Phytochemical and Antibacterial activity of the Pods and Leaves extracts of *Moringa stenopetala* and Docking studies of stigmasterol.

BY

Brehan Areaya

A Thesis Submitted to Applied Chemistry Department

School of Applied Natural Science

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

Office of Graduate Studies

Adama Science and Technology University

September, 2018

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Advisor: Yadessa Melaku (PhD)

Co- advisor: Rajalakshmanan Eswaramoorthy (PhD)

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APPROVAL PAGE

BY _____ _____ _____

Student Name Signature Date

As thesis advisor, we hereby certify that we have read and evaluated this thesis paper under our guidance, by Brehan Areaya entitled: Phytochemical and Antibacterial activity of the Pods and leaves extracts of *Moringa stenopetala* and docking studies of stigmasterol**.** We recommended as fulfilling the thesis requirement.

1._____ _____ _____

Advisor Signature Date

2. _____ _____ _____

Co- Advisor Signature Date

As members of the board of examiners for the MSc. thesis open defense examination, we certify that we have read and evaluated the thesis paper prepared by Brehan Areaya and examined the candidate. We recommend the thesis paper to be accepted as fulfillment for the requirements of the thesis guideline of Adama Science and Technology University /Department of Applied Chemistry.

3._____ _____ _____

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4._____ _____ _____

External examiner Signature Date

5._____ _____ _____

Chair person Signature Date

6. _____ _____ _____

Department head Signature Date

7._____ _____ _____

School head Signature Date

DECLARATION

I hereby declare that the thesis entitled "Phytochemical and Antibacterial activity of the Pods and leaves extracts of *Moringa stenopetala* and docking studies of stigmasterol**.**" has been carried out by me under the supervision of Dr. Yadessa Melaku and Dr. Rajalakshmanan Eswaramoorthy, during the year 2017/2018 as part of Master of Science Program in medicinal Chemistry. I further declare that this work has not been submitted to any other Universities or institutions for the award of any degree. All quotations and their sources are specifically acknowledged by means of references.

Name: Brehan Areaya

Signature: _____

This M.Sc Thesis has been submitted for examination with our approval as thesis advisors.

Advisor: Yadessa Melaku (PhD**)** Signature: _____

Co- advisor: Rajalakshmanan Eswaramoorthy (PhD) Signature: _____

Date of submission:_____

DEDICATION

This M.Sc. thesis is dedicated to all my families, especially to my Fathers Mr G/stadike G/ medhin and Mr Areaya G/silase and to my brother Aeamar G/ stadik and also my sister Lielt Areaya whose advice, support, prayers and helped me all along on the right way and made me who I am today. All my family will always be in my heart.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial Resistance
AR	Analytical Reagent
CDC	Centres for Disease Control and Prevention
DM	Diabetes Mellitus
M. oliefera	*Moringa Oliefera*
M. stenopetala	*Moringa Stenopetala*
SPP.	Species
PDB	Protein Data Bank
TLC	Thin Layer Chromatography
TM	Traditional Medicine

ABSTRACT

Moringa stenopetala is an endemic vegetable tree in east Africa and used in southern Ethiopia as food and medicine. Through the plant has significant contribution as medicine, there is no prior chemical and pharmacological reports on the pods of this species. Therefore the main objective of this research is to conduct phytochemical, antibacterial and docking studies of the pods of Moringa stenopetala. In view of this the pods and leaves were successively extracted with n-hexane, EtOAc and MeOH. The phytochemical screening of the n-hexane, EtOAc and MeOH extracts of pods of Moringa stenopetala shows the presence flavonoids, alkaloids and saponins. The EtOAc extract of the pods after silica gel column chromatography furnished three compounds identified as oleic acid, n-octacosane and stigmasterol while rutin was isolated from the leaves of Moringa stenopetala. The isolated compounds were characterized with IR and NMR. The extracts and isolated compounds were also evaluated for their antibacterial activities using gram negative and gram positive bacteria. The n-hexane extract of the pods showed inhibition zone of 18 mm and 13 mm against S. pneumonia and K. pseudomonas at the 2 µg/mL, respectively. This is significant compared with the positive control. On the other hand the ethyl acetate extract displayed zone of inhibition by 13 mm and 10 mm against S. aureus and S. pneumonia at 10 µg/mL, respectively. Methanol extract of the pods showed inhibition zone of 9, 10, 11, 13 and 14 mm against Shigella boydii, S. pneumonia, E. coli, S. aureus and K. Pseudomonas at 4µg/mL respectively. Docking study was performed using stigmasterol which was active against S. aureus bacteria and the ligand has 60% binding affinity with the protein. Therefore, the antibacterial activity displayed by the pods extracts of M. stenopetala corroborates the traditional use of this plant against bacteria.

***Key words**: Ethiopia; M.stenopetala; medicinal value; nutritional value*

1 INTRODUCTION

1.1 Background

Finding healing powers in plants is an ancient idea. Since the inception of human civilization man has been searching for food which can keep him health. In this modern era of research and technology this futile search still continues to discover novel herbal drugs and alternate source of nutritional supplements. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on human body. Plants are produce a variety of secondary metabolites (alkaloids, flavonoids, glycosides, tannins, volatile oils and phenolic compounds) to protect themselves against a variety of their own pathogens and therefore can be considered as potential source of different classes of antimicrobial substances [1]. According to World Health Organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs [2]. The phytochemical research based on ethno pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants [3].

Medicinal plants are important sources of TM for millions of people and additional inputs to modern medicine in terms of exploring and producing new drugs to meet the need for the overgrowing population of the world [4]. It is reported that more than 3.5 billion people rely on plants for the treatment of both human and livestock diseases [5]. *Moringa* is a tropical plant belonging to the family Moringaceae that grows throughout the tropics. *Moringa* was highly valued in the ancient world which reveals that the ancient kings and queens preferred *Moringa* leaves and fruit in their diet to sustain mental alertness and healthy skin [6]. About 33 species have been reported in the family Moringaceae. Six species of *Moringa* occur in Ethiopia those are *M. borziana* (South Ethiopia), *M. lingituba* (Sidamo and Hararge), *M. oleifera* (introduced), *M. rivae* (Indigenous), *M. ruspoliana* (indigenous) and *M. stenopetala* (endemic) [7].

M. stenopetala is the second most important domesticated *Moringa* species after *M. oleifera*. It is endemic to East Africa, where it predominantly occurs in northern Kenya and southern Ethiopia. *M. stenopetala* is often called "cabbage tree" and is an important endemic vegetable in south western Ethiopia where it is cultivated as a food crop. The Gofa, Konso, Burji, and Gamo tribes consume its leaves as a vegetable, especially during the dry season [8].

The pods of *M. stenopetala* are also used as food in the rural area in east Africa especially in southern nation and nationality of Ethiopia. It is eaten after cooking like vegetable in addition to its use as soup. Despite the traditional use of this plant as food and medicine, the pods are not well explored for its chemical constituents and biological activities. Hence this thesis attempt to explore the chemical constituents and antibacterial activities of the pods extract of *M. stenopetala*. The chemical constituent of the leave extract of *M. stenopetala* was also studied.

1.2 Statement of the problem

Bacteria are listed at first position among the microorganisms causing opportunistic diseases. Bacterial infections are widespread throughout the world, especially in developing countries where in most cases lack of good sanitary practices are seen and primary health care programs are expensive making the fight against diseases difficult [9]. These diseases are the leading causes of death accounting for approximately one-half of all deaths in tropical countries. They are also becoming a significant problem in developed nations. It is estimated that infectious diseases are the underlying causes of death in 8% of the deaths occurring in the US [10]. Antimicrobial resistance (AMR) is a widely recognized and growing problem that causes over 700,000 deaths each year worldwide. At the same time, millions of people cannot access the antimicrobial medicines they need, despite having curable infections [11]. In recent decades, AMR has become widespread, irrespective of countries' level of income. In Europe, it has been estimated that 25,000 people die every year from antibiotic-resistant [12]. A recent report by the US Centre's for Disease Control and Prevention (CDC) conservatively estimated that at least 2 million illnesses and 23,000 deaths a year in the USA could be attributed to antibiotic resistance [13].

Especially in today; multidrug resistance in gram-negative bacteria (MDR-GNB) has become a particularly serious challenge for healthcare professional because of gram-negative bacteria (GNB) differ from Gram-positive bacteria with respect to the structure of the cell wall. These results make differences in the penetration and retention of chemical agents [14]. A survey of

recently reported antibiotics showed that 90% lacked activity against *Escherichia coli, Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter spp* [15, 16]. Therefore, development of new antimicrobials is among the proposed solutions to curb this problem. Regarding this; plants could provide a good alternative in search for new chemical agents with a wide-ranging antimicrobial activity. Among them *Moringa* has been used by various societies such as Roman, Greek, Egypt, India and many others tropical and sub tropical countries for thousands of years. Hence an attempt will be made to check the antibacterial activity against gram-negative and gram positive bacteria and isolate active antimicrobials from the pods extract of *Moringa stenopetala.* Furthermore the leaves extract was also be analyzed for its chemical constituents.

1.3 Significance of the study

The results from this work may help to arrive at new bioactive compound and it's may add new compound to natural product data base as well as it may give us an insight into the chemical profile of the pods of *M. stenopetala*. Similarly it may give information on docking study of the isolated compounds. Besides, it will be helpful for other researchers who will be interested in doing advance work on same topic.

1.4 Objectives

1.4.1 General objective

The main objective of this research is to assess the chemical constituents and antibacterial activities of pods and leaves extracts of *Moringa stenopetala.*

1.4.2 Specific objectives

- To successively extract the pods and leaves of *Moringa stenopetala* using n-hexane, EtOAc and MeOH
- To conduct phytochemical screening of the pods extracts of *M. stenopetala*
- To isolate compounds from pods and leaves of *Moringa stenopetala* extracts using chromatographic techniques including CC.
- To elucidate the structures of isolated compounds from the pods and leaves of *Moringa stenopetala* using various spectroscopic methods such as, IR, NMR.
- To evaluate the antibacterial activities the pods extracts of *Moringa stenopetala*
- To study the molecular docking analysis of the active sites of protein and the isolated compounds from the pods of *Moringa stenopetala*.

2 LITERATURE REVIEW

2.1 *Moringa*

Moringa is a tropical plant belonging to the family Moringaceae that grows throughout the tropics. About 33 species have been reported in the family *Moringa*ceae. Among those, thirteen species namely, *M. arborea*, *M. borziana*, *M. concanensis*, *M. drouhardi*, *M. hildebrandtii*, *M. longituba*, *M. oleifera*, *M. ovalifolia*, *M. peregrina*, *M. pygmaea*, *M. rivae*, *M. ruspoliana*, *M. stenopetala* are well known and found worldwide. Six species of *Moringa* occur in Ethiopia those are *M. borziana* (South Ethiopia), *M. lingituba* (Sidamo and Hararge), *M. oleifera* (introduced), *M. rivae* (Indigenous), *M. ruspoliana* (indigenous) and *M. stenopetala* (endemic [7, 17]. Almost all *Moringa* species are native to Africa, Arabia, South Asia, South America, Himalaya region, India, Pakistan, the pacific and Caribbean Islands [18].

2.2 *M. oleifera*

M. oleifera is the best known species of the genus widely cultivated in India. It has also been introduced throughout the tropics and subtropics and has become popular in many African countries. The plant is commonly named as Drumstick tree (from the appearance of the long, slender seed pods), Ben oil tree (from its pressed seed oil), and Horseradish tree (from the taste of the roots which resembles Horseradish or because its roots are used as horseradish substitute). *M. oleifera* is a small or medium sized tree, about 10 m high. The bark is white, grey or pale brown. The branches are fragile bearing two or three pinnate leaves. It is drought tolerant and grows best in the hot, semi-arid tropics with rainfalls of 250-1500 mm and an altitude of less than 1000 m [19].

2.3 *Moringa stenopatala*

M. stenopetala is the second most important domesticated *Moringa* species after *M. oleifera*. *M. stenopetala* is endemic to East Africa, where it predominantly occurs in northern Kenya and in Ethiopia. *M. stenopetala* grows in the low lands of West of the Rift Valley lakes from arid to semi-humid areas in the altitudinal ranged from 390-2200 m above sea level. The coverage includes South Ethiopia, North Kenya and East Somalia from 1–7°N and 35-42°E [20]. *M. stenopetala* known by different names including Cabbage tree (English), Shiferaw (Amharic) and by different vernacular names: Aleko (Gamo Gofa), Shalkayda (Konso), Haleko (Burji, Derashe), and Halakwa (Wollayta) [8]. *M. stenopetala* grows best in well-

drained soils, and it does not grow in waterlogged or swampy soils. It is a fast growing tree, well adapted to semi-arid areas with annual rainfall as little as 500 mm. It is quite drought tolerant & it has around 6-12 m tall with a diameter of 60 cm and a smooth bark. Its pods are elongated, reddish with grayish blooms and twisted when the fruit is fresh. It has a white flower important in honey production [21]. The leaves, seeds and pods of *M. stenopetala* are larger in contrast to *M. oleifera*.

M. stenopetala is a highly valued plant in Ethiopia indeed in the last few decades there have been a rapid increase in demand of this plant. It is cultivated as agro forestry in southern Ethiopia where the leaves are eaten as vegetables [22]. In Konso area, *Moringa* leaves are eaten almost every day like spinach together with cereal balls. It was reported that about 50% of the people in the Konso district of southern Ethiopia get their food from *M. Stenopetala.* People in the Gamo Gofa zone have a long tradition of consumption of *M. stenopetala*. The leaf of the *M. stenopetala* is eaten after cooking it like a cabbage. The flowers of *M. stenopetala* can be eaten or used to make tea [23, 24]. The seedcake powder is reported as a potential for water treatment through coagulation [25] with its water clarifying properties reported to be more effective than *M. oleifera.* Furthermore *Moringa* seedcake can also be used to reduce the incidence of water borne disease such as diarrheal diseases in which its effectiveness was related to the glucosinolate profile [26].

a. the leaves of *M. stenopetala*

b. the pods of *M. stenopetala*

Figure-1: *M. stenopetala* (leaves and pods) collected from Adama town, picture by Brehan A. (Nov. 8, 2017).

2.3.1 Biological activities of *Moringa stenopetala*

The various parts of *M. stenopetala* are extensively employed in Ethiopian, Kenyan and Somalia in traditional medicine for treating a range of illnesses such as, diabetes, hypertension, stomach pain, malaria, asthma, colds and wound healing [27]. Pharmacological reporting showed that the leaves of *M. stenopetala* showed antispasmodic [28], anti-hyperglycemic [29, 30, 31], antibacterial [1], and blood pressure lowering properties [32].

2.3.2 Antibacterial property

The methanol and n-hexane extracts of *M. oleifera* and *M. stenopetala* seeds as potential antimicrobial agents against some human pathogenic bacteria (*Salmonella typhii, Vibrio cholera,* and *Escherichia coli*) that are known to cause water-borne diseases [1]. The recent findings also indicated that the roots of *Moringa stenopetala* also have antibacterial properties. The acetone extracts of the roots of *M. stenopetala* showed antibacterial activity against *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*, and *Pseudomonas aeruginosa* [33].

