Phytochemical Analysis, Antibacterial and Radical Scavenging Activities of Root Extracts of *Cucumis Prophetarum* L.

By

Wario Galma Dida

Thesis Submitted to the

Department of Applied Chemistry

School of Applied Natural Science

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

Office of Graduate Studies
Adama Science and Technology University

July, 2019

Adama, Ethiopia

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Advisor: Yadessa Melaku (Ph.D)

Co-advisor: Milkyas Endale (PhD)

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Approval of Board of Examiners

We, the undersigned, members of the Board of Examiners of the final open defense by Wario Galma Dida have read and evaluated his thesis entitled "**Phytochemical Analysis, Antibacterial and Radical Scavenging Activities of Root Extracts of *Cucumis prophetarum*"** and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Masters in Applied chemistry (Medicinal Chemistry)

_____	_____	_____
Advisor	Signature	Date
_____	_____	_____
Chairperson	Signature	Date
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Internal Examiner	Signature	Date
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External examiner	Signature	Date

Declaration

I hereby declare that this MSc Thesis is my original work and has not been presented for a degree in this or other university, and all sources of material used for this thesis have been duly acknowledged.

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This MSc Thesis has been submitted for examination with my approval as thesis advisor.

Name: _____

Signature: _____

Date of submission----------

Approval Sheet

This is to certify that the thesis entitled **"Phytochemical Analysis, Antibacterial and Radical Scavenging Activities of Root Extracts of *Cucumis Prophetarum*"** submitted in partial fulfillment of the requirements for the degree of Master of Science in Applied chemistry with specialization in Medicinal Chemistry of School of Applied Natural Science, M.Sc. program of Department of Applied Chemistry, Adama Science and Technology University and is the record of original research carried out by Wario Galma (ID.No. A/PR15317/10) under my supervision. Therefore, I recommend that the student has fulfilled the requirements and hence hereby he can submit the thesis to the department.

Name of major Advisor	Signature	Date
Name of Co-advisor	Signature	Date

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LIST OF ABBREVIATIONS

CC	Column Chromatography
$CDCl_3$	Deuterated chloroform
^{13}C-NMR	Carbon 13-Nuclear Magnetic Resonance
COSY	Correlation Spectroscopy
2D	2-dimension
DEPT	Distortion less Enhancement Bipolarization Transfer
DMSO	Dimethyl sulphonamide
DPPH	1-1- diphenyl-2-picrylhydrazyl
GC-MS	Gas chromatography–mass spectrometry
HMBC	Hetero nuclear Multi Bond Correlation Spectroscopy
^{1}H-NMR	Proton-Nuclear Magnetic Resonance
HPLC	High Performance Liquid Chromatography
HSQC	Hetero nuclear Single Quantum Coherence
RMSD l.b	Root mean standard deviation least bond
TLC	Thin layer chromatography
TMS	Tetra methyl silane
UV-Vis	Ultra Violet- Visible

ABSTRACT

*Cucumis prophetarum is among plants traditionally used by Borena people for the treatment of wide array of diseases including lung disorders, heart failure, back pain, gonorrhea, intestinal problems and abnormal menstrual cycle. Considering the absence of prior scientific report that supports ethno-medicinal uses of the roots of the plant, this study focused on isolation of chemical constituents, evaluation of the anti-bacterial and radical scavenging activities of the roots of C. prophetarum. In view of this, the roots were successively extracted on maceration with hexane, $CHCl_3$ and MeOH to furnish 4 g (1% w/w), 15 g (3.75% w/w), and 23 g (5.75% w/w) yield respectively. The hexane extract, after silica gel column chromatography, afforded two new compound namely compound **32** and **33** along with three known compounds such as myristic acid (**29**),* 24-ethyl-3-hydroxylcholesta-5,25-diene (***30***) *and α-spinasterol (**31**).The extracts and isolated compounds were evaluated for their antibacterial activity using Agar disc diffusion against four strains of bacterial pathogens including E. coli, S. thyphimurium, S. aureus and B. subtilis. Results showed that all extracts and isolated compounds showed inhibition diameter beyond 9 mm. The hexane extract, methanol extract, compound **31** and compound **32** exhibited inhibition diameter of 15±1.41, 14.6±1.7, 13±0 and 13.6±0.94, respectively, against E. coli. Whereas compound **32** and **33** showed inhibition diameter 13±1.41 against S. thyphimurium. Both the crude extracts and isolated compounds investigated herein were in inhibition diameter range (9-12 mm) against S. aureus and compound **33** also exhibited inhibition diameter of 13.3±0.54 against B. subtilis. Molecular docking of isolated compounds was done using Autodock vina software. Among the investigated ligands compound **32** exhibited better docking efficiency with DNA gyrase with binding affinity -7.3. The extracts and isolated compounds were also assessed for their radical scavenging activity using DPPH assay. The methanol extract displayed promising radical scavenging activity of 70.57% (IC_{50} = 28.87). The antibacterial activity presented herein supports the traditional use of this plant against bacteria.*

Keywords: Antibacterial activity, Chemical constituent, *Cucumis prophetarum*

1. INTRODUCTION

1.1. Background

Plants are vital to human being beginning from the prehistoric times as has been used as food, shelter and not the least of which is medicine. A plant which has active constituents of medicinal properties and is used to treat disease in different systems of medicine or traditionally used for the treatment of disease is considered as medicinal plant. Many people especially in the developing countries are still using medicinal plants as a remedy against various life threatening diseases. This is because of the fact that medicinal plants are cheap, easily available and affordable (Rajasree et al., 2016). Medicinal plants are widely and successfully used on every continent. Plant parts and plant products served as the materials for the preparation of medicine and these medicinal plants and plant parts constitute an important natural wealth all over through the world. They also play a significant role in primary health care service to rural people (Tripti and Jyoti, 2015; Rajasree et al., 2016). Worldwide it is estimated that 80% of the population uses herbs; in the developing world rates could be as high as 95% (Rout et al., 2009). According to the WHO, more than 80% of the world's population rely more often on traditional drugs, mainly plants, serving as the main source of health care (Fatemeh et al., 2018). The active constituents responsible for the biological activity of these plants are accounted to the presence of phytochemicals.

Naturally occurring compounds are divided into two broad categories. The first class of compounds are known as primary metabolites. They occur in all cells and play a central role in the metabolism and reproduction of cells. Primary metabolites include the nucleic acids, common amino acids, sugars and the high molecular weight polymeric materials such as cellulose, lignin and proteins which form the cellular structures. Most primary metabolites exert their biological effect within the cell or organism that is responsible for their production (Cseke et al., 2006).The second class of compounds are secondary metabolites. Such compounds are characteristic of a limited range of species and occur in plants in a high structural diversity. The major classes of secondary metabolites include tannins, glycosides, flavonoids, alkaloids, terpenoids, steroids, quinones and saponins (Renuga, 2015).

Secondary metabolites produced by plant are biologically active, naturally occurring chemicals in various parts of a plant, providing health benefits for humans further than those attributed to macronutrients and micronutrients (Uzuazokaro et al., 2018). Their functions are diverse and include provision of strength to plants, attraction of insects for pollination and feeding, while some are simply waste products (Ibegbulem et al., 2003). They give plants colour, flavour, and smell and are part of plants' natural defence system, protecting plants' cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attacks (Ejele and Akujobi , 2011). These compounds have been linked to human health by contributing to protection against degenerative diseases (Dandjesso et al., 2012).
Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Afsar et al., 2018). Due to chemical diversity in screening programs, interest has now grown throughout the world for making therapeutic drugs from natural products (Sasidharan et al., 2011).

In Ethiopia, about 80% of the human population and 90% of livestock is said to be dependent on traditional medicine for primary healthcare services and most of this comes from plants (Giday and Teklehaymanot, 2013). Due to the advantageous of their chemical diversity, considerable number of research works has been done on the various aspects of traditional medicinal plants of Ethiopia (Giday and Teklehaymanot, 2013). However, many more medicinal plants of Ethiopia which are found in lesser studied areas still anticipate scientific studies (Anteneh and Nigussie, 2014).

Cucumis prophetarum L. family Cucurbitaceae, which is locally called as "**Gonnee**" in Afan Oromo, "**yemder-inbuyi**" in Amaric is used by traditional healers in different part of the world to treat certain number of disease. For example, in Saudi folk medicine the plant is used for the treatment of liver disorders (Alsayari et al., 2018). Traditional healers around Mega (Dire), Borena, Oromo of Ethiopia use the root of *Cucumis prophetarum* for the treatment of lung disorders, heart failure, back pain, gonorrhea, intestinal problems and abnormal menstrual cycle. Despite the extensive traditional use of this plant there is no prior report on the chemical constituents, antibacterial and radical scavenging activities on the root extract of same species. Therefore this study is designed to investigate the antibacterial, radical scavenging activities and phytochemical constituent of the root extracts of *Cucumis prophetarum* L.

1.2. Statement of the problem

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

1.3. Objectives

1.3.1. General objective

The overall objective of this study was to conduct phytochemical investigation and evaluate the antibacterial and radical scavenging activities of the root extracts of *Cucumis prophetarum.*

1.3.2. Specific objectives

The specific objectives of the study were:

- To successively extract the root of *Cucumis prophetarum* using *n*-hexane, chloroform and methanol.
- To isolate compounds from the *n*-hexane, chloroform and methanol extracts of the roots of *C.prophetarum* using silica gel column chromatography.
- To elucidate the structures of isolated compounds using NMR spectroscopic method and in comparison with literature data.
- To evaluate antibacterial activities of the extracts and isolated compounds from the root of *Cucumis prophetarum* via plate agar disc diffusion method against *Staphylococcus aureus, Bacillus Subtilis*, *Escherichia coli* and *Salmonella typhimurium*.
- To examine molecular docking study of the isolated compounds using Auto doc version 4.2 software on selected proteins.

- To assess the DPPH radical scavenging activity of the extracts and isolated compounds of the root of *Cucumis prophetarum.*

1.4. Significance of the study

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

2. LITERATURE REVIEW

2.1 Family Cucurbitaceae

Cucurbitaceae consists of about 130 genera and 800 species (Jamuna et al., 2015). The family is predominantly distributed around the tropical Africa, where edible fruits are grown and to some extent in temperate area also. It contributes highest rank among the plant families in human food conception. The major crop species of this family are cucumber, melon and water melon. In nutritional point of view, cucurbits are one among prime dieting foods (Jamuna et al., 2015). The important genera belonging to the family are *Trichosanthes*, *Lagenaria*, *Luffa*, *Benincasa*, *Momordica*, *Cucumis*, *Citrullus*, *Cucurbita*, *Bryonopsis* and *Corallocarpus* (Rajasree et al., 2016). Some of the important plants that have been extensively studied are *Momordica charantia*, *Cucurbita pepo*, *Cucumis sativus*, *Cucumis melo*, *Citrullus colocynthis*, *Luffae chinata*, *Trichosanthes kirilowii*, *Lagenaria siceraria*, *Benincasa hispidaetc* (Shweta et al., 2013). In general, members of this family have always been considered as a subject of research due to the fact that they are rich source of proteins, with many biological activities like anti-fungal, anti-bacterial, antiviral, anti-diabetic, anti-tumor and anti-AIDS (Rajasree et al., 2016).

Cucurbit plants were also known to contain several bioactive compounds addition to cucurbitacins such as triterpenes, sterols and alkaloids (Siddig et al., 2011). Ergosterol (**1**) from *Mukia maderaspatina*, kaempferol (**2**) from *Lagenaria siceraria*, Isoquercetin (**3**) and Forskolin (**4**) from *Solena amplexicaulis* are some of the compound reported from the family (Jamuna et al., 2015).

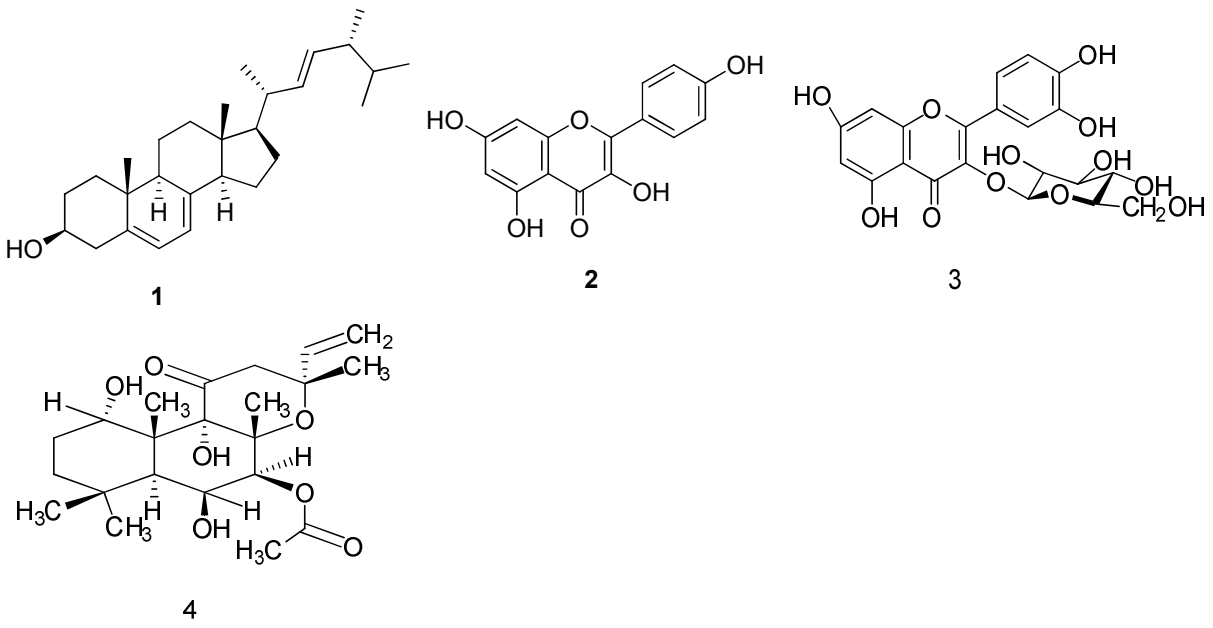

Figure 1. Reported compound from some medicinally important Cucurbitaceae family

2.2. Genus *Cucumis*

Cucumis, one of the major genus of Cucurbitaceae Family, are medium-sized, botanically highly specialized genus mainly climbing, hairy and both wild and cultivated plants. It is well represented in the moist and moderately dry tropics of the World, particularly in grass and bush land areas of Africa including Ethiopia (Worku, 2016). It is a genus of more than 52 species, indigenous mainly to tropical Africa, Asia, Australia and some islands in the Pacific. A lot of works that have been done by the researchers throughout the world on various plants of this genus which showed the presence of triterpene like compounds namely cucurbitacins (Rajasree et al., 2016).

Cucurbitacins are a group of highly oxygenated tetracyclic triterpenoids existing widely in the plant kingdom, especially in the *Cucumis* genus (Chung et al., 2015). A total of 12 classes of cucurbitacins have been recognized based on their structural characteristics and designated alphabetically from A to T with over 200 derivatives (Cai et al., 2015). This genus are used actively as traditional herbal remedies as anti-inflammatory, antitumor, hepato protective, cardio vascular and immuno regulatory activities and also reported to possess purgatives and anti-helminthic properties due to its secondary metabolite cucurbitacin content (Shweta et al., 2013).

2.2.1. Ethnomedicinal use of genus *Cucumis*

The species of this genus *Cucumis* is traditional used by the people of various regions and tribes in the world. It's used to treat different disease like bed wetting in children, bleeding from external cuts and wounds, burns, cancer, cholera, diabetes, ear disorders, eye disorders, fever, stomach ache, gastro-intestinal, goiter, heart disorders, hepatic disorders, infections, infertility, inflammation, malaria, menstrual disorders, mumps, paralysis, pox, respiratory tract disorders, skin disorders, sexual disorders, sexually transmitted diseases, sun stroke, tetanus, tuberculosis, typhoid, and vomiting (Dwivedi et al., 2010).

The whole parts of plant Bitter gourd or bitter melon (*Cucumis)* are used in the treatment of malaria in the south-western regions of Nigeria. The fruit of *Cucumis melo* is tonic, laxative, diuretic diaphoretic and galactagogue and useful in chronic eczema and medicinally used to promote skin hydration, to treat light burns and scrapes or wounds (Robyn and Burnham, 2013). The flowers are expectorant and induce vomiting and the seeds are used as cough suppressant, fever reducer, and a digestive aid. Its seed powder is mixed with water and used as a vermifuge (Robyn and Burnham, 2013).

Regular intake of cucumber fruit promotes healthy hair growth. It is also useful in skin problems, sunburn and swelling under the eye. Its juice is also efficient to soften the skin texture. Placing the two slice of cucumber on eyes for 10 minutes can decrease the inflammation and cure skin infection significantly. Its fruit is also considered traditionally for weight loss, intestinal worms and tapeworms. Leaves are boiled, mixed with cumin seeds, roasted, powdered and administrated in throat infections in the doses of 30 grams to relieve pain. Due to elevated content of potassium (50-80 mg/100g), cucumber can also significantly be helpful for blood pressure (Shrivastava et al., 2013; Kashif et al., 2008 ; Osuagwu and Ejikeme, 2015).

Cucumis sativus leaf is used for headaches; the seeds used as cooling and diuretic, the fruit juice is used as a nutritive and as a demulcent in anti-acne lotions; Juice of leaves used as an emetic in acute indigestion in children (Karthiyayini et al., 2009).

Cucumis prophetarum is one of the medicinally used plants in different part of the world, particullary in Ethiopia. The fruit of the plant is used for the treatment of liver disorder in Saudi (Alsayari et al., 2018). Borena Oromo of Ethiopia also uses the aqueous filtrate of the

boiled root of this plant to cure from disease like lung disorders, heart failure, back pain, gonorrhea, intestinal problems and abnormal menstrual cycle.ethno-botanical use of some species of *Cucumis* was summarized in Table 1

Table 1. Ethno-botanical use of genus *Cucumis*

Species name	Part used	Treatment for	Reference
Cucumis melo	Whole part	Malaria	Robyn and Burnham, 2013
	Fruit	chronic eczema, skin hydration and light burn/ wound	
	Flowers	Induce vomiting	
	Seeds	Cough suppressant, fever reducer and digestive aid	
C. sativus	Leaf	headaches	Karthiyayini et al., 2009
	Seed	As cooling and diuretic	
	Fruit	Nutritive and demulcent	
C. dispaceus	Stem	Anti-emetic	Latta and mittal, 2015
	Fruit	Gasto intestinal disease, diarrhoea, stomach pain and constipation	
	root	Hepatitis, snake bit and carnivore bit	
	Leaf	Haemorrhoids for rabies and wound treatment	
C.prophetarum	Fruit	Liver disorder	Alsayari et al., 2018
	Root	Lung disorder, haert failure, back pain, gonorrhea, intestinal problem and abnormal menstrual cycle	Borena, traditional healer

2.2.2. Pharmacological activity of genus *Cucumis*

Cucurbitacins and their derivatives are triterpenoids found in medicinal plants mostly in the *Cucumis*, known for their diverse pharmacological activities, including anti-cancer effects,

throughout human history. Although initial attention to cucurbitacin as a potential anti-drug withered for decades, recent discoveries showing that cucurbitacin is a strong cancer inhibitor and reclaimed the attention of the drug industry one more time. The increasing evidence shows that some cucurbitacins not only inhibiting disease causing pathogens but also affect other signaling pathways. Moreover, some reports have shown the synergistic effect of cucurbitacins with known chemotherapeutic agents, such as doxorubicin and gemcitabine (Thoennissen, 2010).

Cucumis sativus showing various pharmacological activities such as anti-bacterial activity, antifungal activity, cytotoxic activity, Antacid & Carminative activity, Activity against ulcerative colitis, Hepeto protective activity, Hypoglycemic and Hypolipidemic activity, wound healing activity etc. due to its presence of various active constituents all over the parts of plants (Tripti and Jyoti , 2015).

Crude methanol extract of *cucumber* (*Cucumis sativus*) stem and reported compound from chloroform fraction of methanol extract of *C. sativus* wich possess antifungal and antibacterial activity on selected microorganisms including four fungal and three bacterial species (Tripti and Jyoti , 2015). Particular concentration of seed extract of *Cucumis sativus* showed highest zone of inhibition against *S. aureus*. These pathogens were highly sensitive to the methanol extract also, except *E. coli* (enter pathogen) and *P. aeruginosa* (Tripti and Jyoti, 2015). Different researchers reported on pharmacological evaluation of wound healing potential of *Cucumis sativus* and concluded that aqueous extracts of *Cucumis sativus* have good effect on wound healing (Patil et al., 2012).

Dose dependent cytotoxic activities exhibited by aqueous fruit extract of *Cucumis melo* in human prostate carcinoma cells. As the dose of the extract increased, the number of viable cells decreased. This shows the anti-cancer and cytotoxic potential of the fruit of *C.melo* (Sibi et al., 2018). The fruit extract has a high Superoxide Dismutase Activity (SOD) wich is responsible for the in-vitro and anti-inflammatory properties of the extract (Vouldoukis et al., 2004). The whole parts of *Cucumis melo* also possess anti-fungal, anti-inflammatory, and anti-parasitic and act as a digestive stimulant (Sibi et al., 2018). It also reported that *Cucumis melo* has therapeutic potential and a possible treatment effect for varieties of inflammation-mediated diseases due to one of its compound, cucurbitacin E(**14**) (Siddig et al., 2011).

Methanol fruit extracts of *Cucumis dipsaceus* has antibacterial activity against different bacterial strains like *E-coli* (Gram negative), *Bacillus subtilis* (Gram positive), *Staphylococcus aureus* (Gram positive) (Vansanth et al., 2013). Petroleum ether, dichloromethane, methanol and ethanol extract of *Cucumis dipsaceus* (fruit) showed high analgesic and anti-inflammatory activity as reported by different researchers but dichloromethane and methanolic extract showed highest analgesic effect and anti-inflammatory activity (Salama et al., 1999). The ethanolic extract of aerial part of *Cucumis dipsaceus* showed high percentage cytotoxic effect against K562 and Hep-2 which are human tumoral cellular line. Anti tumor effect was performed by method carrot disk against the tumor produced by the microorganism *Agrobacterium tumefaciens* due to presence of sterol, triterpenes and flavonoids (Salama et al., 1999).

Anti-microbial activity tests on compounds reported from *C. maxima* (spinasterol and 24-ethyl-5α-cholesta-7, 22, 25-trien-3β-ol) indicated that it was slightly active against the fungi (*Aspergillus niger* and *Candida albicans*) and the bacteria (*Bacillus subtilis* and *Pseudomonas aeruginosa*). It was inactive against *Escherichia coli*, *Staphylococcus aureus*, and *Trichophyton mentagrophytes* (Consolacion and Kathleen, 2005).

