Phytochemical Investigation and Antibacterial Activities of the Roots Extract of *Dracaena steudneri*

By :AdisuSakuna

A Thesis Submitted to

Applied Chemistry program

School of Applied Natural Science

Presented In Partial Fulfillment of the Requirement for the Degree of Master of Science In Chemistry

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APPROVAL OF BOARD OF EXAMINERS

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We, the undersigned, members of the Board of Examiners of the final open defense by Adisu Sakuna have read and evaluated his thesis entitled by "Phytochemical Investigation and Antibacterial Activities of the Roots Extract of *Dracaena steudneri* " and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Master of science in chemistry.

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This is to certify that the thesis entitled by "Phytochemical Investigation and Antibacterial Activities of the Roots Extract of *Dracaena steudneri"*submitted in partial fulfillment of the requirements for the degree of Master's in science, the Graduate program of the department of chemistry and has been carried out by AdisuSakuna Id. No GSS/0204/05, under our supervision. Therefore,we recommend that the student has fulfilled the requirements and hence hereby he can submit the thesis to the department.

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DECLARATION

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

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LIST OF ABBREVIATIONS

ATCC	American Test Culture Collection
CC	Column Chromatography
DEPT	Distortionless Enhancement by Polarization Transfer
DS	*Dracaena steudneri*
IR	Infra-Red Spectroscopy
mp	Melting point
NMR	Nuclear Magnetic Resonance
R_f	Retention factor
s	singlet
t	Triplet
m	multiplet
TLC	Thin Layer Chromatography

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ABSTRACT

The plant D.steudneri enjoys a wide ethno medicinal use for the treatment of various diseases which is distributed in east and south Africa. These include its use for fungal and bacterial infections, including healing cuts and wounds, to treat black leg, rabies, trypanosomiasis, and emaciation'cryptococcal meningitis, tuberculosis. This study was directed towards organic solvents (n-hexane, chloroform, EtOAc and methanol) extraction, isolation, and structural elucidation of compounds from the roots of a known medicinal plant Dracaena steudneri by using chromatographic and spectroscopic techniques. Besides, antibacterial activity determination of crude extracts and isolated compounds were carried out against some selected bacterial stains. The antibacterial test was performed by disc diffusion method against selected gram-positive and gram-negative bacterial strains by observing the zone of inhibition. The methanol extract showed promising spots on TLC and hence subjected to fractionation over silica gel column chromatography which led to the isolation of three compoundscompound 1, compound 2, compound3 and identified as azetidine-2-carboxylicacid, kaempferol 3-O-β-glucopyranoside and palmitic acid respectively. These compounds were hitherto unreported from this plant. In the antimicrobial tests, the methanol extract has shown superior activities against all the strains.

Key Words:*D.steudneri, Antibacterial activity, phytochemical screening, azetidine-2- carboxylic acid,*kaempferol 3-O-*β*-glucopyranoside*, palmitic acid*

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1. INTRODUCTION

Medicinal plants are the integral part of the variety of cultures in the world. Thus, traditional medicine is used throughout the world as it is dependent on locally available plants, which are easily accessible, simple to use and affordable [1].There is no pharmaceutical compound on the market today which can completely satisfy all the human needs and that fit their interest [2]. In recent times, focus on plant research has increased all over the world, and a large body of evidence has been collected to show the immense potential of medicinal plants used in traditional systems [3]. Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to mankind. Medicinal plants are used for treating various diseases since prehistory. Around 250 to 500 thousand plant species are estimated to be present on the planet, out of which 1 to 10% of them are used as food by human beings and other animals [4].

The majority of population in the developing countries still relies on herbal preparations to help enhance health. This is also true in case of Ethiopia where the majority of the population depends on plants for their healing action in various health problems. Among the 7000 higher plant species that are known to exist in Ethiopia, about 800 of them are employed in the traditional health care delivery system to prevent and treat nearly 300 physical and mental disorders [5].

Despite its significant contribution to society, traditional medicine has experienced very little attention in modern research and development, and less efforts have been made to upgrade the practice. The part of the medicinal plants collected also poses a serious threat to survival of the species. Loss of knowledge has been aggravated by expansion of modern education, which has made younger generation underestimate its traditional values. Moreover, the continued deforestation and environmental degradation of habitats in many parts of the country has brought about the depletion of medicinal plants and the associated knowledge [6].

