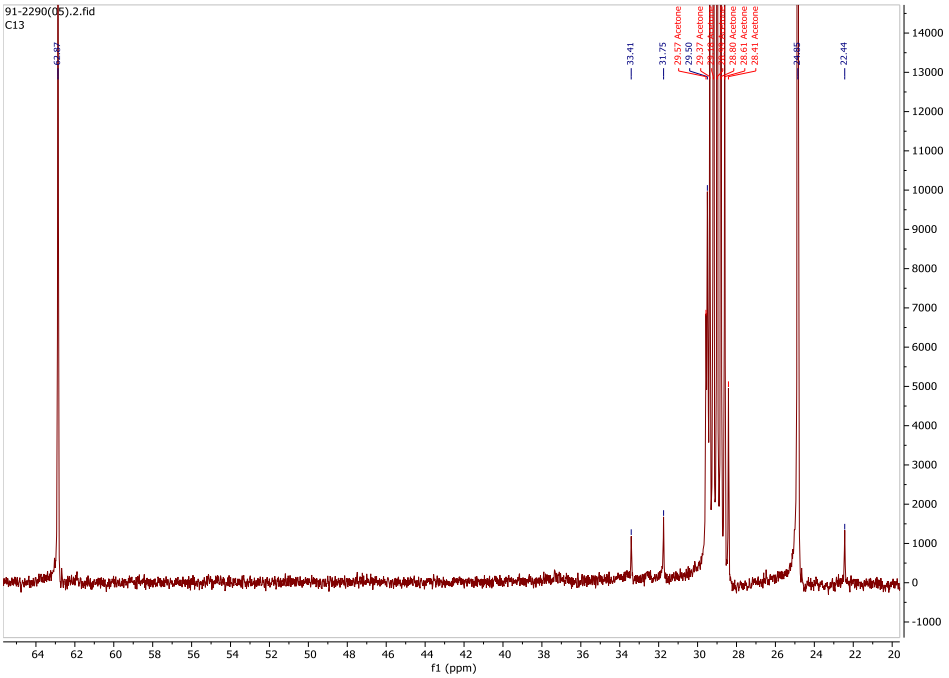
Appendix 6: DEPT-135 Spectrum (400 MHz, $CDCl_3$) Compound **100**



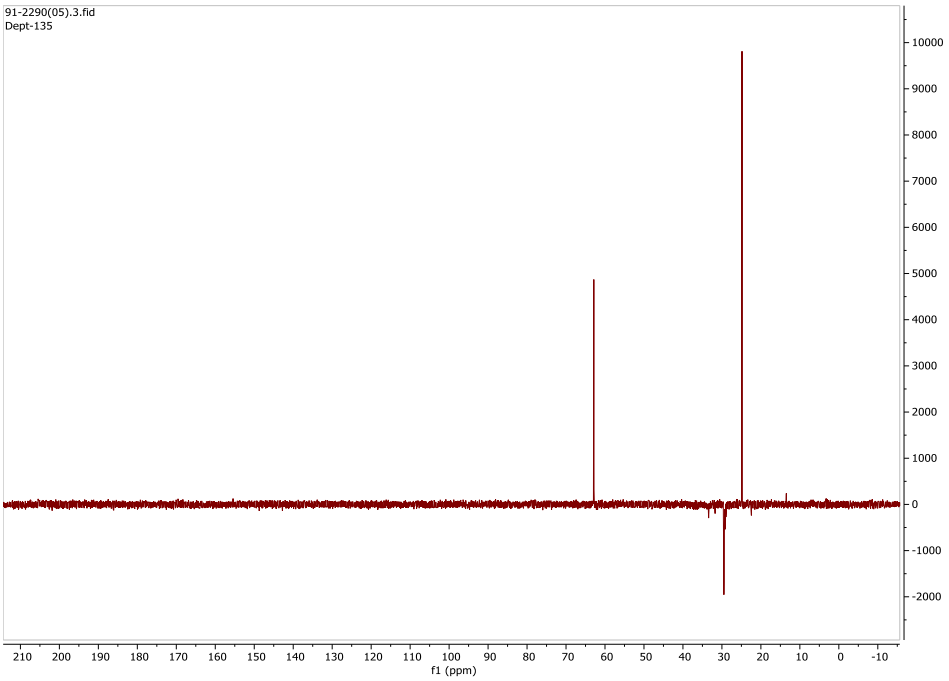
Appendix 7: ^{1}H-NMR Spectrum (400 MHz, Acetone-D_6) of Compound **101**