Table-1: Antibacterial inhibition zones (mm) of crude extracts of root wood of *M. stenopetala*

Types of extract	*E. coli*	*P. aeruginosa*	*s. typhimurium*	*S. aureus*	*Cipro*
Pet. ether	10.0mm	12.5mm	13.5mm	12.0mm	31mm
Chloroform	-	-	-	-	33mm
Acetone	15mm	25mm	27mm	23mm	30mm
Methanol	10mm	12mm	-	-	32mm
Water	-	8mm	12mm	7mm	30mm

*Cipro = ciproflaxacin

Therefore, the seed extracts of *M. stenopetala and M. oleifera* shows that promising natural antimicrobial agents with potential applications in controlling bacteria that cause water-borne diseases. The acetone extracts of the roots of *M. stenopetala* also showed good antibacterial activity. More ever the seed of *M. stenopetala and M. oleifera* and roots of *M. stenopetala* we can use as traditional medicine to treat bacterial cause disease.

2.3.3 Blood pressure lowering

Hypertension or high blood pressure is a worldwide problem that affects approximately 15-20% of all adults. Hypertension known as silent killer as it showed no symptom.

Hypertension is associated with cardiovascular disease, insulin resistance, obesity and carbohydrate tolerance. Hypertension affects the structures and functions of small muscular arteries, arterioles and other blood vessels and can cause damage at variable rate to various target organs including kidney, brain and eye, related with the end stage of renal disease. Traditionally in the southern Ethiopia, People with high blood pressure boil the leaves and drink the water to get relief from their aliment. A recent report indicated that leaves *M. stenopetala* has blood pressure lowering effects. These researchers showed that crude aqueous leaf extract of *M. stenopetala* caused a significant drop in systolic blood pressure, diastolic blood pressure, and mean arterial blood pressure. Similarly, experiments conducted on animal models showed that butanol fraction of the ethanol extract of *M. stenopetala* leaves has anti-hyperglycemic and anti-hyperlipedemic effects, and as a result, can be used to treat diabetes [23, 33]. Therefore the leaves of *Moringa stenopetala* were reported to have good pharmacological properties including reduction hypertension and Blood pressure.

2.3.4 Anti-diabetic and cholesterol lowering property

Diabetes is a chronic metabolic disorder with impaired glucose tolerance and high risk of cardiovascular disease. The main symptom of DM is hyperglycemia, which leads to many complications classified in to “microvascular” and “macrovascular”. Microvascular complications, such as diabetic nephropathy and diabetic retinopathy, result from damages to the small blood vessels, whereas the macrovascular complications are caused by damages to arteries, leading to coronary artery and periphery artery diseases, and stroke. Basically, hyperglycemia is the result of relative insulin deficiency, insulin resistance or both [34, 35]. Leaf extracts of *M. stenopetala* are used to lower blood glucose and cholesterol levels. The aqueous leaf extract of *M. stenopetala* is shown to increase body weight and reduce serum glucose and cholesterol levels in mice. Serum glucose and serum cholesterol levels decreased significantly after six weeks of treatment [36].

2.3.5 Acute toxic effects of fraction of *M. stenopetala*

Because of the widespread use of *M. stenopetala* as a food and medicinal plant in the southern Ethiopia, It was evaluated for the potential toxicity in experimental animals. This study was, aimed to evaluating the effects of the butanol fraction of the leaves of *M. stenopetala* on behavior, general appearance, body weight, gross pathology of the liver and kidney in laboratory-bred rats. The acute toxicity study was conducted at the doses of 500, 1000, 2000, and 5000mg/kg body weight. Administration of the fraction of *M. stenopetala*,

up to the dose of 5000 mg/kg body weight, did not produce significant changes on the general appearance (condition of fur, general cleanliness) and behavioural profile (such as alertness, aggressiveness, irritability), as compared to the controls. Moreover, no death was observed in the treated groups up to the dose of 5000mg/kg during the 14 days of the experimental period. This indicates that the LD_{50} of the fraction could be greater than 5000mg/kg body weight. According to the privies report any compound or drug with the oral LD50 estimate greater than 1000 mg/kg could be considered low toxic and safe [37, 38].

2.4 Nutritional Profile of *Moringa*

Moringa is a super food tree with a remarkable source of proteins, calcium (Ca), Iron (Fe) and vitamin C. It contains the essential nutritional elements that are vital for human beings and livestock [39, 40]. In addition, it contains vitamin B, chromium, copper, magnesium, manganese, phosphorus and zinc. Being a rich source of proteins *Moringa* leaves are recommended by doctors, nutritionists and community health workers to cope with the problems of malnutrition worldwide [39, 41. The nutritional value of *Moringa* leaves is of utmost importance because a single gram of leaf powder is packed with 25 times the iron of spinach, 17 times the calcium of milk, 15 times the potassium of bananas, 10 times the vitamins of carrots, 9 times the protein of yoghurt, and 0.5 times the vitamins of oranges. Apart from vitamins and minerals *Moringa* leaves have been characterized to contain a desirable nutritional balance of minerals, amino acids, and fatty acids [39, 42]. Due to these assets, there are several *Moringa* supplements now available in the market [43]. However many pharmaceutical and herbal healthcare industries sell various *Moringa* products like *Moringa*-capsules, *Moringa*-tea, *Moringa*-oil, *Moringa*-soap, *Moringa* shampoo, etc. In general *Moringa* is important as human food because the leaves persist throughout the year, including the dry season when few other sources of green vegetables are available.

2.5 Water Purifying Properties of *M. stenopetala*

Water is a resource that is essential for life and it's required by almost every living organism. About 75% of the present world population lives in the developing countries of the world. About 1.2 billion people still lack safe drinking water and more than 6 million children die from diarrheal in developing countries every year [44]. Potable water is scarce in third world countries. Hence, the natural coagulants, such plant parts, rocks, sands and microorganisms are an alternative treatment for coagulation in the distillation process of turbid water and to remove microorganisms.

The seeds of *M. stenopetala* have natural flocculating properties, the experiments showed that whole crushed seeds of *M. stenopetala* were effective in removing turbidity from waters with high initial turbidity and bacterial contamination was reduced by 90 to 99.9%. *M. Stenopetala* seeds have better water purifying properties than *M. oleifera*. The active coagulating substances are found in the cotyledons of the seeds. A recent study also indicated that proteins extracted from the seeds of the *M. stenopetala* tree are effective flocculent for particles dispersed in water and are attractive as a natural and sustainable product for use in water purification [45, 46].

2.6 Review on Chemistry of the Genus *Moringa*

An extensive survey on the previous phytochemical studies on the genus *Moringa* resulted as the plant is rich in metabolites such as alkaloids, flavonoids, glycosides, phenolics, glucosinolates, isothiocyanate, steroids, triglycerides and fatty acids. So far, many compounds have been reported from *Moringa* species.

Previously reported amides include marumosides (**1**) and (**2**) from the leaves [47] and aurantiamide acetate (**3**) from the root of *M. oleifera* [48].

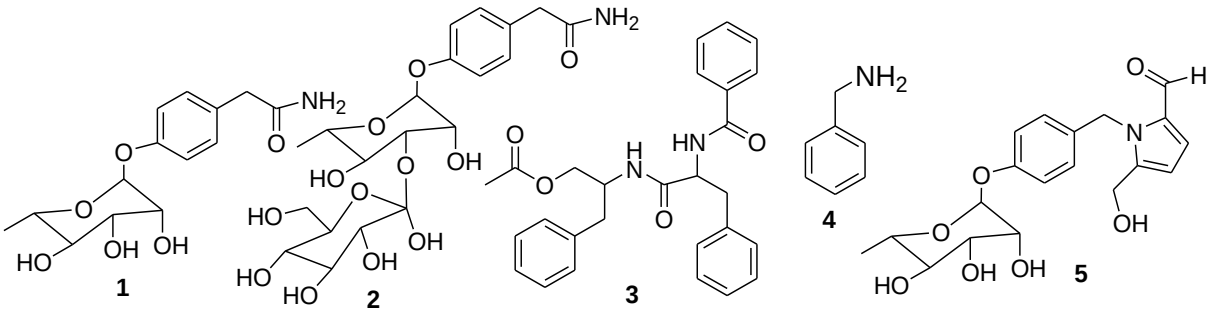


Figure-2: Amines, Amides and Alkaloids reported from *M. oleifera*

Alkaloids previously reported from *M. oleifera* include Moringine (**4**) and Moringinine. Glycoside of pyrrole alkaloid, pyrrolemarumine 4-O-α-L-rhamnopyranoside (**5**), was also reported from leaves of *M. oleifera* [52]. Among carbamates and thiocarbamate glycosides reported include (*E*)-*O*-cyano 4-(R-L-rhamnosyloxy)-benzenethiocarbamate (**6**), (*E*)-*O*-cyano 4-(O-acethyl-R-L-rhamnosyloxy)-benzenethiocarbamate (**7**), benzyl thiocarbamate (**8**) from pods of *M. oleifera*; 4-(*α*-L-rhamnopyranosyloxy) benzylcarbamate **(9**) from leaves of *M. oleifera* [47]; O-Methyl-4-(4'-O-acethyl-*α*-L-rhamnosyloxy)benzyl thiocarbamate (**10**) from

the seeds of *M. oleifera* [49; and benzylcarbamothioethionate (**11**) from the root bark of *M. oleifera*

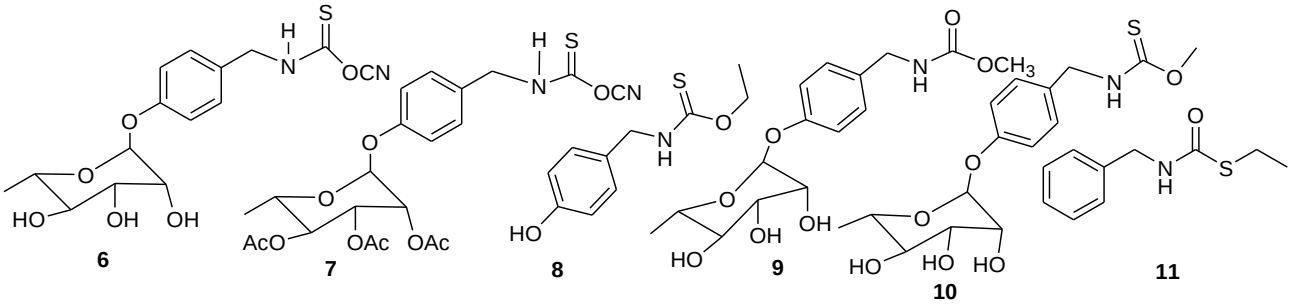


Figure-3: Carbamates and thiocarbamate glycosides reported from *Moringa*

Previously reported esters from the stem bark of *M. oleifera* include *n*-heptacosanyl n-octadec-9, 12, 15-trieneonate (moringyl linoleate) (**12**) and n-docas-4-en-11-one- 1-yl-n-decanoate (oleiferyl capriate) (**13**) [54]. The root of *M. stenopetala* was reported to have 1,3-dilinoleoyl- 2-olein (14) and 1,3-dioleoyl-2-linolein (**15**).

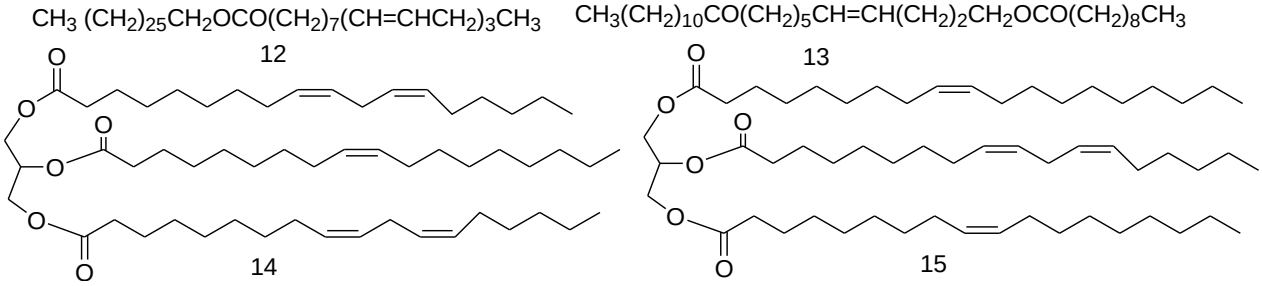
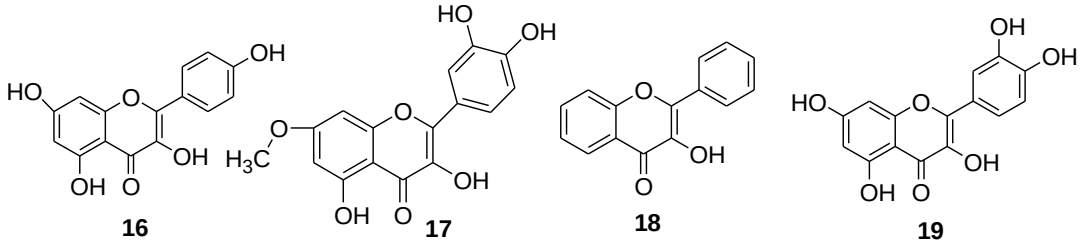
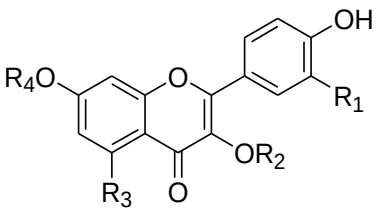


Figure-4: Esters reported from *Moringa*

Flavonoids reported from the leaves of *M. oleifera* include kaempferol (**16**), rhamnetin (**17**), 3-hydroxy-4-phenylchromen-4-one (**18**) and quercetin (**19**). Flavonoid glycosides previously isolated from *M. oleifera* leaves include isoquercitrin (**20**), kaempferitrin (**21**), D-(6''-O-malonyl)-glucoside (**22**), astragalin (**23**), and kaempferol 3-O-*β*-D-(6''-O-malonyl)-glucoside (**24**) [49]. The only flavonoid glycoside so far isolated from *M. stenopetala* was rutin (**25**)



[37]



20. R_1 = OH, R_2 = rham, R_3 = H, R_4 = rham
21. R_1 = OH, R_2 = Glu, R_3 = OH, R_4 = H
22 R_1 = OH, R_2= Glu-malonyl, R_3 = OH, R_4 = H
23. R_1 = H, R_2 = Glu, R_3 = OH, R_4 = H
24. R_1 = H, R_2 = Glu-malonyl, R_3 = OH, R_4 = H
25. R_1 = OH, R_2 = Glu-rham, R_3 = OH, R_4 = H

Figure-5: Flavonoids and Flavonoid glycosides reported from *Moringa*

Fatty acids such as palmitic acid (**26**), and oleic acid (**27**) were reported from the root wood of *M. stenopetala* along with *n*-octacosane (**28**) [35] while octacosanoic acid (**29**) was reported from the seeds of *M. oleifera*

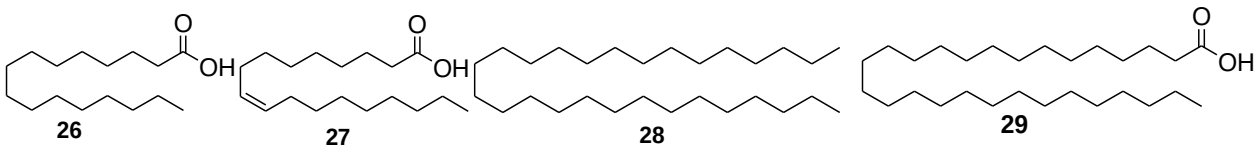


Figure-6: Fatty acids alkane reported from *Moringa*

4-(*α*-L-rhamnopyranosyloxy) benzyl glucosinolate (**30**) was reported from the seeds of *M. oleifera* and *M. stenopetala*. Isocyanate reported isothiocyanatomethylbenzene (**31**), 4-(4'-O-acethyl-α-L-rhamnosyloxy)-benzylisothiocyanate (**32**), 4-(*α*-L-rhamnopyranosyloxyl)-benzylisothiocyanate (**33**), 4-(*β*-D-glucopyranosyl-1→4-*α*-L-rhamnopyranosyloxy)-benzyl isothiocyanate (**34**) from seeds of *M. oleifera* [34, 54, 55, 50] with compound **32** also reported from the fresh leaves of *M. stenopetala* leaves. Furthermore 4-(4'-O-acethyl-*α*-L-rhamnosyloxy)-benzaldehyde (**35**) was also reported from fresh leaves of *M. stenopetala* [50].

