JIMMA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF CHEMISTRY

M. Sc THESIS

PHYTOCHEMICAL INVESTIGATION ON THE STEM BARKS OF *Brucea antidysenterica* AND EVALUATION OF ITS ANTIMICROBIAL ACTIVITIES

BY

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JUNE 2019

JIMMA, ETHIOPIA

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES JIMMA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

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DECLARATION

The data included in this thesis are the result of my investigation, and this thesis is my original work and has not been presented for award of any degree or diploma in any other university.

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List of Abbreviation and Acronym

^{13}C-NMR	Carbon Nuclear Magnetic Resonance
DEPT-135	Distortion less Enhancement by Polarization Transfer
DMSO	Dimethyl Sulphoxide
IR	Infrared
NMR	Nuclear Magnetic Resonance
^{1}HNMR	Proton Nuclear Magnetic Resonance
TLC	Thin-Layer Chromatography
UV	Ultraviolet
TMS	Tetra Methyl Silane
WHO	World health organization
CC	Column Chromatography
NI	No inhibition
mm	millimeter

Acknowledgement

First of all, I would like to thank the Almighty God who gives me full health, peace and wisdom to realize this thesis work and for still be on my side forever. I am very much grateful to my advisors; Mr. Tsegaye Girma and Mr. Dale Abdissa for their remarkable professional support during the course of this study, their constructive and critical feedbacks added a great value to this thesis. It is also my great pleasure to thank the Department of Chemistry, Jimma University for their main financial support for the accomplishment of this thesis and cooperation throughout the study period. My thanks also extend to members of Department of Biology, Jimma University, particularly Dr. Dereje Denu for his support during plant identification. My special thanks also go to my friends. I am also highly grateful to my family, especially my wife Tirunesh Mekonnon; my sister Alemitu Fite, for unconditional support, heartfelt moral and unreserved support at different stage of my academic life had a significant effort on the success of my present day study.

Abstract

Medicinal plants have played crucial role as a source of traditional medicine in Ethiopia from the time immemorial to combat different ailments and human sufferings. The plant sample was collected; air dried, powdered and then soaked in petroleum ether, chloroform, acetone and methanol by using sequential method at room temperature. The crude extracts were evaluated against four bacterial strains *Escherichia coli (*ATCC 25922*) Bacillus subtilis* (ATCC 6633) *Pseudomonas auriginosa (*ATCC 27853*) Staphalococous aureus (*ATCC 25923*)* and two fungal strains *Candida albicuns* and *Fusarium spp*. The chloroform crude extract exhibited highest antimicrobial activity. Thus, this extract was subjected to column chromatography over silica gel eluted with *n*-hexane with increasing amounts of ethyl acetate and further purification was done by sephadex LH-20 chromatography, which resulted with the isolation of two compounds: compound (**NF1**) and compound (**NF2**) .The structure of the isolated compound was established using spectroscopic techniques ^{1}HNMR, DEPT-135 and ^{13}C-NMR. therefore, the partially characterized compounds were identified as compound (**NF1**) *6-(5-ethyl-6-methylheptan-2-yl)-4a-methyl-1,2,3,4,4a,4b,5,6,8a,9-decahydrophenanthren-2ol* and compound (**NF2**) *(5E,9E)-(((6E,11E)-2-oxooctadeca-6,11-dien-1-yl)oxy)methylheptadeca-5,9-dienoate*. The project work has shown that the stem barks of *Brucea antidysenterica* are rich sources of compounds with good potential for further biological and chemical research. Thus, the researchers recommend further studies on isolation of other fractions.

1. Introduction

1.1 Background of the study

Medicinal plants include various types of plants used in medicinal activities [1]. Medicinal plant is any plant which in one or more of its organs contains substances that can be used for therapeutic purposes [2]. Each and every community has its own system of traditional medicine and utilize natural resources around their habitats for various medicinal purposes. This traditional knowledge is handed-down orally from one generation to the other [3]. Traditional medicine is not only a vital source of health care, but also an important source of income for many communities and an integral part of a community's identity [4]. According to the WHO, approximately 80 % of world population rely on traditional medicinal plants for their primary healthcare requirements [5]. In Ethiopia more than 80 % of the population depend on traditional medicine for primary health care [6]. Medicinal plants are currently in considerable significance view due to their special attributes as a large source of therapeutic phytochemicals that may lead to the development of novel drugs [7].

Natural products are organic molecules that are formed by living organisms. They are broadly divided into primary and secondary metabolites [8]. Primary metabolites includes proteins, carbohydrates, lipids and nucleic acids. They are responsible for growth and development of organisms, which means they have direct roles in the processes of photosynthesis, respiration and major metabolic process of plants [9]. In contrast secondary metabolites include flavonoids, alkaloids, phenols, Saponins, triterpenoids. They are limited occurrences, unique to particular species and usually produced for the purposes of defense [10]. Bioactive natural products are the most successful sources of potential drug leads and have pharmacological activities for use in pharmaceutical drug discovery and drug design. Plants are the principal source of raw materials for plant-based medicine since ancient time the traditional herbal medicines are receiving great importance in healthcare sector over the world [11].

The use of traditional medicine is more substantial in the developing world. Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say 'Return to Nature'. The demand for plant based medicines, health products, pharmaceuticals, food supplement, cosmetics, etc. are increasing in both developing and developed countries, due to

the growing recognition that the natural products are non-toxic, have less side effects and easily available at affordable prices [12]. In Africa, traditional medicine is a part of the people's culture despite the fact that this form of medicine is not as well organized as, for example, in India and China. In developing countries, ethno botanical research has a potential role to local communities and to regional and national stakeholders [13].

In Ethiopia, the use of traditional medicine is widely practiced. Ethiopia is the land of vegetation as well as livestock. Like other places in Ethiopia, people of Tiro Afeta district have traditional practices which they accumulated for generations to treat both human and livestock ailments. They use different parts of plants to prepare remedies (roots, barks, leaves, and other plant structures). The elders, who know more about medicinal plants, may die without sharing their traditional knowledge to the young generation [14]. Since the knowledge of traditional medicine is transferred orally from generation to generation. Therefore, documentation of medicinal plants and the knowledge associated with them are important [15].

In order to pass the knowledge to the next generation since the plant material and the indigenous knowledge can be basis for the invention of modern drugs. Herbal medicines have the test of time for their safety, efficacy, cultural acceptability and less side effects [16]. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. Herbal medicines are important for which no modern medicine or only palliative therapy is available. These drugs are made from renewable resources of raw materials by eco-friendly processes and will bring economic prosperity to the masses growing these raw materials [17].

Many plant species have been recognized to have medicinal properties and beneficial impact on health [18]. The powdered leaves of *B. antidysenterica* mixed with butter are highly valuable for the treatment of itch and wounds [19]. The boiled bark of the root and fruits are used as medicine against dysentery and the inner part of the stem bark is also used to treat toothache. For this purpose, extracts of different plants have been tried with success as antibacterial agents. Thus, the study was focused on phytochemical investigation and evaluation of antimicrobial activity of the stem barks of *B. antidysenterica.*

1.2 Statement of the problem

Infectious diseases are the second leading cause of death worldwide, and the third leading cause of death in developed countries in both children and adults the burden of infectious diseases falls most heavily on people in developing countries. These infectious diseases are caused by viruses, bacteria, fungus, and parasites which results in respiratory infections, diarrheal tuberculosis, malaria, gonorrhea, meningitis and, tropical parasitic diseases [20]. The challenge associated with fighting these diseases has become an increasingly complex one, because of the fast development of resistance to the classic antibiotics and multiple drug resistant human pathogenic microorganisms [21].

In Ethiopia several plants are claimed to have antimicrobial activities, only few of them are tested for their activities and investigated for their chemical constituents. This demands the search for new sources of antimicrobial substances mainly from medicinal plants. Different researchers conducted a research on seeds and leaves of *B. antidysenterica* and identified the plant is rich in alkaloids, flavonoids, phenols, saponins, triterpenoids and steroids [22]. It is similarly used in traditional medicine for multiple purposes [23]. Peoples of Tiro Afeta district uses stem barks of *B. antidysenterica* to treat toothache, dysentery, wound and itch but, they do not know what chemical constituent present in this plant. Thus, this study was focused on phyto chemical investigation and evaluation of antimicrobial activity of crude extracts obtained from the stem barks of the *B. antidysenterica*.

1.3. Objectives of the study

1.3.1. General objective

The main objective of the research was to investigate the phytochemicals in the stem barks of *B. antidysenterica* and evaluate their antimicrobial activities.

1.3.2. Specific objectives

The specific objectives of the study are:

- To extract the stem barks of *B. antidysenterica* using different solvents
- To evaluate the antimicrobial activities of the crude extracts of *B. antidysenterica*
- To isolate the chemical constituents present in the stem barks of *B. antidysenterica* using chromatographic techniques
- To elucidate the structures of isolated compounds using spectroscopic techniques

1.4 Significance of the study

In Ethiopia, *Brucea antidysentrica* which is commonly known as *Abalo* (in Amharic), *Qomonyoo* (in Afan Oromo) has been used as traditional medicine to treat different human and livestock ailments. The study was focused on phytochemical investigation and evaluation of antimicrobial activities of extracts of the stem barks of B. antidysenterica that was collected from Tiro Afeta district, Jimma zone, Oromia regional state, Ethiopia.