Since 1978, there have been some ethno botanical studies conducted in different parts of the country to document medicinally important plants. Compared with the huge knowledge of medicinal plants available in the country, very little information is so far collected and documented. Since knowledge of traditional medicine is transferred from generation to generation by word of mouth especially in countries like Ethiopia, where there is little

accessibility to written documents and records on medicinal plants, there is loss of information on the use of traditional medicinal plants through time [7, 8].

In an attempt to conserve traditional medicine knowledge, it is necessary that inventories of plants with therapeutic values are carried out and the knowledge related to their use documented in systematic studies. Furthermore, studies related to herbal medicines can help to stimulate confidence in traditional medicine and enhance appreciation of herbal medicines among local communities [9].

Medicinal plants are various plants thought by some to have medicinal properties, but few plants or their phytochemical constituents have been proven by rigorous science to have medicinal effects. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties [10]. They are chemical compounds formed during the plants' normal metabolical processes. These chemicals are often referred to as secondary metabolites of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, phenolic compounds, tannins, terpenes and terpenoids and steroids which are capable of producing definite physiological action on disease causing organisms [11,12].

Medicinal plants represents rich source from which antimicrobial agents may be obtained. According to World Health Organization [13], more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. In developing countries, low-income people such as farmers, people of small isolated villages and native communities use folk medicine for the treatment of common infectious diseases.

These plants are ingested as decoctions, teas or juice preparations. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Making antibacterial therapy effective, safe and affordable has been the focus of interest during recent years [14].

The different parts used include root, stem, flower, leaves, fruit, twigs exudates and modified plant organs. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [15]. The antimicrobial activities of plant extracts may reside in a variety of

different components. The beneficial medicinal effects of the plant materials typically result from the combinations of secondary product present in the plant [16].

In Ethiopia, there are several medicinal plants. *D.steudneri* is one of the Ethiopian plants which is distributed in Oromia, Amahara , Southern nation and nationality traditionally used for the treatment of various ailments including woundhealing.Despite the traditional medicinal use of this plant, to the best of my knowledge there is no published report on isolation of chemical constituents and evaluation of biological activities of any part of this plant.

1.1. Statement of the Problem

In Ethiopia various plants are commonly used for the disease treatment by traditional healers and community people. The acceptance of traditional medicine as an alternative form of health care and the development of bacterial resistance to the available synthetic drug have led to investigate active antibacterial agent from natural products.

In Ethiopia, there are several indigenous medicinal plants. *D. steudneri*is an indigenous Ethiopian plant with wide ranging ethno medicinal applications. Despite the traditional medicinal use of this plant, there is no information that dwells on the chemical constituents and biological activities of the roots of the plant which initiate us for the study.

1.2 Significance of the Study

Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents present in the plant play a significant role in the identification of crude drugs. The treatment of infectious diseases and others still remains an important and challenging problem because of a several factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens.

The study on the extracts of the roots of *D. steudneri*would help to identify new natural products that display significant antimicrobial activities. This may lead to further investigations to arrive at new drugs. Identified products could be used as candidates in the discovery of new antimicrobial agents.

1.3. Justification of the Study

This thesis has a practical application to know the biologically active compounds present in this plant.This plant is effective traditional medicine for wound healing.Thus,knowing the chemical constituent of this plant help us to promote this traditional medicine to modern medicine and documenting of knowledge for drug industry.So, this thesis helps to fill that gap.