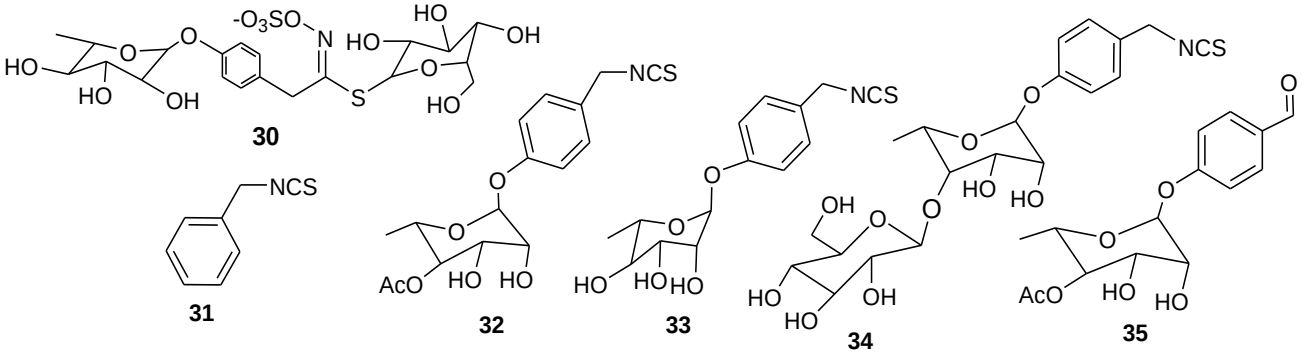


Figure-7: Other compounds reported from *Moringa*

Nitriles such as niazirin (**36**), niaziridin (**37**) [56] and nitrile ester (**38)** were previously reported from *M. oleifera* pods [48, 51, 52]. 4-Hydroxyphenyl acetic acid (**39**) was phenolic compound reported from seeds of *M. oleifera* [49].

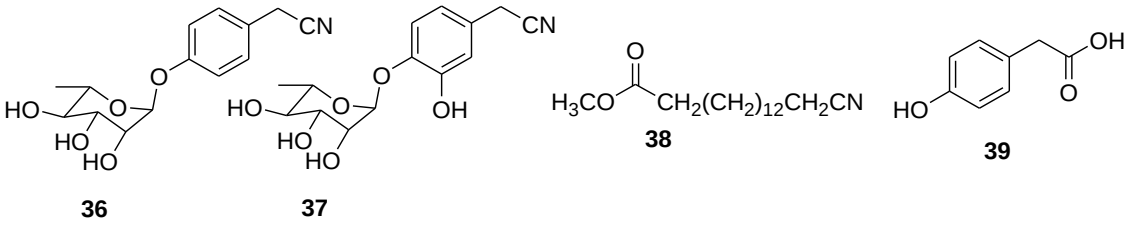


Figure-8: Nitriles and Phenolic compounds reported from *Moringa*

Quinic acid derivatives previously reported from the leaves of *M. oleifera* include 4-O-(4-O-*α*-D-glucopyranosyl) caffeoyl quinic acids (**40**) and 4-O-(3-O-*α*-D-glucopyranosyl) caffeoyl quinic acids (**41**), chlorogenic acid (**42**) and 4-O-caffeoyl quinic acid (**43**), 5-O-caffeoyl quinic acid (**44**) [53].

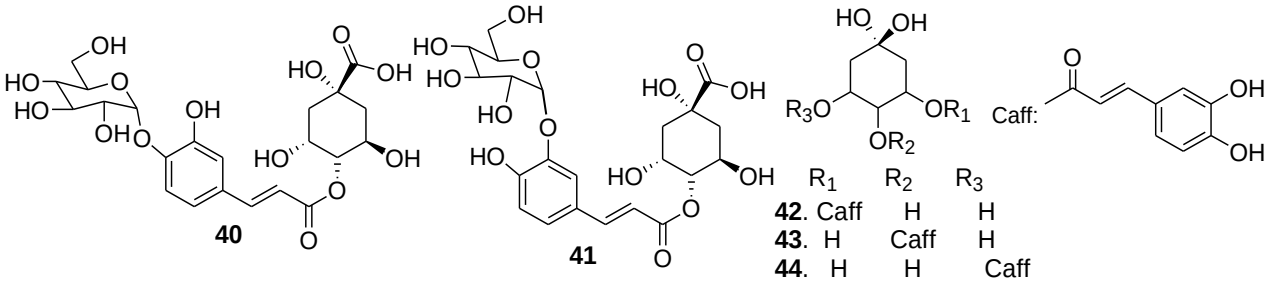


Figure-9: Quinic acid derivatives reported from *Moringa*

Another secondary metabolites reported from the genus *Moringa* are steroids such as *β*-sitosterol (**45**) from the root wood of *M. stenopetala* [35] and 3-*O*-(6'-*O*-oleoyl-*β*-D-glucopyranosyl)-*β*-sitosterol (**46**), and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside from the seeds of *M. oleifera* (**47**) [54].

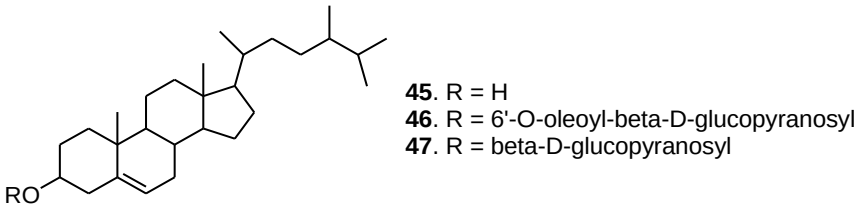


Figure-10: Steroids reported from *Moringa*

3 MATERIALS AND METHODS

3.1 Materials/Apparatus

The materials/ apparatus used in this study include beaker, measuring cylinder, funnel, filter paper, aluminum foil, stirrer, crusher, spatula, pencil, labeler, ruler, scissor, TLC plat, Autodock - 4, PTLC plat, Vials, Column, NMR, IR, UV and capillary tube.

Silica gel (250–400 mm mesh size) and TLC plate (silica gel, visible in UV254 and 365 and pre-coated on aluminium sheets) were used for chromatographic analyses. Compounds on TLC plates were detected using UV spectroscopy, iodine and vanillin chambers. The pure compounds evaporate the solvents at room temperature to make solid compound. The pure solid compounds interpret with IR and NMR spectroscopy. Infra red (IR) data were recorded as KBr pellets using Perkin-Elmer BX spectrometer (400–4,000 cm^{-1}). Nuclear magnetic resonance data (1H-NMR, 13CNMR, and DEPT-135) were recorded using Bruker Avance 400 MHz spectrometer.$CDCl_3$ and DMSO was used as solvent in nuclear magnetic resonance (NMR) analyses. All NMR and IR analyses were carried out at Addis Ababa University.

3.2 Chemicals

The chemicals/reagents listed herein were used for extraction, isolation and identification; n-hexane (99%) AR, ethyl acetate (99.5%) AR, chloroform (95%) AR and 25% ammonia solution were purchased from Sigma-Aldrich. Methanol (99.8%) HPLC grade and acetone (90%) AR was purchased from Alfa chemicals. Ethanol (97%) reagent was purchased from Ethiopia. Distilled water was used from ASTU chemistry laboratory. Iodine, vanillin powder and silica gel also used for identification and purification technique.

3.3 Plant material collection, authentication and preparation

The fresh pods and leaves of *Moringa stenopetala* were collected from Adama town in November 8/2017. The plant was authenticated by Mr. Wege Abebe and voucher specimen B001 and deposited at the National Herbarium of Addis Ababa University. The pods were cleaned by using a clean cotton cloth to remove the dust and other foreign particles. Then it was made into pieces by using scissors and dried in an open air at room temperature. The dried sample was powdered using mortar and finally packed properly and placed in fridge.

3.4 Methods

3.4.1 Extraction

The pods of *Moringa stenopetala* (500 g) was successively extracted each with 2 L n-hexane, EtOAc and MeOH at room temperature for 72 hours on maceration. Each extract was filtered using filter paper (Whitman No.1) and concentrated under reduced pressure on rotary evaporator. The waxy residues obtained from each extracts were further dried at room temperature and analyzed with TLC.

Likewise the leaves (80 g) were extracted successively each with 0.5 L of n-hexane, EtOAc and Methanol following similar extraction procedure as above

3.4.2 Isolation

The EtOAc extract (3.6 g) of the pods of *M. stenopetala* was adsorbed and subjected to silica gel column chromatography with n-hexane: EtOAc and EtOAc:MeOH of increasing polarities as eluent to furnish 45 fractions, each 50 mL was collected. Similar fractions were combined based on similarity of their TLC profiles.

Table- 2: CC fractions of EtOAc extract of the pods of *M. stenopetala.*

Fractions	Eluent (Ratio)	Amount obtained in mg	Remark
Fr1-4	100% n-hexane	60 mg	One spot (Compound 28)
Fr5	n-hexane/ EtOAc (9:1)	45 mg	Mixture
Fr6 &7	n-hexane/ EtOAc (9:1)	38 mg	Three spots
Fr8-10	n-hexane/ EtOAc (8:2)	24 mg	Two spots
Fr11	n-hexane/ EtOAc (8:2)	19.9 mg	Mixture
Fr12-14	n-hexane/ EtOAc (7:3)	51 mg	Mixture
Fr15&16	n-hexane/ EtOAc (6:4)	20 mg	One spot (Compound 27)
Fr17&18	n-hexane/ EtOAc (1:1)	23 mg	One spot (Compound 48)
Fr 19&20	n-hexane/ EtOAc (1:1)	13.3 mg	Two spot
Fr21 & 22	n-hexane/ EtOAc (4:6)	43 mg	Mixture
Fr23	n-hexane/ EtOAc (3:7)	15 mg	Two spots
Fr24	n-hexane/ EtOAc (2:8)	6 mg	Two spots
Fr25	n-hexane/ EtOAc (2:8)	5.6 mg	three spots

Fr26	n-hexane/ EtOAc (2:8)	11 mg	Mixture
Fr27 & 28	n-hexane/ EtOAc (1:9)	200 mg	Three spot
Fr29	100% EtOAc	10.38 mg	two spot
Fr30 & 31	EtOAc/MeOH (9:1)	15 mg	Three spots
Fr32	EtOAc/MeOH (8:2)	11 mg	Two spots
Fr33 &34	EtOAc/MeOH (7:3)	19 mg	Five spots
Fr35 & 36	EtOAc/MeOH (6:4)	8 mg	Two spots
Fr37	EtOAc/MeOH (1:1)	24 mg	Three spots
Fr38	EtOAc/MeOH (4:6)	12 mg	Three spots
Fr39 & 40	EtOAc/MeOH) (3:7)	33 mg	Six spots
Fr41 &42	EtOAc/MeOH (2:8)	17 mg	Four spots
Fr43 & 44	EtOAc/MeOH (1:9)	21 mg	Two spots
Fr45	100% MeOH	9 mg	Two spot

Three compounds were isolated from the pods of *M. stenopetala* labeled as compound 27, 28 and 48 which were shown to have one spot on TLC and hence subjected to IR and NMR.

An attempt was also made to isolate compounds from the leaves of *M. stenopetala*. In this regard, the extracts were analyzed with TLC. The methanol extract showing good TLC profile was suspended in n-hexane followed by EtOAc. The insoluble solid (compound 25) was shown to have one spot on TLC and hence subjected to NMR.

3.4.3 Phytochemical Screening

Phytochemical screening of the pods extracts of *Moringa stenopetala* was conducted to investigate the presence of alkaloids, flavonoids, anthraquinones, Saponins and phenolics for qualitative detection by the following procedures [33].

- **Alkaloids**

Alkaloids test were determined based on Wagner's test: 2 mg/mL of the extracts were measured and added into test tube with Wagner's reagent (1.27 g of iodine and 2 g of KI along within 100 mL of distilled water). Reddish brown colored precipitate indicated the presence of alkaloids in the pods extracts of *M. stenopetala*.

- **Phenolics**

Phenolics were detected using the ferric chloride test: 1mg/mL of each extracts was replaced in test tube and 1mL of 5% $FeCl_3$ reagent added in to the extract and does not appeared deep

blue color therefore the pods extracts of *M. stenopetala* does not contain phenolics compounds.

- **Flavonoids**

From each extracts 0.5 mg/mL were added in to 10 mL of distilled water and it was treated with concentrated H_2SO_4 and the solution was shaken properly. The formation of orange color was indicated the presence of flavonoids in the pods extracts of *M. stenopetala*.

- **Anthraquinones**

From each extracts 2 mg/mL were added in to 10 mL of benzene and shaken properly; the mixture was filtered and 5 mL of 10 % ammonia solution was added in to the filtered solution, and then shaken properly. But doesn't show or observed any pinkish color during the experiment therefore, the absences of anthraquinones in the pods extracts of *M. stenopetala***.**

- **Saponins**

Saponins were detected using the froth test: 1.5 mg/mL of the each extracts were dilute with 2 mL of distilled water in a test tube and the mixtures were vigorously shaken and left to stand for 10 min and it's show development of foam on the surface of the mixture this indicates the presence of saponins in the pods extracts of *M. stenop.*

3.4.4 Characterization

The structures of isolated compounds were characterized using spectroscopic methods such as IR and NMR.

3.4.5 Antibacterial Activity

3.4.5.1 Disc Diffusion Method

The antibacterial activity the pods crude extract of *M. stenopetala* was determined using by disc diffusion method on Muller Hinton agar (MHA) medium against gram negative bacteria such as *E. coli, Klebsiella Pseudomonas and shigella boyidi* and gram positive bacteria *Staphylococcus aureus and Streptococcus pneumonia* and the Muller Hinton Agar medium was weighed as 37 gm and dissolved in 1000 mL of distilled water. Then the medium was kept for sterilization at 121^{o}C for 20 min. After sterilization the media was poured in to sterile petri dish and it was allowed to solidify. After the medium solidification, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The extract was prepared with different solvent in different concentration solution(2 µg of n-hexen extract, 10 µg of ethyl acetate extract and 4 µg of methanol extract) then using a Kirby

power disc diffusion techniques, a 20µL from each extract was added on a 6mm diameter filter paper disk including a control(positive control: 15µg of erythromycin, negative control distill water) and incubated for 18hrs at 37^{o}C to allow the bacterial growth and the antibacterial activity read by measuring the diameter of the zone of inhibition. The antibacterial potential of the different extracts was evaluated by comparing their zones of inhibition [33].