Therefore, the findings of this research would be useful:

- To give information about the chemical constituents of the stem barks of *B. antidysenterica*.
- To provide the obtained result for further study on this plant.
- To synthesize derivative compounds which may have highest antimicrobial activity

2. Review of Related Literature

2.1 Botanical Information

The family Simaroubaceae comprises about 32 genera and 170 species of trees distributed widely in tropical America and West Africa. It is characterized by its content of bitter substances, mostly responsible for its pharmaceutical [24] The genus *Brucea* comprises 10 species occurring in Africa, Asia and Australia. In Ethiopia two species are found namely *Brucea ferruginea and B. antidysenterica* [25] *B. antidysenterica* (Fig.1) is the most well-known of these species and up-land (1400 to 2800 m) high evergreen forest and forest margins are very suitable area for the plant [25] It is a shrub or small tree up to (7-15) meters tall. Sometimes branching from the base bark grey to grey-brown with heart-shaped leaf scars, alternative leaves usually crowded at the end of younger twigs,10-65 cm long very common shrub at the edge of semi-humid forests or overgrazed mid highland areas [26]

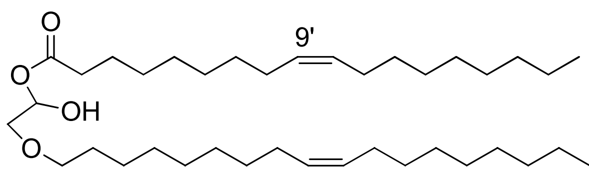
Figure 1. Picture of *Brucea antidysentrica*

2.2 Ethno medicinal uses of *Brucea antidysentrica*

From the beginning of humanity, indigenous community has developed their own local specific knowledge on plant utilization, protection and management [27]. Medicinal plants would be the best source to obtain variety of drugs [28]. They contain bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, and steroids. The bioactive compounds are synthesized by secondary metabolism of living organisms [29]. Due to the cost effectiveness, safety, increasing failure of chemotherapy and antibiotic resistance exhibited by pathogenic microbial agents, search for plant products has increased for their potential antimicrobial activity [30]. *B. antidysenterica* has been used locally for toothache, eczema, hookworm, tineanigra retained placenta and also as an abortifacient and in the management of asthma, headache, alopecia, bronchitis, bruise, chest cold, constipation, itch, diarrhea, skin sore, vermifuge, dysentery, dysmenorrheal, erysipelas, fever, inflammation, liver disease, poisoning, tumor, venereal disease, sedative, anti-fertility and rheumatism [31].

2.3 Review of the chemistry of Brucea species

Plants from Simaroubaceae are known to contain compounds with highly oxygenated triterpenens and bitter taste called as quassinoids [32]. So far, Many compounds have been isolated from this genus, including quassinoids, alkaloids, triterpenoids, and flavonoid. Previous chemical investigations of different Brucea species have reported the following compounds: glycerol1,2-bisoleate (**1**), Brusatol (**2**), Bruceantin (**3**), Bruceantinol (**4**), Bruceantarin (**5**), Dehydrobruceantin (**6**). Dehydrobruceantarin (**7**), Dehydrobrucein B (**8**), Dehydrobruceantinol (**9**), Bruceanic acid B (**10**), Bruceanic acid C (**11**), Bruceolline C (**12**), Bruceolline E (**13**), octatriacontan-1-ol (**14**), Bombiprenone (**15**), 5-Hydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-ylβ-Dglucopyranoside (**16**) [33] Bruceolline A (**17**), Bruceolline B (**18**), Bruceolline C (**19**), Bruceolline D (**20**) (Fig. 2) [25]. In the case of glycosidic alkaloids, glycosilation occurs at different positions on the compounds. The common position of glycosilation observed for the quassinoids were C-1, C-2, C-3 of the compounds.



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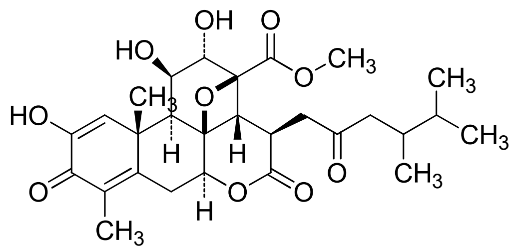

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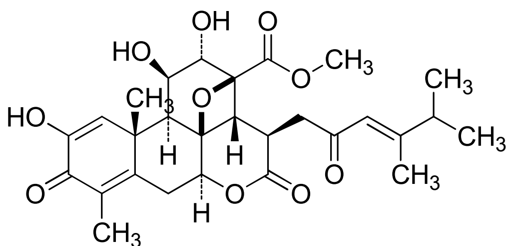

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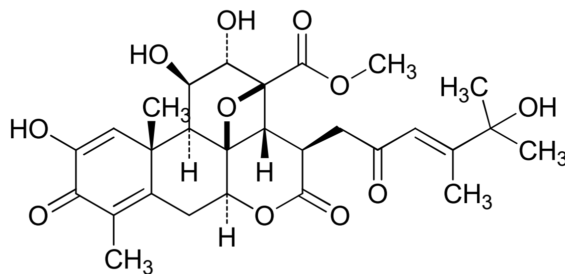

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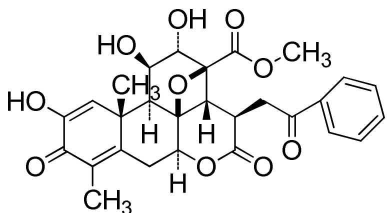

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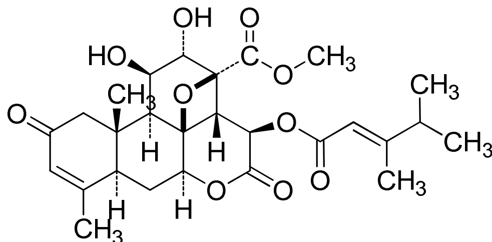

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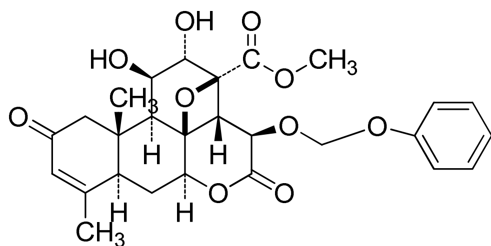

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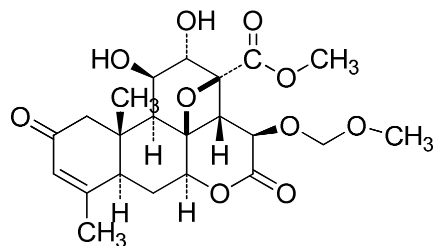

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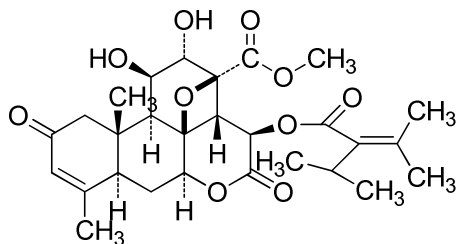

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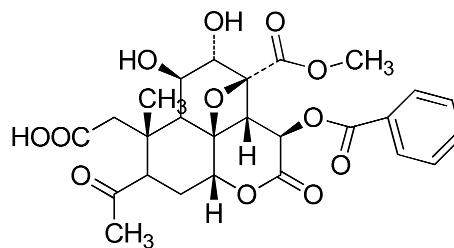

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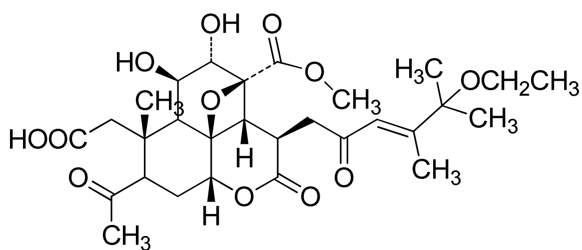

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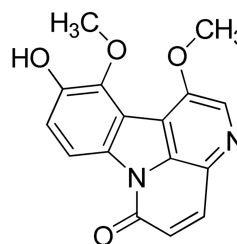

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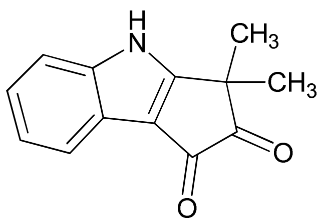

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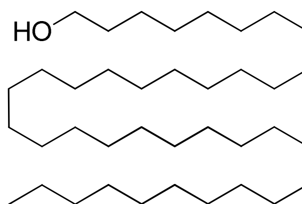

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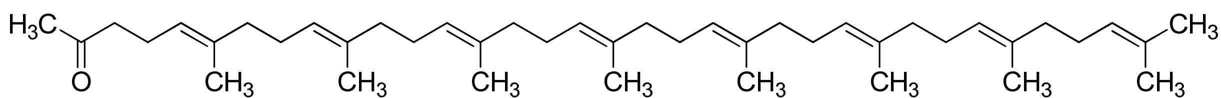