Chloroform seeds extract and some isolated compounds of *C. prophetarum*, showed cytotoxicity towards human cancer cell lines, as tested on mouse embryonic fibroblast and exposed positive result. Cucurbitacin B(**15**) one of the compound of the species is most widely used for in-vitro studies on tumor inhibition (Alsayari et al., 2018). Accumulated evidences have shown that cucurbitacin B (**15**)inhibits the growth of numerous human cancer cell lines and tumor xenografts, including breast, prostate, lung, uterine cervix, liver, skin, and brain cancers. Combination therapy with multiple drugs is a common practice in the treatment of cancer to get an additive or synergistic effect and to reduce toxicity to the host (Tingyan et al., 2008).

Even though, the root part of the *C. prophetarum* has been used traditionally for the treatment of different alignments including tumor infection, the previous phytochemical analysis and biological activities report was limited to the fruit. Therefore, the present study was focused on the root of the plant to identify its secondary metabolites and anti-bacterial activities against selected bacterial strains.

2.2.3. Phytochemistry of genus *Cucumis*

2.2.3.1. Phytochemical constituent of *Cucumis melo*

The essential oil of the seeds of *C. melo* (tibish) was analyzed with GC-MS and found to have cyclohexane, 1-methyl-4- (5-methyl-1- methylene-4-hexenyl) (**5**), benzene, 1-(1, 5-dimethyl-1-hexenyl)-4-methyl (**6**), a new phenyl ethyl chromenone, cucumin S [(R)-5, 7-dihydroxy-2-[1-hydroxy-2-(4-hydroxy-3- methoxy phenyl) ethyl] chromone] (**7**), along with five known compounds: 5, 7-dihydroxy-2-[2-(4- hydroxyphenyl) ethyl] chromone (**8**), 5,7-dihydroxy-2-[2-(3,4-dihydroxyphenyl) ethyl] chromone (**9**), 7-glucosyloxy-5-hydroxy-2-[2-(4-hydroxyphenyl) ethyl] chromone (**10**) luteolin (**11**), quercetin (**12**) and (Figure 2) (Azhari et al., 2012).

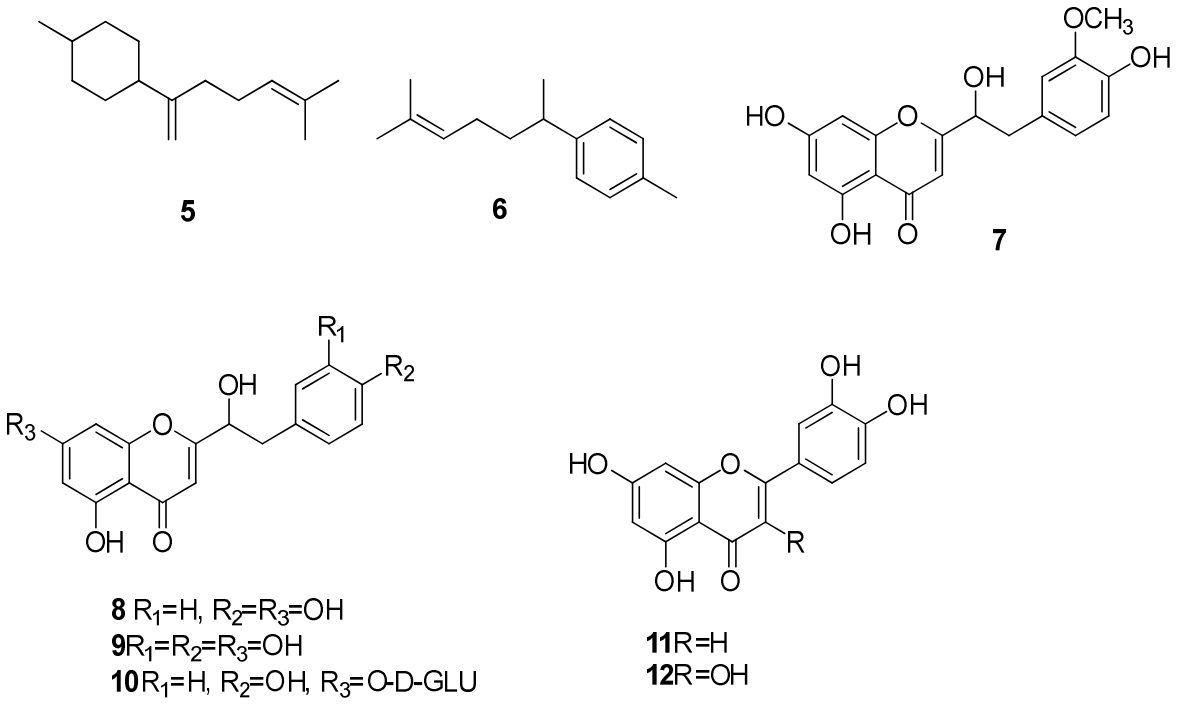

Figure 2. Some reported compounds from *Cucumis melo*

2.2.3.2. Phytochemical constituent of *Cucumis sativus*

Cucumis sativus is a widely cultivated plant in the genus *Cucumis*. It is a creeping vine bearing cylindrical fruits that used as culinary vegetables. It contains several phytoconstituents in its different parts which are responsible for its various pharmacological activities and toxicities, Cucumber fruit is composed mostly of water; more than 96% of edible unpeeled fruit is water. Other constituents of *Cucumis sativus* are vitamins, minerals, amino acids, phytosterols,

phenolic acids, fatty acids, curcurbitacin. Traces of essential oil, pectins, starch, sugars and vitamin C are found in cucumbers (Tripti and Jyoti, 2015). Glycosides, steroids, flavonoids, carbohydrates, triterpenoid, and tannins were identified in an aqueous extract of the cucumber fruit (Ankita et al., 2012). Isolated compound from the chloroform fraction of the crude methanol extract of *Cucumber* (*Cucumis sativus*) stems were (2S, 3S, 4R, 10E)-2[(2'R)-2-hydroxytetra-cosanoylamino]-1, 3, 4-octadecanetriol10-ene, 1-*O*-β-D-glucopyranos, (2S, 3S, 4R, 10E)-2-[(2'R)-2-hydroxy-tetracosanoylamino]-1, 3, 4-octadecanetriol-10-ene and soyacerebroside I ((Tripti and Jyoti , 2015).

The yellow solid compound which was soluble in hot water and organic solvents but insoluble in cold water has been identified from flowers of *C. sativus* (5,7-di-*O*-methyl luteolin-6-*C*-(3''-*O*-benzoyl)-β-D-xyloside) (**13**)(Figure 3) (Senthamilselvi et al., 2016).

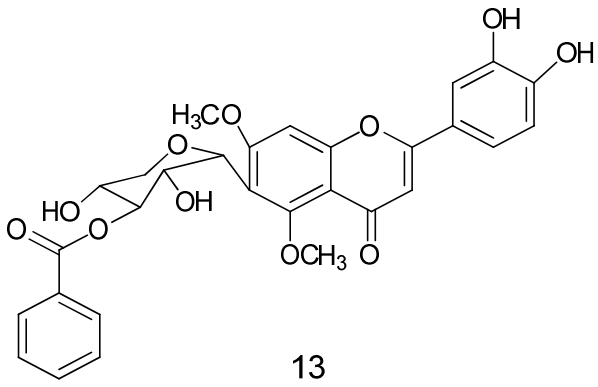

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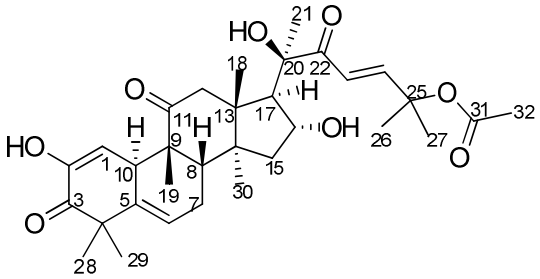
Figure 3. Compound reported from the flowers extract of *Cucumis sativus*

2.2.3.3. Phytochemical constituent of *Cucumis prophetarum*

Cucumis prophetarum var. prophetarum (Cucurbitaceae), which is locally called as Shari-al-deeb, in arabic is used in Saudi folk medicine for the treatment of liver disorders and grows widely between Abha and Khamis Mushait City, Saudi Arabia (Alsayari et al., 2018).

Figure 4. *Cucumis prophetarum* L. from borena Zone (picture taken by Wario on April, 2019)

Cucurbitacin E (**14**), cucurbitacin B (**15**), hexanor cucurbitacin D (**16**), cucurbitacin D (**17**), cucurbitacin F 25-O-acetate (**18**), 23,24- dihydrocucurbitacin D (**19**), isocucurbitacin D (**20**), cucurbitacin E glycoside (**21**) and 23,24- dihydrocucurbitacin B (**22**), cucurbitacin I (**23**), cucurbitacin O (**24**), curcurbitacin p (**25**), isocucurbitacin B (**26**), dihydroisocucurbitacin D (**27**) and dihydroisocucurbitacin E (**28**)(Figure 5) have been reported from fruit of *Cucumis prophetarum* L (Alsayari et al., 2018).



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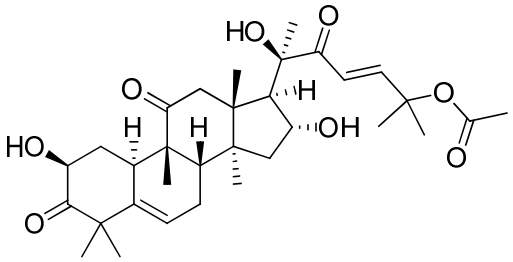

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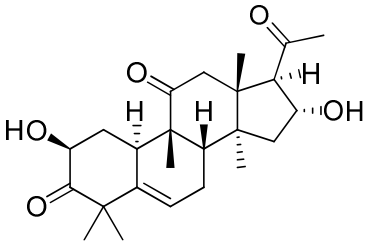

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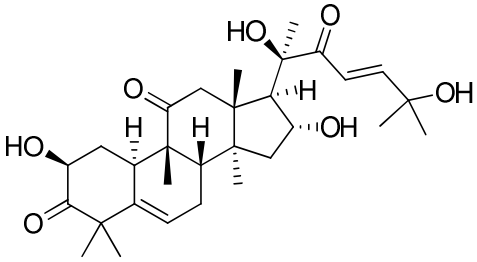

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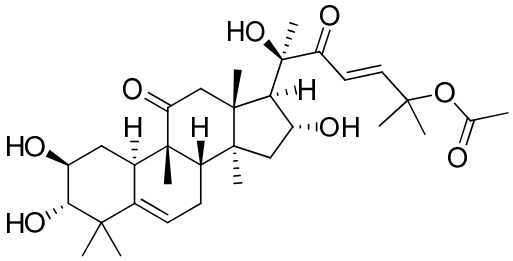

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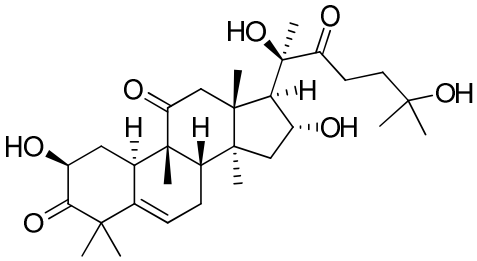

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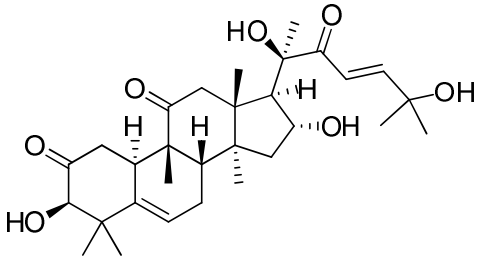

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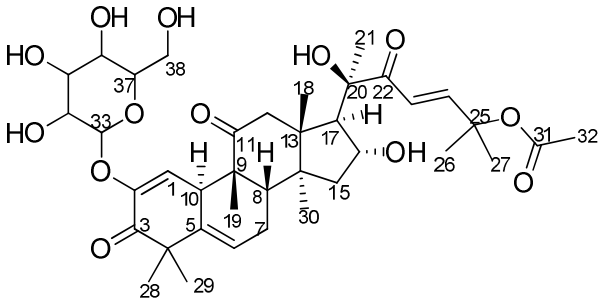

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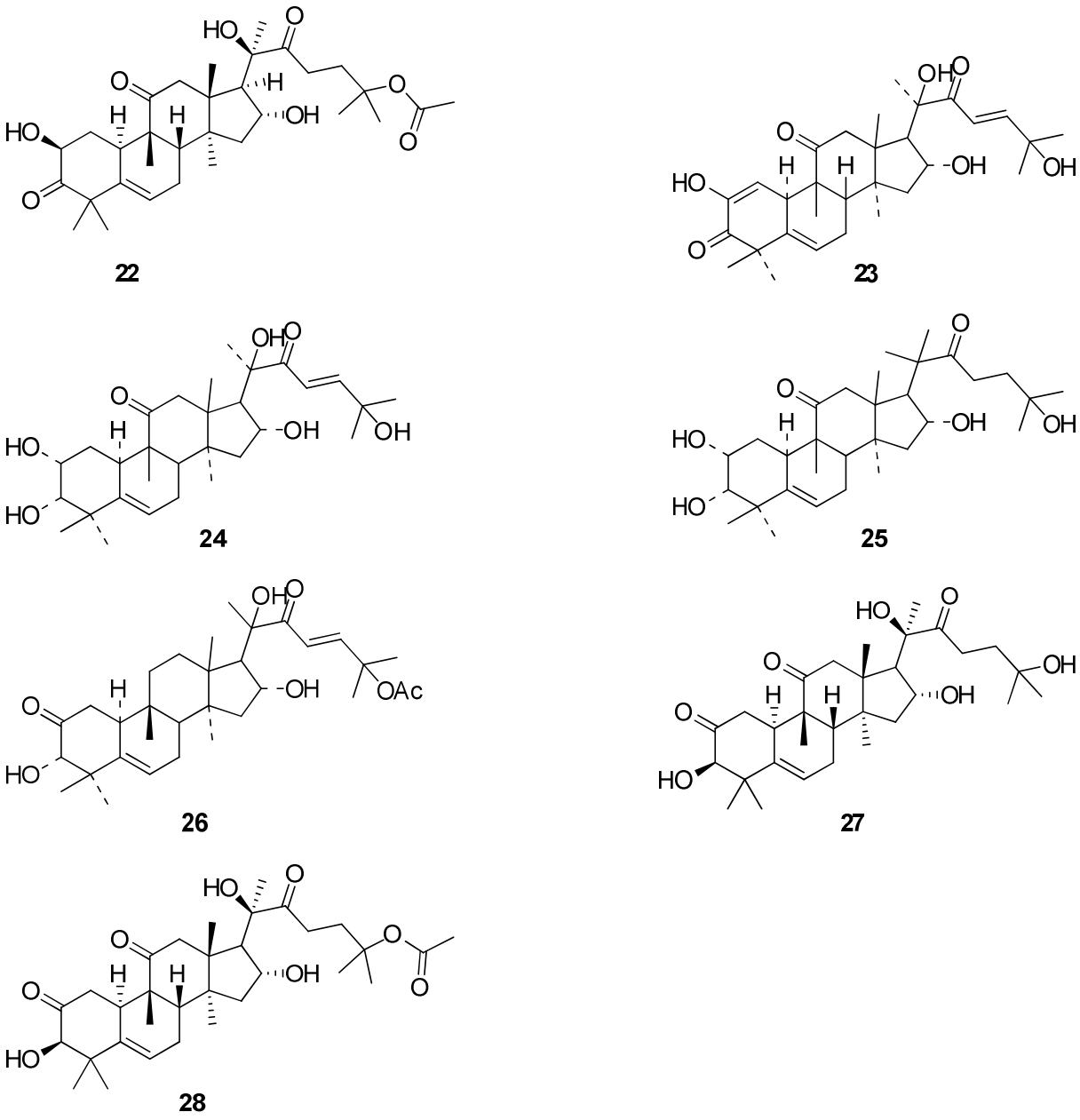


Figure 5. Compounds reported from fruit extract of *Cucumis prophetarum*

3. MATERIALS AND METHODS

3.1. Description of the sample collection site

The roots of *Cucumis prophetarum* were collected in April, 2019 from Dheka Dima which is located at (04° 05′ 46.4″ N latitude and 038° 39′ 47.3″ E longitudes) Dire woreda of Borena zone, Oromia region, Ethiopia.

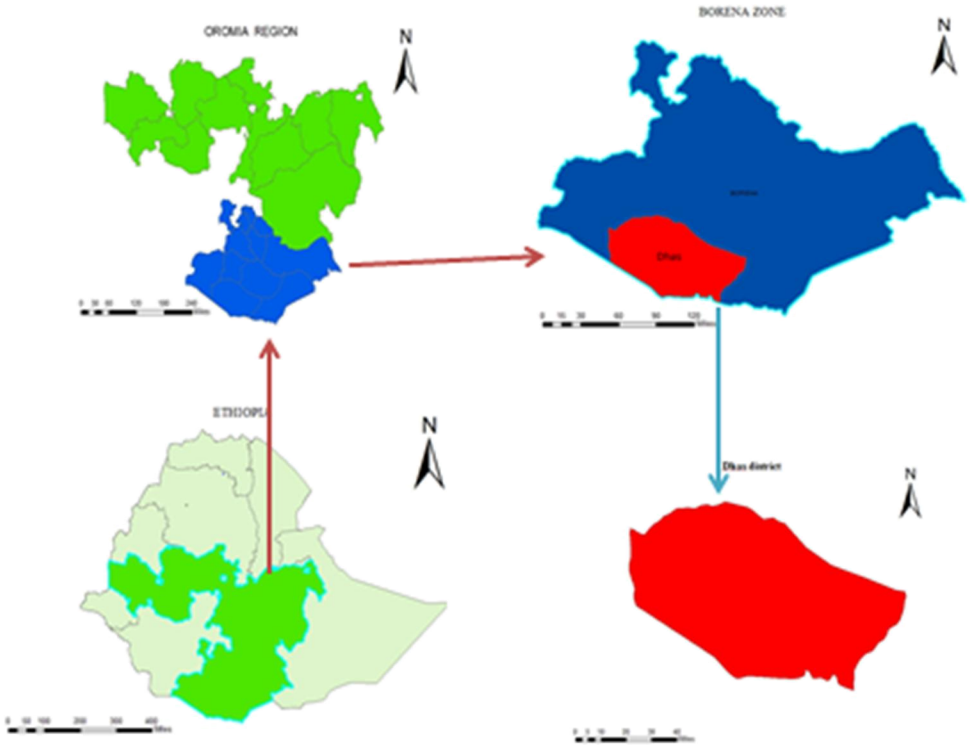


Figure 6. Map showing location of sample collection site (Ethio Arc map 10.4.1)

3.2. Materials and chemicals

Electrical grinder, Rotary evaporator, digital measuring balance, 1000 mL Erlenmeyer flask, 100 mL beaker, TLC plate (pre-coated aluminum sheet, 20 × 20 cm), silica gel (60 F254), UV lamp (254 nm and 365 nm) were used. Melting points were measured in glass capillaries using Thiele tube. Techniques employed for purification of compounds were silica gel column chromatography (CC) which was performed using silica gel (60-120 mesh size) as stationary

phase and organic solvent as mobile phase. Vanillin in H_2SO_4 was also employed for visualization purpose. COSY, HSQC, HMBC, ^{1}H and ^{13}C-NMR spectra were recorded using a Bruker Avance 400 MHz NMR spectrometer, in deuterated chloroform ($CDCl_3$) in the NMR laboratory, Addis Ababa University, Ethiopia. UV-Vis spectral measurements were done using T60 UV-Vis spectrometer. (Hexane 65-70deg C (85%) (Fraction from petroleum)), Ethyl acetate (99.5%) (Methanol (99.8%) (HPLC grade)) And chloroform (99.8%) of LOBA CHEMIE PVT. LTD (India), Auto dock vina software used for molecular docking study.

3.3. Plant materials collection and identification

The root of *Cucumis prophetarum* was collected from Dheka Dima, 150 km from Yabalo, Borena zone of Oromia region, Ethiopia. The authentication of the plant specimen was made with the help of Botanist (Mr Shambel Alemu) and voucher specimen (number WA- 001) was deposited at the National Herbarium of Ethiopia, Addis Ababa University, Ethiopia.

3.4. Extraction and isolation

3.4.1. Extraction

The air dried and powdered roots of *C. prophetarum* (400 g) were macerated in *n*-hexane (2 L) for 72 hrs at room temperature, filtrated and concentrated with rotary evaporator at 35^{o}C. The marc was successively extracted with chloroform (2 L) and methanol (2 L) and the corresponding extracts were filtered and concentrated at 40°C. All crude extract were stored in refrigerator at 4°C until required for further analysis. The extracts were analyzed with TLC for further fractionation.

3.4.2. Isolation of compounds

3.4.2.1. Fractionation of the hexane extract

The TLC profile of the *n*-hexane and $CHCl_3$ extracts were found to be similar. The *n*-hexane extract (4 g) of the roots of *Cucumis prophetarum* was dissolved and subjected to silica gel column chromatography over silica gel, (150 g) using *n*-hexane for packing. Elution was carried out with increasing polarities of EtOAc in *n*-hexane to afford 60 fractions (Each 100 mL) which were combined to 16 fractions on the basis of TLC profile. The eluent, ratio of solvents used for fractionation, volume of eluent collected and amount obtained are depicted in Table 2.

Table 2. Fractionation of the hexane extract of the root of *Cucumis prophetarum*

Fractions	Eluent (ratio)	Volume(mL)	Amount(mg)	Remark
Fr1-10	hexane (100%)	1000		
Fr11-18	hexane:EtOAc (95:5)	800		
Fr19-20	hexane:EtOAc (95:5)	200		
Fr21-23	hexane:EtOAc (95:5)	300		
Fr24	hexane:EtOAc (90:10)	100	25	single spot labeled as compound **29**
Fr25-26	hexane:EtOAc (90:10)	200		
Fr27-29	hexane: EtOAc (85:15)	300	167	re-chromatographed and labeled as compound **30**
Fr30	hexane: EtOAc (80:20)	100		
Fr31-33	hexane: EtOAc (75:25)	300	158	Single spot and labeled as compound **31**
Fr34-36	hexane: EtOAc (70:30)	300		
Fr37-38	hexane: EtOAc (65:35)	200		
Fr39-42	hexane: EtOAc (65:35)	400	49	Single spot and labeled as compound **32**
Fr43	hexane: EtOAc (65:35)	100		
Fr44-47	hexane: EtOAc (60:40)	400		
Fr48-53	hexane:EtOAc (55:45)	600	238	single spot and labeled as compound **33**
Fr54-60	hexane:EtOAc (50:50)	700		

Purification of fraction 27-29: Fractions 27-29 (167 mg) that showed one major spot on TLC were combined and re-chromatographed over silica gel column chromatograph with *n*-hexane and EtOAc (9:1) as eluent to furnish 14 sub-fractions. Sub fraction 5-6 showed one single spot and yielded compound **30** (67 mg).