3.5.5 Docking studies

The two dimensional structure of confirmed organic compound was drawn and the structure was 3D optimized after adding hydrogen atoms using ACD / Chemsketch. The crystal structure of protein of selected bacterial was downloaded from Protein Data Bank (PDB). The active site of the receptor and protein of the selected bacteria which was used in the antibacterial test was obtained from the PDB. Molecular docking analysis was carried out to find the binding affinity of the ligand-organic compound for the active sites of protein. Docking analysis was carried out using Autodock - 4. Onto an autodock-4 platform, the tertiary structure of protein was loaded for the purpose of docking studies to find out the potential binding sites.

4 RESULTS AND DISCUSSION

4.1 Extraction yield

The pods of *M. stenopetala* on extraction with n-hexane, EtOAc and MeOH furnished 9 g (1.8 %) yellow solid, 4.5 g (0.9 %) black solid and 18.7 g (3.74 %) red dark jell solid respectively. The data obtained revealed that the pod contains mainly polar secondary metabolites.

The leaves of *M. stenopetala* the extraction performed with n-hexane, EtOAc and MeOH furnished 3.6 g (4.5 %) yellow solid, 6.2 g (7.75 %) green solid and 11 g (13.75 %) pale yellow jell solid respectively. The leaves extracts also contains mainly polar secondary metabolites.

4.2 The qualitative phytochemical analysis of the pods extracts of *Moringa stenopetala*

This study reveals that *Moringa stenopetala* pods shows the presence of phytochemical constituents like alkaloids, saponins and flavonoids, in different solvents extracts as shown in Table-3. Alkaloids are naturally occurring chemical compounds that often have pharmacological effects and used as medications and recreational drugs [55]. Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes [56].

Table- 3: Qualitative phytochemical analyses of the pods extracts of *M. senopetala.*

Solvents used for extraction	Alkaloids	Phenolics	Flavonoids	Anthraquinones	Saponins
n-hexane	-	-	-	-	+
EtOAc	-	-	+	-	+
MeOH	+	-	+	-	+

(+) indicates presence while (–) indicates the absence of the components

Literature report shows that the phytochemical screening of *Moringa oleifera* seeds the hexane, ethyl acetate and methanolic extracts contain alkaloid, glycoside, flavonoid, saponin, steroidal ring, tannin and reducing sugar [57]. The recent report also shows that the phytochemical screening of *Moringa oleifera* leaves the ethanol and water extracts contain alkaloid, flavonoid, saponin, steroid, tannin and volatile oil [58]. The pods extracts of *M.*

stenopetala also contain alkaloid, flavonoid and saponin (Table 3). Therefore the pods also contain important secondary metabolites which are powerful against various human diseases.

4.3 Structure elucidation of compounds

In the course of this work, three compounds from the pods and one compound from the leaves of *M. stenopetala* were isolated. Compound 27 and 28 were obtained as a white solid and compound 48 was obtained as a white needle solid from the pods of *M. stenopetala*. Compound 25 was isolated as pale yellow solid from the methanol leaves extract of *M. stenopetala*. Herein are the structure elucidations of the isolated compounds using ^{1}H-NMR, ^{13}CNMR, DEPT-135 and IR spectra.

Compound 28

Compound 28 was isolated as a white solid from the EtOAc extract of the pods of *M. stenopetala* eluted from the column with 100% n-hexane. The IR spectrum (KBr) (Appendix1) indicates medium peaks at 1466 cm^{-1} ($-CH_2$) or C-H stretch and the weak band at 721 cm^{-1} indicates C-C stretching of an alkane. The ^{1}H-NMR spectrum (Appendix 2, Table 4) of compound 28 exhibited a peak at δ 0.92 (3H, *t*) indicate the presence of terminal methyl ($-CH_3$) group. The peak at δ 1.28 ppm indicates the presence of many overlapping methylene protons. The ^{13}C-NMR spectrum (Appendix 3, Table 5) of compound 28 showed peaks solely in the aliphatic region alone. In view of this the signal at 14.1 ppm is due to methyl ($-CH_3$) while the signal in the range between 22.7 to 31.9 ppm is as a result of methylenes ($-CH_2$) groups. The DEPT-135 NMR spectrum (Appendix 4, Table 5) shows the presence of CH_3 and CH_2. Therefore the observed data suggested that compound 28 is a long chain alkane named as n-octacosane whose structure is shown in Figure 11 (Table 4 and 5).

Table-4: ^{1}H-NMR data of compound 28

Position		^{1}H-NMR data	Multiplicity	Reference of ^{1}H, [59, 60, 61]
1	H-1 & H-28	0.92	(6H, *t*, *J* = 6.4 Hz)	0.91
2	H-2 - H-27	1.28	(52, br, s)	1.28

Table-5: ^{13}C-NMR and DEPT-135 NMR data of compound 28

^{13}C-NMR data		^{13}C-NMR	DEPT-135	^{13}C-NMR reference [59, 60, 61]
1	C-1 & C-28	14.1	CH_3	14.1
2	C-2 & C-27	22.7	CH_2	22.7
3	C-3 & C-26	31.9	CH_2	31.9
4	C-4 & C-25	29.3	CH_2	29.4
5	C-5 - C-24	29.6	CH_2	29.6

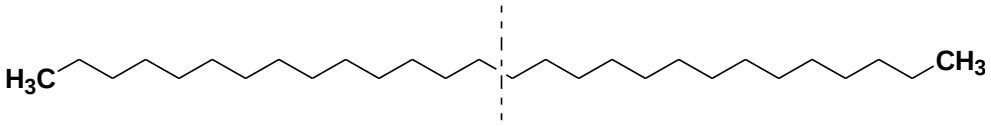


Figure 11: Chemical structure of n-octacosane

Compound 27

Compound 27 was obtained as a white solid from the EtOAc extract of the pods of *M. stenopetala* which was eluted with n-hexane and ethyl acetate in the ratio 6:4. In the IR spectrum (KBr) (Appendix 5) the band at 2900 cm^{-1} indicates the C-H stretch on the olefinic carbon and medium band at1700cm^{-1} indicates the presence of C=O stretch of carbonyl groups.The IR spectrum further revealed a broad band centred at 3425 cm^{-1} likely accounting for the that indicates the presence of carboxyl group.

The ^{1}H-NMR spectrum (Appendix 6, Table 6) of compound **27** showed peak at δ 0.99 (3H) due to the presence of protons of methyl (-CH_3) groups; the peak at 1.32 & 1.63 ppm indicates protons of aliphatic methylene (CH_2) group while a peak at 2.02 ppm (H-8 and H-11) indicates presence of protons of a methylene group that is bonded to C=C bond; the peak at 2.36 ppm indicate of protons of methylene that is bonded to a carbonyl group; the peak at 2.77 ppm indicates presence of protons of methylene group that is flanked by two C=C bonds where as the peak at 5.4 ppm indicates presence of olefinic protons in the structure 27.

In the analysis of ^{13}C-NMR spectrum (Appendix 7, Table 6), the peaks at 130.0 and 129.7 ppm indicated the presence of C=C bond in the compound; a single peak at 180.1 ppm

indicated a quaternary carbon signal of carbonyl carbon of carboxylic acid. On the other hand, the chemical shift values in the range of at 14.0 to 34.0 ppm indicates the presence of methyl (-CH_3) and methylene (-CH_2) carbons. The DEPT-135 spectrum (Appendix 8, Table 6), showed a single peak for the presence of methyl (-CH_3) carbon at 14.0 ppm, methylene carbons at 22.7 to 34.0 & olefinic methine carbons.

Table- 6: ^{1}H- NMR, ^{13}C- NMR and DEPT-135 NMR data compound **27**

Position	**^{1}H-NMR**	**^{1}H-NMR Lit.**	**^{13}C-NMR**	**DEPT-135**	**^{13}C-NMR reference [62, 63]**
1	-	-	180.1	Quaternary C	180.1
2	2.36	2.36	34.0	CH_2	34.2
3	1.63	1.63	24.6	CH_2	24.6
4	1.26	1.27	29.1	CH_2	29.1
5	1.25	1.27	29.2	CH_2	29.0
6	1.26	1.27	29.4	CH_2	29.4
7	1.32	1.32	29.6	CH_2	29.6
8	2.04	2.02	27.1	CH_2	27.1
9	5.36	5.34	129.7	CH	129.7
10	5.36	5.34	130.0	CH	130.0
11	2.02	2.02	27.2	CH_2	27.2
12	1.26	1.27	29.5	CH_2	29.7
13	1.26	1.27	29.3	CH_2	29.3
14	1.26	1.27	29.1	CH_2	29.5
15	1.32	1.32	29.2	CH_2	29.2
16	1.32	1.32	31.9	CH_2	31.9
17	1.26	1.27	22.7	CH_2	22.7
18	0.99	0.88	14.1	CH_3	14.1

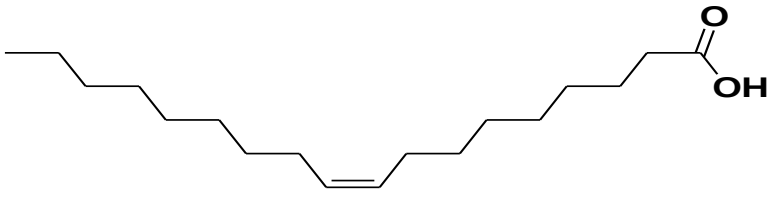


Figure-12: Structure of oleic acid isolated from pods of *M. stenopetala*

Compound 48

Compound 48 was obtained as a white needle solid when the column was eluted with n-hexane and ethyl acetate in the ratio 1:1. The IR spectrum (KBr) (Appendix 9) shows a broad band at 3400 cm^{-1} that indicates the presence of O-H stretching. The absorption band at 1075 cm^{-1} signifies indicate the presences of cycloalkane while the band at 2957 cm^{-1} is assigned to C-H structure. The weak and sharp band at1380 cm^{-1} and 1618 cm^{-1} indicate the presence CH_2 and CH=CH stretching, respectively.

The ^{1}H NMR (δ ppm, DMSO) (Appendix 10, Table 7) spectrum of compound **48** showed the presence of two methyl singlet's at δ 0.71, and 1.03; three methyl doublets that appeared at δ 0.80, 0.82, and 0.91; and a methyl triplet at δ 0.83, Protons at δ 4.98, 5.14, and 5.31 suggesting the presence of three protons corresponding to that trisubstituted and disubstituted olefinic bond. The proton corresponding to the H-3 of a sterol moiety was appeared as a triplet of doublet of doublets at δ 3.51.The ^{13}C-NMR (δ ppm, DMSO) (Appendix 11 Table 7) spectrum has shown recognizable signals at 140.7 ppm and 121.7 ppm which are assigned C5 and C6 double bonds, respectively. The value at 19.0 ppm corresponds to angular carbon (C-19) 138.3 ppm for C-20 and 129.2 ppm for C-21.The DEPT-135 spectrum (Appendix 12, Table 7) spectrum show twenty nine carbon signal including six methyls, nine methylenes, eleven methine and three quaternary carbons.

Table-7: ^{1}H- NMR, ^{13}C-NMR and DEPT-135 NMR data of compound 48

Position	**^{1}H-NMR**	**Multiplicity**	**^{13}C-NMR**	**DEPT-135**	**^{13}C-NMR reference [64]**
1			37.2	CH_2	37.6
2			33.9	CH_2	32.1
3	3.54	(*tdd*, 1H *J*=4.5, 4.2, 3.8 Hz)	71.8	CH	72.1
4			42.3	CH_2	42.4
5			140.7	Quaternary C	141.1
6	5.37	(*t*, 1H, *J* = 6.1 Hz)	121.7	CH	121.8
7			31.7	CH_2	31.8
8			31.8	CH	31.8
9			50.1	CH	50.2
10			36.5	Quaternary C	36.6
11			21.2	CH_2	21.5
12			39.8	CH_2	39.9
13			42.2	Quaternary C	42.4
14			56.8	CH	56.8

15			24.3	CH_2	24.4
16			29.1	CH_2	29.3
17			56.0	CH	56.2
18			40.4	CH	40.6
19	0.94	(*d*, 3H, *J*=6.2 Hz)	21.2	CH_3	21.7
20	5.04	(*m*, 1H)	138.3	CH	138.7
21	5.16	(*m*, 1H)	129.3	CH	129.6
22			45.8	CH	46.1
23			25.4	CH_2	25.4
24	0.86	(*t*, 3H, *J* =7.1 Hz)	12.0	CH_3	12.1
25			29.1	CH	29.6
26	0.84	(*d*, 3H, *J* = 6.6 Hz)	19.8	CH_3	20.2
27	0.82	(*d*, 3H, *J* = 6.6 Hz)	19.4	CH_3	19.8
28	0.69	(*s*, 3H)	19.0	CH_3	18.9
29	1.00	(*s*, 3H)	12.0	CH_3	12.2

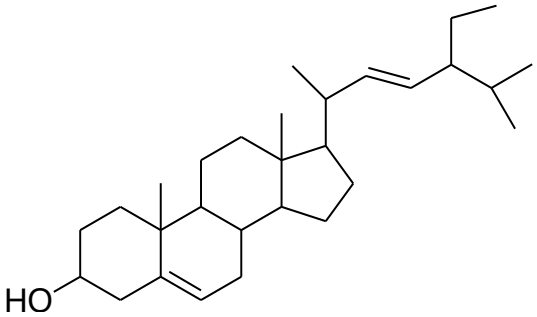


Figure-13: Chemical structure of stigmasterol

Compound 25

Compound 25 was obtained as a pale yellow solid. ^{1}H NMR (δ ppm, DMSO) (Appendix 13 Table 8); ^{1}H-NMR: A singlet peak at 12.60 ppm indicates the signal of 5-OH group. The ^{1}H-NMR spectrums also showed a peak at 7.56 ppm; these were assigned to H-2’ and H-6’. One proton doublet at 6.84 ppm corresponds to H-5’ while one proton doublets at 6.18 and 6.38 ppm were assigned to meta related H-6 and H-8 respectively. Many peak appeared between 3.50-3.06 (m, 10H sugar) ppm which indicates the presence of a sugar moiety. The presence of L-rhamnose was indicated by 6”’ –CH_3 at 0.95 ppm and H-1”’ at 4.38 (s) ppm; it is connected to ß-D-glucose, whose H-1” appears as a doublet at 5.34 ppm. The absence of a singlet in the 6.40-7.10 ppm region strongly suggests that C-3 is substituted. Other proton signals were also observed at 6.18 (1H, *d*, *J* = 2 Hz, H-6), 6.38 (1H, *d*, *J* = 2 Hz, H-8), 6.88 (1H, *d*, *J*= 8 Hz, H-5’), 7.63 (1H, *d*, *J* = 8 Hz, H-6’) and 7.72 (1H, *d*, *J* = 2 Hz, H-2’).