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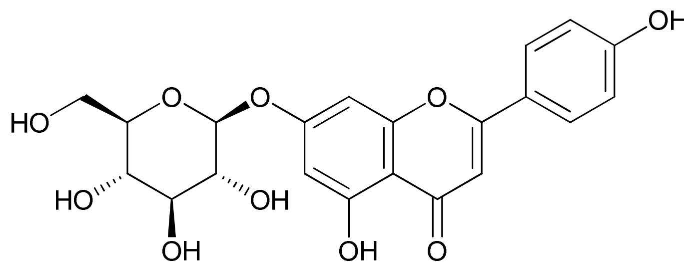

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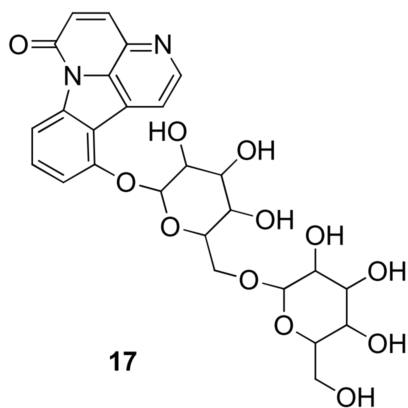
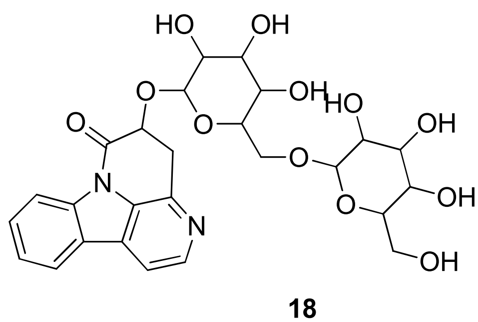
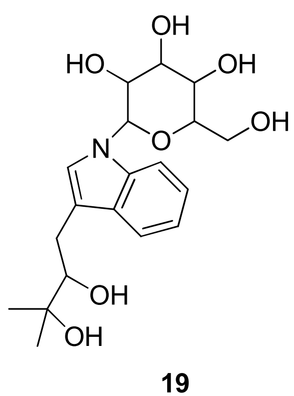






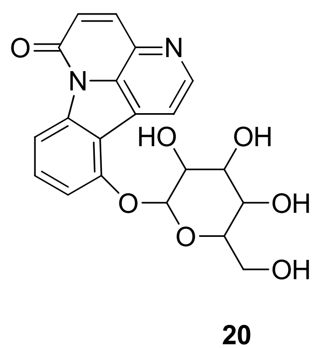


Figure 2 .Structure of some compounds isolated from Brucea species

Table 1. Summary of compounds isolated from the Brucea species

No	Compounds	Species	References
1	Glycerol 1,2-bisoleate	*B. javanica*	[34]
2	Dehydrobruceantarin	*B. antidysenterica*	[35]
3	Dehydrobrucein	‘’	‘’
4	Dehydrobruceantol	‘’	‘’
5	Isobruceine B	‘’	[36]
6	Yadanizolide B	*B .jjavanica*	‘’
7	Yadanziolide S	*B.aantidysentrica*	‘’
8	Bruceanic acid A	‘’	‘’
9	Methyl ester of Bruceanic acid A	‘’	[37]
10	Bruceanic acid B	*B. antidysenterica*	‘’
11	Bruceanic acid C	‘’	‘’
12	Bruceanic acid D	‘’	[38]
13	Bruceins A	‘’	‘’
14	Bruceanols A	*B. sumatrana*	[39]
15	Bruceanols C	*B. antidysenterica*	[40]
16	Bruceanols D	‘’	[41]
17	Bruceanols E	‘’	‘’
18	Bruceanols F	‘’	‘’
19	Bruceanols G	‘’	[42]
20	Bruceanols H	‘’	‘’
21	Brucantinoside A	‘’	‘’
22	Brucantinoside B	‘’	[43]
23	Brucantinoside C	‘’	[44]
24	Yadanzioside P	*B. javanica*	[45]
25	yadanziolide A	‘’	[46]

2.4 Microbial infection

Infectious diseases represent 41 % of total health problems of the world along with non-infectious (43 %) and injuries (16 %) [47]. Microbial infection is a major health problem all over the world. Infection by bacteria happens when harmful bacteria start reproducing at a fast rate in the body causing mild to severe infections [48]. There are Gram positive and Gram negative bacteria based on their constituent of the cell wall in the polymer of N-acetyl glucosamine. Gram positive bacterial cell wall contains a thick layer of peptidoglycan, Gram-negative cell walls contain a thin layer of peptidoglycan between the cytoplasmic membrane and the outer membrane [49].

Gram negative bacteria are like *Escherichia coli and Pseudomonas aeruginosa. E. coli* are commonly found as intestinal flora. *Pseudomonas aeruginosa* widely distributed in soil and water [50]. Gram positive bacteria is like *Staphylococcus aureus and Bacillus subtilis*. *Staphylococcus aureus* is found on dermal and mucosal flora. The genus *Bacillus* is found in soil, water and air and on vegetation. Plants used to treat microbial infections, an estimated 6 % have been screened for specific anti-microbial activities and only a small proportion of these have been studied phyto chemically to identify the active constituents [51]. Extracts of stem bark of Ailanthus excels family of Simaroubaceae showed significant fungicidal activity against *Aspergillus fumigatus*, *Penicillium notatum* and inhibited the growth of *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* [52].

3. Materials and Methods

3.1 Chemicals and Apparatus

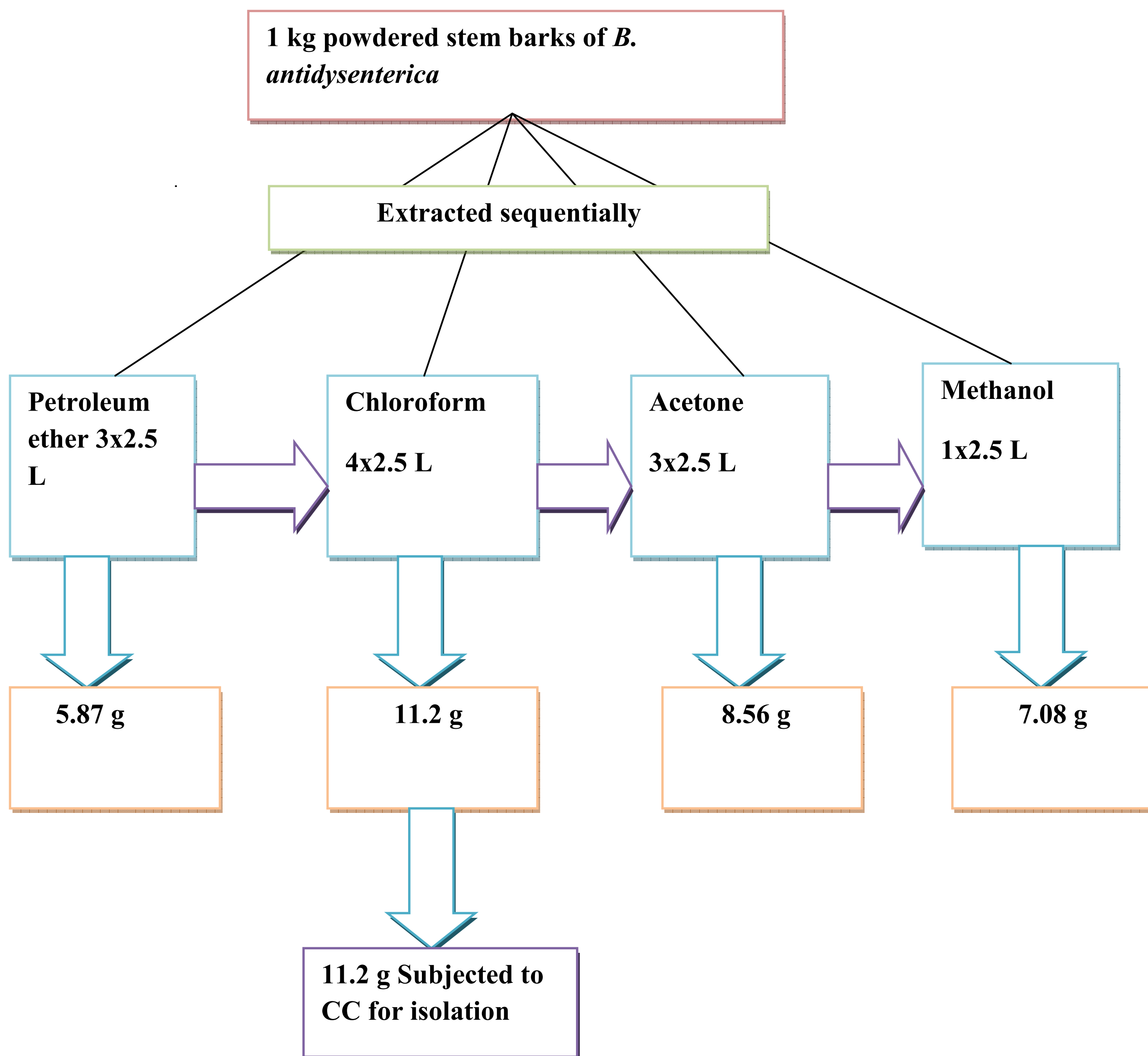
Analytical grade of *n*-hexane acetone chloroform methanol petroleum ether and ethyl acetate were used for extraction and isolation (Loba Chemie, Mumbai, India) silica gel 60-120 mm mesh size (Loba Chemie, Mumbai, India) Deuterated chloroform for obtaining NMR spectra and Dimethylsulfoxide (Loba Chemie, Mumbai, India) for antimicrobial activity test. Apparatus such as rotary evaporator (Heidolph, Germany, laborota 4000, No 519-0000-00-2) was used for solvent evaporation, round bottom flask of different volumes (500 mL and 1000 mL), measuring cylinder of different size, mortar and pestle for grinding, filter papers ,weighing balances (model No WT100001X), oven for drying purpose, glass columns chromatography (500 mm size) for elution , Uv-Tec (254 and 365 nm) chamber for detection of spots, Analytical TLC was performed on pre-coated silica gel 60 F_{254} 20cm x20cm size plates (Sigma-Aldrich), ^{1}H NMR (400MHz) and ^{13}C NMR (100MHz) were obtained on a Bruker Avance 400 spectrometer at Addis Ababa University, the spectra were processed using MestReNova software, autoclave (Astell ,model No AMA44DBT) Vertical Laminar Flow Cabinet hood (CLB-201-04)and Genlab incubator were used for antimicrobial activity test were used in the study.