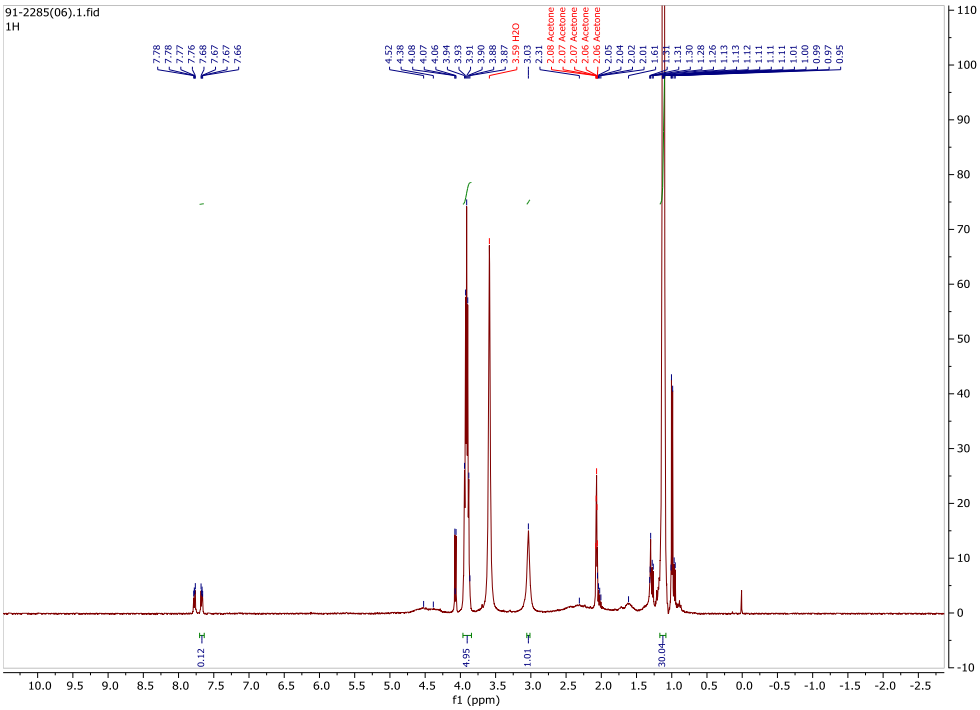
Appendix 8: ^{13}C-NMR Spectrum (400 MHz, Acetone-D_6) of Compound **101**



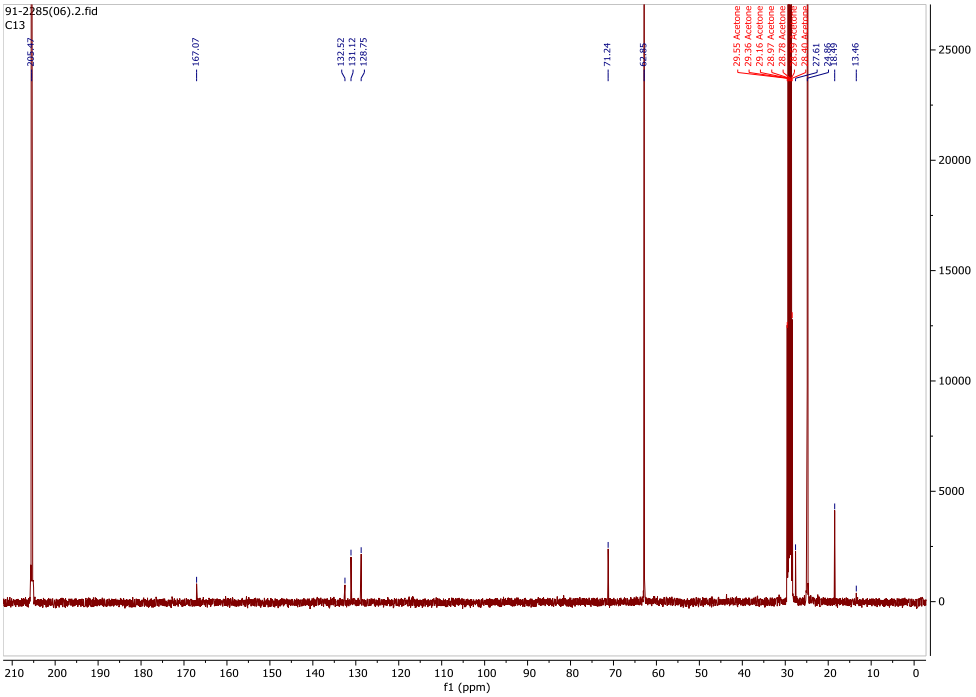
Appendix 9: DEPT-135 Spectrum (400 MHz, Acetone-D_6) of compound **101**