1.4. Objectives

1.4.1 Generalobjective

- To conduct phytochemical and antibacterial studies on the extracts of the roots of *D. steudneri*

1.4.2 Specific objectives

The specific objectives of the study are:

- To extract the roots of the plant using n-hexane, chloroform, ethyl acetate and methanol.
- To carry out phytochemical screening test of the crude extracts
- To isolate compounds from the selected crude extracts using chromatographic techniques
- To characterize isolated compounds employing Melting point and spectroscopic methods (IR and NMR).
- To study the antibacterial activities of the crude extracts and isolated compounds using agar disc diffusion method

2. LITERATURE REVIEW

2.1 The Genus *Draceana*

Draceana is a genus comprising more than 60 species which are mainly found in tropical and subtropical Africa[17]. Linnaes circumscribed *Draceana* in 1767 which refers to about sixty species. Some species, for example *D. fragrans* and *D. ellenbeckiana* have forms with variegated leaves which are major foliage ornamentals particularly as indoor plants, while others, such as *D. steudneri* are popular as landscaping aids in tropical gardens. The fragrant flowers and fleshly berries of some species are diet for animals. Among the species in the genus, *Draceana*include *D. fragrans, D. afromontana, D. ombet*, *D.ellenbeckiana*, *D. laxxisima*, *D. manni*, *D. arboria*, *D. draco*,*D. marginata*,*D. cochinchinensis,D. cinnabari*and many more [18].

2.1.1. Biological activities of the genus

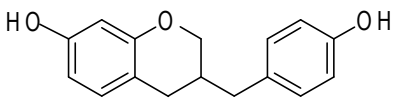
2.1.2. Antimicrobial activity

Extracts obtained from leaves and roots of *D. spicata*were examined for their antimicrobial activities against some gram positive bacteria such as *Bacillus sereus, Bacillus megaterium*,and also gram negative strains of *Escherichia coli, Salmonella typhi,* and fungus *Aspergillusniger*. Agar disc diffusion method was applied to observe the antibacterial efficacy of the extracts. Results indicated that plant extracts (600 µg /disc) displayed antibacterial activity against tested microorganisms *E.coli*and *Aspergillusniger*. These results were also compared with the zones of inhibition produced by commercially available standard antibiotic, Kanamycin at concentration of 30 µg/disc. The methanol extract of *D. spicata*showed good antimicrobial activity with the average zone of inhibition 9-12mm. Observed antimicrobial properties of the methanolic extract of *D. spicata*showed that plant might be useful sources for the development of new potent antimicrobial agents [19].

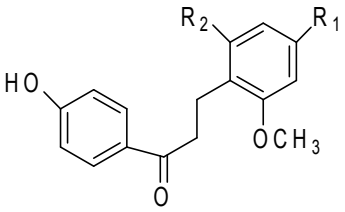
Antimicrobial activity of exudes of red resin of *D. cinnabari* collected from India against *Baccilluscereusvarmycoides* (ATCC11778), *Bacillus pumilus* (ATCC14884), *Bacillus subtilis* (ATCC6633), *Bordetellabronchiseptica* (ATCC4617) was reported .which indicates that *D.* resin could be a rich source of antimicrobial agents with possibly novel mechanism of action [20].