Table 8: ^{13}C-NMR and DEPT-135 NMR data of compound 25

Position	^{13}C-NMR	DEPT-135	^{13}C-NMR reference [65, 66]
2	156.8	Quaternary C	156.5
3	133.7	CH	135.7
4	177.8	Quaternary C	179.4
5	161.6	Quaternary C	163.3
6	99.1	CH	100.0
7	164.5	Quaternary C	166.0
8	94.0)	CH	95.0
9	157.0	CH	100.4
10	104.4	Quaternary C	105.7
1'	122.0	CH	123.7
2'	116.6	CH	117.8
3'	145.2	Quaternary C	145.9
4'	148.8	Quaternary C	148.8
5'	115.6	CH	116.1
6'	121.6	Quaternary C	123.2
1"	101.6	CH	104.8
2"	74.5	CH	75.8
3"	76.3	CH	77.2
4"	70.4	CH	71.5
5"	76.8	CH	78.2
6"	67.4	CH	68.6
1"'	101.1	CH	102.5
2"'	70.8	CH	72.3
3"'	71.0	CH	72.2
4"'	72.2	CH	74.0
5"'	68.7	CH_2	69.8
6"'	18.1	CH_3	18.0

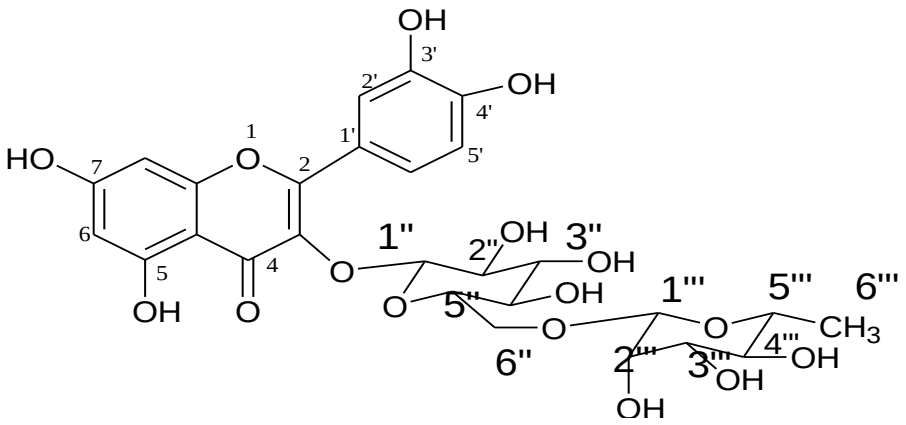


Figure 13: Chemical structure of rutin

Antibacterial activity the pods extracts of *Moringa stenopetala*

The antibacterial activity of n-hexane, ethyl acetate and methanol extracts were investigated using disk diffusion method, against the selected bacterial species such *as S. aureus, S. pneumonia, E. coli, K. Pseudomonas* and *Shigella boydii.* All the examined extract showed varying degrees of antibacterial activities against bacteria.

Table 9: Antibacterial activity of n-hexane, ethyl acetate and methanol the pods extracts of *Moringa stenopetala* medicinal plants against gram positive and gram negative spp.

		Inhibition zone (mm)				
Plant	**Extracts**	***S. aureus***	***S.pneumonia***	***E. coli***	***K. Pseudomonas***	***Shigella boyidi***
M. stenopetala	n-hexane 2µg/mL	6	18	6	13	6
M. stenopetala	EtOAc 10µg/mL	13	10	6	6	6
M. stenopetala	MeOH 4µg/mL	13	10	11	14	9
Methanol		6	6	6	6	6
EtOAc		6	6	6	6	6
n-Hexane		6	6	6	6	6
Erytomycine positive control 15 µgm		25	25	6	6	6
Distilled Water negative control		6	6	6	6	6

Where: 3= MeOH extract, 2 = EtOAc extract and 1= n-hexane extract

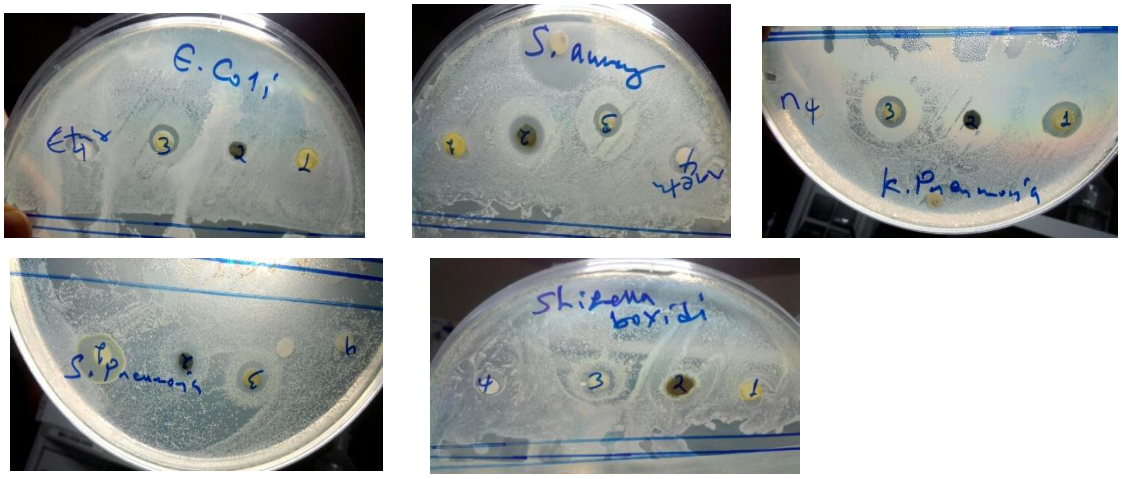


Figure-14: Antibacterial test of pods crude extracts of *M. stenopetala*

The antimicrobial effect of many medicinal plants is well documented and the results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant. In this study, using the disk diffusion method, it was observed that the pods crude extracts of *M. stenopetala* produce antibacterial activity against both Gram negative and Gram positive bacterial. The result from table 9 showed that, the pods crude extract of *M. stenopetala* showed good antibacterial activity against Gram positive and Gram negative bacterial. The antibacterial activity of n-hexane extract of *Moringa stenopetala* showed zone of inhibition at 18 and 13 mm against *S. pneumonia* and *K. Pseudomonas* with the 2 µg/mL concentrations respectively. In the other hand the n-hexane extract did not showed any zone of inhibition against *Staphylococcus aureus, E. coli* and *Shigella boydii*. Therefore the n-hexane extracts were active against the selected bacteria species.

The antibacterial activity of ethyl acetate extract showed zone of inhibition at 13, 10 mm which inhibit the growth of *S. aureus* and *S.* pneumonia within 10 µg/mL concentration respectively. The ethyl acetate extracts didn't show any zone of inhibition against *E. coli, K. Pseudomonas* and *Shigella boydii*. Therefore the ethyl acetate extract was only inhibit the growth of Gram positive bacteria while for Gram negative bacteria showed lowest activity or does not inhibit the growth of any Gram negative bacteria at all. The antibacterial activity of methanol extract also showed zone of inhibition at (9, 10, 11, 13 and14 mm) and inhibit the growth of *Shigella boydii, S.* pneumonia, *E.* coli, *S. aureus* and *K. Pseudomonas* within 4 µg/mL concentration respectively. Therefore this indicates that methanol extract shows good activity against Gram negative and Gram positive bacteria than n-hexane and ethyl acetate extract against the given bacteria species *S. Pneumonia, E.* coli, *K. Pseudomonas, Shigella boydii* and *S. aureus.* In other word, methanol extracts have more potential to extract active compounds than n-hexane and ethyl acetate extract.

previously reported literature shows that the antibacterial activity of leaves extract of *Moringa olifera* against four bacteria spp., the ethanol and water extract showed higher antibacterial activity against *S. aureus, S. typhii* (11, 8)mm & (13, 6)mm respectively. But Chloroform extract did not show any antibacterial activity at all. In addition to this, it was also reported that the antibacterial activity of root barks of *M. oleifera* against four bacterial species. Ethyl acetate extracts showed higher antibacterial activity than chloroform extract against *P. Aeruginosa and S. aureus* with inhibition zone of (18,12)mm and (11,8)mm

respectively. Based on the above report the pods extracts of *M. stenopetala* have good potential to inhibit the growth of bacterial compared to the root barks extract and leaves extract of *M. oleifera.* Therefore the pods extracts of *Moringa stenopetala* have almost similar antibacterial activity within leave & root barks extracts of *M. oleifera.* However, the pods extracts of *M. stenopetala* we can use as alternatives medicinal plants instead of the leave & root barks extracts of *M. oleifera.*

In the other hands; the antibacterial activity of seed, leave, stem bark and root barks of *M. stenopetala* was analyzed against four bacteria spp. this study shows that the Seeds of *M. stenopetala* methanol extract showed inhibition zone at (18.66, 11.66 and 14.66)mm against *S. aureus, E. coli and S. boydii* respectively. *The* leaves extract of *M. stenopetala* within methanol, Chloroform and ethyl acetate didn't show any inhibition zone against all bacteria spp. Stem barks of *M. stenopetala* methanol and ethyl acetate extracts shows inhibition zone at(13.33, 11.00)mm respectively against *S. aureus.* The root bark of *M. stenopetala* ethyl acetate extracts have zone of inhibition at(16.00, 13.00, 15.33)mm against *S. aureus, E. coli* and *S. boydii* respectively. Therefore the pods extracts of *M. stenopetala* compared with reaming parts *M. stenopetala* (leaves, seeds, stem bark and root bark); the pods have almost similar activity within seed and root bark of *Moringa stenopetala* and more active than the leave and steam barks. This indicates that the pods extracts of *M. stenopetala* we can use as alternative traditional medicine in study of the seed and the root of *M. stenopetala* in addition it's more potential than root bark and leaves of *M. stenopetala* based on the above reports.

4.4 Stigmasterol Molecular Docking Study

1) Protein (Receptor) -The 3D structure of protein is downloaded is from PDB.

A ligand database for target proteins in this study was downloaded from protein data bank (PDB). Initial databases did not include the activity (sit) data. After downloading the pdb format of the protein, remove the solvent and water molecules to make computational molecular matching easier and clear the binding pocket. After removing the water molecule it is to easy search and creates multiple favorable contacts to the protein of molecular docking.

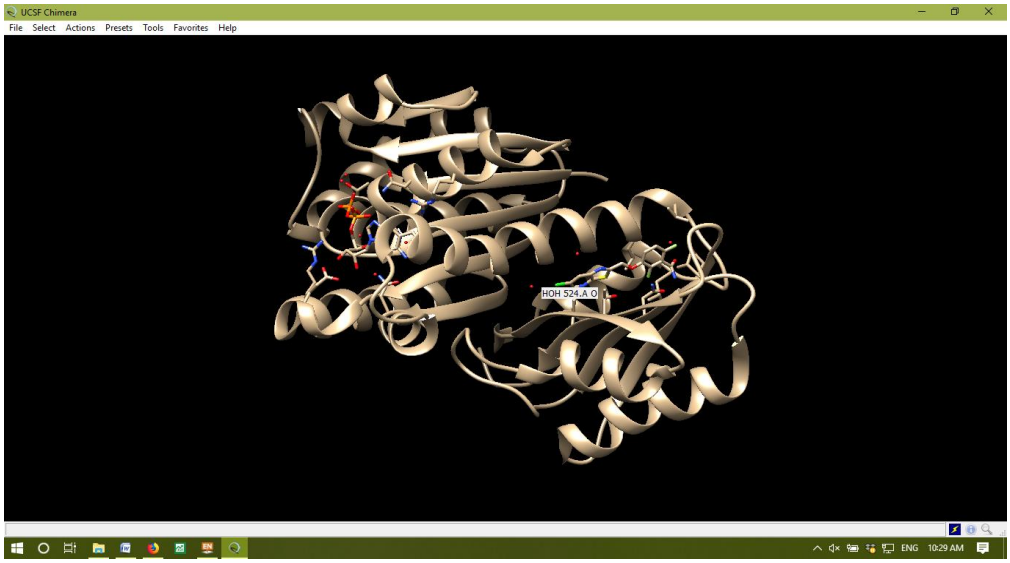


Figure-15: The receptor protein

2nd protein-ligand activity

Both the protein and the ligand are treated as a rigid structure and this is the simplest and quickest method. Both of these approximations constitute major simplifications of the real environment in which ligands and proteins interact.

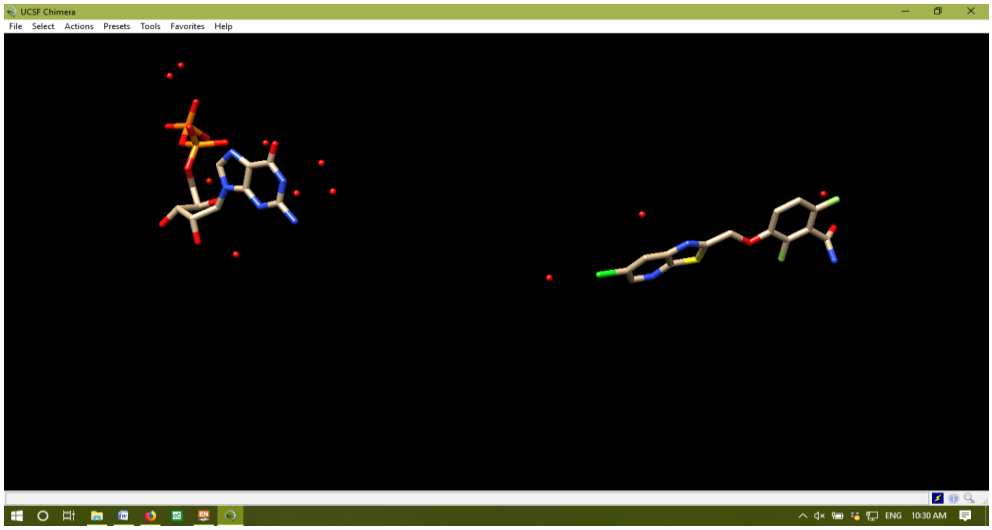


Figure-17: Ligand and protein structure.

3rd Energy minimization

First, minimize the energy of fragment size ligands with a single rotatable bond as part of a protein mapping method developed for the identification of binding hot spots. Second,

consider energy minimization for docking a rigid ligand to a rigid protein receptor, an approach frequently used in existing method. It shows the hydrogen bond is favorable to this ligand. The studies presented in this thesis have to a large extent with describing and quantifying hydrogen bonds between molecules. Drugs are typically small molecules that interact with proteins in our bodies by binding to important areas on or inside the protein.

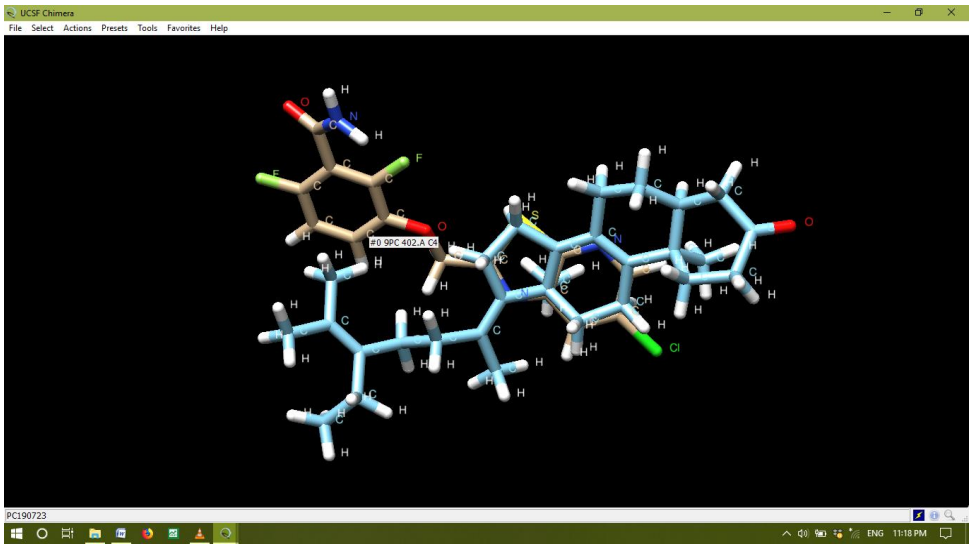


Figure-18: Ligand minimization energy.