3.4.2.2. Fractionation of the methanol extract

The methanol extract (7g) was adsorbed on equal amount of silica gel and subjected to column chromatography over silica gel (150 g) packed with *n*-hexane. Elution was carried out with increasing polarities (5% increment as eluent) of *n*-hexane, EtOAc and methanol to afford 145 fractions. Compounds isolated from the methanol extract were similar with those obtained from the hexane extract as revealed from TLC.

3.5. Anti-bacterial activities

3.5.1. Disc diffusion Assay

Anti-bacterial activities of the crude extracts and isolated compounds were investigated using agar disc diffusion method against four bacterial strains (two Gram-positive bacteria; *S. aureus* and *B. subtilis* and two Gram-negative bacteria; *E. coli* and *S. typhi*) obtained from Biology laboratory of Adama Science and Technology University. Appropriate colonies of required bacterial strains were selected and picked using an inoculating loop and Inoculum suspension was prepared and standardized with 0.5 McFarland standards. The surface of the medium plate was inoculated with standardized suspension by streaking back and forth from edge to edge and the discs containing crude extract and isolated compounds of concentration (2.5, 5 and 7.5 in mg/mL), standard drugs (chloramphenicol) of concentration 2.5 mg/mL and negative control (DMSO) were applied within 15 minutes of inoculation. It was incubated in ambient air at 35°C for 16–18 hours. The samples were analyzed in triplicates. Zones of inhibitions were measured from the back of the plate by ruler and interpreted as partially active, active and very active depending on literature data.

3.5.2. Molecular Docking study of isolated compounds for Antibacterial Activity

Molecular docking studies were performed in order to predict the interaction of isolated compounds with the binding sites of DNA-gyrase. Topoisomerases are interesting antibacterial drug targets, since bacteria express two is forms, DNA gyrase and topoisomerase IV, which

were both required for maintenance of proper DNA topology during transcription and replication (Trott and Olson, 2010).

The chemical structure of NMR identified compounds namely, as myristic acid (**29**), 24-ethyl-3-hydroxylcholesta-5,25-diene (**30**), α-spinasterol (**31**), compound **32** and compound **33** were drawn using Chem Bio Draw tool (Chem Bio Office Ultra 14.0 suite) assigned with proper 2D orientation, and structure of each was checked for structural drawing error. Energy of each molecule was minimized using ChemBio3D. The energy minimized ligand molecules were then used as input for AutoDock Vina, in order to carry out the docking simulation. The protein data bank (PDB) used crystal structure of the enzyme as receptor molecule. All the water molecules were removed from the receptor. The graphical user interface program Molecular graphics lab tool was used to set the grid box for docking simulations. The grid was set so that it surrounds the region of interest in the macromolecule. The grid box volume was set to 8, 14, and 14 Å for x, y, and z dimensions, respectively, and the grid center was set to 3.194, 43.143, and 69.977 for x, y, and z center, respectively, which covered all the ten amino acid residues in the considered active pocket. The docking algorithm provided with AutoDock Vina was used to search for the best docked conformation between ligand and protein. During the docking process, a maximum of ten conformers were considered for each ligand. The ligands are represented in different colour, H-bonds with their respective distances are represented with yellow colour and the interacting residues are represented in ball and stick model representation (Trott and Olson, 2010).

3.6. Evaluation of the DPPH radical scavenging activity

Free radical scavenging activity of crude extract and isolated compounds from the roots of *Cucumis prophetarum* were measured by 1-1- diphenyl-2-picryl hydrazyl (DPPH). Sample was prepared by dissolving 4 mg of DPPH in 100 mL of MeOH. Four different concentrations of the samples were mixed with DPPH solution to furnish 12.5, 25, 50 and 100 µg/mL. The mixture was shaken vigorously and incubated at 37^{o}C for 30 min. Then, absorbance was measured at 517 nm by using UV-Vis spectrophotometer. The % inhibition was calculated using the following formula from literature (Rajani et al., 2013). Inhibition % = Ac-As/Ac×100 where Ac is the absorbance of the control and As is the absorbance of the sample.

4. RESULTS AND DISCUSSION

4.1. Extract yield

Powdered roots of *C. prophetarum* were extracted successively using n-hexane, chloroform and methanol and yielded a yellow *n*-hexane crude extract (4 g, 1% w/w), dark-yellow chloroform crude extract (15 g, 3.75% w/w) and dark-green methanol crude extract (23g, 5.75% w/w). The results showed that the percentage yields increase with increasing the extraction solvent polarity suggesting polar compounds are found in the root of the plant.

4.2. TCL profile of the extracts

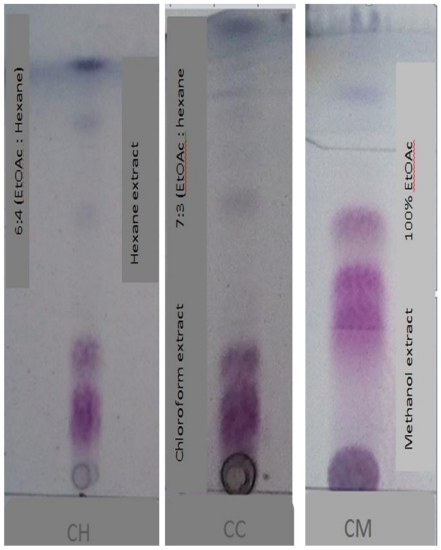


Figure 7. TLC profile of the extracts of the roots of the *C. prophetarum*

Hexane, chloroform and methanol crude extracts of the roots of *C. prophetarum* were analyzed for their TLC profile using different solvents as a mobile phase and visualized after dipping in vanillin/H_2SO_4. Their TLC profile (Figure 7) revealed similarity between hexane and chloroform extracts. However, there is a little bit difference with that of methanol crude extract. The results of the TLC profile of the crude extract demonstrated the presence of different spots which confirmed the existence of different compounds in the crude extract. Therefore, the hexane and methanol extracts were subjected to silica gel column chromatography for further isolation of compounds.

4.3. Characterization of isolated compounds

In the course of this work, five compounds (compound **29**-**33**) were isolated from the *n*-hexane root extracts of *Cucumis prophetarum*. The detailed characterizations of these compounds are presented below.

4.3.1. Compound 29

Compound **29** was isolated as a white solid (25 mg) from the *n*-hexane extract of the root of *Cucumis prophetarum*. It melted at 49-50°C. The TLC profile showed black spot with Rf value of 0.7 (*n*-hexane: EtOAc (8.5:1.5) as eluent).

The ^{1}H-NMR spectrum (Table 3, Appendix 1.1) showed the presence of one terminal methyl protons at δ_H 0.90 (3H, t, J = 6.1Hz). The spectrum also displayed methylene signals at δ_H 2.36 (2H, t, J = 7.4) and 1.64 (2H, m).Where the former suggests a methylene protons α to a carbonyl of carboxylic acid. The proton signal centered at δ_H 1.27 integrating for 20 hydrogens were diagnostics for the presence of many overlapping methylene groups. The spectrum also showed proton signal accounted to aliphatic carboxyl hydrogen at δ_H 5.36.

The ^{13}C-NMR spectrum (Table 3, Appendix 1.2) of compound **29** with the help of DEPT-135 revealed the presence of twelve well resolved carbon resonances of which one carbonyl carbon, one methyl and the remaining ten carbons are due to methylene. The carbonyl carbon of carboxylic acid appeared at δ_C 179.2. The terminal methyl group is evident at δ_C 14.2 and the remaining carbons appeared at δ_C 22.7, 24.7, 29.0, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9 and 34. The intense signal observed at δ_C 29.7 was likely due to the presence of three overlapping methylene groups. Thus based on the above spectral data the compound was found to be identical to known fatty acid named as myristic acid (Figure 8).

Table 3. ^{1}H and ^{13}C-NMR spectral data of compound **29**

position	δ_H (multiplicity, J in Hz)	^{13}C NMR (δ_C in ppm)	DEPT-135 (δ_C in ppm)
1		179.2	-
2	2.36 (2H, t, J = 7.4)	34.0	34.0 (CH2)
3	1.64 (2H, m)	24.7	24.7 (CH2)
4	1.27 (2H, m)	29.0	29.0 (CH2)
5	1.27(2H, m)	29.4	29.4 (CH2)
6	1.27(2H, m)	29.6	29.6 (CH2)
7	1.27(2H, m)	29.7	29.7 (CH2)
8	1.27(2H,m)	29.7	29.7(CH2)
9	1.27(2H, m)	29.7	29.7 (CH2)
10	1.27(2H, m)	29.5	29.5 (CH2)
11	1.27(2H, m)	29.3	29.3 (CH2)
12	1.27(2H, m)	31.9	31.9 (CH2)
13	1.27(2H, m)	22.7	22.7 (CH2)
14	0.90 (3H, t, J = 6.1Hz)	14.1	14.1

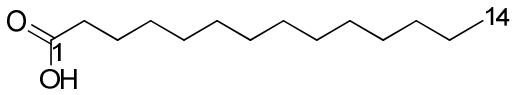


Figure 8. Proposed structure of compound **29**

4.3.2. Compound 30

Compound **30** was isolated as pale yellow solid (67 mg) from the *n*-hexane extract of the roots of *Cucumis prophetarum.* It melted at 140-141°C. The TLC profile showed black spot with Rf value of 0.6 (*n*-Hexane:EtOAc (8:2) as eluent).

The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of compound **30** (Table 4 Appendix 2.1) displayed a pair of olefinic proton signals at δ_H 4.68 (1H, brd) and 4.73 (1H, brd). The appearance of a proton signal at δ_H 5.13 as broadened doublet (J = 5.6 Hz) was also assigned to an olefinic proton. The spectrum also displayed a multiplet signal at δ_H 3.66 due to methine protons on

carbon bearing oxygen. Two methyl singlet signals at δ_H 0.83 and 0.99, one methyl triplet signal at δ_H 0.91 and one methyl doublet signal at δ_H 1.04 were also observed from spectrum. The spectrum also showed proton signals at δ_H 1.27 (8H, m), 1.62 (4H, m), 1.70 (1H m) and 2.06 (1H, brd m).

The ^{13}C-NMR spectrum (Table 4, Appendix 2.2) of compound **30** with the aid of DEPT-135 revealed the presence of twenty eight well resolved carbon resonances of which four were olefinic carbons at δ_C 105.9(C-26), 125.3(C-6),130.9(C-25) and 156.9(C-5). The spectrum also displayed the carbon signal at δ_C 78.9 (C-3) which corresponds to carbon bearing hydroxyl group (also supported by methine proton (H-3) signal at δ_H 3.34 on ^{1}H-NMR). The spectrum showed five carbon signals at δ_C 14.0(C-29), 17.7(C-18), 18.0(C-19), 18.2(C-27) and 19.3(C-21) attributed to methyl carbons. The remaining carbon signals were displayed at δ_C 21.1, 24.9, 26.5, 28.1, 29.7, 31.9, 32.9, 33.8, 35.6, 35.9, 36.3, 40.5, 47.1, 48.0, 48.8 and 52.3. The intense signal at δ_C 29.7 indicated the overlapping of two methylene carbon signals. Thus, based on the above data generated for the characterization of compound **30,** the compound was found to be 24-ethyl-3-hydroxylcholesta-5, 25-diene with its structure shown in Figure 9.

^{I}H and ^{13}C-NMR spectral data of compound **30** was summarized in Table 4

Table 4. ^{1}H and ^{13}C-NMR spectral data of compound **30**

position	δ_H,(multiplicity *J*(Hz))	δ_C	position	δ_H,(multiplicity *J*(Hz))	δ_C
1	1.02 (2H)	36.3	**21**	1.04 (3H d, J = 2)	19.3
2	1.30 (2H, m)	35.6	**22**		31.9
3	3.66 (1H, m)	78.8	**23**		29.7
4	1.27, 1.70 (2H, m)	47.1	**24**	1.59 (1H, m)	48.0
5		156	**25**		130.9
6	5.13 (1H,t, J= 5.6)	125	**26**	4.68 (1H, brd s) 4.73 (1H, brd s)	105.9
7	1.27, 1.62 (2H, m)	29.7	**27**	0.83(3H, s)	18.2
8	1.31 (1H, m)	33.8	**28**	1.05, 1.27 (2H, m)	26.5
9	1.62 (1H, m)	52.3	**29**	0.91 (3H t, J= 4.6)	14.0
10		40.5			
11		21.1			
12	1.27 (2H)	32.9			
13		48.8			
14	1.99 (1H, t, J= 6.4)	48.0			
15	1.27, 1.62 (2H, m)	24.9			
16	1.27 (2H)	28.1			
17	1.59 (1H, m)	52.3			
18	0.83 (3H,s)	17.7			
19	0.99(3H, s)	18.0			
20	2.06 (1H, m)	35.9			

Further confirmation of the structure of compound **30** was made by comparing with the literature reported for related compound ((24S)-24-ethylcholesta-5, 25-diene-7α-hydroxy-3-one) (Gamze et al., 2002).

Taking into consideration the position and number of hydrogens from the ^{1}H-NMR and carbons from ^{13}C-NMR spectrum, the proposed structure of compound **30** was depicted in Figure 9.

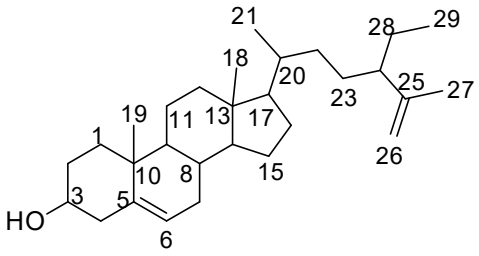


Figure 9. Proposed structure of compound 30

4.3.3. Compound 31

Compound **31** was isolated as a white solid (158 mg) from *n*-hexane extract of the root of *Cucumis prophetarum*. It melted at 172-173°C (literature 169-171°C) (Consolacion and Kathleen, 2005). The TLC profile (Figure 10) showed purple spot with Rf value of 0.46 (*n*-Hexane: EtOAc (7:3) as eluent).

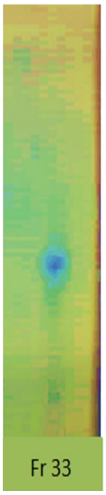


Figure 10. TLC profile of compound 31

The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of compound **31** (Table 5, Appendix 3.1) indicated three olefinic proton signals at δ_H 5.11 (1H, m), 5.16 (1H, dd J = 2.4) and 5.17 (1H, brd s). The multiplet proton signal appearing at δ_H 3.61 (1H, m) suggest methine proton on carbon bearing oxygen. Methyl proton signals were observed at δ_H 0.56 (3H, s), 0.8 (3H, s), 0.81 (3H, m) 0.83 (3H, d, J= 1.6), 0.85 (3H) and 1.03 (3H, s) on the spectrum manifest the presence of methyl group. The spectrum also showed a proton signal resonated at δ_H 1.04 (2H), 1.27 (6H m) , 1.82 (1H, m), 1.30 (2H, m), 1.78 (1H, m), 1.58 (2H, m), 1.42 (2H m), 1.79 (1H, t J= 2.8), 1.38 (1H, m), 1.5 (1H,m), 2.03 (1H, m) and 1.56 (2H, m) which accounted for methylene and methine protons.

The ^{13}C-NMR (400 MHz, $CDCl_3$) spectrum data (Table 5, Appendix 3.2) of compound **31** with the help of DEPT-135 revealed the presence of well resolved twenty nine carbon resonances of which four olefinic carbons signals were observed at δ_C 117.4, 129.4, 138.2 and 139.6. The earlier olefinic carbon signal is diagnostic for Δ^7 sterols. The signal due to carbon bearing oxygen (C-3) appeared at δ_C 71.1. The carbon signal displayed at δ_C 12, 12.3, 13.1, 19.8, 21.1 and 21.4 were due to methyls, in good agreement with ^{1}H NMR spectrum. The remaining carbon signals appeared at δ_C 37.1, 31.4, 38, 40.3, 29.7, 49.5, 34.2, 21.5, 39.5, 43.3, 55.14, 23, 29.6, 55.10, 40.8, 51.2, 31.9 and 25.4. Thus, based on the above spectral data and comparison with literature value the structure of the compound is in good agreement with α-spinasterol (31) whose structure is depicted in Figure 11

Table 5. NMR spectral data of compound **31** and literature report of α-spinasterol (Consolacion and Kathleen, 2005).

NMR spectral data of compound 31			**literature reported of α-spinasterol**	
position	δ_C	δ_H, multiplicity J(Hz)	δ_C	δ_H, multiplicity J (Hz)
1	37.1	1.04 (2H)	37.2	1.02 (2H)
2	31.4	1.30 (2H, m)	31.5	1.36 (2H)
3	71.1	3.61 (1H, m)	71.1	3.52 (1H, m)
4	38	1.27 &1.82 (2H, m)	38.1	1.27& 1.70 (2H)
5	40.3	1.38 (1H, m)	40.3	1.4 (1H)
6	29.7	1.27& 1.78 (2H, m)	29.7	1.22 & 1.74 (2H)
7	117.4	5.17 (1H, t)	117.5	5.15 (1H, br s)
8	139.6	q	139.6	q
9	49.5	1.58 (1H)	49.5	1.66 (1H)
10	34.2	q	34,2	q
11	21.5	1.42 (2H, m)	21.6	1.48 (2H)
12	39.5	1.27 (2H)	39.5	1.23 (2H)
13	43.3	q	43.3	q
14	55.1	1.79 (1H, t J = 2.8)	55.1	1.81 (1H, t)
15	23	1.58 & 1.38 (2H, m)	23.1	1.40 & 1.52 (2H)
16	29.6	1.27 (2H)	28.5	1.25 (2H)
17	55.1	1.5 (1H, m)	55.9	1.5 (1H)
18	12	0.56(3H, s)	12.1	0.55 (3H, s)
19	13.1	0.8 (3H, s)	13.1	0.8 (3H, s)
20	40.8	2.03 (1H,m)	40.8	2.05 (1H, m)
21	21.4	1.03 (3H, s)	21.4	1.02 (3H)
22	138.2	5.16 (1H, dd J= 2.4)	138.2	5.17 (1H, dd)
23	129.4	5.11 (1H, m)	129.5	5.09 (1H, dd)
24	51.2	1.56 (1H, m)	51.3	1.55 (1H)
25	31.9	1.56 (1H, m)	31.9	1.55 (1H)
26	19.9	0.85 (3H)	21.1	0.85 (3H, d)
27	21.1	0.83 (3H, d J= 1.6)	20.9	0.84 (3H, d)
28	25.4	1.04 &1.42 (2H)	25.4	1.18 &1.42 (2H)
29	12.3	0.81 (3H, t)	12.2	0.81(3H, t)

Taking into consideration the number of hydrogen from the ^{1}H-NMR and number of carbons in the ^{13}C-NMR spectrum, the proposed structure of compound **31** is as depicted in Figure 11.

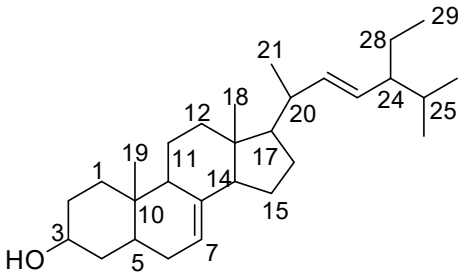


Figure 11. Proposed structure of compound **31**

4.3.4. Compound 32

Compound **32** was isolated as a yellow solid (49mg) from *n*-hexane extract of the root of *Cucumis prophetarum,* It melted at 180-181 °C. The TLC profile (Figure 12) showed purple spot with Rf value of 0.4 (*n*-Hexane: EtOAc (6:4) as eluent).

Figure 12. TLC profile of compound 32

The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of compound **32** (Table 6, Appendix 4.1) indicated the presence of three olefinic protons at δ_H 5.79 (1H, m), 5.95 (1H, s) and 5.97 (1H, m). The spectrum also displayed the proton signal at δ_H 3.92 (1H, brd s) which is evident for methine

proton of oxygen bearing carbon. The spectrum showed the presence on ten methyl singlet signals at δ_H 0.81 (3H s), 1.00 (3H s), 1.03 (3H, s), 1.10 (3H s), 1.14 (3H s), 1.25 (3H, s), 1.26 (6H, s), 1.34 (3H, s), 1.38 (3H, s) and 3.56(3H, s) which were accounted to methyl groups on quaternary carbons and one methyl proton signal at δ_H 1.27 (3H, d J= 5.2) which accounted to the presence of methyl adjacent to methine carbon. The remaining proton signals were observed at δ_H 2.02 (2H, m), 1.98(2H), 2.54(2H, brd s), 2.29(2H, m), 2.47(2H, brd s), 2.39(1H, s), 3.08 (1H, s) and 2.25 (1H, s).

The ^{13}C-NMR (400 MHz, $CDCl_3$) spectral data of compound **32** (Table 6, Appendix 4.1) with the support of DEPT-135 revealed the presence of well resolved forty three carbon signals of which five are due to carbonyl carbons with their signals at δ_C 198.5, 210.0, 210.3, 211.1 and 215.9. The spectrum also exhibited the presence of six olefinic carbons at δ_C 114.4, 120.2, 121.3, 136.9, 138.3 and 144.6. Three of six olefinic carbons are quaternary carbons as their signals intensity were suppressed in DEPT-135, which also agreed with ^{1}H-NMR spectrum that displayed only three olefinic signals. The carbon signal observed at δ_C 80.2 is obvious for carbon (C-3) bearing oxygen as supported by proton signal at δ_H 3.92 from ^{1}H-NMR spectrum. As shown from DEPT-135 spectrum, the compound displayed sixteen quaternary carbons, nine CH_2, seven methine and 11 methyl groups. The spectrum also showed signals for methyl groups at δ_C 18.5, 19.1, 20.0, 20.1, 20.2, 20.9, 24.1, 24.2, 24.3, 27.8 and 34.8. The signals due to methylene carbons are evident at δ_C 23.9, 24.2, 38.9, 45.6, 45.9, 49.1, 49.2, 50.0 and 50.2. The remaining carbon signals were displayed at δ_C 36.3, 42.1, 43.3, 44.2, 44.3, 44.8, 45.1, 46.7, 47.6, 49.8 and 50.3. The ^{1}H and ^{13}C NMR data of compound **32** were summarized in Table 6

Table 6. ^{1}H and ^{13}C-NMR spectral data of compound **32**

position	δ_H (multiplicity, *J* in Hz)	δ_C in ppm	Position	δ_H (multiplicity, *J* in Hz)	δ_C in ppm
1	5.95 (1H, brd s)	114.4	**23**	2.47 (2H, brd s)	23.9
2		144.6	**24**		211.1
3		198.5	**25**		45.1
4		47.6	**26**	1.10 (3H, s)	20.0
5		136.9	**27**	1.03 (3H, s)	24.3
6	5.79 (1H, m)	120.1	**28**	3.56 (3H, s)	34.8
7	2.07 (1H, m) 2.25 (1H, m)	50.2	**29**	1.26 (3H, s)	27.8
8	2.29(1H, m)	42.1	**30**	1.25 (3H, s)	20.1
9		50.3	**1′**	2.29 (1H, m)	43.3
10		49.8	**2′**		23.9
11		215.9	**3′**		24.2
12	2.07 (2H, m)	50.0	**4′**	2.79 (1H, m)	36.3
13		44.8	**5′**	1.27 (3H, d *J* = 5.2)	18.5
14		44.3	**1″**		44.2
15	3.08 (1H, s) 2.39(1H, s)	45.6	**2″**	2.54 (1H, brd s) 1.98 (1H, d *J*= 3.2)	49.1
16		210.3	**3″**	2.02 (2H, m)	45.9
17		46.7	**4″**	5.97 (1H, m)	121.3
18	1.14 (3H, s)	19.1	**5″**		138.3
19	1.98(1H, brd s) 2.54 (1H, brd s)	49.2	**6″**	1.34 (3H s)	24.1
20	1.38 (3H, s)	20.2	**7″**	0.81 (3H, s)	20.9
21	3.92 (1H, brd s)	80.2	**8″**	1.00 (3H, s)	24.2
22		210.0			

The spectral data presented above (Table 6) led us to propose the structure of compound **32** as depicted in Figure 13.