2. 1.3.Phytochemistry of the genus

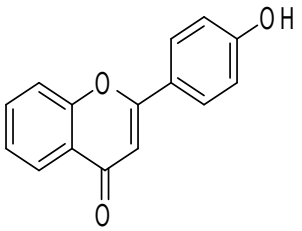
Phytochemical investigations on the genus of the plant have been reported. Thus, the presence of biologically active compounds like steroidal saponins [21], flavanoids[22], phenolic compounds [23] were reported. The exracts of different species of the plant were screened for phytoconstituents indicating the presence of biologically active compounds like alkaloids, carbohydrates, glycosides, amino acids, proteins, steroids, diterpenes, flavanoids, phenolics and tannins [24]. A cytotoxic steroids sponin from *D. afromontana*, antimicrobial steriodialdracogenun, and several flavanoids from *D. draco*, n-heptacosane and palmitic acid from *D. manni* have been reported. From the stems of *D. loureiri,* 16 flavanoids derivatives were isolated [25].Several steroidal saponins with antifungal [26] antituberculosis [27], antiproliferative [28] and cytotoxic [29] activities have been reported from *D. angustifolia.* Nine steroidal saponins were isolated from *D. marginata*. Steroidal saponins are class of natural compounds that provide to exhibit several biological and pharmacological properties [30, 31]. Seven benzenoids were reported from methanol extracts of *D. angustifolia* .Some structures of compounds isolated from *D.loureiri*and *D.angustifolia*were 3-(4-hydroxybenzyl)-3,4-dihydro-2H-chromen-7-ol (**1**), 1-(4- hydroxybenzyl)-3-(2,4-dimethoxyphenyl)propan-1-one(**2**), 1-(4-hydroxybenzyl)-3-(2,4,6-trimethoxyphenyl) propan-1-one(**3**), 3-(4-hydroxy-2-methoxy phenyl)-1-(4-hydroxyphenyl) propan-1-one(**4**), 3-(2,4-dihydroxy-6-methoxy phenyl)-1-(4-hydroxy phenyl) propan-1-one(**5**), 2-(4-hydroxy phenyl)- 4H-chromen-4-one(**6**), (S)-2,3-dihydro-2-phenyl chromen-4-one(**7**), (S)-2,3-dihydro-5-hydroxy-2-phenyl chromen-4-one(**8**), (S)-2,3-dihydro-2-(4- hydroxy phenyl)-5-methoxy chromen-4-one(**9**), (E)-1-(4-hydroxy -2- methoxy phenyl)-3-(4-hydroxy phenyl) prop-2-en-1-one(**10**), 3,4-dihydro-2-(4- hydroxy phenyl)-2H-chromen-7-one (**11**), 4-(3-4-dihydro-7-methoxy-2H-chromen-2-yl) phenol (**12**), 3-(4-methoxyl benzyl)-2,3-dihydro-3,7-dihydroxy chromen-4-one (**13**), 3- (4- methoxyl benzyl)-3,4-dihydro-3-hydroxy-4-oxo-2H-chromen-7-yl- hydrogen carbonate(**14**), 4-hydroxy benzaldehyde(**15**), 3-oxo-2-phenyl acrylaldehyde(**16**), 2-(4-hydroxy phenyl) pent-1-ene-1,3-dione(**17**), ethyl 4-hydroxy benzoate(**18**), benzoic acid(**19**), 4-hydroxy benzoic acid(**20**), methyl 4-hydroxy-2,3-dimethoxy benzoate (**21**)[32].



1.



	R1	R2
2.	OCH_3	H
3.	OCH_3	OCH_3
4.	OH	H
5.	OH	OH



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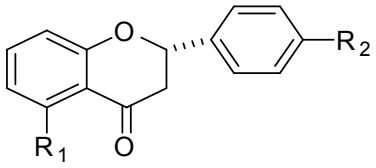
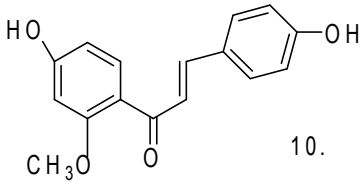
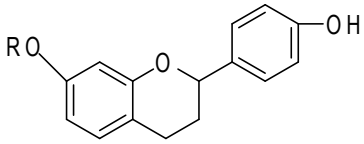


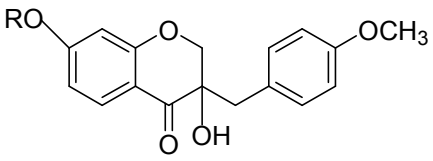
	R1	R2
7.	H	H
8.	OH	H
9.	OCH_3	OH



10.

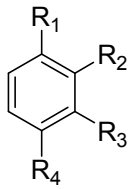


	R
11.	H
12	CH_3



	R
13.	H
14.	AC

Figure 1. Structures of compounds isolated from *D.loureiri*



	R1	R2	R3	R4
15.	CHO	H	H	OH
16.	C═ CCOOH	H	H	H
17.	C═CCOOCH$_2$CH$_3$	H	H	OH
18.	COOCH$_2$CH$_3$	H	H	OH
19.	COOH	H	H	H
20.	COOH	H	H	OH
21.	COOCH$_3$	OCH$_3$	OCH$_3$	OH