Molecular matching of docking requires a 3D structure of the ligand and protein as input. Typically, the software was generating 3D conformations of the ligands and optimizes their interactions with the protein by computing the binding affinity between the two.

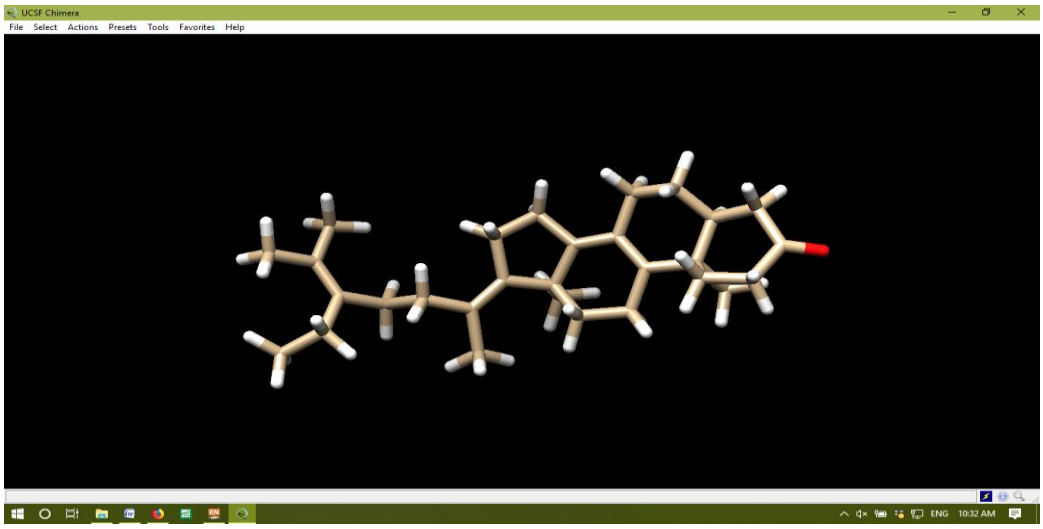


Figure-19: 3Dstructure of stigmasterol.

4th Molecular matching ligand and protein

This step is very important to know either the ligand is used as alternative drug or not. The protein –ligand maching below show good bindinig abilitiy.

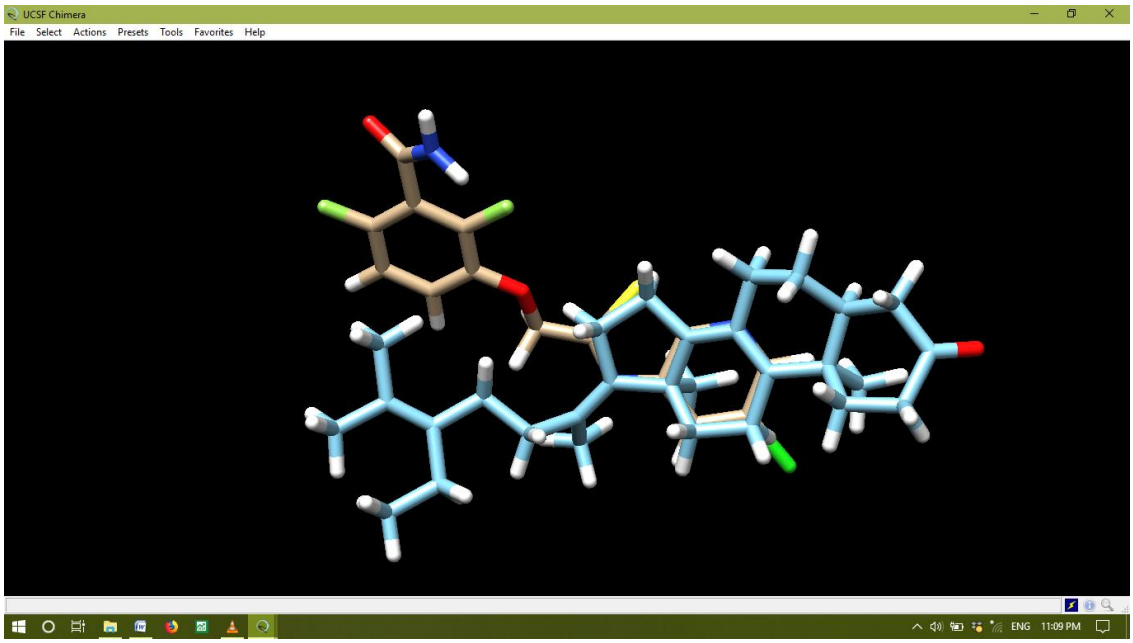


Figure-20: Molecular matching of stigmasterol with protein activation ligand.

Binding affinity experiments performed based on quantum mechanics or molecular mechanics the factors that affect binding between proteins and small ligands. Common interactions found between ligands and proteins have been assembled in the overall ligand protein binding affinity. The molecular matching studies of this ligand with protein show 60% matching with protein activation ligand. The stigmasterol was performed against S. *aureus bacteria* based on the antibacterial activity and it show molecular matching with protein activation ligand. Therefore the drug designing from the stigmasterol will be doing effectively against S. *aureus* bacteria case disease. In general molecular docking method has been used to predict potent drug molecules in order to inhibit growth of bacteria cells.

5 CONCLUSION

In this study the phytochemical investigation has shown the presence of alkaloid, flavonoid and saponin in *M. stenopetala*, which justifies the contribution of the plant to folk medicine. Three compounds isolated from the pods of *M. stenopetala* (two compounds are long chain fatty acid and one sigmasterol).

The pods extracts of *Moringa stenopetala also* showed good antibacterial activity against the selected bacteria species; especially the methanol extract had good activity for all selected bacteria. Based on the inhibition zone *S.* pneumonia was containing the highest zone of inhibition and followed by *K. Pseudomonas*. The common observation that Gram-negative bacteria are more resistant to many compounds than Gram-positive but in this research the methanol extract of the pods of *Moringa stenopetala* showed good inhibition zone against all Gram negative bacteria. This is one positive attributes of this plant. Therefore, *M. stenopetala* could be used as a good source of useful drugs for treatment of various ailments and could also be recommended as a food supplement due to its medicinal potential.

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site and an optimization problem, which would describe the “best-fit” orientation of a ligand that binds to a particular protein of interest. Molecular docking approaches have been applied in the identification of novel bioactive compounds. In drug discovery for one target protein million of compounds can be screening therefore, this methods able to screen large compound databases at low cost, time-saving and cost-effective. In generally Molecular docking has been able to identify promising compounds that might represent future solutions in critical areas of human health.

6 RECOMMENDATION

Based on these findings the following are recommended;

- The antibacterial testing was conducted on limited number of bacteria. therefore, the same work should be carried out on large variety of fungal and bacterial strains so as to have a clear picture of the spectrum of antimicrobial activity of the herbal drugs
- Investigation of antioxidant property also important.
- Finally I recommended to further study for the reaming biological activity like anticancer, antivirus and anti-malaria activity its needed additional study.

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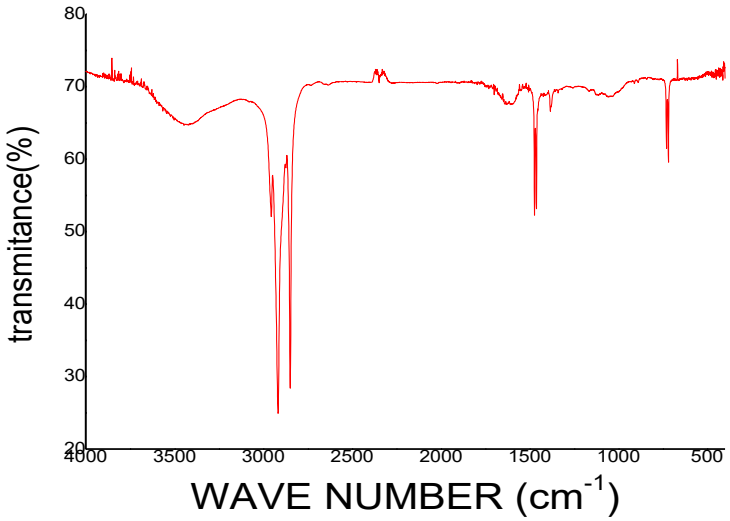
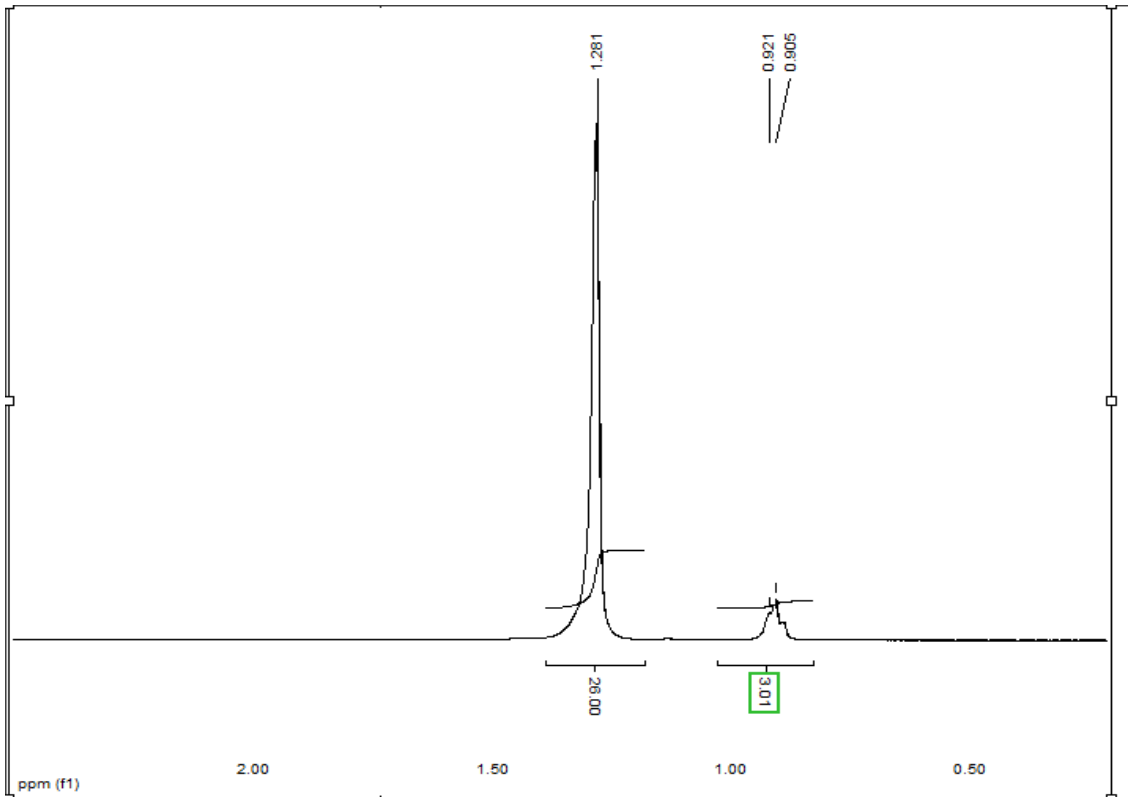
APPENDIXES