3.2 Collection and Preparation of the plant specimens

The plant materials were collected from their natural habitat, uncultivated land in Tiro Afeta district 84 km from Jimma town and 384 km from Addis Ababa, Oromia regional state, South West of Ethiopia in June 2018. Identification of the plant species were made by a plant botanist Dr. Dereje Denu and voucher specimens were deposited at botanical science laboratory herbarium, Biology department, Jimma University. The stem barks of the plant were air-dried under shade for three weeks and powdered to suitable size by using mechanical grinder to improve the subsequent extraction by rendering the sample more homogenous, increasing the surface area and facilitating the penetration of the solvent in to the cell .

3.3 Extraction

The air dried and powdered stem barks of *B. antidysenterica* (1 kg) was soaked in petroleum ether, chloroform, acetone and methanol each extracted sequentially. The filtrate was then separated from the mark using fresh cotton plug and filter paper. The extracts were concentrated using a rotary evaporator under reduced pressure. The resulting semi-solid extracts were stored in desiccators until use.



3.4 Antimicrobial Activity Test

3.4.1 Test strain

Microbial strains used in the study were selected based on their availability and previous use reported in similar studies. The selected strains were *Escherichia coli* (ATCC 25922) *Bacillus subtilis* (ATCC 6633) *Pseudomonas auriginosa* (ATCC 27853) *Staphalococous aureus* (ATCC 25923) Candida Albicuns and Fusarium spp were obtained from microbiology laboratory, Biology department, Jimma University.

3.4.2 Bioassay Method

Bioassay is a very crucial stage in assessing the pharmacological actions of plant extracts and their ethno medical uses. There are different methods to assess antimicrobial activities [53]. Disc diffusion method is one of these method that uses discs, which are treated with the test sample dissolved in a suitable solvent. The dried discs are kept in contact with the inoculated media to allow diffusion before being incubated to promote bacterial growth [54]. The bioactivity of the crude extracts of stem barks of *B. antidysenterica* were tested on four types of bacterial strains and two fungal strains. The antimicrobial activity was performed following the disc diffusion method. The nutrient agar was inoculated with 100 µL of the inoculums and poured into Petri plate. The paper discs 6 mm size were prepared from filter paper. Then, 200 mg crude extract of petroleum ether, chloroform, acetone and methanol were dissolved in 1mL DMSO.

The stock solution were prepared with concentrations of 200 mg mL^{-1} for each solvent extracts. Chloramphenicol and ketoconazole as positive control and pure solvent (DMSO) as the negative control were used. The blotting paper discs (6 mm) was soaked in chloramphenicol, ketoconazole, DMSO and all stock solution. The discs were placed on a plate with standardized culture suspension at equidistance to one another to avoid overlap of zones of growth inhibitions. Antimicrobial activities of potential plant extract against bacterial and fungal pathogens were determined after incubation of the plates for 24 hr at 37 ^{o}C, by measuring the diameter of zone of inhibition of growth. The diameter of zone of growth inhibition surrounding the discs was measured and expressed in millimeter using transparent ruler. The mean of the inhibition zone of each test sample were taken for evaluating the antimicrobial activity.

3.5 Isolation

The chloroform extract (11.2 g) was adsorbed and then applied on top of column chromatography packed with 200 g of silica gel. Elution was done with 100 ml of *n*-hexane, followed by increasing amounts of ethyl acetate gradient. The following ratio of solvent combination was used in the elution process 100:0 to 0:100 to give 256 fractions .Each fraction was collected in 30 mL beakers. The level of separation was monitored by TLC analyses and detection was done using Uv teci (254 and 365 nm). After TLC analysis, fractions having similar R_f value collected together as follows:

Figure 3. Fractions ($F_{134-140}$) on TLC

After TLC analysis, fractions from $F_{100-110}$ were collected with 20 % ethyl acetate and fractions from $F_{134-140}$ were collected with 26 % ethyl acetate in n-hexane was subjected to sephadex LH-20 column chromatography using chloroform: methanol (1:1) for further purification to give 13.9 mg of compound (**NF1**) and 69 mg of compound (**NF2**). The characterization was done by spectroscopic technique, Nuclear Magnetic Resonance (^{1}HNMR,^{13}CNMR, DEPT-135) at Addis Ababa university.

4. Results and Discussion

4.1 Percentage of crude extracts

The air dried and powdered stem barks of *B. antidysenterica* (1 kg) was soaked sequentially in petroleum ether, chloroform, acetone and methanol. The filtrate was then separated from the mark using fresh cotton plug and filter paper, the extracts were concentrated using a rotary evaporator under reduced pressure. The chloroform extract was subjected to column chromatography for further purification following its antimicrobial activity. In this work it has been possible to isolate the compound (**NF1**) and compound (**NF2**) using different chromatographic techniques. Table 2 lists the amounts of extracts obtained in the various extraction procedures with the percentage yield calculated with reference to the dried plant material used.

$$\text{percentage yield} = \frac{weight\,of\,crude}{weight\,of\,sample}\,x\,100$$

Table 2. Extracts and their percentage yield

Solvent used	Crude extracts in (g)	% yield
Petroleum ether	5.87	0.587
Chloroform	11.2	1.12
Acetone	8.56	0.856
Methanol	7.08	0.708

Chloroform extracts were highest in yield (1.12 %) and petroleum ether extracts were the least in (0.587 %).

4.2 Partial Characterization of Isolated Compound (NF1)

Compound (NF1) is a brown amorphous solid obtained from chloroform extract. Thin layer chromatogram was run for this compound (Rf = 0.6) in eluting 20 % ethyl acetate with n-Hexane. The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum revealed the presence of one proton singlet signals at (δ_H 5.35) showed the presence of hydroxyl proton on cyclic ring, there is also fifteen protons on five methyl group, sixteen protons on methylene group and ten proton on methine group. The ^{13}C-NMR (100MHz $CDCl_3$) spectrum revealed that five methyl

carbons of which one methyl on cyclic ring, eight methylene carbon, two quaternary carbons which is attributed to cyclic ring, ten methine carbons ,one oxygenated carbon at 71.76, two double bonds at δ_C (140.75 and 121.68) δ_C (129.28 and 138.31) there are 25 carbons in the spectral data as shown in table 3 and (Appendix 1,2 and 3).

Table 3. The ^{13}C NMR and ^{1}H NMR spectral data of compound (**NF1**)

Carbon position	Experimental ^{13}C-NMR δ(ppm)	Remark	Experimental ^{1}H-NMR δ(ppm)
1	37.3	CH_2	1.12
2	31.7	CH_2	1.26
3	71.6	CH	3.51
4	41.8	CH_2	1.96
5	140.8	C	-
6	120.5	CH	5.15
7	32.3	CH_2	1.84
8	33.4	CH	2.31
9	45.2	CH	1.65
10	39.3	C	-
11	29.9	CH_2	1.42
12	38.1	CH	1.96
13	131.3	CH	5.18
14	128.4	CH	5.17
15	40.5	CH	1.67
16	33.4	CH_2	1.23
17	26.5	CH_2	1.26
18	46.1	CH	1.46
19	30.4	CH	1.83
20	21.0	CH_3	0.93

21	21.0	CH_3	0.93
22	23.2	CH_2	1.54
23	12.2	CH_3	0.92
24	18.9	CH_3	0.97
25	19.4	CH_3	1.70

Based on the above spectroscopic data compound (**NF1**) was partially characterized and named 6-(5-ethyl-6-methylheptan-2-yl)-4a-methyl-1,2,3,4,4a,4b,5,6,8a,9-decahydrophenanthren-2-ol.