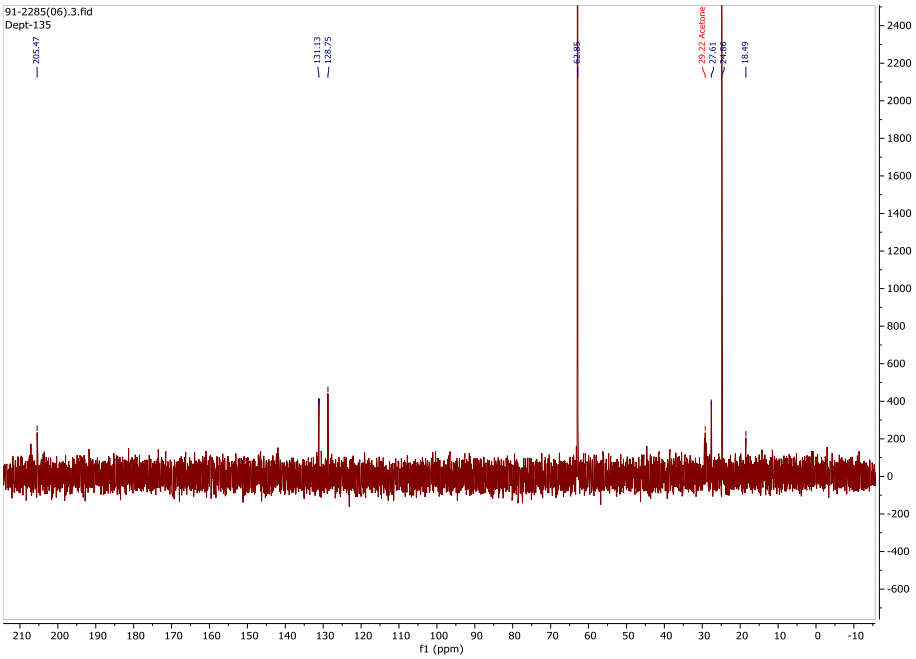
Appendix 10: ^{1}H-NMR Spectrum (400 MHz, Acetone-D_6) of Compound **102**