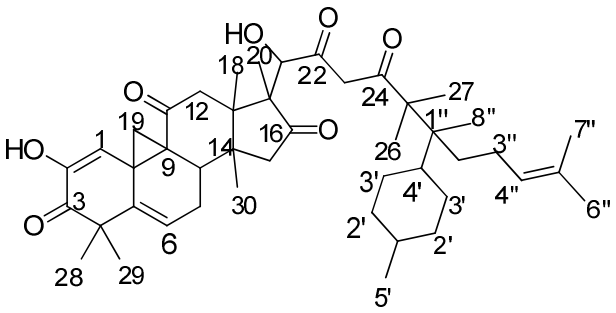


Figure 13. Proposed structure of compound **32**

The structure of compound **32** was further confirmed using 2D NMR including COSY, HSQC and HMBC. The COSY spectrum (Appendix 4.4) of compound **32** showed that the proton at δ_H 5.79 (H-6) was correlated with proton at δ_H 2.07 (H-7). The proton signal at δ_H 5.97(H-4″) had strong correlation with the methylene signal at δ_H 2.02 (H-3″).

The HSQC spectrum (Appendix 4.5) showed correlation between the olefinic proton signals at δ_H 5.79 (H-6), 5.95 (H-1) and 5.97 (H-4″) with the olefinic carbons at δ_C 120.1 (C-6), 114.4(C-1) and 121.3(C-4″) respectively. In the HSQC spectrum the proton at δ_H 3.92 (H-21) correlated with the carbon at δ_C 80.2 (C-21). The spectrum also displayed the correlation of proton signals at δ_H 3.56 (H-28), 3.08 (H-15) and 2.39 (H-15), 2.79 (H-4′), 2.29 (H-8,1′), 2.07(H-7) and 2.25(H-7), 2.07(H-12), 1.26(H-29), 1.38(H-20), 1.34(H-6″), 1.24(H-30), 1.10(H-26), 1.04(H-27), 1.00(H-8″), 0.81(H-7″) with carbon signals at δ_C 34.9 (C-28), 45.6 (C-15), 36.3(C-4′), 42.2(C-8) and 43.3(C-1′), 50.2(C-7), 50.0(C-12), 27.8(C-29), 20.2(C-20), 24.1(C-6″), 20.1(C-30), 20.0(C-26), 24.3(C-27), 24.2(C-8″), 20.9(C-7″) respectively.

The long range correlations between hydrogen and carbons were established with HMBC spectrum (Appendix 4.6). In view of this, the proton signal at δ_H 5.95(H-1) was strongly correlated with carbonyl carbon at δ_C 198.5(C-3), two olefinic carbon at δ_C 144.6(C-2) and 136.5(C-5) and two quaternary carbon at δ_C 49.8(C-10) and 50.3(C-9) (Figure 14).

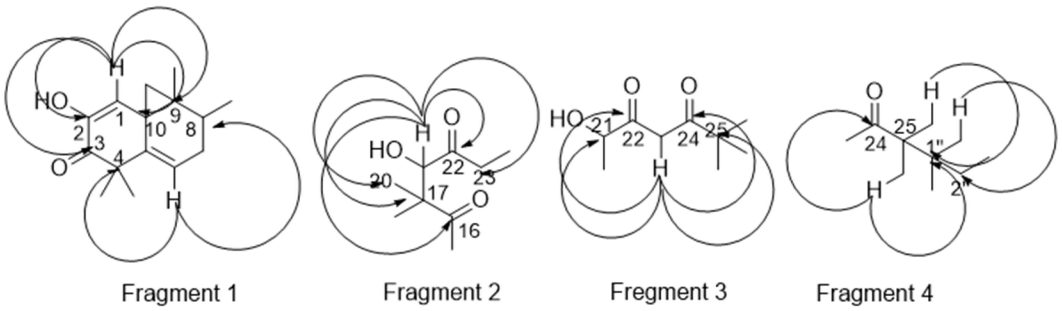


Figure 14. Partial skeleton of compound **32**

The HMBC spectrum also revealed the long range correlation of proton signal at δ_H 5.79(H-6) with quaternary carbon at δ_C 47.6(C-4) and methine carbon at δ_C 42.2(C-8). This led us to propose the fragment **1** of partial skeleton shown in Figure 14. The NMR data for the fragment **1** of partial skeleton (Figure 14) is in agreement with the literature reported for structurally related compound (Christoph et al., 2005). The proton signal at δ_H 3.92(H-21) showed strong long range correlations with two carbonyl carbons at δ_C 210.0(C-22) and 210.3(C-16), one quaternary carbon at δ_C 46.7(C-17), one methylene carbon at δ_C 23.9(C-23) and one methyl carbon at δ_C 20.2(C-20) as informed from HMBC spectrum, which also lead us to the fragment **2** of partial skeleton (Figure 14). The HMBC spectrum of compound **32** revealed strong long range correlation of proton signal at δ_H 2.47(H-23) with two carbonyl signals at δ_C 210.0(C-22) and 211.0(C-24), one quaternary carbon signal at δ_C 45.1(C-25) and hydroxy bearing carbon signal at δ_C 80.2(C-21), which led us to propose fragment **3** of partial skeleton (Figure 14). HMBC also showed the long range correlation of proton signal at δ_H 1.10(H-26) with carbonyl carbon signal at δ_C 211.0(C-24) and proton signals at δ_H 1.00(H-8″) and 1.10(H-26) with quaternary carbon signal at δ_C 44.2(C-1″) led us to fragment **4** of partial skeleton (Figure 14).

The data from the 2D NMR is in good agreement with the proposed structure for compound **32** (Figure 13)**.**

4.3.5. Compound 33

Compound **33** was isolated as a pale yellow solid (238 mg) from the *n*-hexane extract of the roots of *Cucumis prophetarum*. It melted at 118-120°C. The TLC profile (Figure 15) showed a purple spot with Rf value of 0.5 (*n*-hexane:EtOAc (1:1) as eluent).

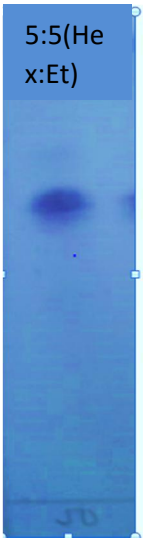


Figure 15. TLC profile of compound 33

The ^{1}H-NMR spectrum of compound **33** (Table 7, Appendix 5.1) displayed one olefinic proton signal at δ_H 5.79 (1H, dd, J = 2, 2 Hz). The spectrum also showed proton signal at δ_H 4.43 (1H, dd, J= 6.0, 6.0 Hz) which was obvious for hydrogen on carbon bearing oxygen. The proton signal appearing at δ_H 1.00 (3H s), 1.14 (3H, s), 1.23 (3H, s), 1.27 (3H, s) and 1.34 (3H, s) were accounted for the presence of five methyl groups on quaternary carbon. The spectrum also revealed the presence of proton signal at δ_H 1.23(1H, d J= 4.4), 1.27 (2H m), 1.95 (1H, s), 2.00 (1H, brd s), 2.07(1H, brd s), 2.21 (H, brd, s), 2.43 (2H, brd s), 2.53 (1H, s), 2.79 (1H, d, J = 5.2), 3.17 (1H, s).

The ^{13}C-NMR (400 MHz, $CDCl_3$) spectrum (Table 7, Appendix 5.2) of compound **33** with the aid of DEPT-135 revealed the presence of 22 carbon signals of which eight quaternary, four methine, five methylene and five methyl groups are clearly evident. Three carbon signals appearing at δ_C 210.3, 212.8 and 216.0 were diagnostic for the presence of three carbonyl groups in the structure of the compound. The carbon resonances in the spectrum at δ_C 140.5 and 119.8 were characteristics of olefinic carbons, out of which the former one is accounted to sp^2 quaternary carbon which was also in good agreement with the ^{1}H NMR spectrum. The spectrum also displayed one methine carbon signal at δ_C 71.5 which was evident for hydroxyl bearing carbon (C-2). The other signal due to methine carbons were observed at δ_C 42.9 and 33.9. The carbon signals displayed at δ_C 36.1, 24.3, 50.0, 49.2 and 45.7 were due to methylene carbons in the structure of the compound. The presences of methyl signals were

evident at δ_C 24.3, 29.2, 21.2, 20.0 and 19.3. The remaining carbon resonances appeared at δ_C 50.3, 45.0, 49.9 and 44.3 are due to quaternary carbons. The ^{1}H and ^{13}C-NMR spectral data of compound **33** is summarized in Table 7

Table 7. ^{1}H and ^{13}C NMR data of compound **33**

position	δ_{H} (multiplicity, J in Hz)	^{13}C NMR (δc in ppm)	DEPT-135 (δc in ppm)
1	1.23 (1H, d, J =4.4)	36.1	36.1 (CH2)
	1.27 (1H, m)		
2	4.43 (1H, dd, J = 6.0, 6.0)	71.5	71.5
3		212.8	q
4		50.3	q
5		140.5	q
6	5.79 (1H, dd J = 2, 2)	119.8	119.8
7	2.43 (1H, brd s)	24.3	24.3 (CH_2)
	1.27 (1H, m)		
8	2.21 (1H, brd s)	42.9	42.9
9		45.0	q
10	2.79 (1H, d, J = 5.2)	33.9	33.9
11		215.9	q
12	2.00 (1H, brd s)	50.0	50.0 (CH_2)
	2.07 (1H, brd s)		
13		49.9	q
14		44.3	q
15	2.43 (1H, brd s)	49.2	49.2 (CH2)
	3.17 (1H, s)		
16		210.3	q
17	1.95 (1H, s)	45.7	45.7 (CH2)
	2.53 (1H, s)		
18	1.23 (3H, s)	19.3	19.3
19	1.14 (3H, s)	20.0	20.0
20	1.34 (3H, s)	21.2	21.2
21	1.27 (3H, s)	29.2	29.2
22	1.00s (3H, s)	24.3	24.3

Taking into consideration the above spectral data (Table 6) the structure of compound **33** is proposed as the one depicted in Figure 16.

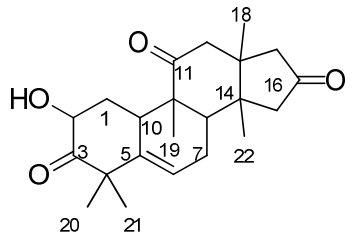


Figure 16. Proposed structure of compound 33

The structure of compound **33** was further confirmed using the spectral data obtained from the 2D-NMR including COSY, HSQC and HMBC. The COSY spectrum (appendix 5.4) revealed that proton signal at δ_H 5.79(H-6) was correlated with two diastereotopic proton signals at δ_H 1.27(H-7) and 2.43(H-7). The COSY spectrum also showed a correlation between a proton signal at δ_H 4.43(H-2) with two diastereotopic proton signals at δ_H 1.27(H-1) and 1.23(H-1).

The one bond correlation between hydrogens and carbon was established with HSQC. The HSQC spectrum (appendix 5.5) revealed that the proton signal at δ_H 5.79(H-6) was attached to carbon at δ_C 119.8(C-6). The spectrum also displayed that the proton signal at δ_H 4.43(H-2) was attached to carbon at δ_C 71.5(C-2) in agreement with ^{1}H-NMR spectrum. The HSQC spectrum also informed that proton signal at δ_H 1.00(H-22), 1.14(H-19), 1.23(H-18), 1.27(H-21) and 1.34(H-20) were attached to the carbon signals at 24.2(C-22), 20.0(C-19), 19.2(C-18), 29.2(C-21) and 21.2(C-20) respectively. The carbon signal at δ_C 24.3(C-7) was correlated with two proton signals at δ_H 2.43(H-7) and 1.27(H-7). The correlation of proton at δ_H 2.79(H-10) with carbon signal at δ_C 33.9(C-10), correlation of proton signals at δ_H 1.23(H-1) and 1.27(H-1) with carbon signal at δ_C 36.1(C-1), correlation of proton at δ_H 2.21(H-8) with carbon at δ_C 42.9(C-8) and correlation of two proton signals at δ_H 3.17(H-15) and 2.43(H-15) with carbon signal at δ_C 49.2(C-15), the correlation of proton signal at δ_H 2.00(H-12) and 2.07(H-12) with carbon signal at δ_C 50.0(C-12) and correlation of carbon signal at δ_C 45.7(C-17) with two proton signals at δ_H 1.95(H-17) and 2.53(H-17) were also observed from HSQC spectrum.

The HMBC spectrum (appendix 5.6) shown that proton at signal δ_H 5.79 has strong long range correlation with two quaternary carbon at δ_C 140.5 and 50.3. The spectrum also revealed that

the same signal correlated long range relation with two CH carbons signal at δ_C 33.9 and 42.9. The HMBC spectrum also shown long range correlation of proton signal at δ_H 4.43 with one carbonyl carbon signal at δ_C 212.8 and one CH_2 carbon at signal 36.1 which led us to fragment **1** of partial skeleton of compound 33 (Figure 17). The spectrum also indicated long range correlation of proton signal at δ_H 2.00 with carbonyl at δ_C 215.9, methyl carbon at δ_C 19.3 and three quaternary carbon signals at δ_C 49.9, 45.0 and 44.3, which is evident for the existence of C-9, C-11, C-13, C-14 and C-18 led to fragment **2** of partial skeleton of compound **33** (Figure 17).

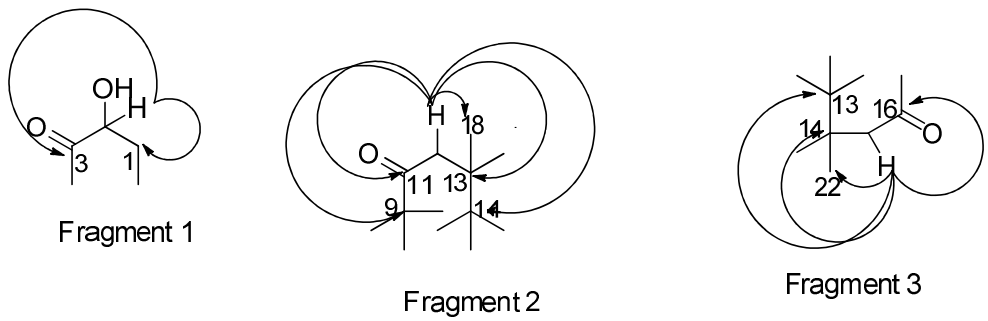


Figure 17. Partial skeleton of compound 33

The HMBC spectrum also revealed the long range correlation of two proton signals at δ_H 2.43 and 3.17 with one carbonyl signal at δ_C 210.3, two quaternary carbon signals at δ_C 49.9 and 44.3 and methyl carbon signal at δ_C 24.3, which is a good evidence for the existence of C-16 and C-22 led to fragment **3** of partial skeleton of compound **33** (Figure 17).

4.4. Antibacterial activity

The antibacterial activity of the extracts and isolated compounds of roots of *C. prophetarum* were investigated against two Gram positive *(S. aureus* and *B. subtilis*) and Gram negative (*E. coli* and *S. thyphimurium*) bacteria using Agar disc diffusion methods. The extracts and isolated compounds were evaluated at three different concentrations (2.5 mg/mL, 5mg/mL and 7.5 mg/mL). Chloramphenicol (2.5 mg/mL) and DMSO were used as positive and negative controls respectively. The results were presented in Table 8.

Table 8. Anti-bacterial activities of extracts and isolated compounds from the root of *C. prophetarum*

Sample	Conc (mg/ml)	Inhibition zone in mm (mean±SD)			
		Gram negative bacteria		Gram positive bacteria	
		E-coli	*Salmonella thyphimurium*	*Staphylococcus aureus*	*Bacillus subtilis*
Hexane extract	5	15±1.41	12±1.41	11.6±0.48	12.6±2.15
Methanol extract	5	14.6±1.7	12.6±2.05	11±0	12.3±0.47
Compound 29	5	11±0.81	10.6±1.24	9±0	10.3±0.47
Compound 30	5	12±0.81	11.6±0.94	10.6±0.47	11.6±1.25
	2.5	11±1.29	10.6±1.7	10.6±0.94	9±0
Compound 31	5	13±0	12.3±0.94	11.3±0.94	10±0
	7.5	13±0.81	12.6±1.25	12.3±1.25	10±1.41
Compound 32	2.5	12.3±0.6	12±0	10.6±0.47	12±0
	5	12.6±0.94	13±0.81	10.6±0.47	12.6±0.47
	7.5	13.6±0.94	13±1.41	11.3±0.94	12.6±0.47
Compound 33	2.5	12±0.81	12.6±0.47	11±1.41	12.3±0.47
	5	12±1.63	12.6±0.47	12±1.41	12.6±0.47
	7.5	12.3±1.25	13±1.41	12±2.16	13.3±0.54
Chloramphenicol (PC)	2.5	20.7±1.63	20±1.63	22±2.08	20.7±0.94

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

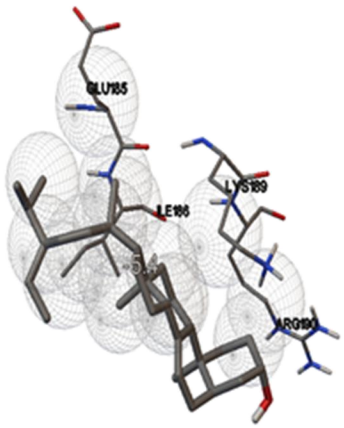
The zone inhibition diameter was measured and interpreted as inactive, partially active, active and very active following literature guide (Alves et al., 2000). Anti-bacterial inhibition zone of any sample can be expressed in terms of the diameter of the inhibition zone, less than 9 mm as inactive, 9-12 mm partially active, 13-18 mm active and greater than18 mm very active (Alves et al., 2000). From (Table 8, Appendix 6.1-6.4) it was observed that all samles diplayed inhibition zone beyond 9 mm which clearly demonestrated the extracts and compounds as active against selected bacterial strains. The hexane extract, methanol extract, compound **31** and compound **32** exhibited inhibition diameter of 15±1.41, 14.6±1.7, 13±0 and 13.6±0.94 against *E. coli*, respectively. While compound **32** and **33** showed inhibition diameter of 13±1.41 against *S. thyphimurium*. Both the crude extract and isolated compounds under

investigated concentration were partially active against *S. aureus* and compound **33** with inhibition diameter (13.3±0.54) was also active against *B. subtilis.*

The comparison of antibacterial activity of the extracts and isolated compounds were done with related work from literature. One of the reported antibacterial activities of the ethyl acetate extracts of the flower of *Cucumis sativus* against *S. typhi* and *E. coli* exhibited zone inhibition diameter of 0 mm, 7 mm for 10 mg/mL and 10 mm, 11mm for 30mg/mL respectively using disc diffusion method (Muruganantham et al., 2016). While this paper result showed zone inhibition diameter of the range 10.6-13mm for concentration range 2.5-7.5 mg/ml and zone inhibition diameter of the range 11-15mm for the same concentration range against *S. typhi* and *E. coli* respectively, using disc diffusion method (Table 8). This clarified that the inhibition zone displayed by the extracts and isolated compounds in the present work was superior compared with the antibacterial ativity reported in the literature for sister species, named as *Cucumis sativus* (Muruganantham et al., 2016). Further comparison was also made with literature reported for the seed extract of *Cucumis melo* at different concentrations (10mg, 20mg and 30 mg /mL) against pathogenic bacterial strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* (Gnanasingh et al, 2017). The literature report revealed zone inhibition diameter of 0mm for 10mg/mL and 12mm, 11mm, 10mm for 20mg//mL against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* respectively. This work showed zone of inhibition diameter of the range 9-13.3mm, 9-12.3mm and 11-15mm against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* respectively for concentration range 2.5-7.5mg/mL (Table 8). Interestngly better antibacterial activity was also obatined in this work even at lower concentration against similar bacterial strain compared with the extract of *Cucumis melo*.