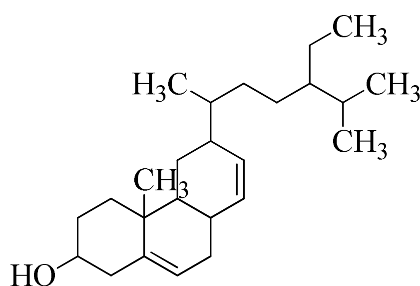


Figure 4. The proposed structure of compound (**NF1**)

4.3 Partial Characterization of Isolated Compound (NF2)

Compound (NF2) is a white amorphous solid obtained from chloroform extract. Thin layer chromatogram was run for this compound (Rf = 0.5) in eluting 26 % ethyl acetate with n-hexane. The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum revealed the presence of singlet proton on methoxy carbon at δ H (10.64,1H,s) protons on methyl carbon δ_H (0.87,3H,t) δ H (0.89,3H,t) protons attached to alkene carbons (olefinic carbons) δ H 5.35 (1H,q) 5.34 (1H,q) 5.40 (1H,q) 5.34 (1H,q) 5.35 (1H,q) 5.37 (2H,q) 5.38 (2H,q) 5.40 (2H,q). The ^{13}CNMR (100MHz, $CDCl_3$) spectrum revealed that one carbonyl carbon of ester group δC (180.44) , olefinic carbons (unsaturated carbons) at δC 130.15 (C-13') 129.97 (C-9) 129.80 (C-8') 129.68 (C-12') 128.05 (C-8) 127.89 (C-7') 129.80 (C-12) 130.15 (C-13). Furthermore, the spectrum demonstrated the presence of two methyl carbons δ C 14.09 (C-20) δ C 14.09 (C-19') twenty four methylene carbons, eight methines and two non hydrogenated carbons as shown in table 4 (Appendix 4, 5 and 6).

Table 4. The ^{13}C NMR and ^{1}H NMR of compound (**NF2**)

Position	^{13}C-NMR	^{1}H NMR	Remark
1	-	-	-
2	62.11	10.64	CH_2
3	-	-	-
4	180.44	-	C
5	34.13	2.33	CH_2
6	24.83	1.66	CH_2
7	31.53	2.19	CH_2
8	128.05	5.40	CH
9	129.97	5.40	CH
10	31.94	2.00	CH_2
11	31.94	2.02	CH_2
12	129.80	5.38	CH
13	130.15	5.37	CH
14	36.16	2.19	CH_2
15	29.71	1.27	CH_2
16	29.69	1.27	CH_2
17	29.45	1.27	CH_2
18	31.80	1.27	CH_2
19	22.70	1.32	CH_2
20	14.09	0.87	CH_3
2’	56.70	4.33	CH_2
3’	-	-	C
4’	42.30	2.35	CH_2
5’	23.05	1.85	CH_2
6’	31.94	2.05	CH_2
7’	127.89	5.34	CH
8’	129.80	5.34	CH

9’	31.92	2.07	CH_2
10’	29.77	1.62	CH_2
11’	31.92	2.19	CH_2
12’	129.68	5.35	CH
13’	130.15	5.35	CH
14’	36.16	2.09	CH_2
15’	29.71	1.27	CH_2
16’	29.38	1.27	CH_2
17’	31.79	1.27	CH_2
18’	22.58	1.32	CH_2
19’	14.09	0.89	CH_3

Based on the above spectroscopic data of 13C-NMR, H-NMR and DEPT-135 spectra Partial characterization was done. Then the following structure was proposed for compound (**NF2)** and named as *(5E,9E)-(((6E,11E)-2-oxooctadeca-6,11-dien-1-yl)oxy)methyl heptadeca-5,9-dienoate.*

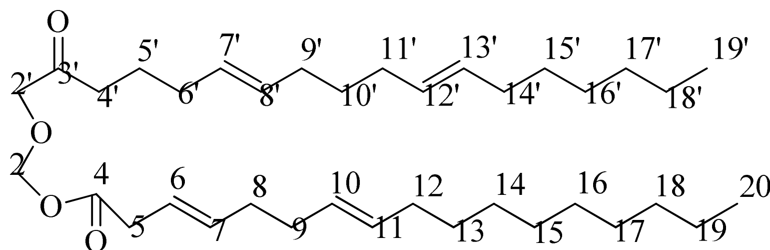


Figure 5. The proposed structure of compound (**NF2**)

4.4 Antimicrobial Evaluation

The crude extracts of petroleum ether, chloroform, acetone and methanol were evaluated for antimicrobial activity on four bacterial strains and two fungal strains respectively. *S. aureus, E. coli, P. aeruginosa ,B. subtilis, C. albicuns and F. spp.* Antimicrobial activity of potential plant extract against bacterial pathogens by disc diffusion technique were identified after incubation for 24 hours. at 37 °C, and the result was obtained by measuring the zone of growth inhibition [55].

The strains were activated for the experiment at 37 °C for 24 hours on nutrient broth for bacterial strains and potato dextrose broth for fungal strains. The culture media was dissolved in distilled water and autoclaved at 121°C for 2 hours. Then, it was poured into sterile petri dishes until use. After the culture media had solidified ,organisms were uniformly seeded with sterile swab. 200 mg/mL of each crude extracts (petroleum ether, chloroform, acetone and methanol) and the same volume of DMSO were impregnated using whatman filter paper disc (diameter 6 mm). Positive control chloramphenicol for bacteria and ketoconazole for fungus were assayed simultaneously. The plates were left for 10 minutes until the extracts diffuse in the medium with the lid closed and incubated at 37 °C for 24 hours for bacteria and 48 hours for fungus. After incubation, the diameter of the inhibition zone was measured in millimeter using transparent ruler.

Table 5. Antimicrobial activity of crude extracts obtained from Brucea antidysentrica.

	Diameter of zone of growth inhibition (mm)					
	Bacterial strains				Fungal strains	
	E.coli	*P.aeriginosa*	*S.aureus*	*B.subtilis*	*C. albicuns*	*F. spp.*
Petroleum ether	8	10	14	12	10	10
chloroform	13	10	12	20	12	18
Acetone	10	13	15	8	16	16
Methanol	10	16	8	7	9	14
Chloramphenicol	27	30	35	18	-	-
Ketoconazole	-	-	-	-	11	10
DMSO	NI	NI	NI	NI	NI	NI

The zone of growth inhibitions (table 5) above are different based on the types of antimicrobial strains and the test samples. The petroleum ether, chloroform, acetone and methanol crude extracts showed a significant difference on inhibition zone diameters against the test of strains which ranged between (7 mm) and (20 mm). The crude extract obtained from chloroform exhibited higher zone of growth inhibition (20 mm) on gram positive bacteria *B. subtilis* and higher zone of growth inhibition(18 mm) on *Fusarium spp*. fungal strain, while the crude extract obtained from methanol exhibited higher zone of growth inhibition (16 mm) on gram negative bacteria *P. aeruginosa*. Among all extracts analyzed minimum antimicrobial activity was exhibited by the methanol extract against *B. subtilis* (7 mm), acetone against *B. subtilis* (8 mm).

5. Conclusion and Recommendation

5.1 Conclusion

Based on the results obtained in this study, all the extracts showed varying degrees of antimicrobial activity against the test strains. However, the activities varied based on the solvents used for extraction. The crude extract obtained from chloroform exhibited higher zone of growth inhibition (20 mm) on gram positive bacteria *B. subtilis* and higher zone of growth inhibition (18 mm) on *F. spp*. fungal strain as compared to the other extracts. These results provide evidence that some of the secondary metabolites present in the studied medicinal plants confirmed as they contained multiple bioactive compounds which might have synergetic impact on inhibition of pathogenic microbes tested.

Due to limitation of time and spectroscopic instruments complete characterization is not done for all isolated compounds obtained from the stem barks of *B. antidysenterica* the structural elucidation of the compounds were done partially for compound (**NF1** and **NF2**) by using spectroscopic data obtained from (^{13}C NMR,^{1}H NMR and DEPT-135) . The [i]characterized compounds were proposed and named as 6-(5-ethyl-6-methylheptan-2-yl)-4a-methyl-1,2,3,4,4a,4b,5,6,8a,9-decahydrophenanthren-2-ol (**NF1**) and compound (**NF2**) *(5E,9E)-(((6E,11E)-2-oxooctadeca-6,11-dien-1-yl)oxy)methyl heptadeca-5,9-dienoate.*

5.2 Recommendation

I. The project work has shown that the stem barks of *B. antidysenterica* are rich sources of compounds with good potential for further biological and chemical research. Thus, the researchers recommend further studies on isolation of other fractions.

II. The clear zone of growth inhibition measured for chloroform extracts on gram positive bacteria *B. subtilis* (20 mm) as compared to the standard chloramphenicol was about 18 mm in diameter, for this crude extracts the minimum inhibition concentration should be recommend further.

III. In this project work isolation of a compound was done only on chloroform extracts, but the antimicrobial activity of acetone extract on Fusarium spp is (16 mm) growth inhibition zone as compared to positive control ketoconazole 10 mm, for this extract isolation of the compound should be done further.