Figure 2.Structures of the compounds isolated from *D.angustifolia*

2.1.4. Medicinal uses of the genus

Many species of the plant are used as traditional medicine. In China, the red resin of *D. cochinchinens*, known as dragon's blood, is used for promoting blood circulation and the treatment of traumatic and visceral hemorrhages [33]. *D. manni* barks decoction is used in Africa against abdominal pains, while the mixtures of its root with palm wine is used for the treatment of male impotency [34]. The decoction of *D. angustifolia*is used as tonic and for the treatment of asthma, diarrhea and inflammation [35]. The pharmacological investigation indicated that the C_{27} steroidal saponins present on the genus showed broad biological activity such as anti-inflammatory, antifungal, antimicrobial, antiviral, analgesic, antioxidative, cytotoxic and hypoglycemic properties [36]. Dragon's blood, the resin extracted from stems of *D. cochinchinensis* has been reported to have several medicinal applications as haemostatic, antidiarrhetic, antiulcer, antimicrobial, antiviral, wound healing, antitumor, anti-inflammatory and antioxidant [37]. Powder of *D. steudneri*root is used for wound healing [38].

2.2. Ethnobotany of *D. steudneri*

The species of *D. steudneri*occurs in different parts of Ethiopia. It is locally called*Afarfattuu* in AfanOromo.It is a shrubby multi-stemmed tree up to 45ft tall with tapering trunks with pale brown bark arise separately from the root stock and may branch occasionally. They bear terminal clusters of large sword-shaped, ribbed green leaves. The inflorescence is a panicle of many small greenish-white flowers followed by clusters of reddish-purple fruit. The plant has several ethnomedicinal uses. For instance, the powder of driedroot of *D. steudneri*is used to treat wound [39]. Freshstem barkjuiceisgivenorallytocattle to improve their health.The bark of *D. steudneri* is used to treat black leg, rabies, trypanosomiasis and emaciation [39]. Bark of *D. steudneri* is used to treat cryptococcal meningitis and tuberculosis [40].

Figure 3.Aeiral parts of *D. steudneri* (photography taken by AdisuSakunafrom study area, Jeno,January 2017)

3. MATERIALS AND METHODS

3.1. Chemicals

Chemicals that were used for extraction and column chromatography in this study include n-hexane (99%, Ranchem industry and trading), chloroform (99%), ethyl acetate (99.9%) and methanol (99.9%).All these chemicals were products of Carloerba reagents, France. Silica gel 250-400 mesh size was used as a stationary phase for packing column.Mueller Hinton agar and nutrient broth were used as culture media for antibacterial activity test.Deuteratedchloroform, deuterated acetone and deuterated water were used as solvents for recording NMR spectra.

3.2. Apparatus and Instruments

Apparatus that were used for this study include rotary evaporator, aluminium plated TLC,round bottom flask (50,100 and 250 ml),measuring cylinder,Erlemneryflask,pestle and mortar, Whatmann No.1 filter papers, funnels, grinder, drying oven, vials, glass wares, refrigerator measuring cylinder, TLC Chamber, and capillary tubes, weighing balances, melting point apparatus,UV lamp,IR (Perkin Elmer FT-IR spectrometer with KBrpellets) and NMR (Bruker 500MHz avance NMR spectrometer).

3.3. Plant Materials Collection and Preparation

The roots of *D. steudneri* were collected in January, 2017 fromJenoKebele,LaloKileWoreda, ,KelemWollega zone, Oromia, Ethiopia. The plant material was then authenticated by a botanist Mr. ShambelAlemuand voucher specimen was deposited as DS001 in the National Herbarium of Addis Ababa University. The plant material was then dried in a shade at the laboratory of Kellem Preparatory School.

3.4. Extraction

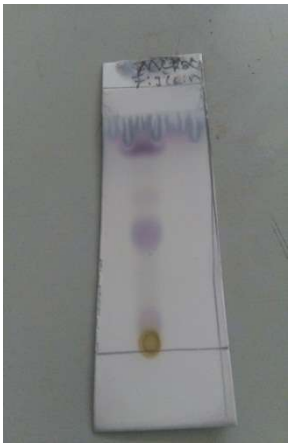
The grounded roots (500 g) of *D. steudneri* were extracted using methanol for 72hrs at room temperature. This was repeated two times and concentrated using a rotary evaporator to afford 40g of a reddish brown crude solid substance. The crude substance obtained was then defatted by n-hexane, filtered and concentrated to give 3g of dark red crude solid substance. The crude methanol extract was also washed by chloroform and ethyl acetate successively, filtered and concentrated under reduced pressure to furnish 4.7g and 3.5g of crude solid substances, respectively.