4.5. Molecular Docking study of isolated compounds for Antibacterial Activity

In association with in vitro antimicrobial activity, it is useful to carry out in silico studies to predict the orientation and binding affinity at the active site of the receptor. The molecular docking of NMR identified ligand molecules 24-ethyl-3-hydroxylcholesta-5,25-diene (**30**), α-spinasterol (**31**), compound **32** and compound **33** with bacterial enzyme DNA gyrase is shown in Figure 18.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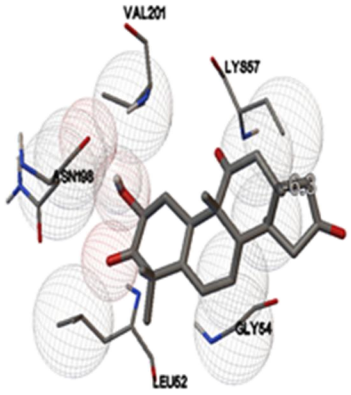
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GLY54
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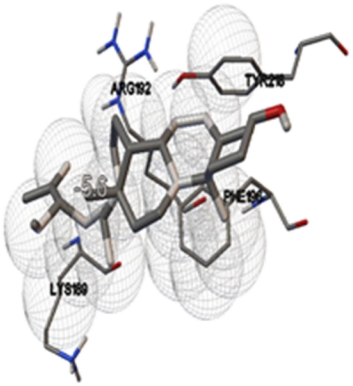
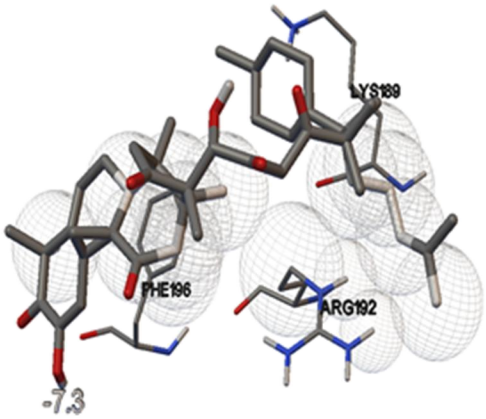
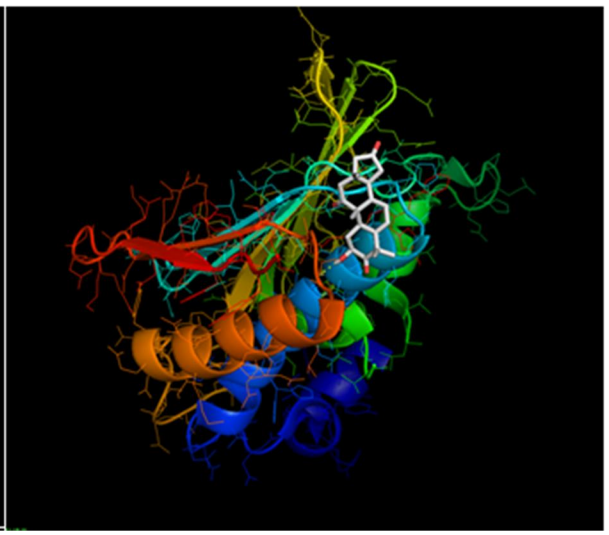

ARG192
-5.6
LYS189


LYS189
PHE196
ARG192
-7.3



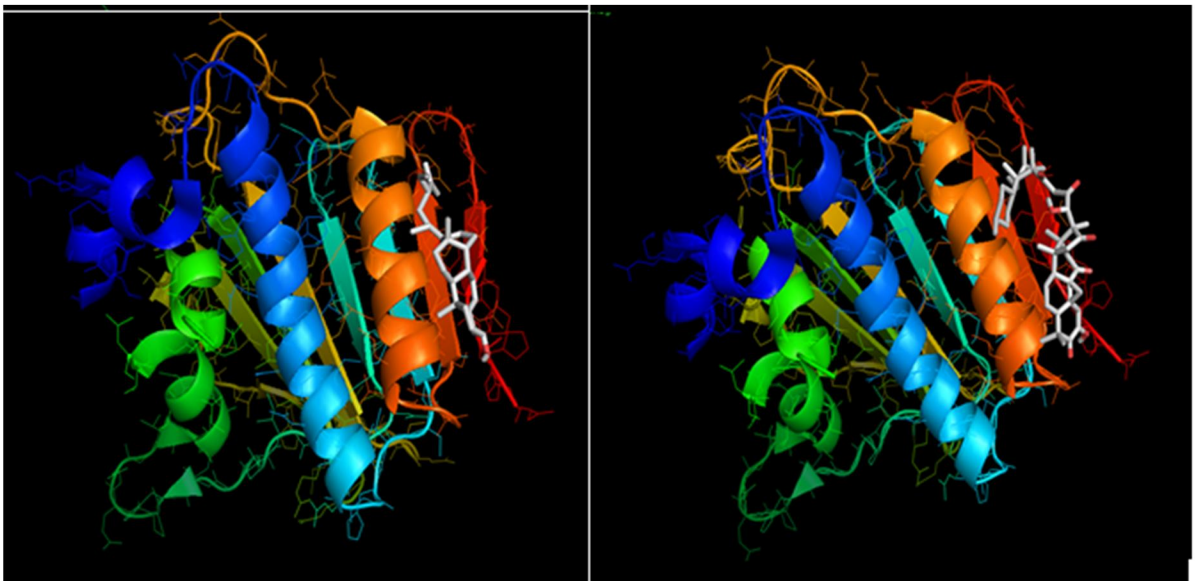


Figure 18. 2D and 3D protein-ligand interaction of DNA gyrase with selected ligands

Among the investigated ligands, compound **32** exhibited better docking efficiency with DNA gyrase. It formed three hydrophobic interactions with amino acids Lys189, Arg192 and Phe 196 in the active site of the target protein with the least binding affinity **-7.3** and hence was considered as the best dock conformation among the investigated ligands (Table 9). Compound **33** formed one hydrogen bonds with Asn 198 amino acids and hydrophobic interaction with Gly54, Lys57 and Val201 with least binding affinity **-6.3**. While 24-ethyl-3-hydroxylcholesta-5, 25-diene (**30**) and α-spinasterol (**31**) formed only hydrophobic interaction with the amino acids of target protein with the least binding affinity **-5.6** and **-5.4** respectively. However, all these docked molecules exhibited more hydrophobic interaction than the standard drug ciprofloxacin of binding affinity **-4.4.** The RMSD has often been used to measure the quality of reproduction of a known binding pose by molecules with ligands. All docked molecules have zero RMSD values as shown in the (Table 9). The binding affinity, distance from RMSD l.b, H-bond and hydrophobic interaction of selected ligands were summarized in Table 9.

Table 9. Molecular docking value of compounds isolated from root of *C. prophetarum*

Ligand	Affinity (kcal/mol)	Distance from rmsd L. b	H-bond with	Hydrophobic interaction with
24-ethyl-3-hydroxylcholesta-5,25-diene (30)	-5.6	0	-	ARG**192**LYS**189** PHE**196**TRY**218**
α-spinasterol (31),	-5.4	0	-	GLU**185** LYS**189** ILE**186**ARG**190**
Compound 32	-7.3	0	-	LYS**189**ARG**192** PHE**196**
Compound 33	-6.3	0	1(ASN198)	GLY**54**LYS**57** VAL**201**
Ciprofloxacin	-4.4	0	1(ARG1122)	ASP**512**SER**1084** SER**1085**(Ravikumar et al., 2018)

4.6. DPPH radical scavenging assay

The radical scavenging activity of the extracts and isolated compounds were assessed using DPPH. DPPH is a simple method used to test the antioxidant activity of extracts and isolated compounds by measuring the reduction of absorbance at 517 nm. Extracts and compounds having radical scavenging activity reduce absorbance at 517 nm which is in fact due to the radical. Once the radical is scavenged by the antioxidants the absorbance at 517 nm is reduced. The results of the radical scavenging activity of the extract and isolated compounds were summarized in Table 10

Table 10. Scavenging effect of the extracts and isolated compound of the root of *C. prophetarum*

Sample	Concentration of sample in µg/mL	% inhibition	IC50
hexane extract	100	63.68	
	50	56.13	56.76
	25	37.55	
	12.5	26.60	
MeOH extract	100	70.57	28.87
	50	62.45	
	25	52.64	
	12.5	38.11	
Compound 29	100	26.32	232.6
	50	22.64	
	25	16.70	
	12.5	10.28	
Compound 30	100	26.23	208.5
	50	15.28	
	25	10.28	
	12.5	6.79	
Compound 31	100	31.98	172.4
	50	24.72	
	25	16.89	
	12.5	10.85	
Compound 32	100	47.17	99.9
	50	38.58	
	25	26.23	
	12.5	14.43	
Compound 33	100	46.13	172.4
	50	41.13	
	25	29.91	
	12.5	24.43	
	Absorbance of DPPH 1.06		

Reference standard compound being used was ascorbic acid (IC_{50} 58.92 µg/ml) and experiment was done in triplicate and different concentration of sample was prepared in series dilution. Increase of % inhibition with increase in concentration from (12.5, 25, 50 and 100 µg/mL) (Table 10) indicated the concentration dependent antioxidant activity of investigated sample. All samples under investigated concentration were shown radical scavenging activity since their absorbance was decreased gradually as compared with absorbance of control (1.06)

as deduced from Table 10. Among the studied samples MeOH extract of (100 µg/mL) with % inhibition and IC_{50} of 70.5 and 28.8, respectively were shown better radical scavenging activity among the investigated samples.

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Successive root extraction of *Cucumis prophetarum* with hexane, chloroform and methanol resulted with the yield of 1% w/w, 3.5%w/w and 5.75w/w% crude extracts, respectively indicating the presence of polar constituents in this species. Silica gel column chromatographic analysis of the *n*-hexane extract furnished five compounds and the NMR structure elucidation of the isolated compound led to propose the structure and labeled as myristic acid (**29**), 24-ethyl-3-hydroxylcholesta-5,25-diene (**30**), α-spinasterol (**31**), compound **32** and compound **33**. The last two compounds were not reported before.

Both the extracts and isolated compounds of the root of *Cucumis prophetarum* had promising antibacterial activity against Gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis*) and Gram negative(*E-coli*, *Salmonella thyphimurium*). The methanol extract of the root of *Cucumis prophetarum* had better DPPH radical scavenging activity than both the hexane extract and isolated compounds from the root of *C. prophetarum*. Compounds; 24-ethyl-3-hydroxylcholesta-5, 25-diene (**30**), α-spinasterol (**31**), compound **32** and compound **33** exhibited promising docking efficiency with DNA gyrase and compound **32** is best among them.

5.2. Recommendation

In this study only NMR spectrum (1D and 2D) and literature data was used for structure elucidation of isolated compounds, but the last two compounds are not reported before and their structure is also complex, so this paper recommend mass spectrophotometry for further confirmation of the structure of compound **32 & 33.**

The results of these study supports the traditional use of the *C.prophetarum* root for disease caused by pathogenic bacteria both Gram negative and Gram positive, since the result of *invitro* and *insilico* studies against bacteria are promisingly collaborated. But the traditional healer uses the plant for wide array of disease including lung disorder, heart failure, back pain and etc. so this work recommend further activity test like anti-cancer, anti-AIDS and anti-diabetic etc.

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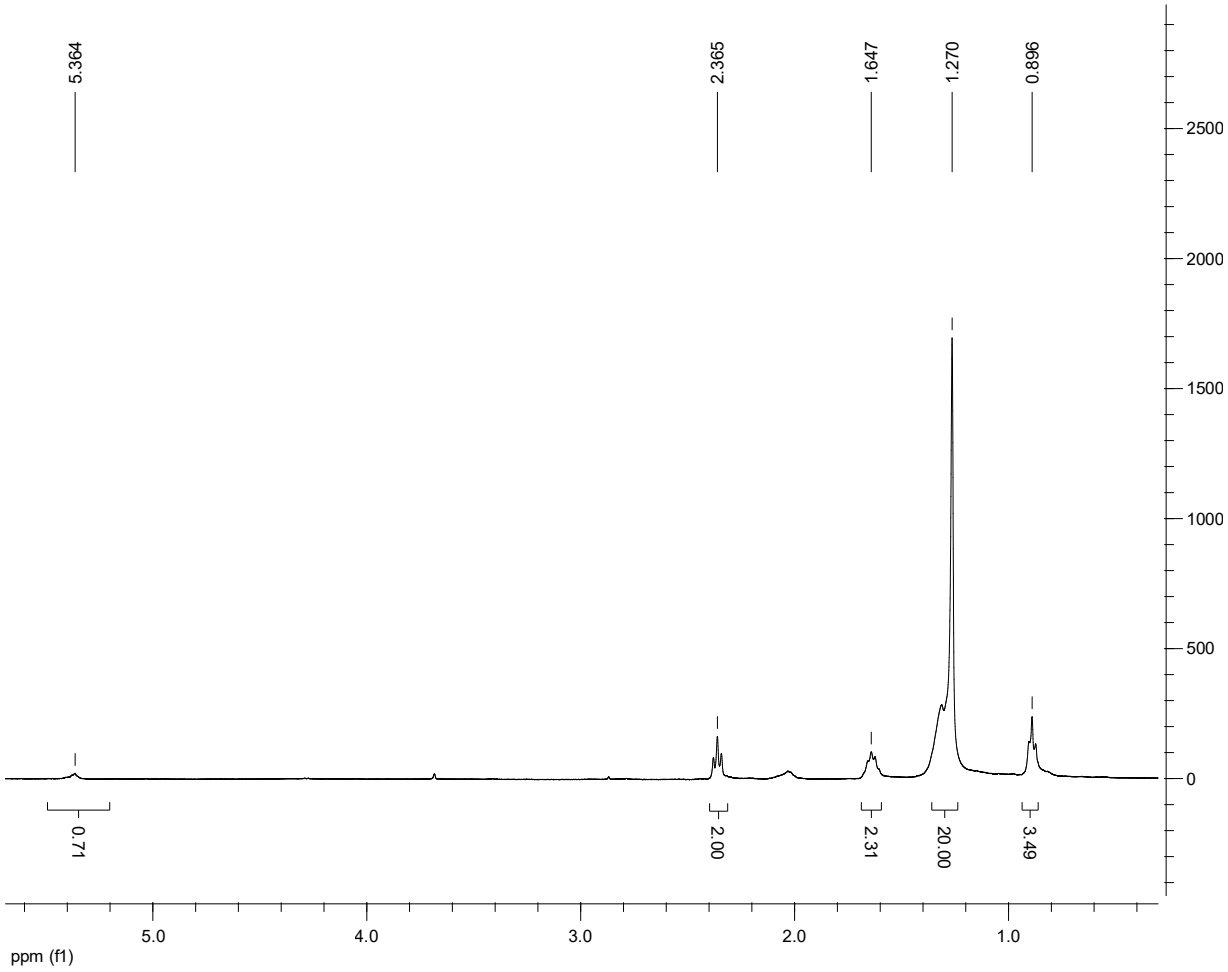
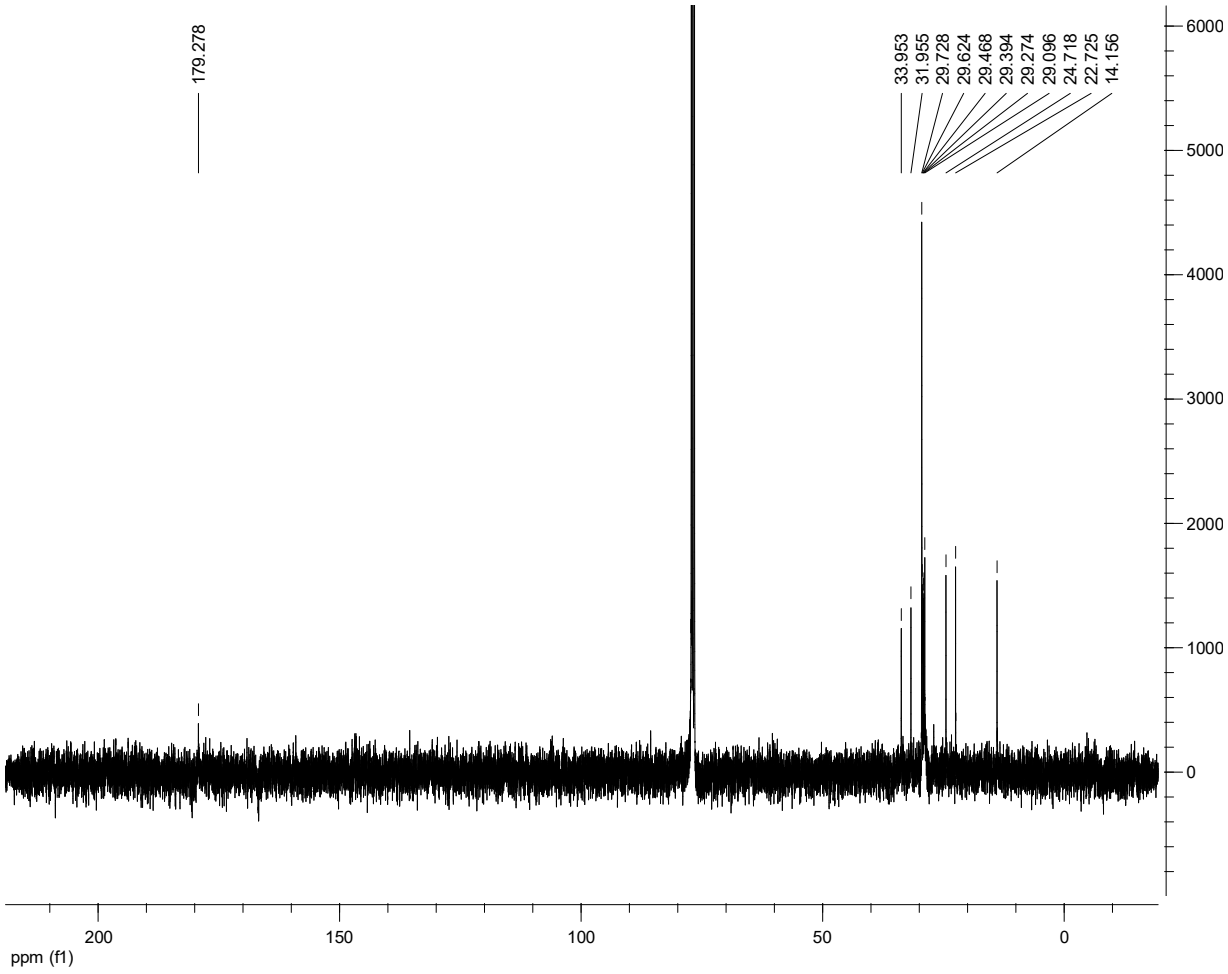
APPENDIXES

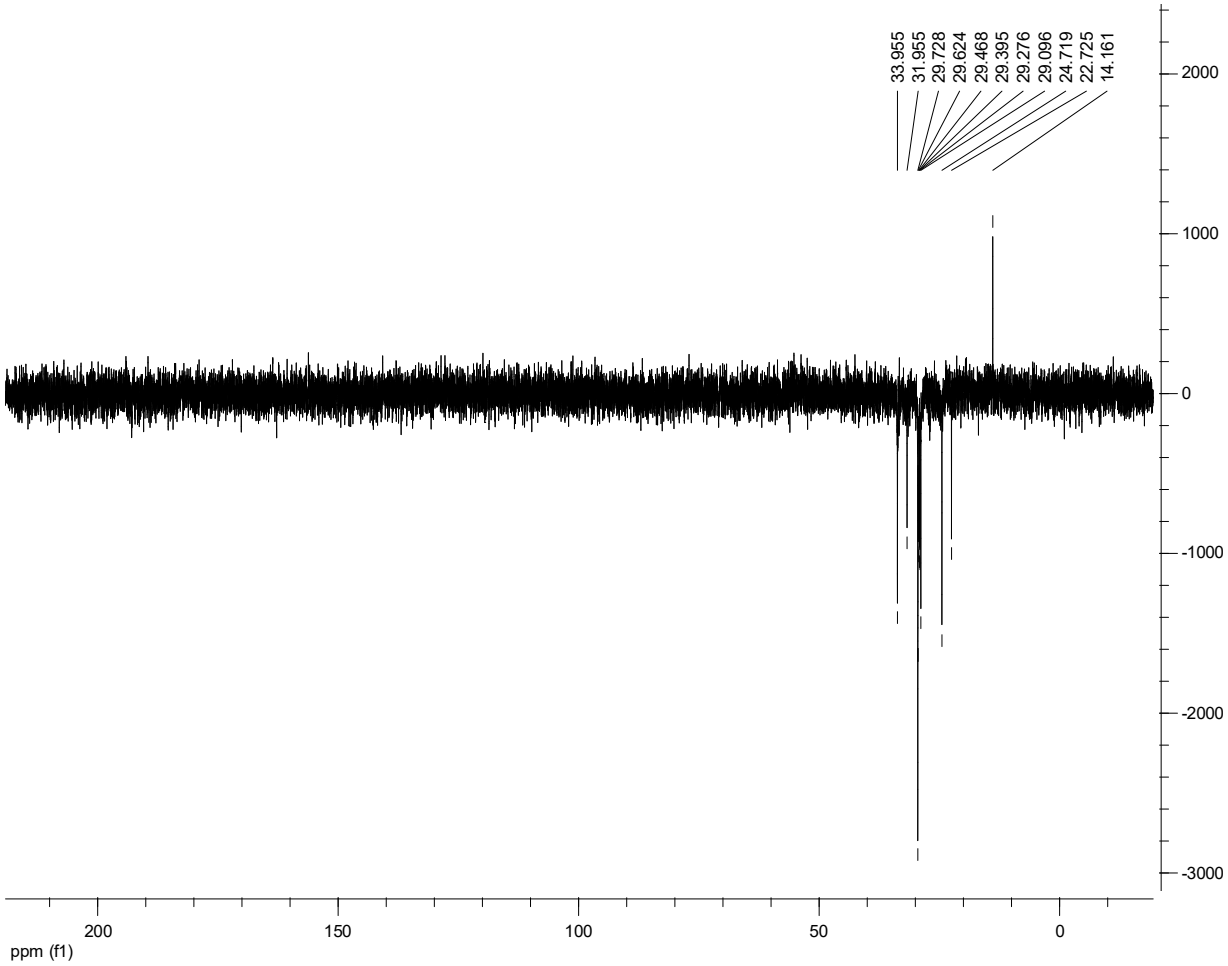
1.1. The ^{1}H-NMR spectrums of compound 29



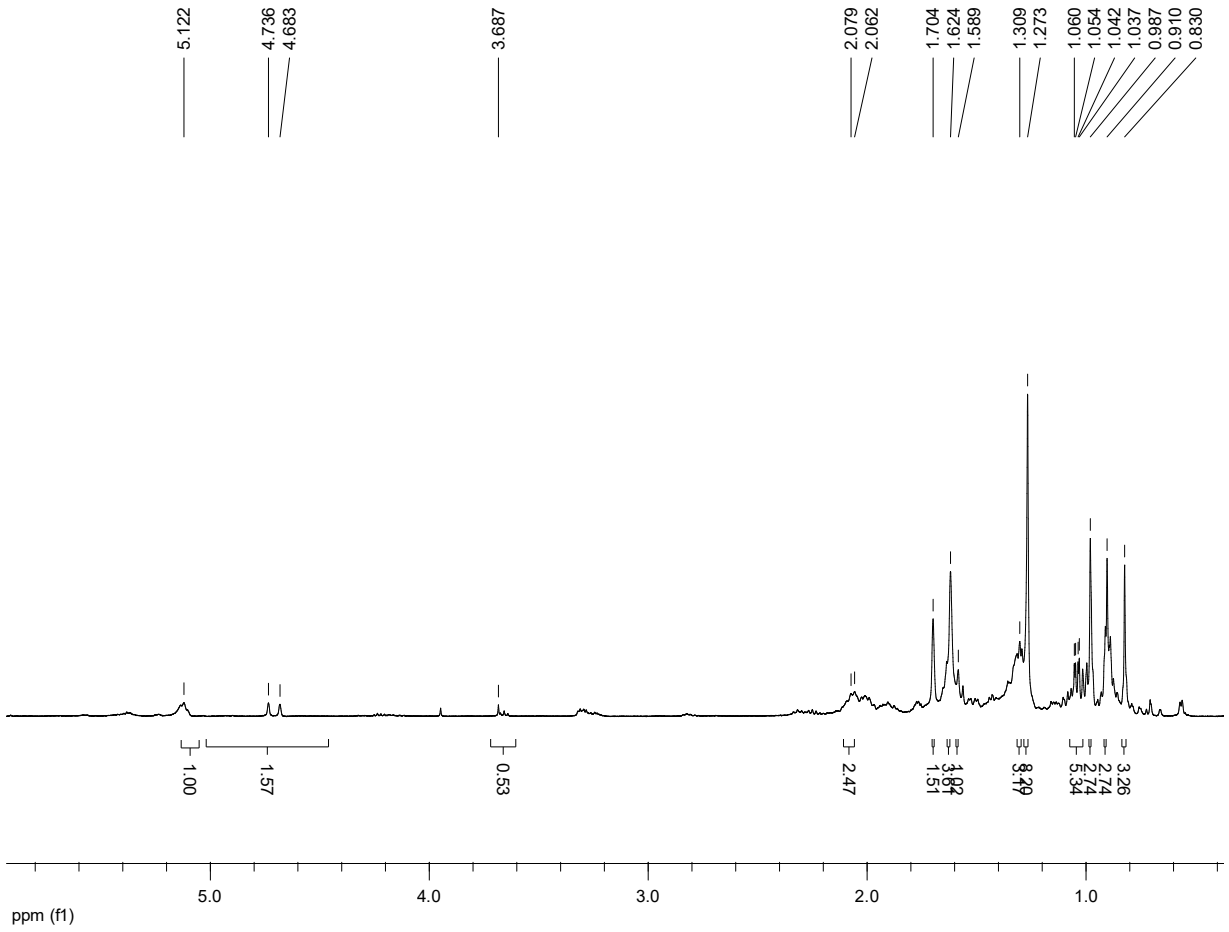
1.2. The ^{13}C-NMR spectrum of compound 29