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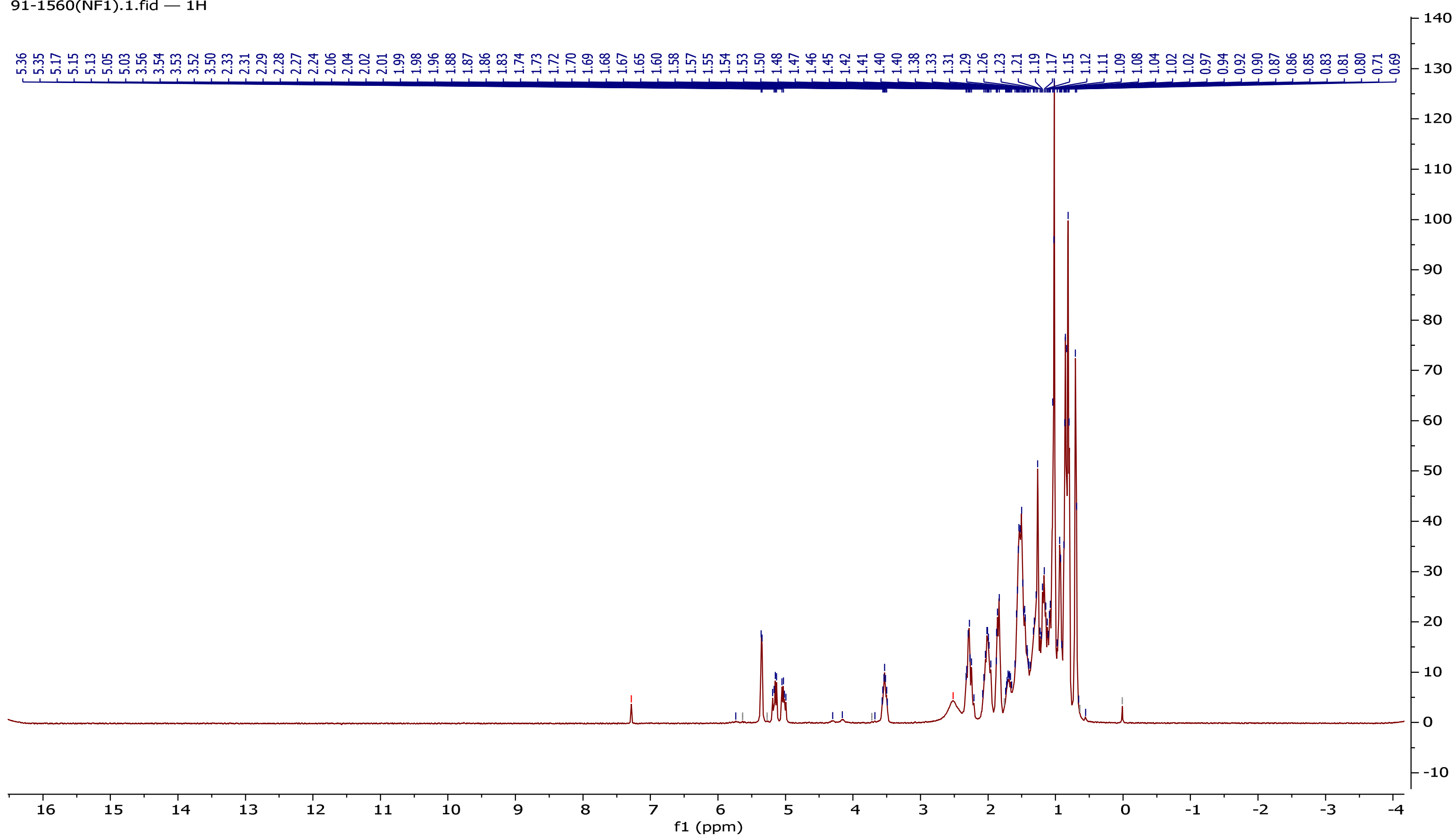
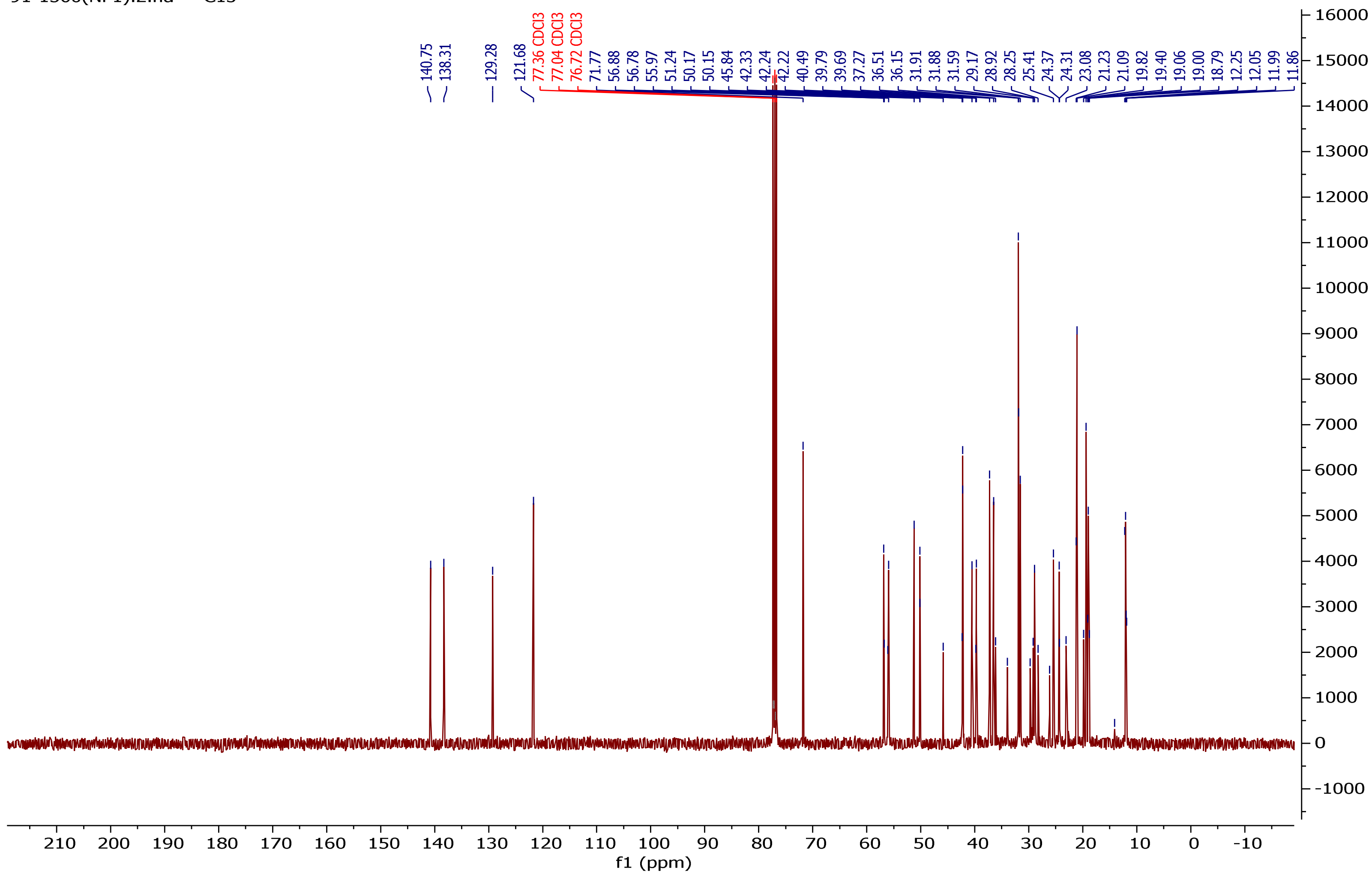
Appendices