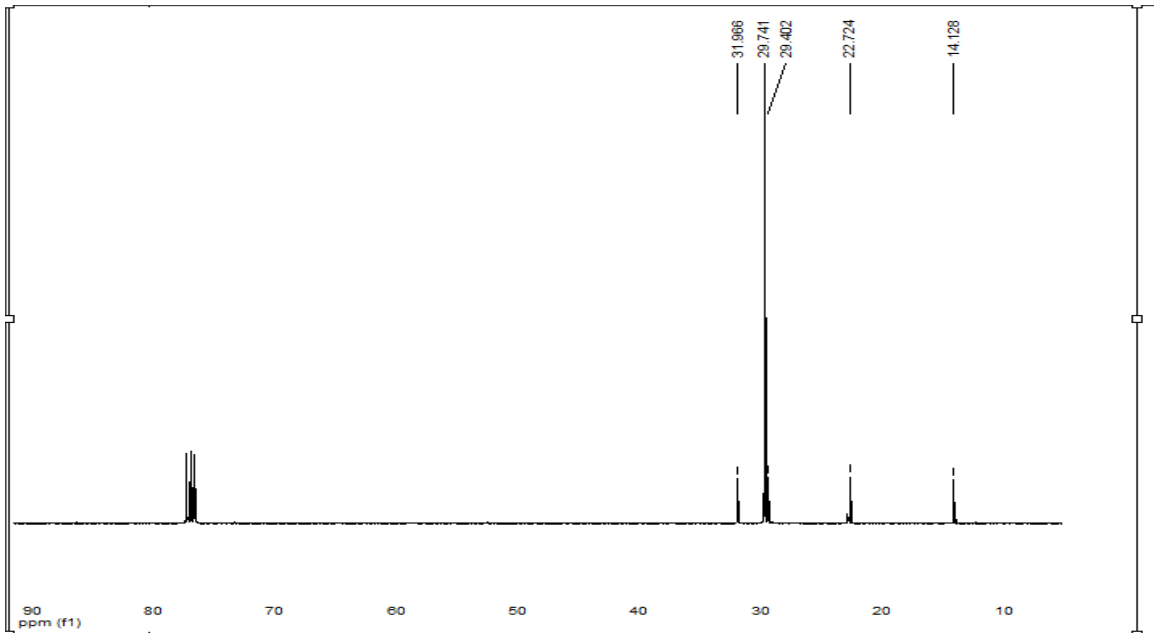
Compound 28



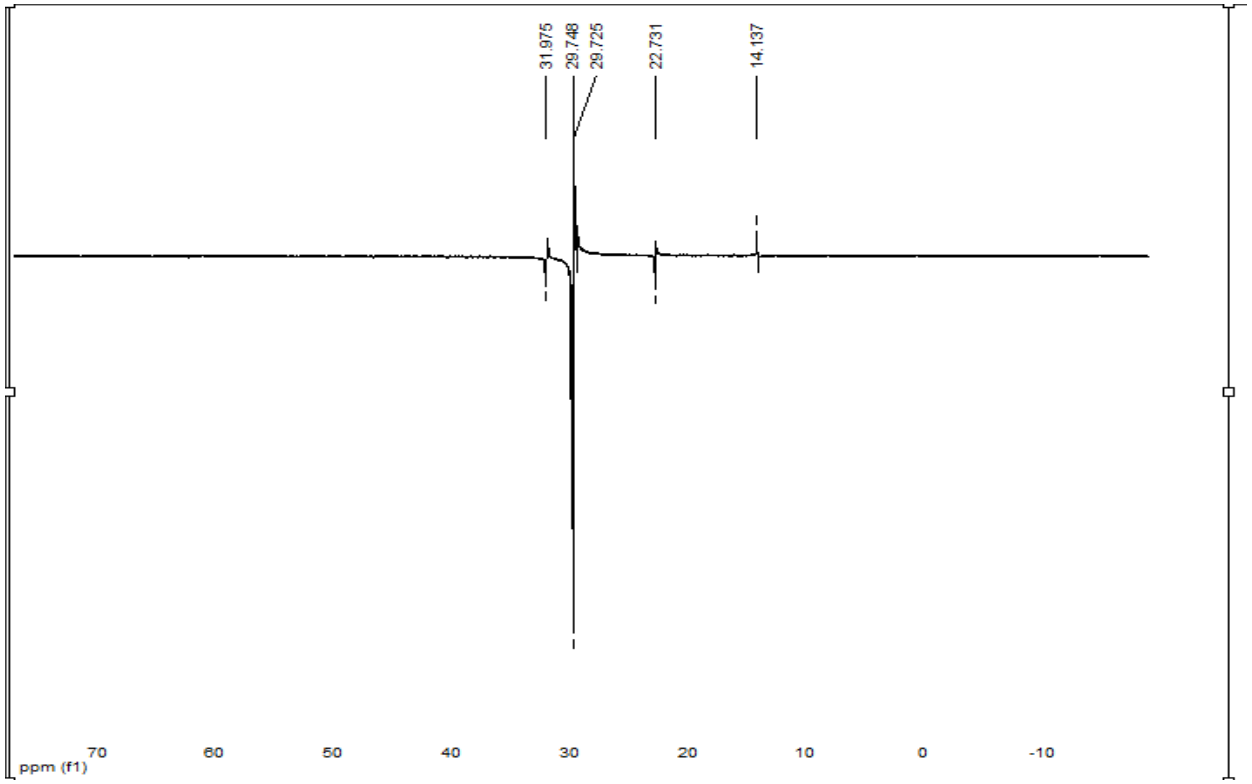
Appendix 1: IR spectrum of compound 28



Appendix 2: [1] H-NMR spectrum of compound 28

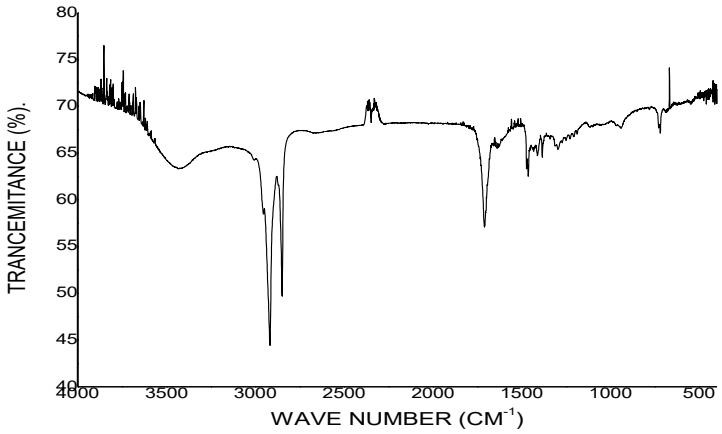


Appendix 3: ^{13}C- NMR spectrum of compound 28

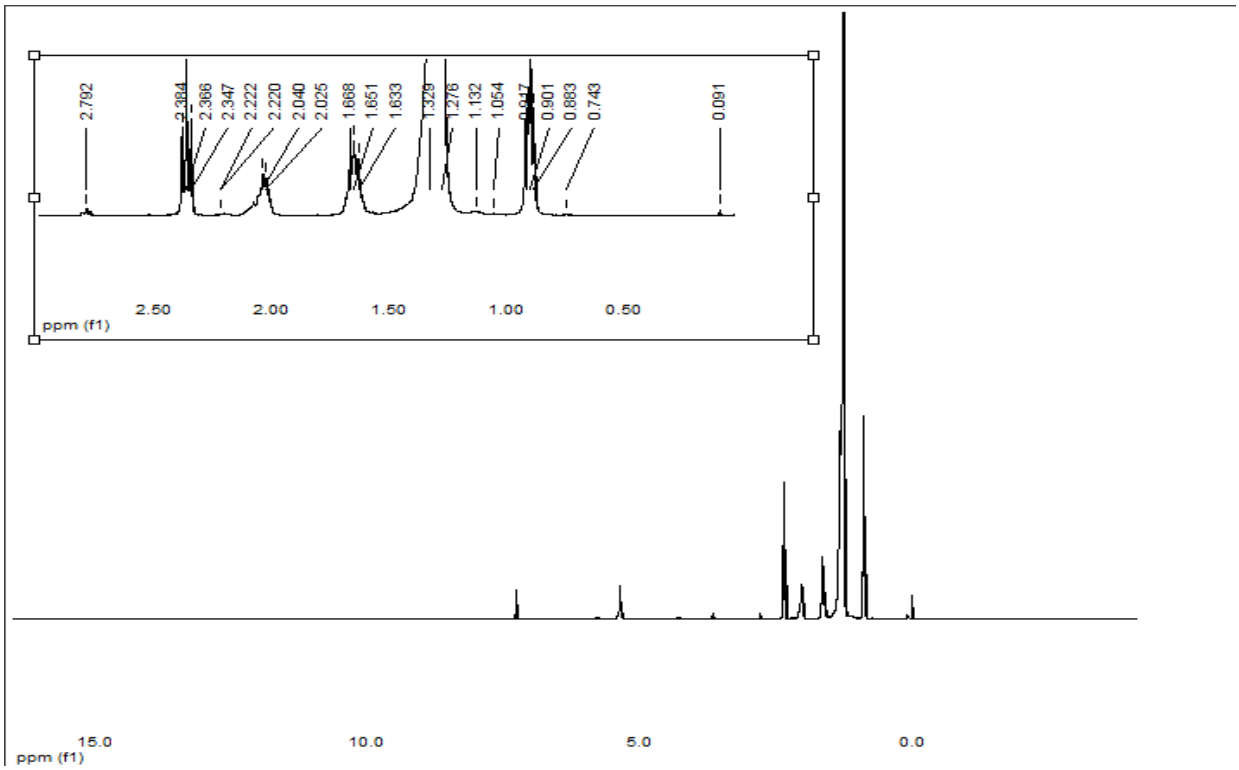


Appendix 4: DEPT-135 NMR spectrum of compound 28

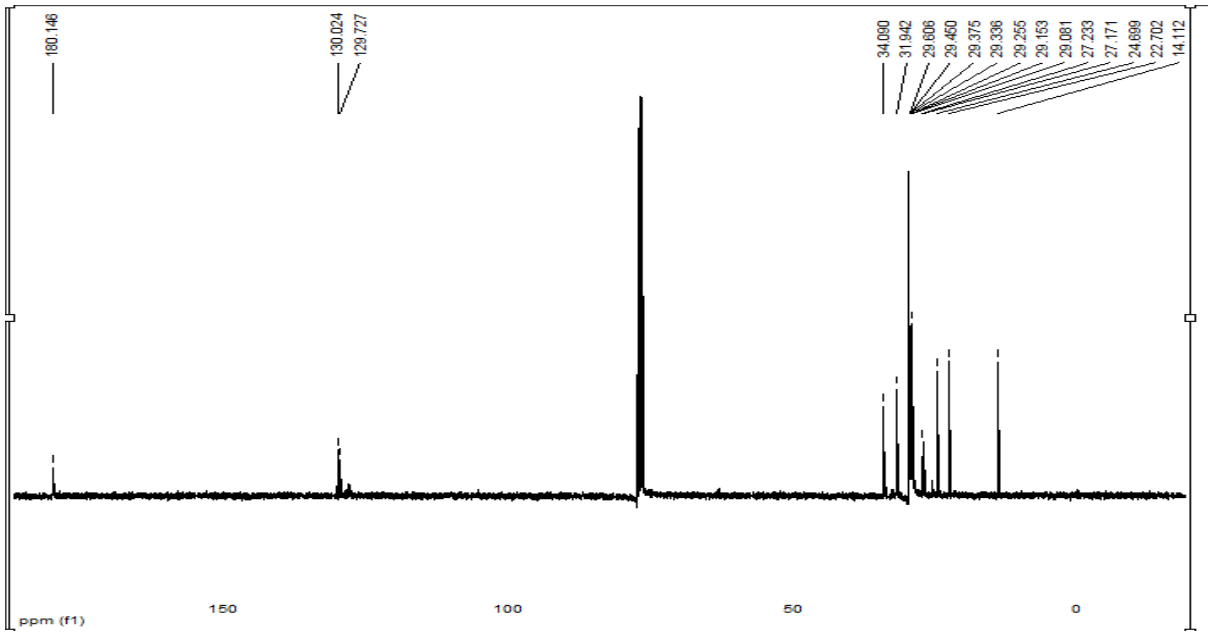
Compound 27



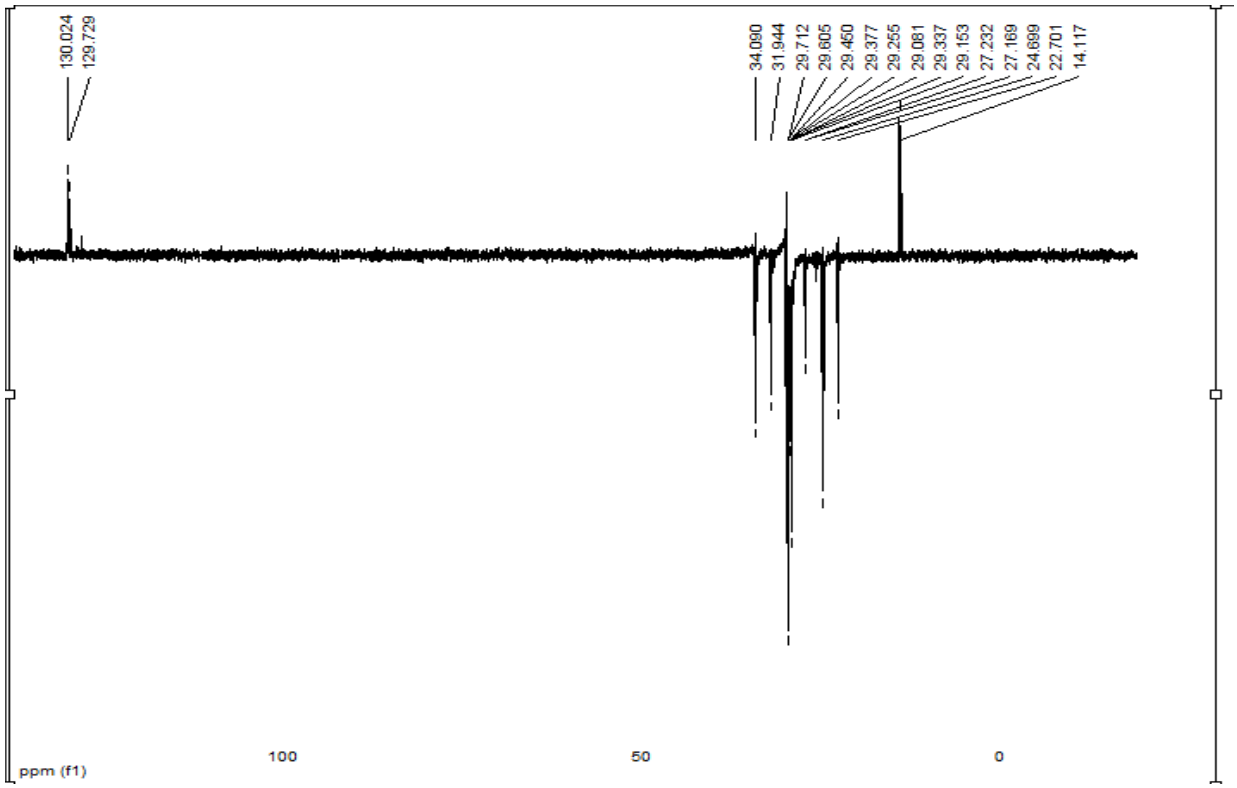
Appendix 5: IR spectrum of compound 27



Appendix 6: ^{1}H-NMR spectrum of compound 27

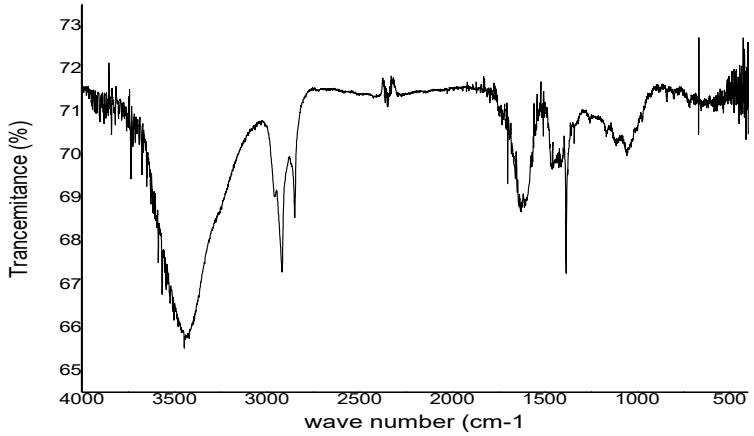


Appendix 7: ^{13}C-NMR spectrum of compound 27