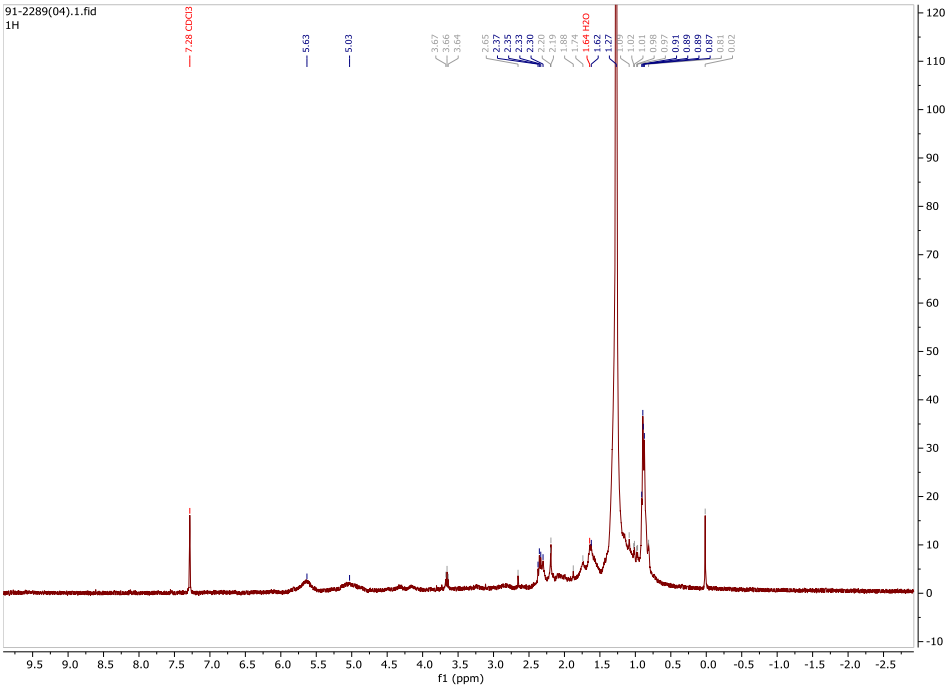
Appendix 11: ^{13}C-NMR Spectrum (400 MHz, Acetone-D_6) of Compound **102**



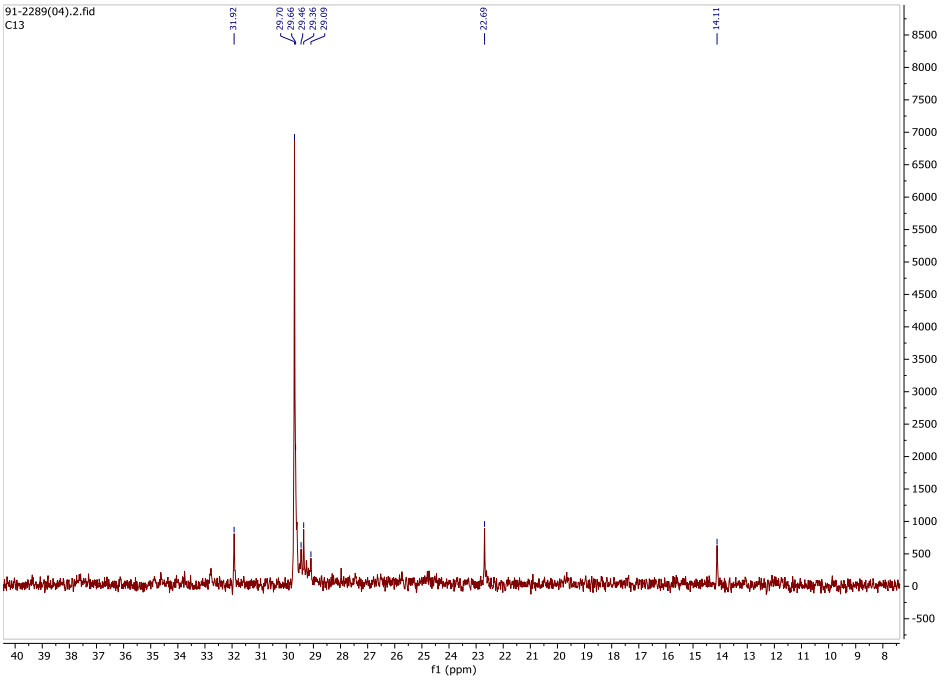
Appendix 12: DEPT-135 NMR Spectrum (400 MHz, Acetone-D_6) of Compound **102**



Appendix 13: ^{1}H-NMR Spectrum (400 MHz, $CDCl_3$) of Compound **103**



Appendix 14: ^{13}C-NMR Spectrum (400 MHz, $CDCl_3$) of Compound **103**



Appendix 15: DEPT-135 Spectrum (400 MHz, $CDCl_3$) of Compound **103**