Appendix 1. ^{1}H-NMR Spectrum of compound (**NF1**) in $CDCl_3$

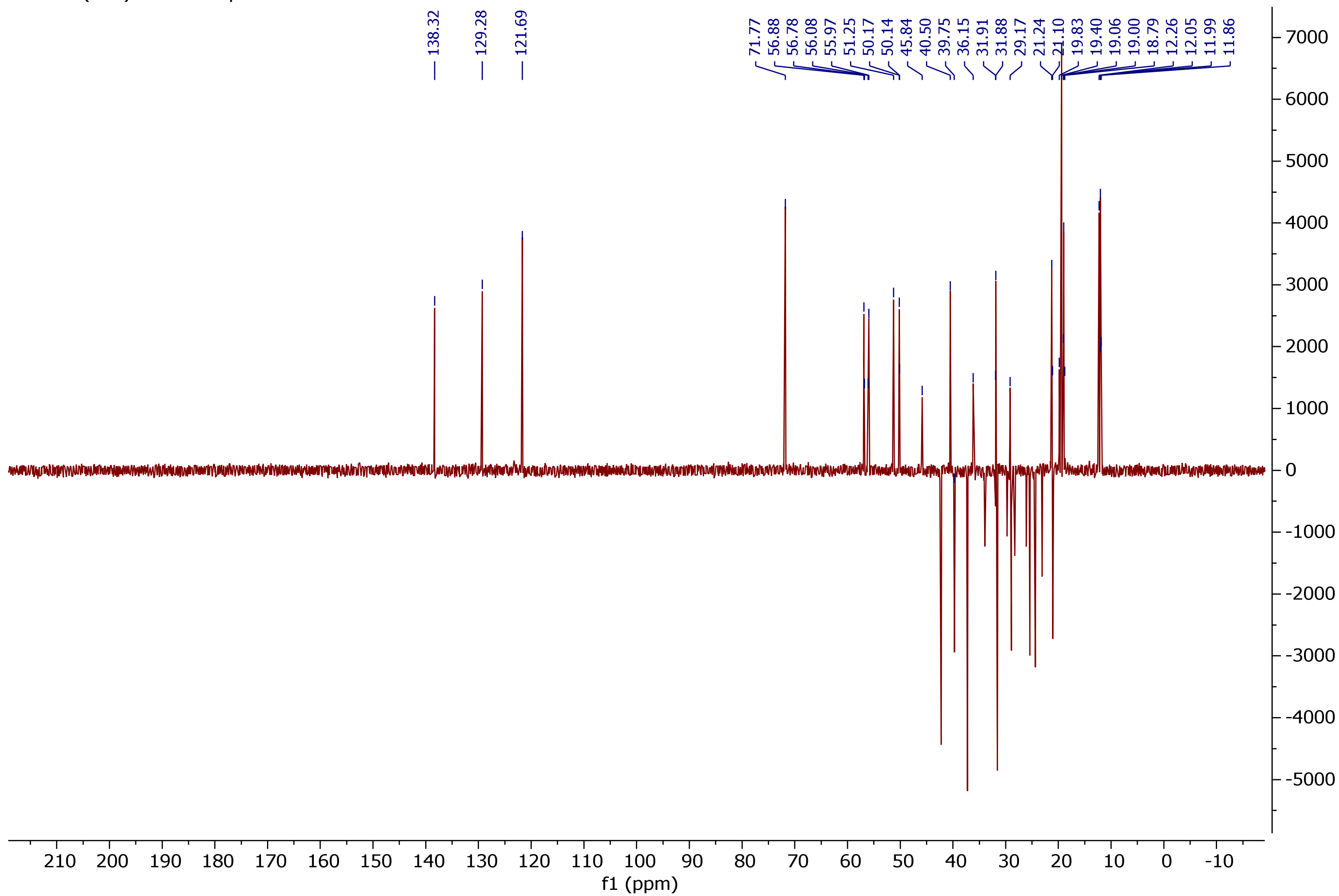


Appendix.2 ^{13}C-NMR Spectrum of Compound(**NF1**) in $CDCl_3$

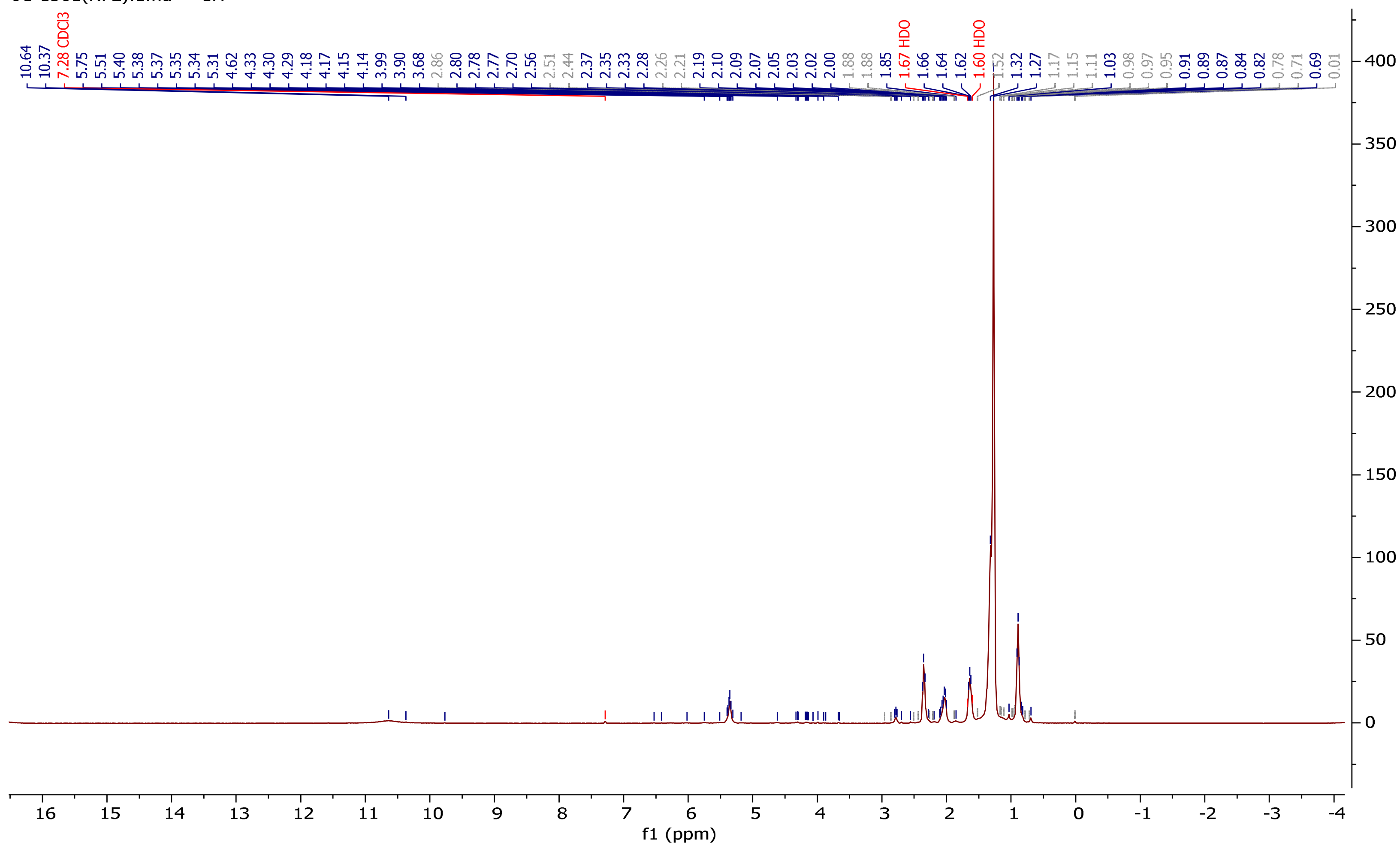
91-1560(NF1).2.fid — C13



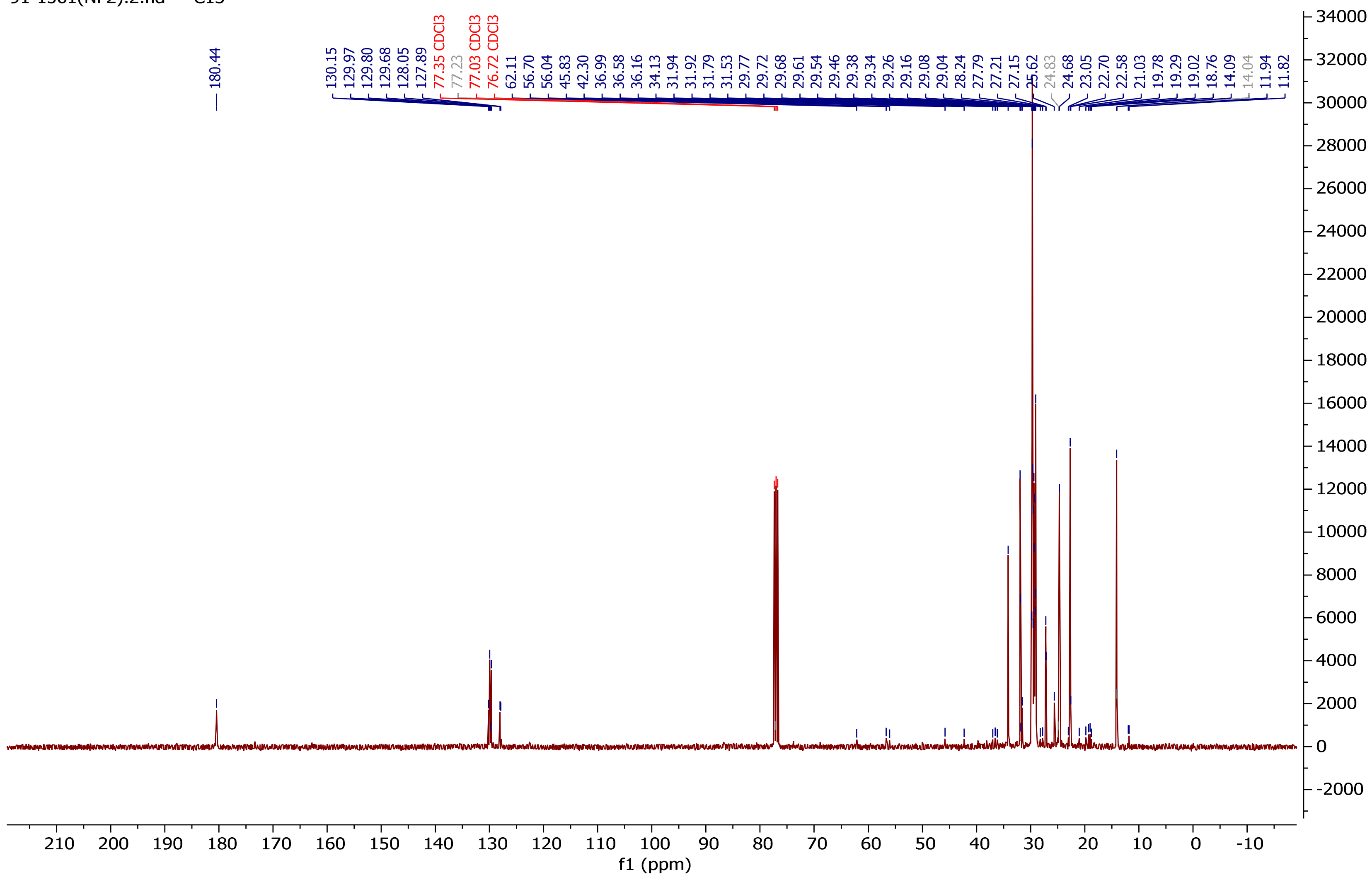
Appendix 3. DEPT-135 spectrum of compound (**NF1**) in $CDCl_3$