1.3. The DEPT-135 spectrum of compound 29



2.1. The ^{1}H-NMR spectrum of compound 30



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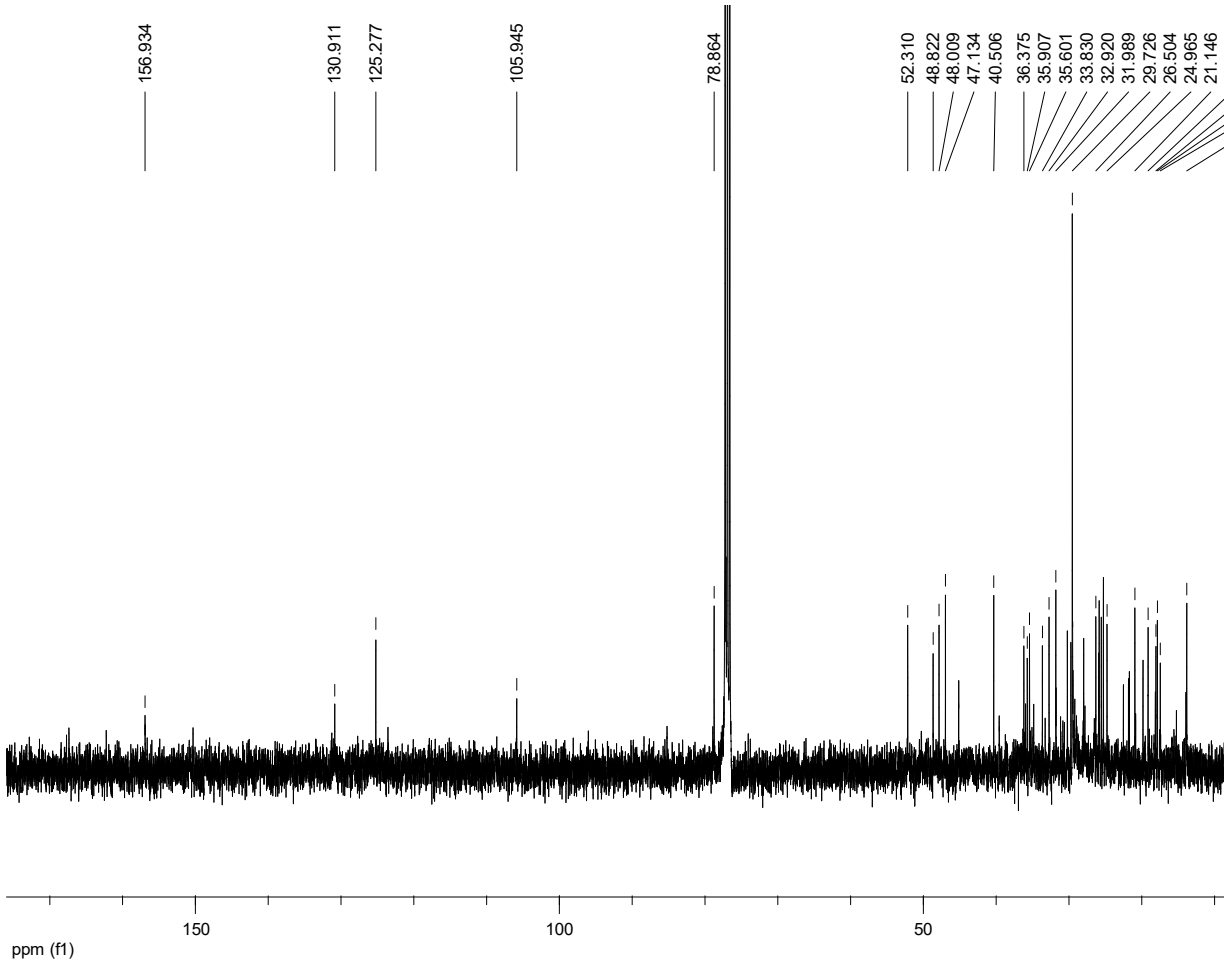

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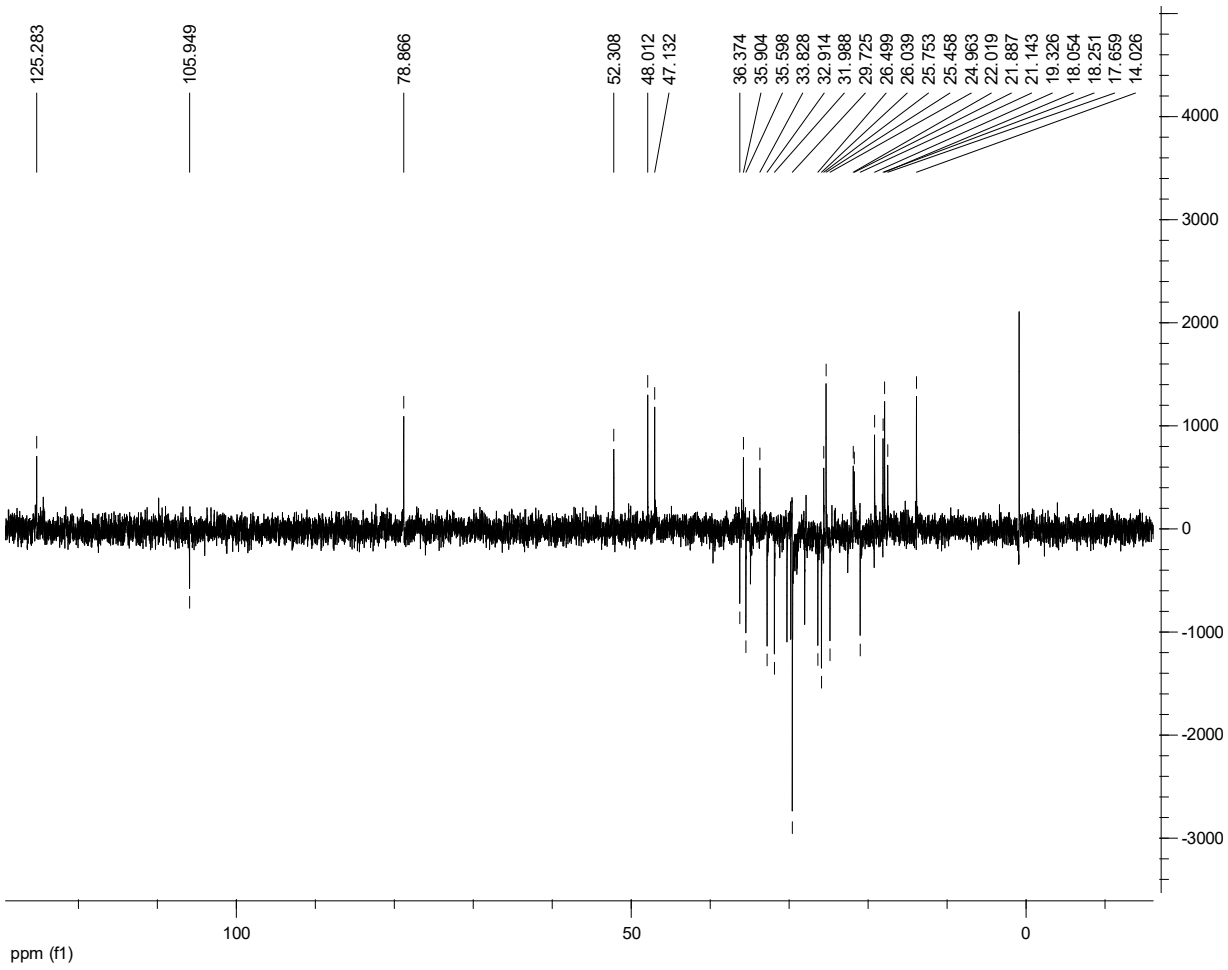

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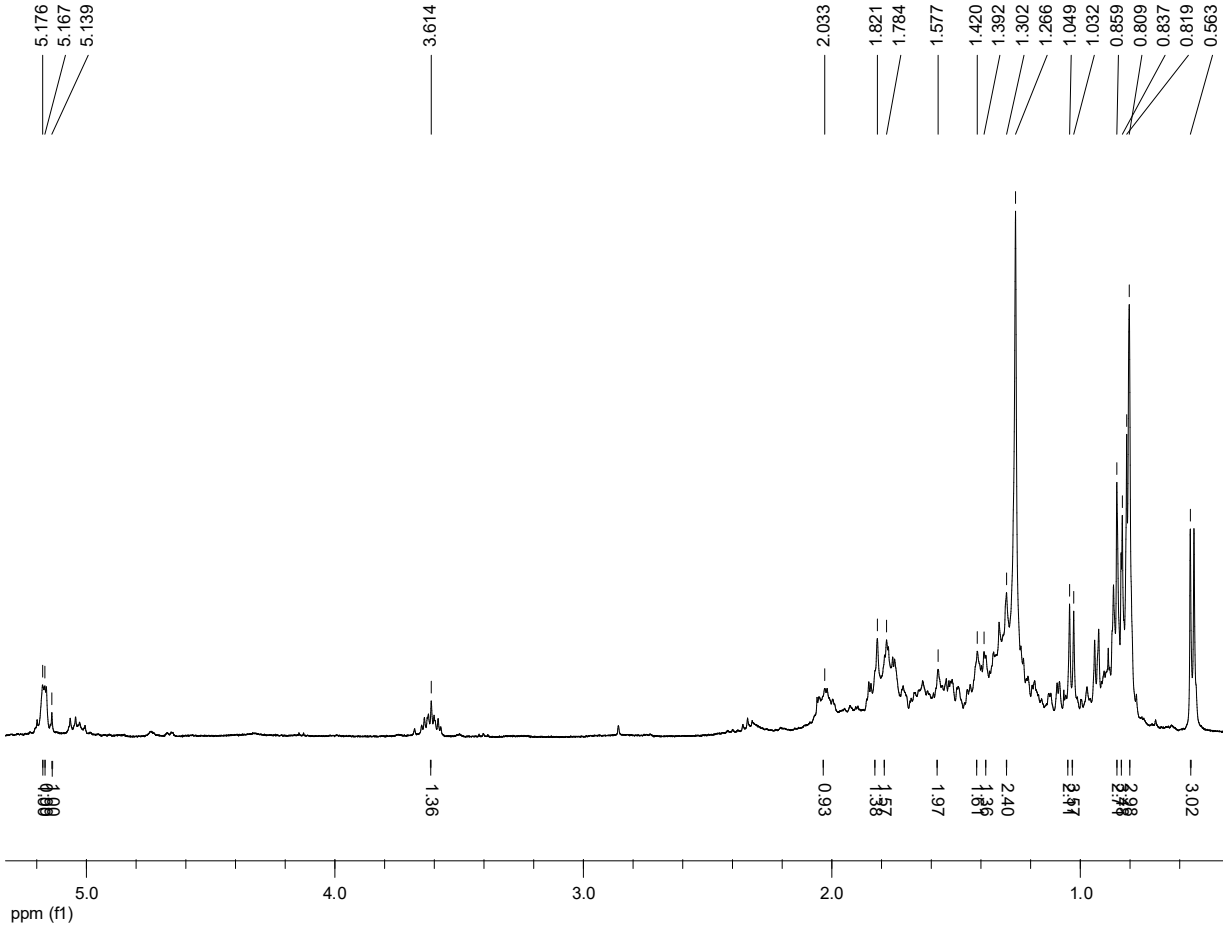

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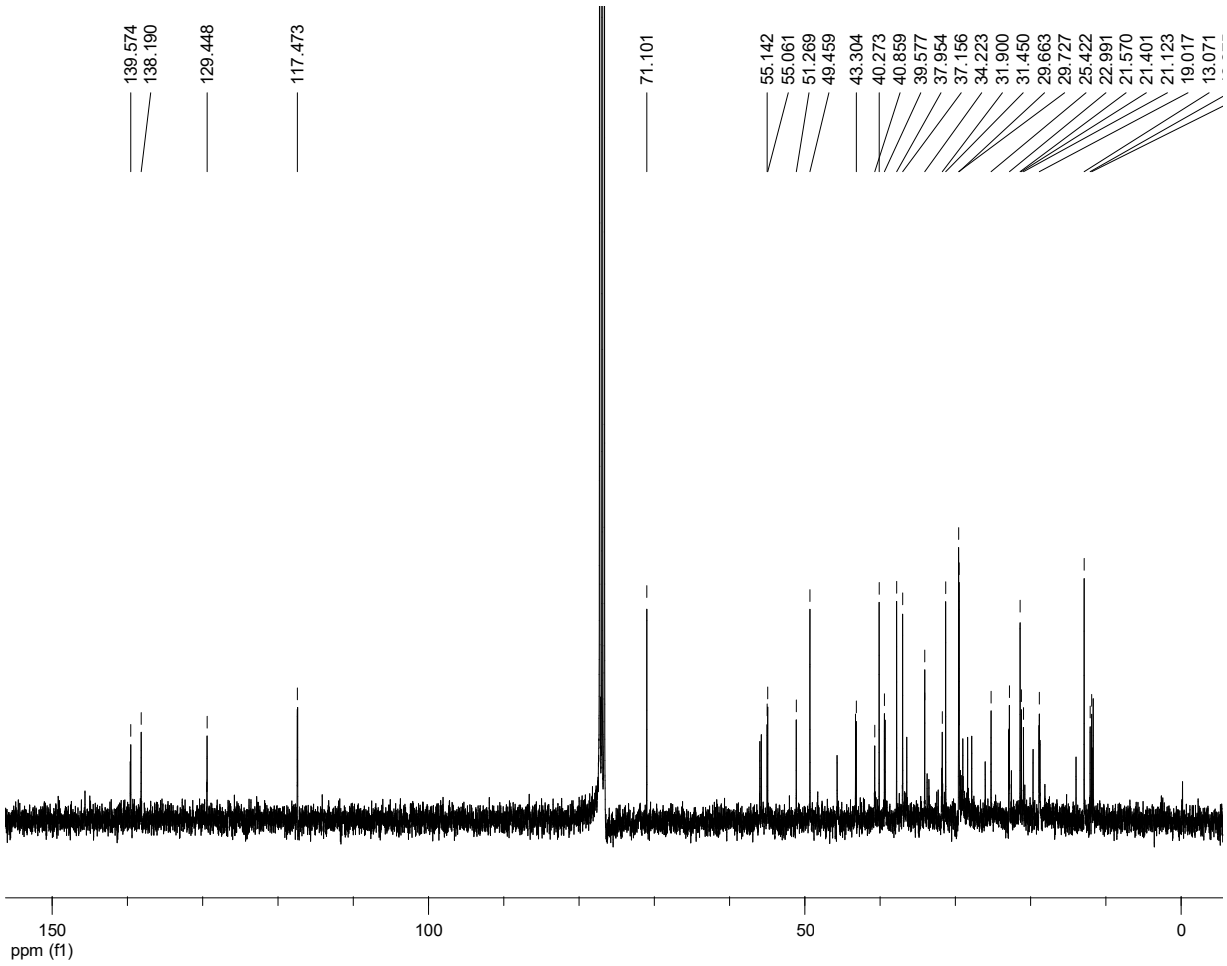
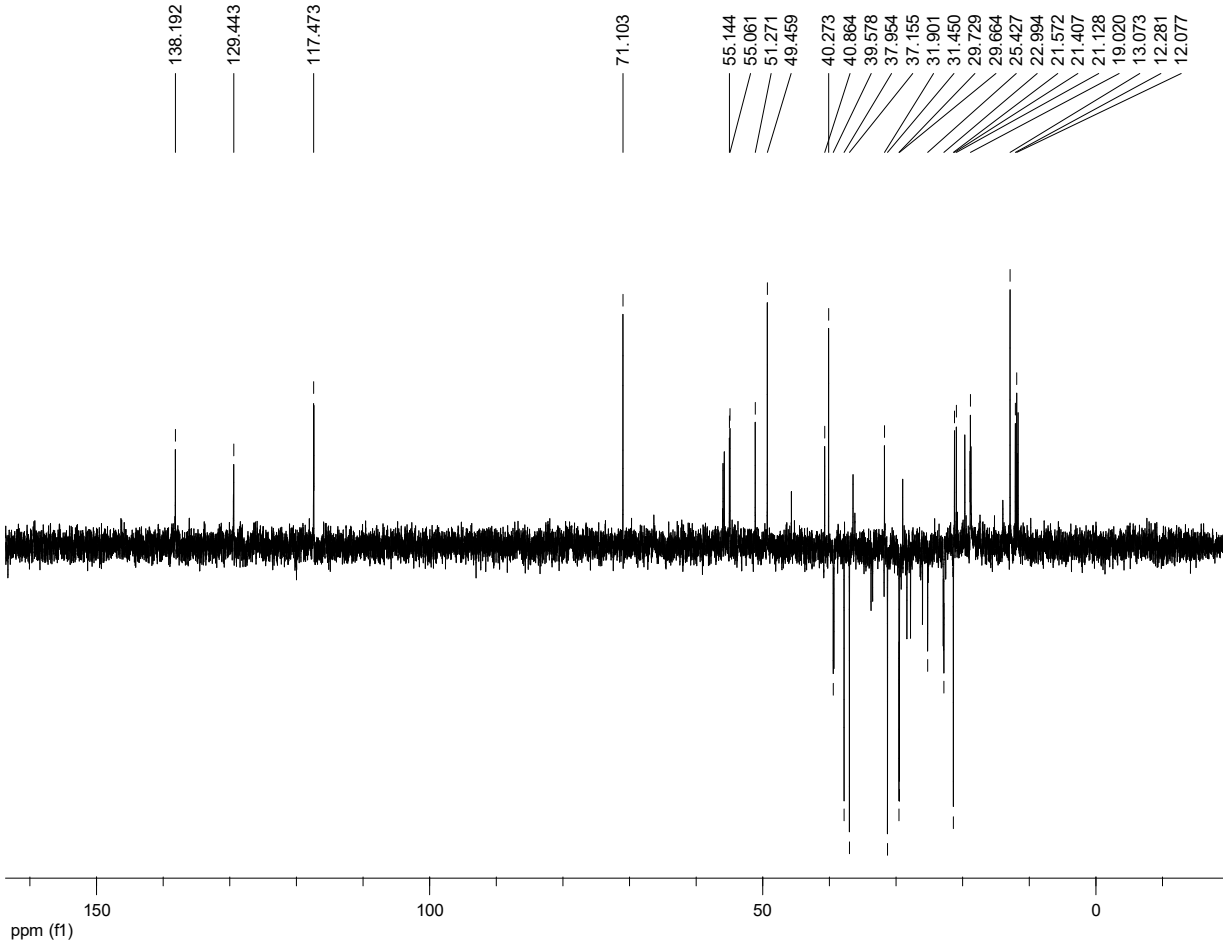
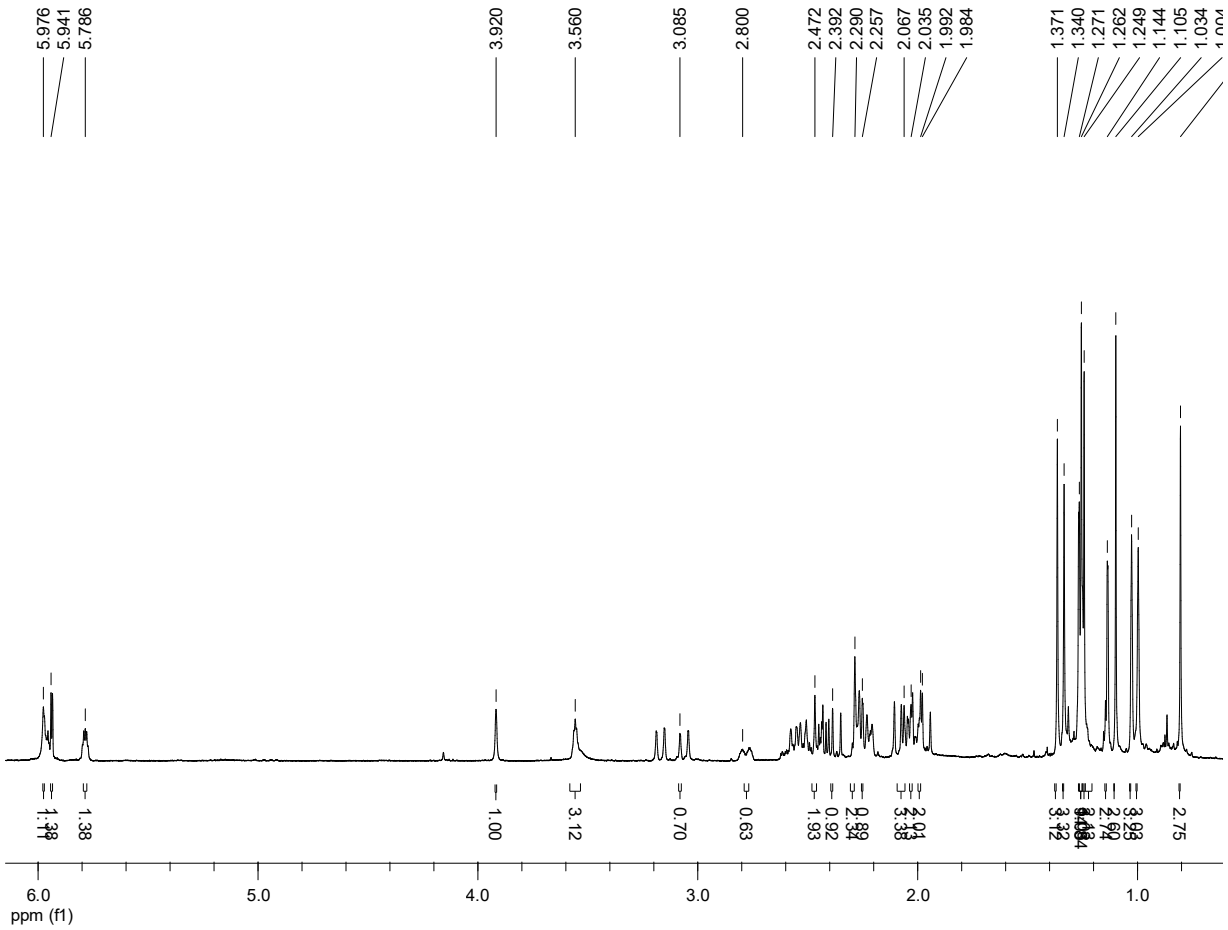