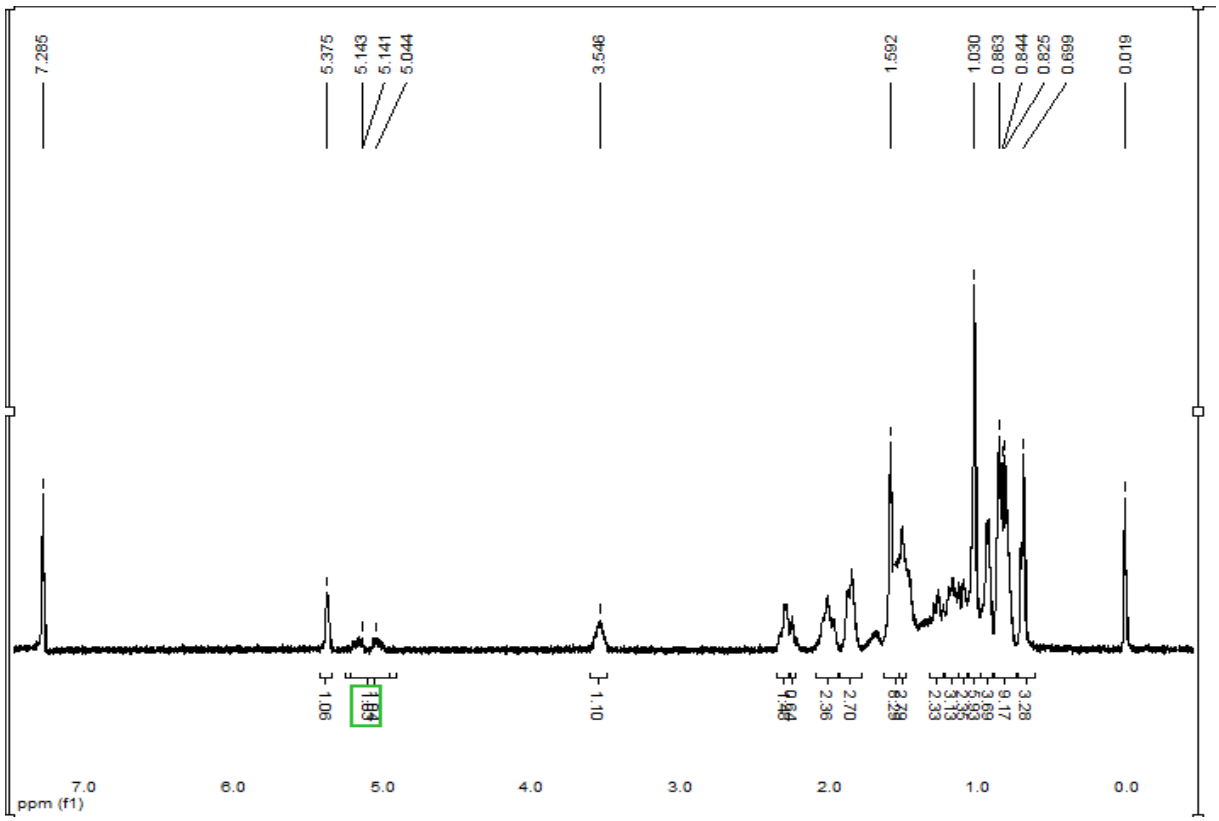


Appendix 8: DEPT-135 NMR spectrum of compound 27

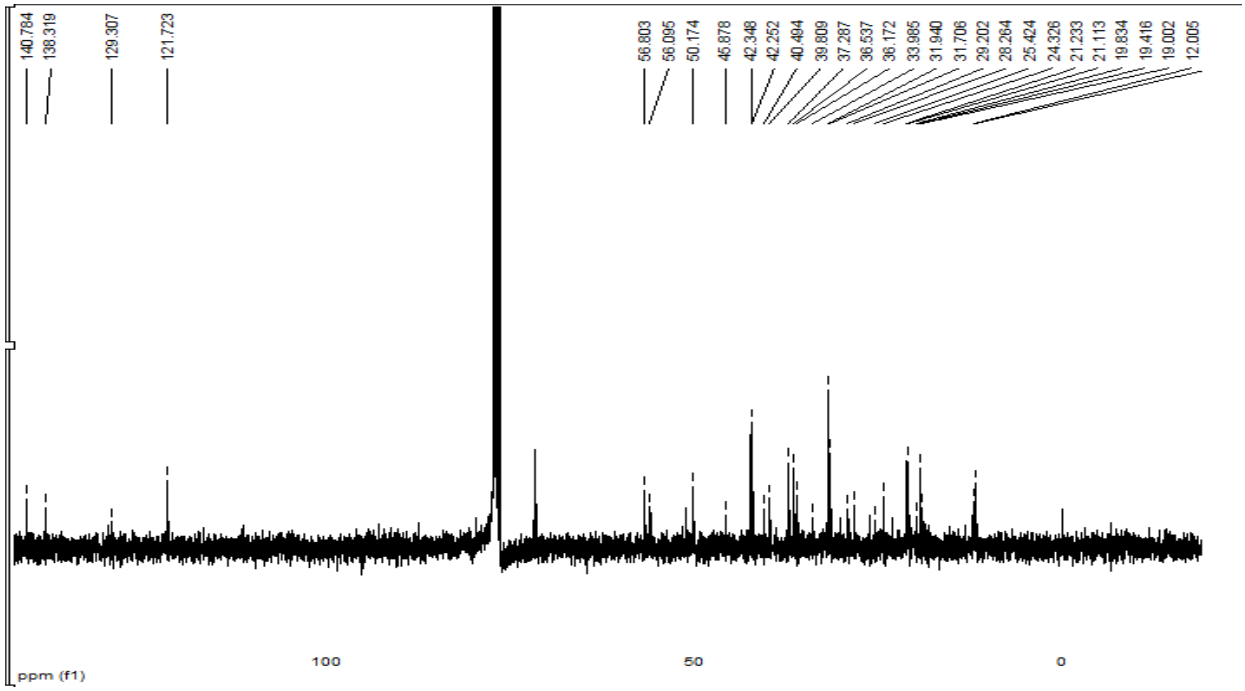
Compound 48



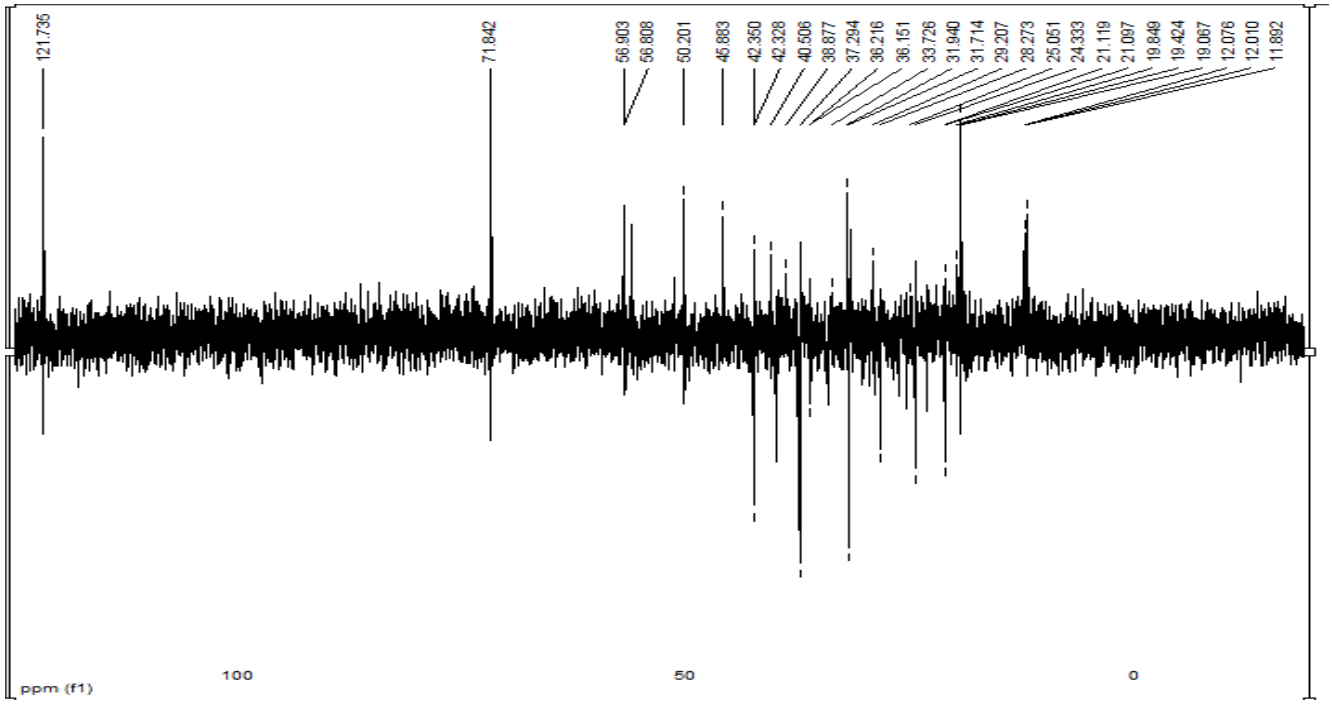
Appendix 9: IR spectrum of compound 48



Appendix 10:[1] H-NMR spectrum of compound 48

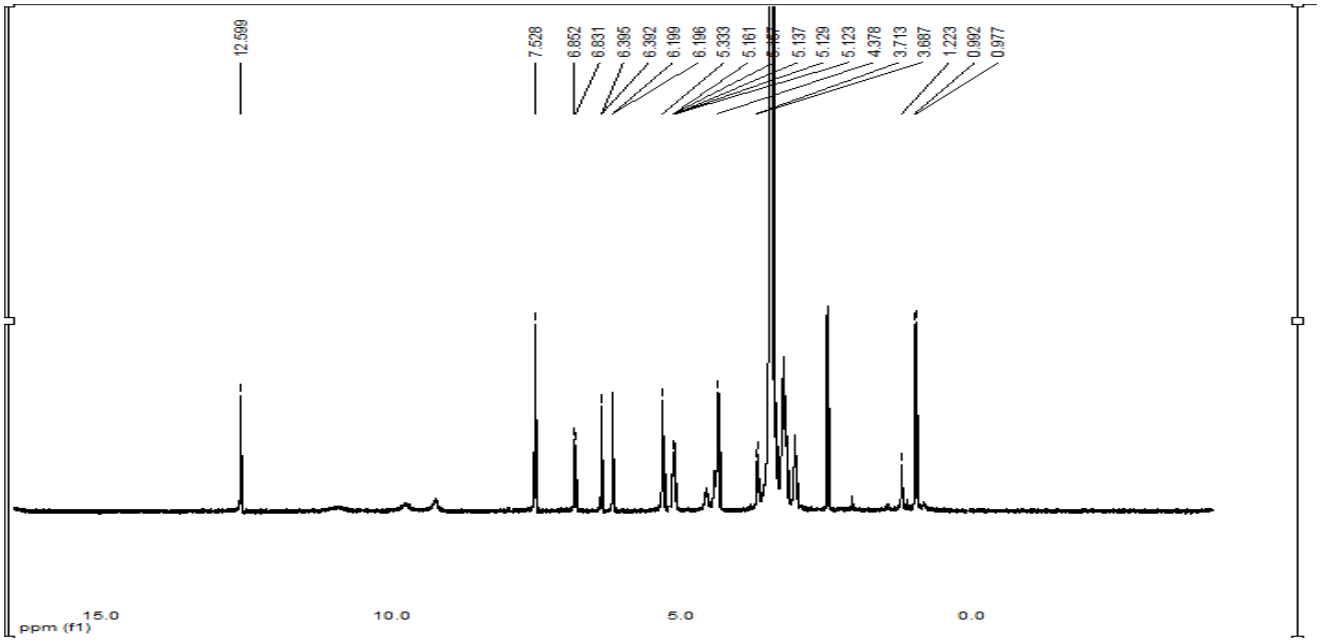


Appendix 11: ^{13}C-NMR spectrum of compound 48

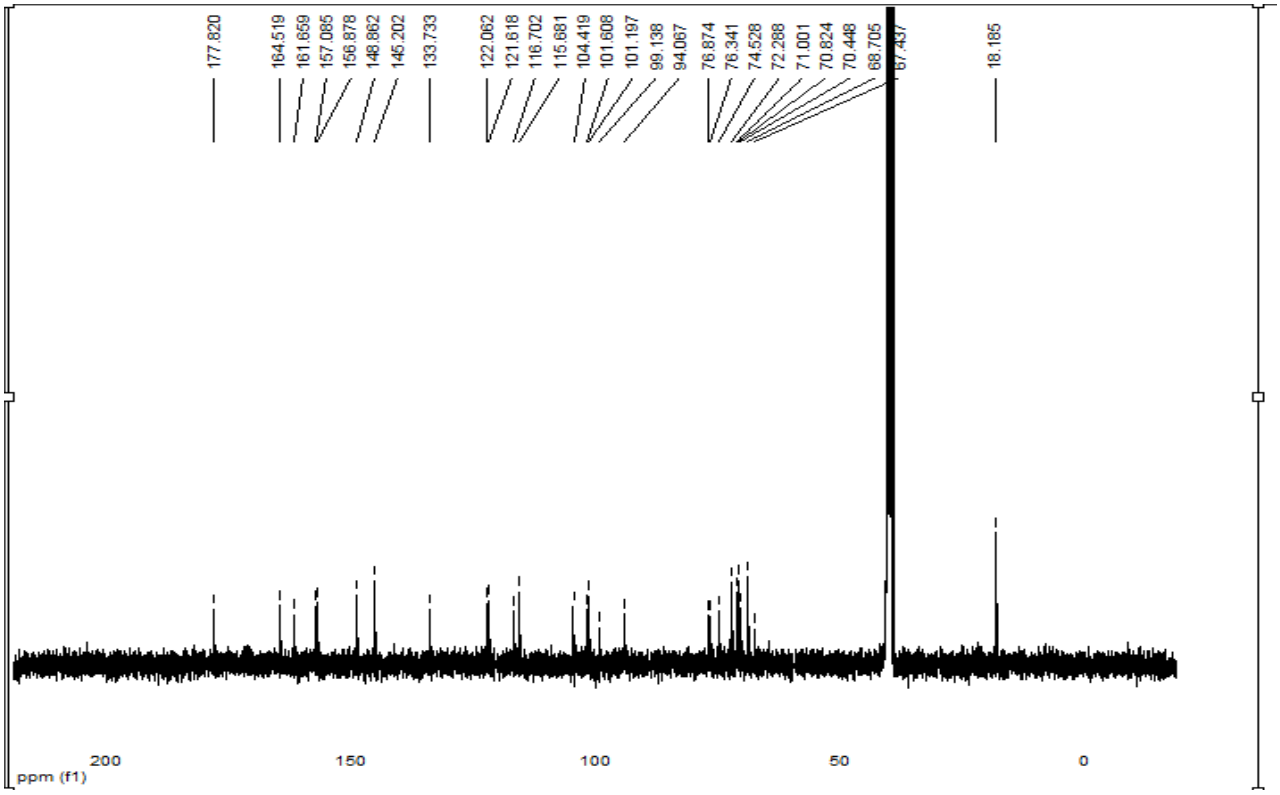


Appendix 12 DEPT-135 NMR spectrum of compound 48

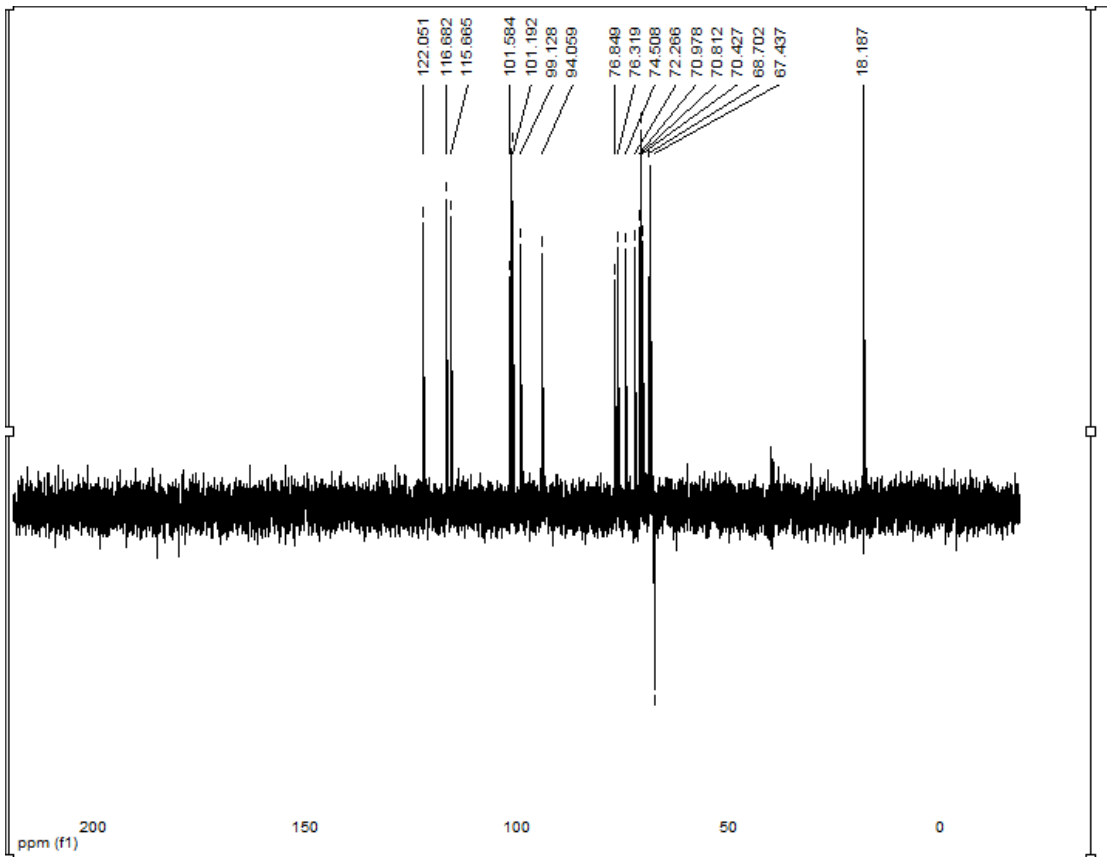
Compound 25



Appendix 13: [1] H-NMR spectrum of compound 25



Appendix 14: [13]C- NMR spectrum of compound 25



Appendix 15: DEPT-135 NMR spectrum of compound 48