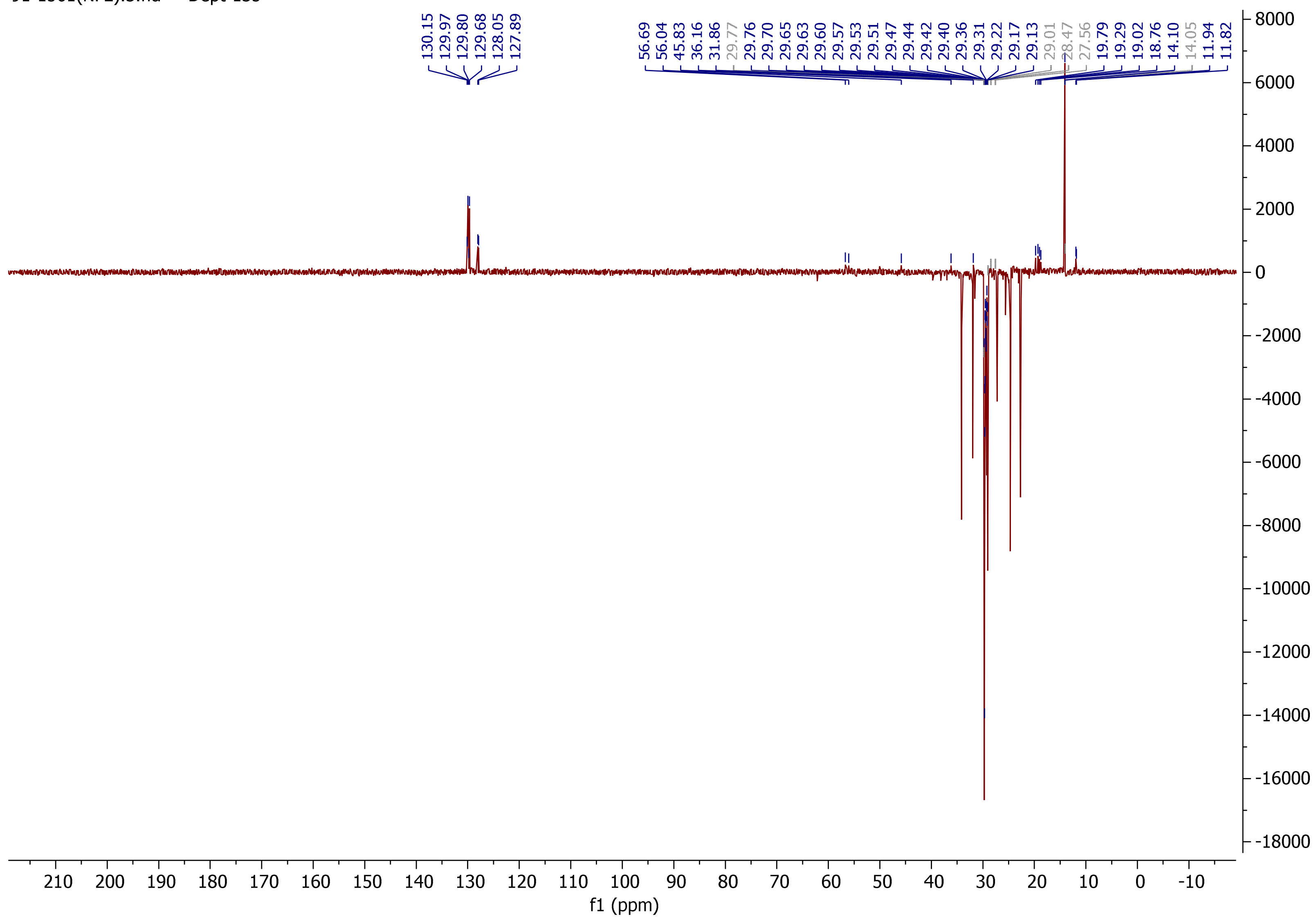
Appendix 4. ^{1}H-NMR Spectrum of compound (**NF2**) in $CDCl_3$



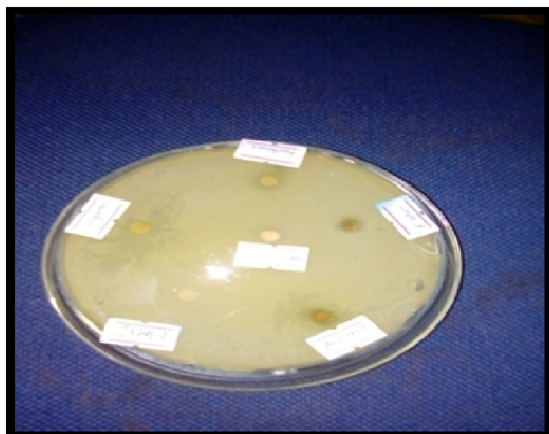
Appendix 5. ^{13}C-NMR Spectrum of compound (**NF2**) in $CDCl_3$



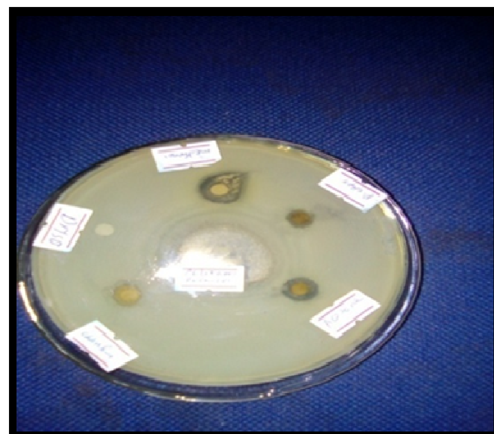
Appendix 6. DEPT-135 Spectrum of compound (**NF2**) in $CDCl_3$



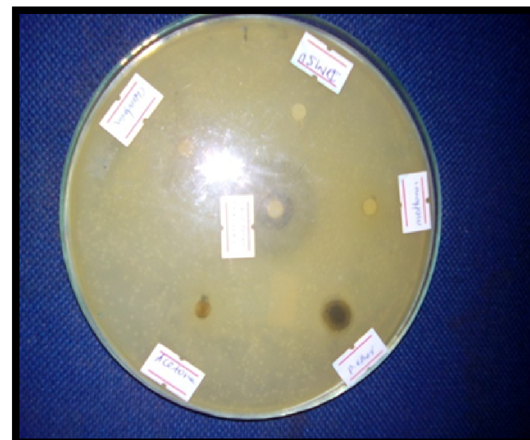
Appendix 7. Antimicrobial activity Tests of Crude Extracts



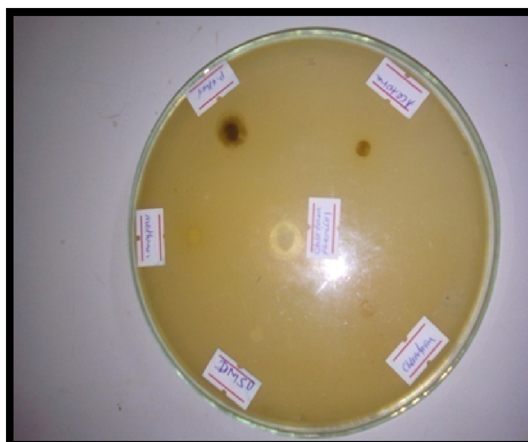
B.subtilis



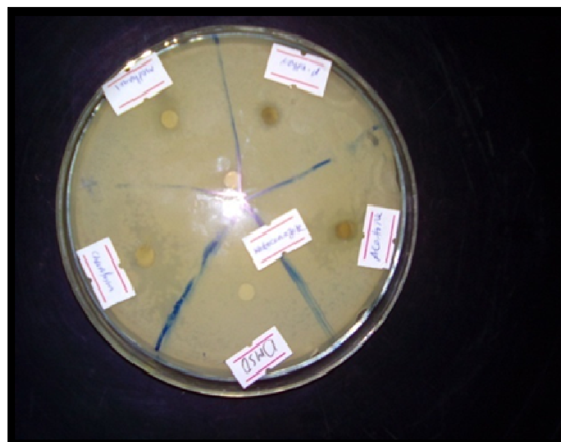
P.aeruginosa



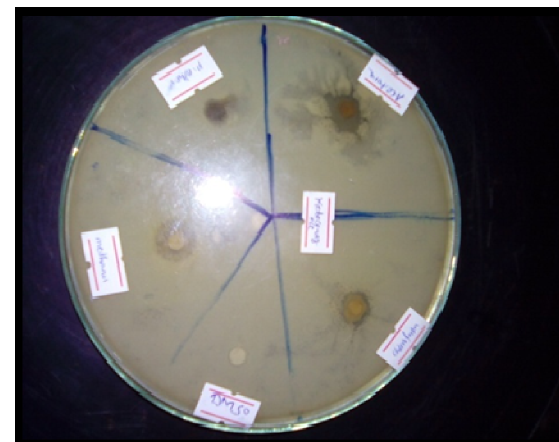
E.coli



S.aureus



Fusarium spp.



Candida albicums