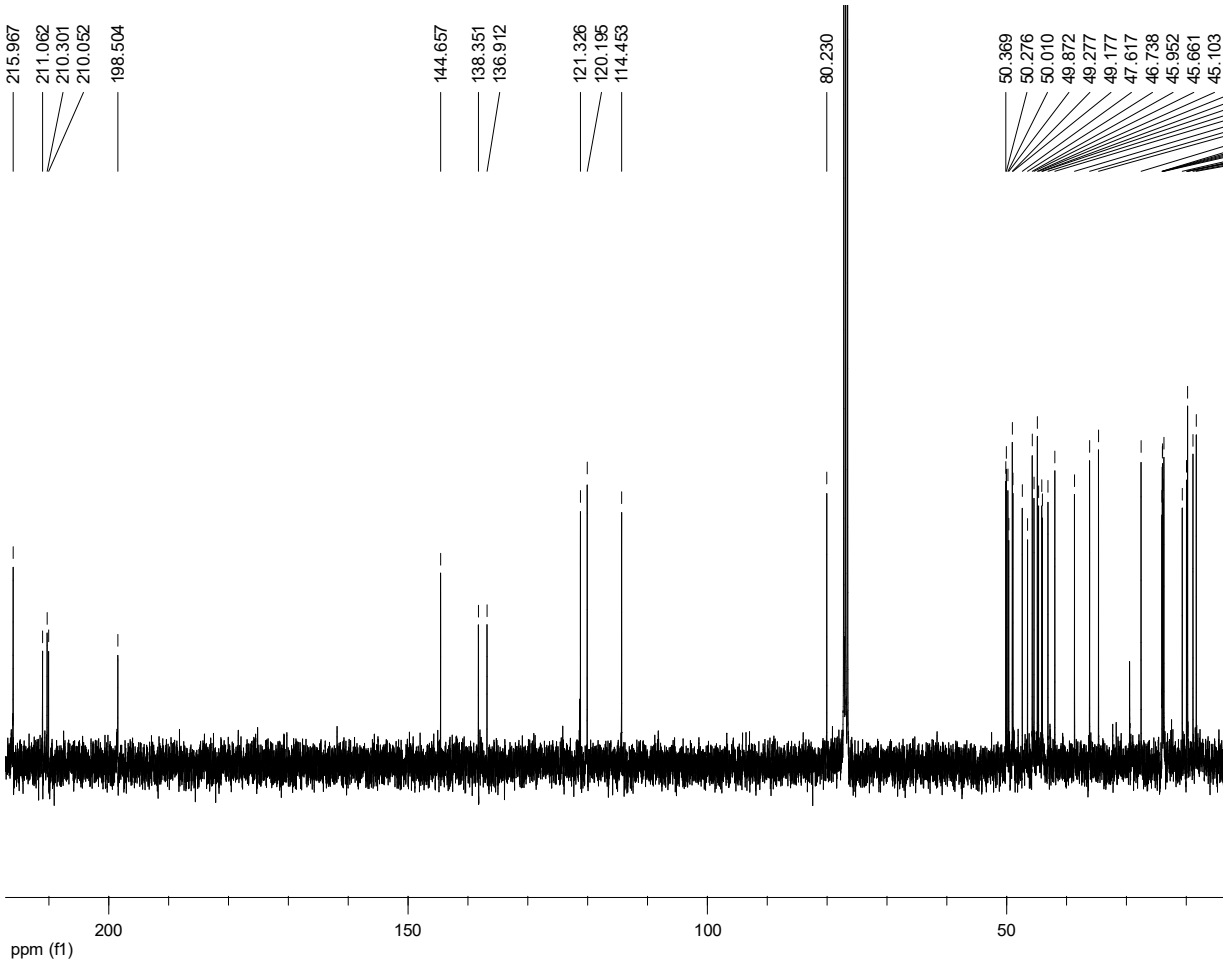
3.3. The DEPT-135 spectrum of compound 31



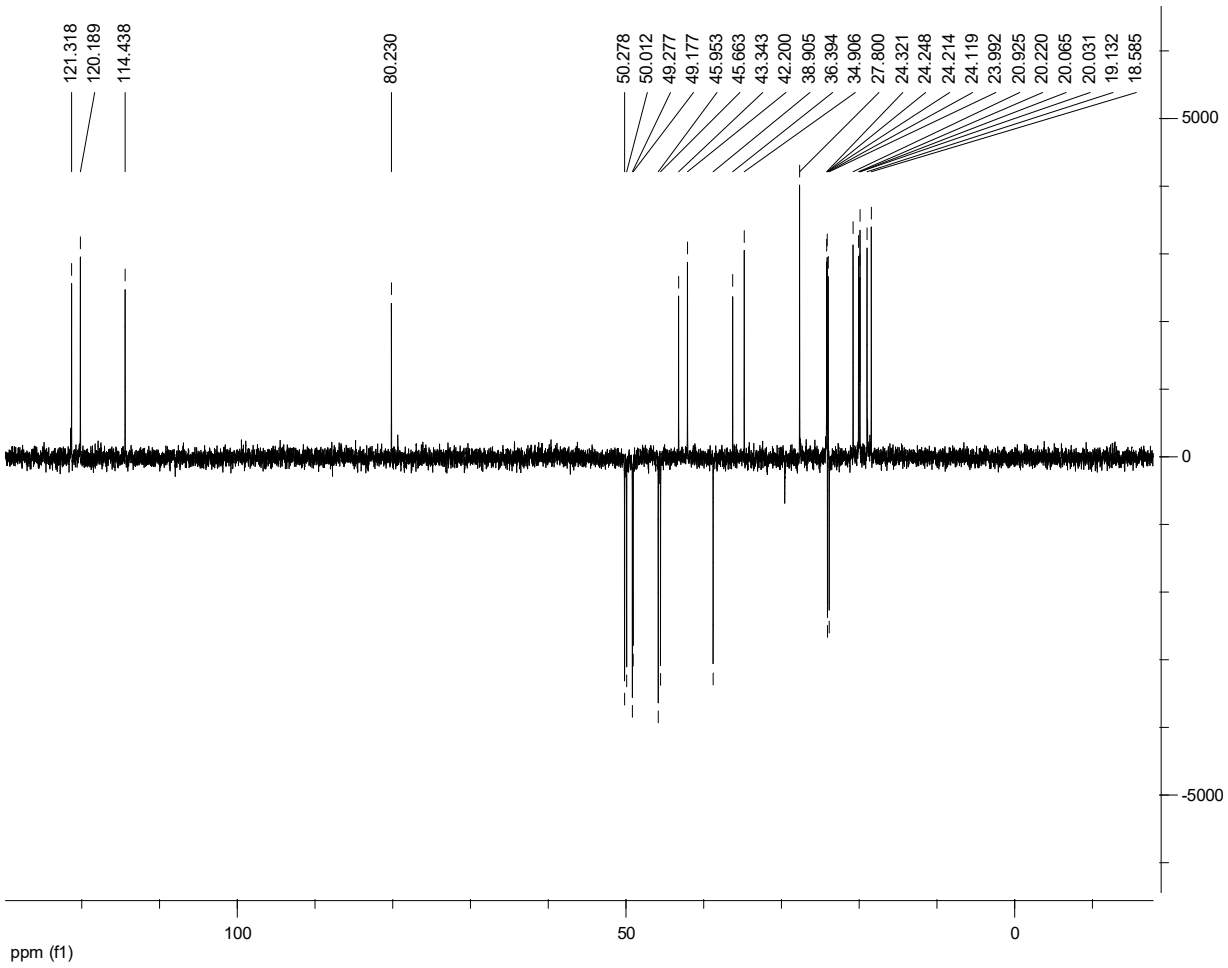
4.1. ^{1}H NMR spectrum of compound 32



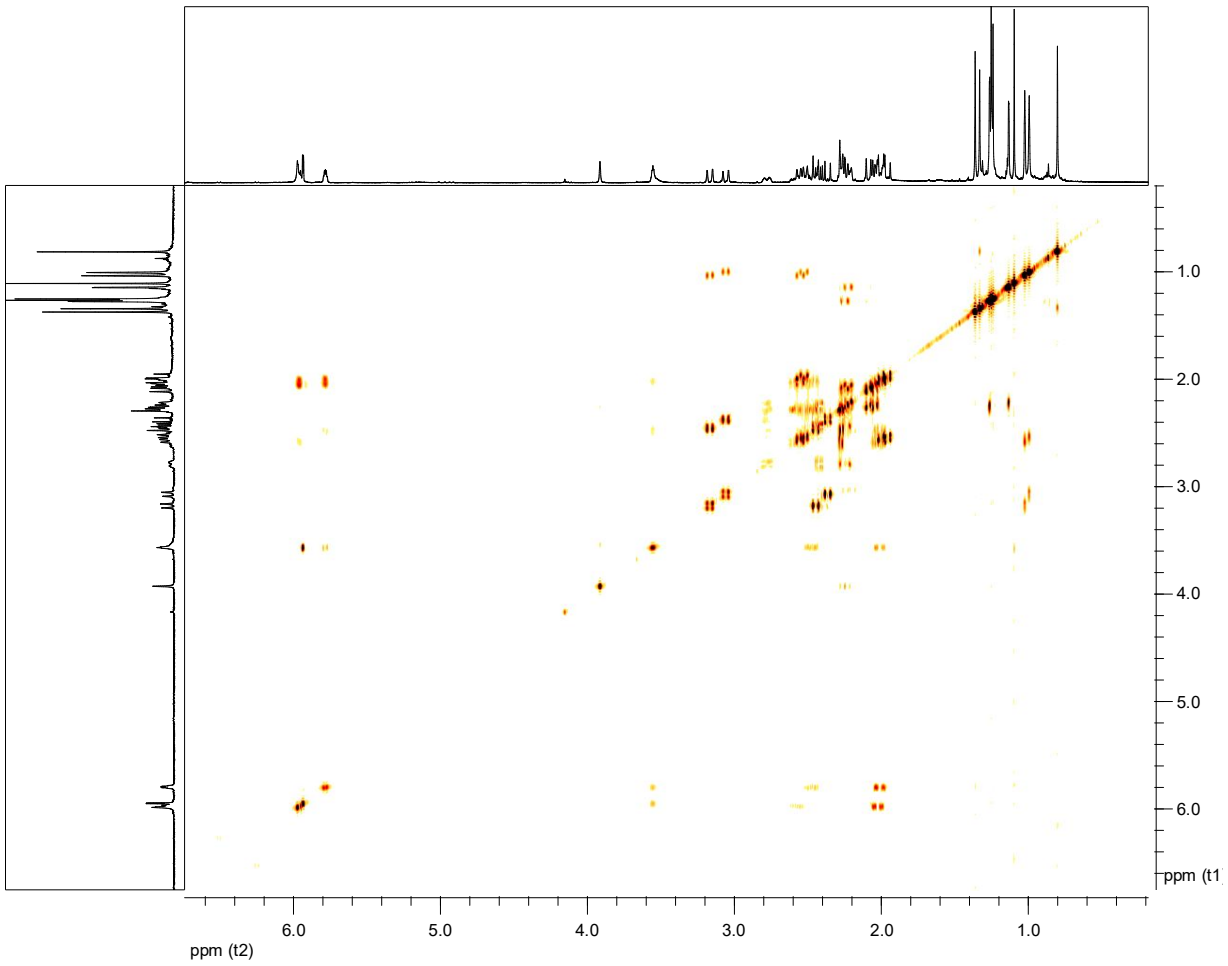
4.2. The 13C NMR spectrum of compound 32



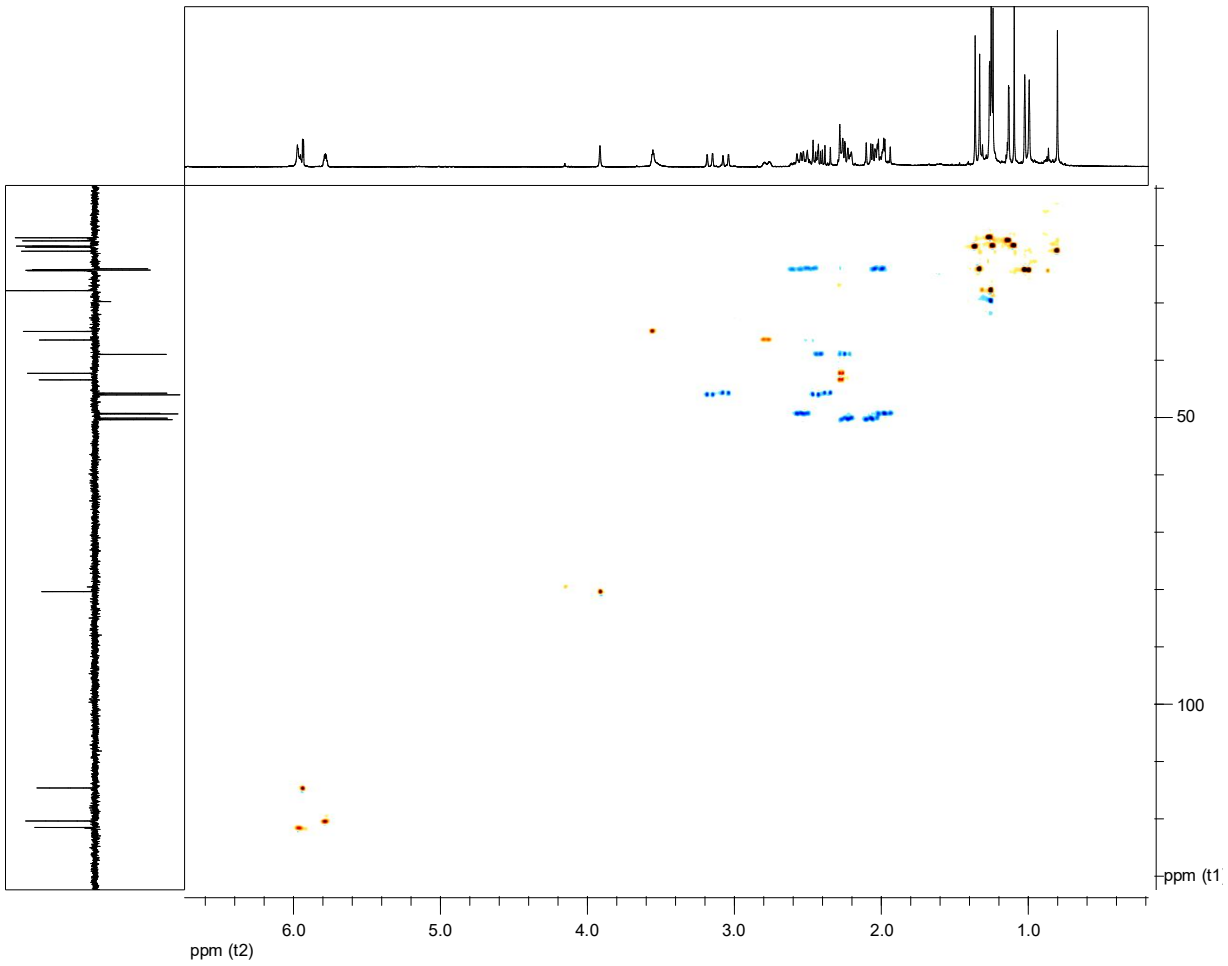
4.3. The DEPT-135 spectrum of compound 32



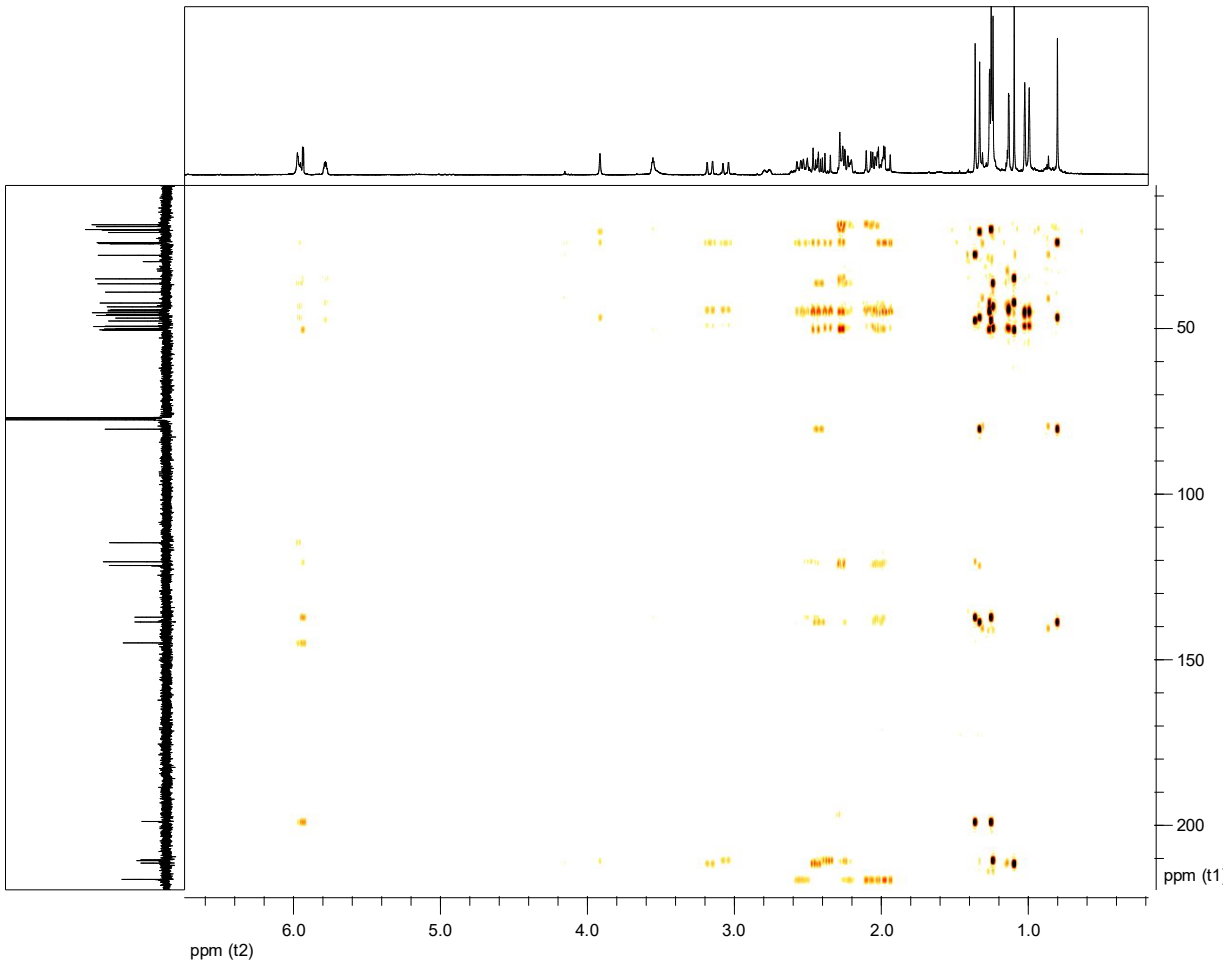
4.4. The COSY spectrum of compound 32



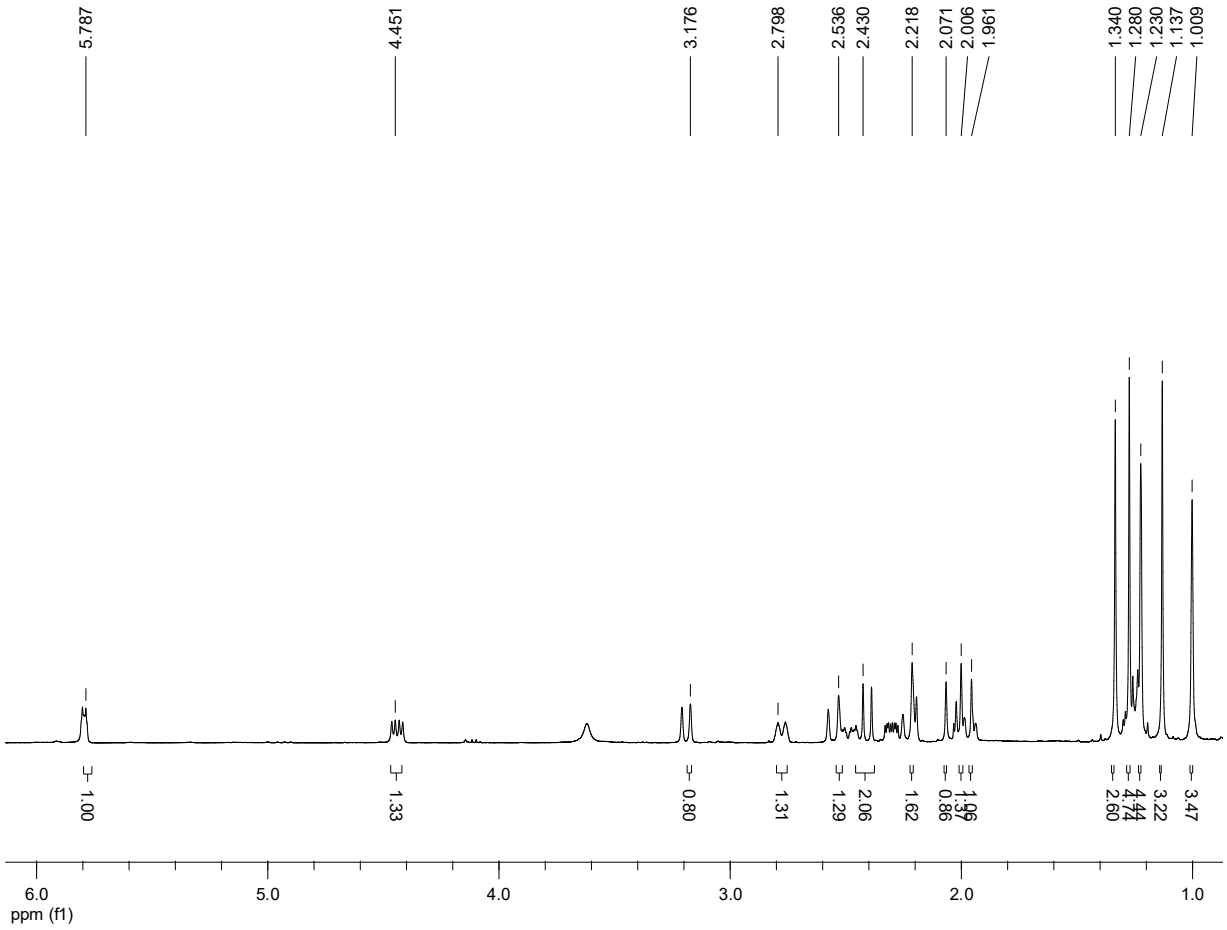
4.5. The HSQC spectrum of compound 32



4.6. The HMBC spectrum of compound 32



5.1. The ^{1}H-NMR spectrum of compound 33



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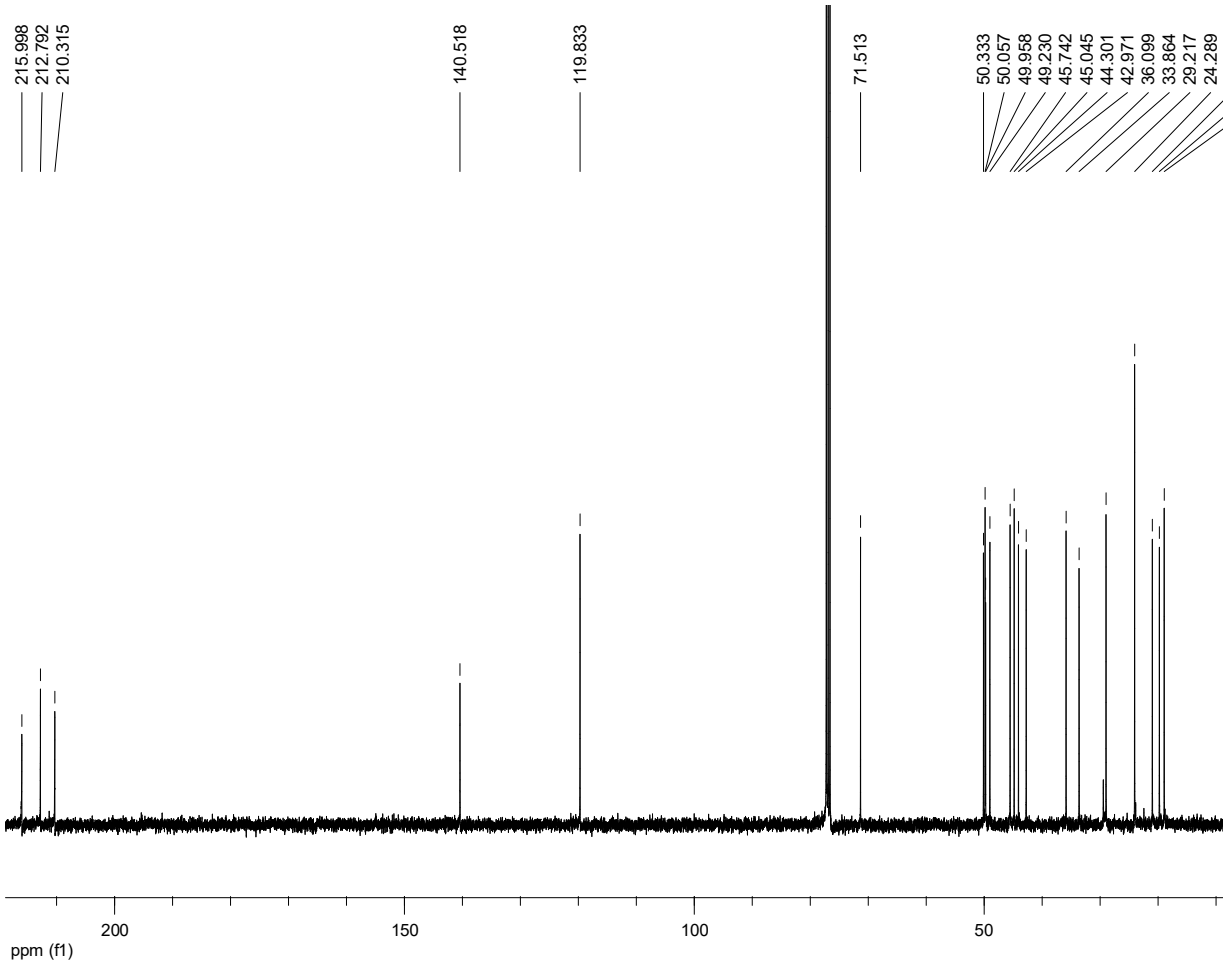
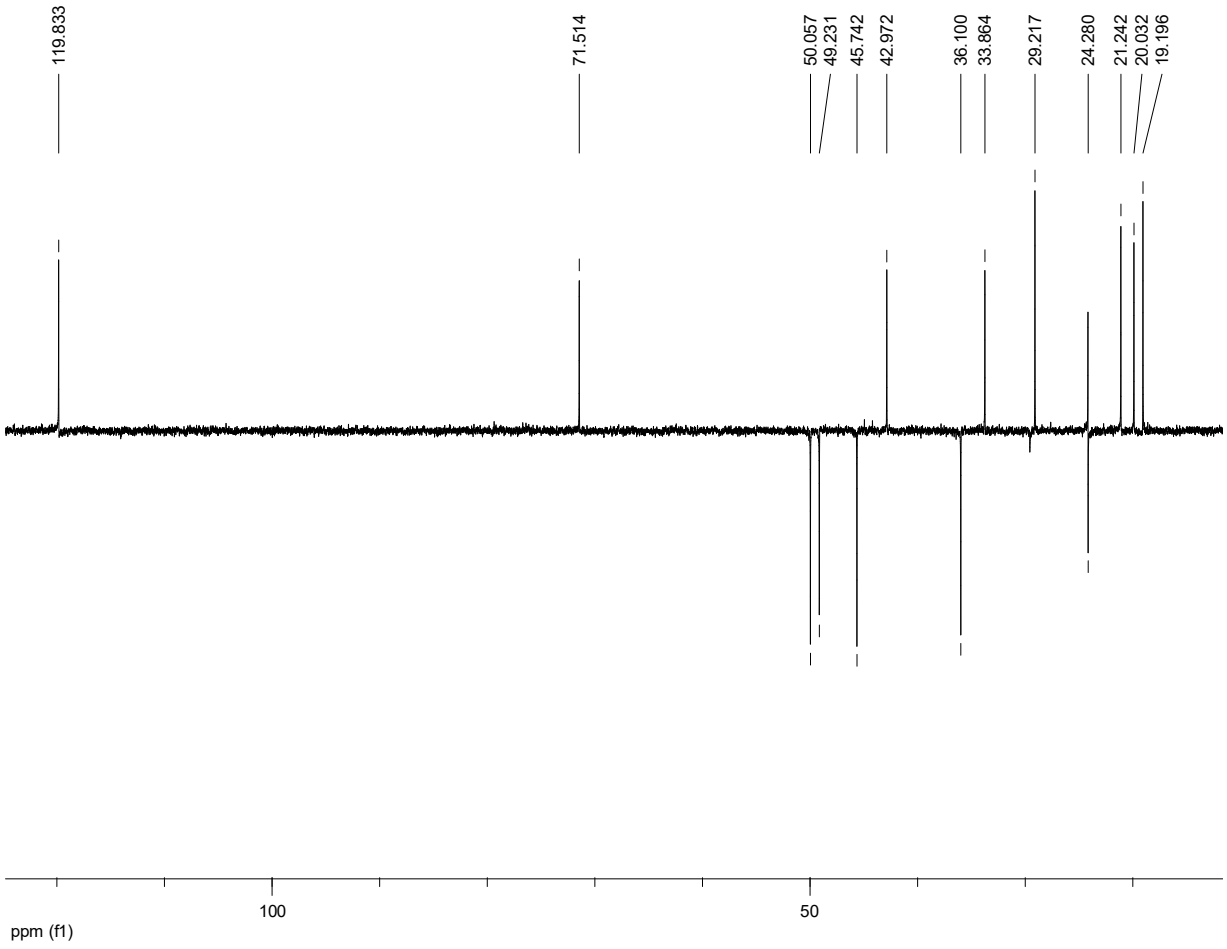
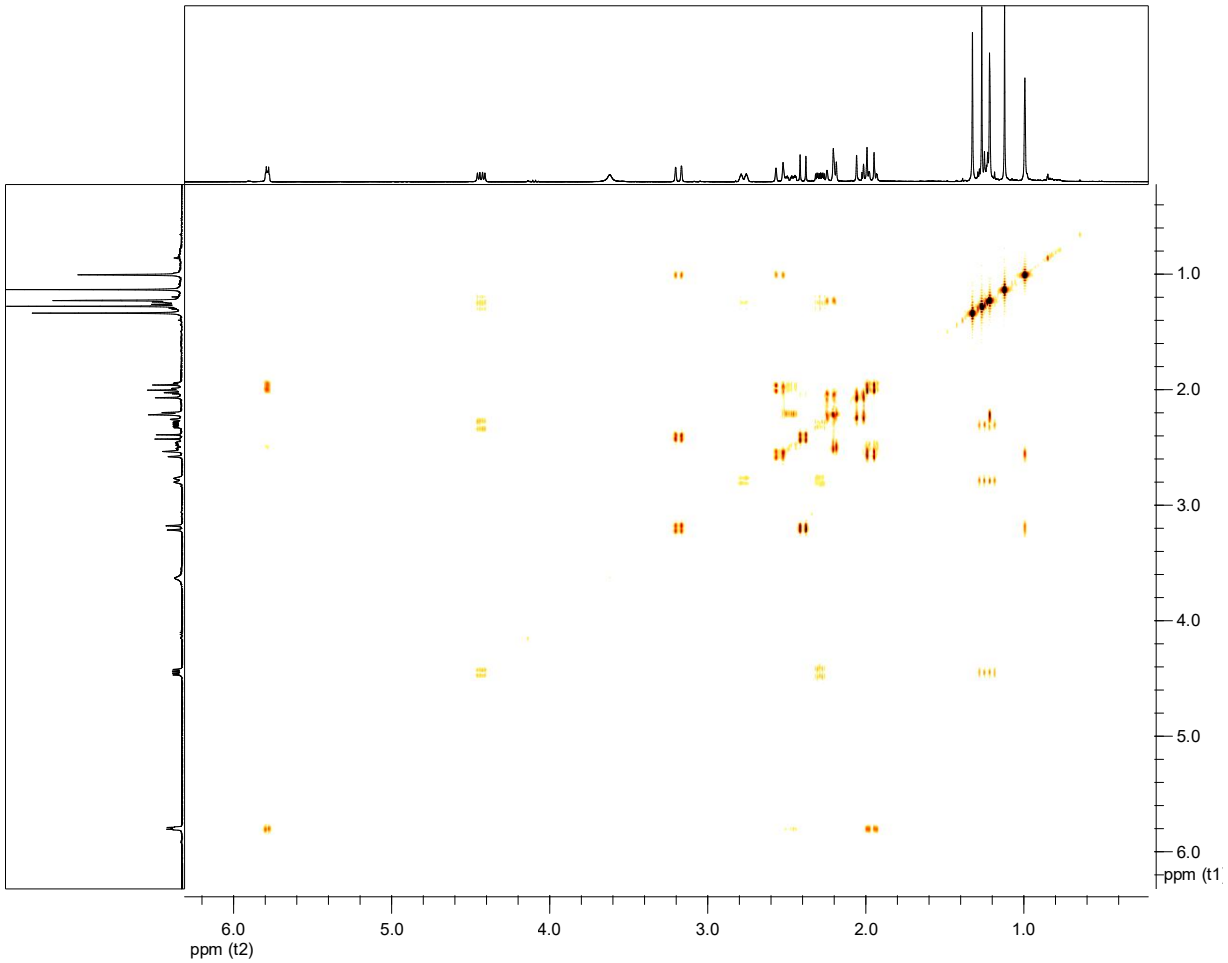


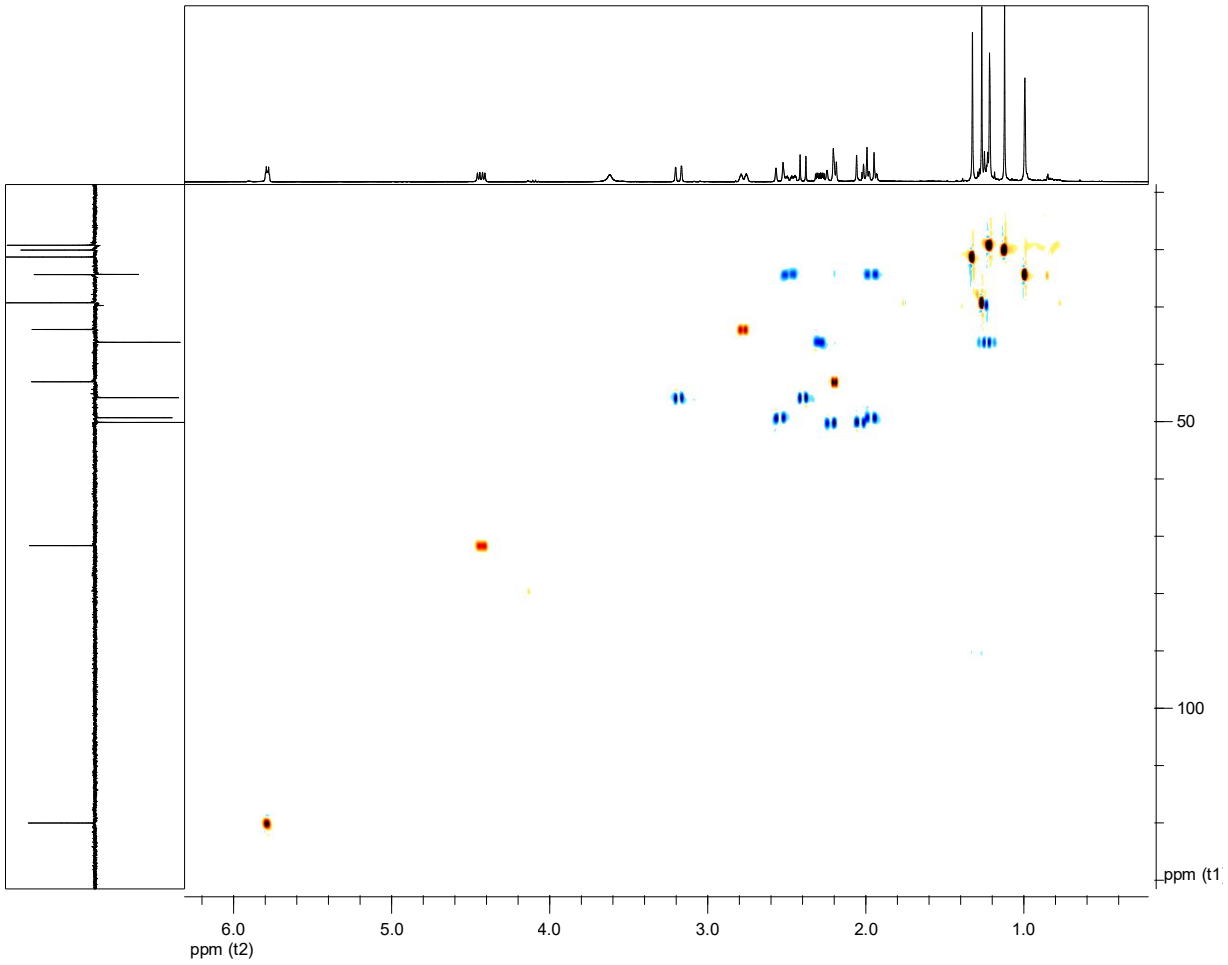
5.3. The DEPT-135 spectrum of compound 33



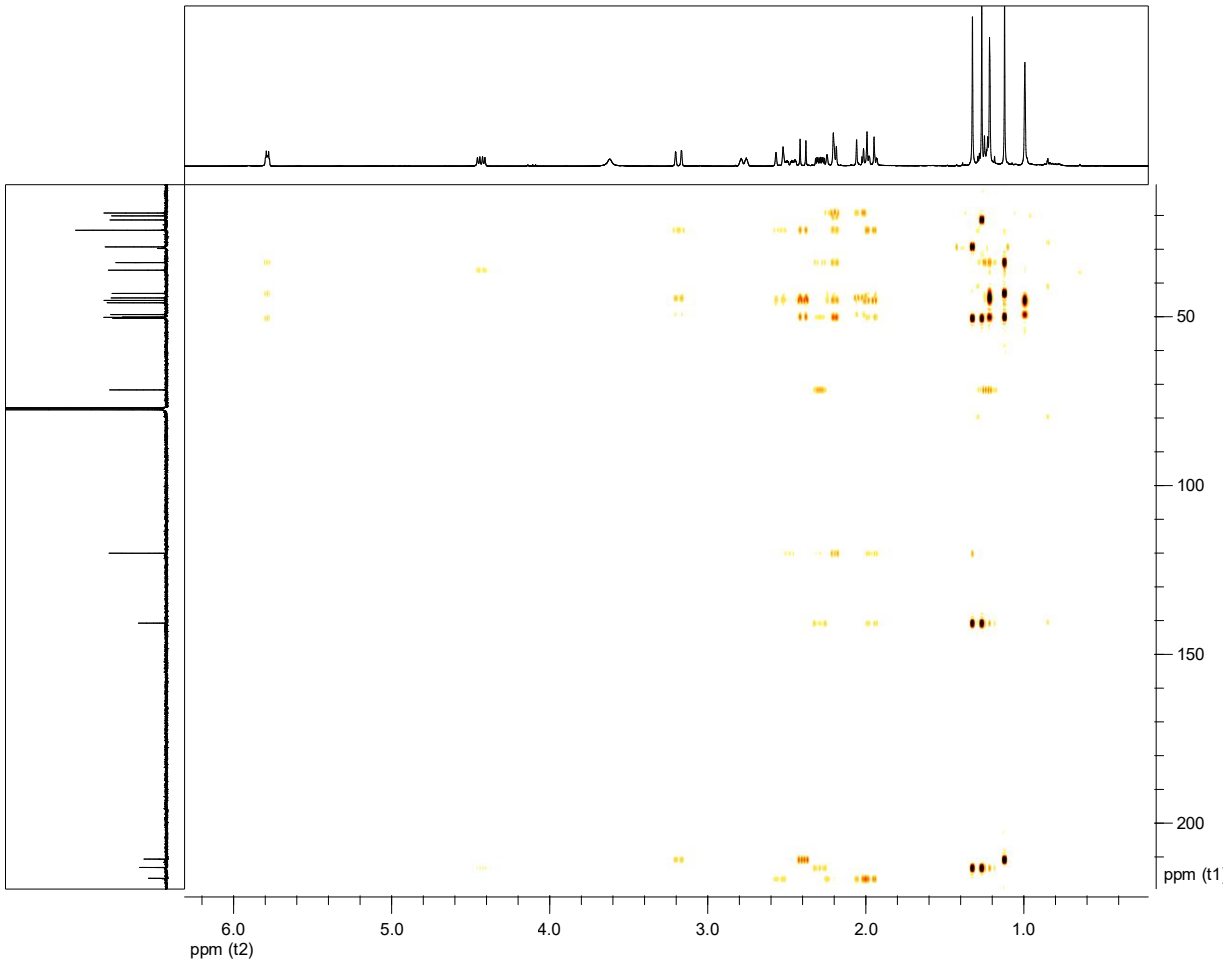
5.4. The COSY spectrum of compound 33



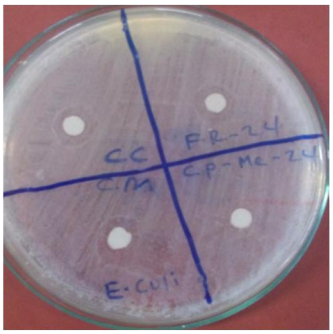
5.5. The HSQC spectrum of compound 33

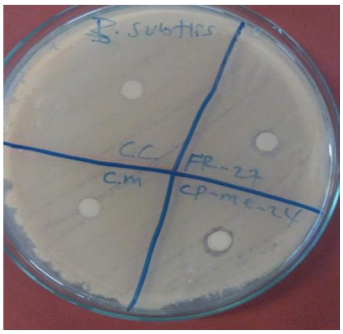


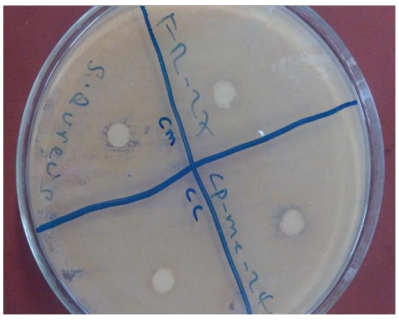
5.6. The HMBC spectrum of compound 33



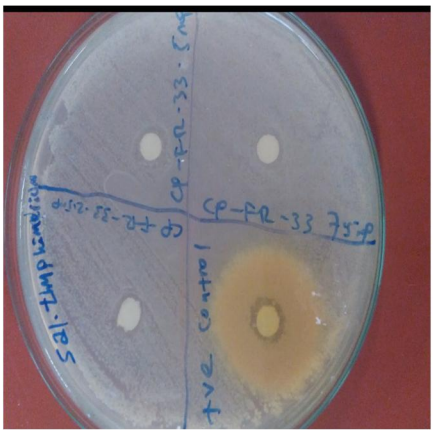
6.1. Zone inhibition of crude extracts, compound 29 and compound 30

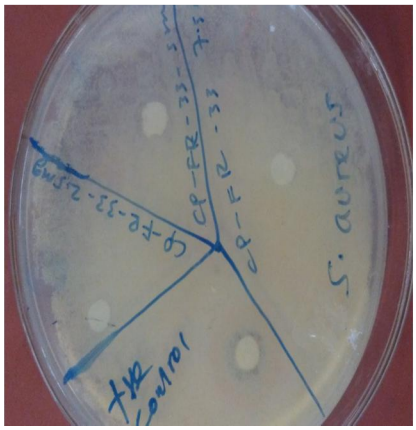




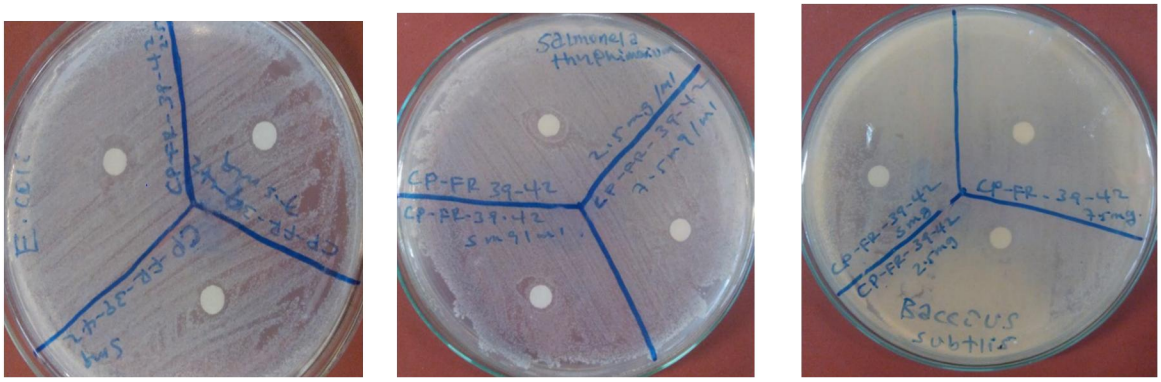


6.2 Inhibition zone of compound 31





6.3. Inhibition zone of compound 32



6.4. Inhibition zone of compound 33