3.5. TLC Analysis of the Crude Extracts

Each crude extract of the plant was analyzed with TLC. The mobile phases used for chloroform, EtOAc and MeOH extracts were n-hexane:ethyl acetate (9:1), chloroform:ethyl acetate (1:1), and ethylacetate:methanol (7:3), respectively.The TLC were visualized after dipping in vanillin-H_2SO_4 followed by heating on heating mantle. Methanol extract TLC was found to have better separation than other extracts (Figure 4) and preferred to be subjected to column chromatography to afford 30 fractions.

a)TLC chloroform extract

b) TLC of ethyl acetate extract



c) TLC of methanol extract

Figure 4 . TLC profile of the crude extracts of the roots of *D. steudneri*

3.6. Isolation of Compounds Using Column Chromatography fromMethanol Crude Extract

The methanolextract (10 g) was adsorbed on equal amount of silica gel and subjected over silica gel (140g) column chromatography using chloroform for packing. The column was eluted with chloroform:EtOAc:MeOH of increasing polarities to afford 30 fractions (Table 1) .

Table 1. Solvent systems and fractions collected from methanol crude extract of *D. steudneri*

Fractions	Eluent	Ratio	Volume in mL	Mass in mg
1-2	chloroform	100%	200	10
3	chloroform:EtOAc	9:1	100	5
4-5	chloroform :EtOAc	8:2	200	11
6	chloroform :EtOAc	7:3	100	3
7	chloroform :EtOAc	6:4	100	5
8	chloroform:EtOAc	1:1	100	5
9	chloroform :EtOAc	4:6	100	22
10	chloroform:EtOAc	3:7	100	38
11	chloroform:EtOAc	2:8	100	9
12	chloroform:EtOAc	1:9	100	11
13	EtOAc	100%	100	10
14	EtOAc:MeOH	9:1	100	12
15	EtOAc:MeOH	8:2	100	14
16	EtOAc:MeOH	7:3	100	21
17	EtOAc:MeOH	6:4	100	20
18	EtOAc:MeOH	1:1	100	167
19	EtOAc:MeOH	4:6	100	133
20	EtOAc:MeOH	3:7	100	44
21	EtOAc:MeOH	2:8	100	56
22-24	EtOAc:MeOH	1:9	300	462
25-27	MeOH	100%	300	422
28	MeOH	100%	100	156
29	MeOH	100%	100	76
30	MeOH	100%	100	56

From the 30 fractions, fractions 1-4 contained three spots with different R_f value. Fractions 5-7 showed three spots having tail with different R_f values on TLC and solvent system chloroform:ethyl acetate (1:1) as mobile phase. Fractions 8-12 showed four spots having different $R_f$values on TLC in solvent system chloroform:methanol (8:2) as eluent. Fractions 15-20 showed four coloured spots having different $R_f$values on TLC inchloroform:methanol (6:4) as eluent. Fraction 28 was recrystallized and washed with methanol several times and compound 1 was obtained.Fractions 8-12 that showed four spots having different $R_f$values on TLC in solvent system chloroform:methanol (8:2) as eluent wasrechromatographedusing isocratic elution method(Table 2) .

Table 2. Fractions that were collected from column chromatography of F8-12 using chloroform/methanol solvent system

No	Solvent	Ratio	Fraction code	Yield (mg)	TLC profile
1	chloroform/methanol	8:2	F_1	7	Not good
2	chloroform/methanol	8:2	F_2	9	Two spot
3	chloroform/methanol	8:2	F_3	12	Two spot
4	chloroform/methanol	8:2	F_4	14	One spot
5	chloroform/methanol	8:2	F_5	8	Not good
6	chloroform/methanol	8:2	F_6	4	Not good

Fraction F_4 was found to have a promising spot on TLC with 14mg and compound 1 was formed.

Fractions 15-20 from the initial column chromatography that showed four spots was rechromatographed using isocratic solvent to collect 14 fractions each 10ml (Table 3)

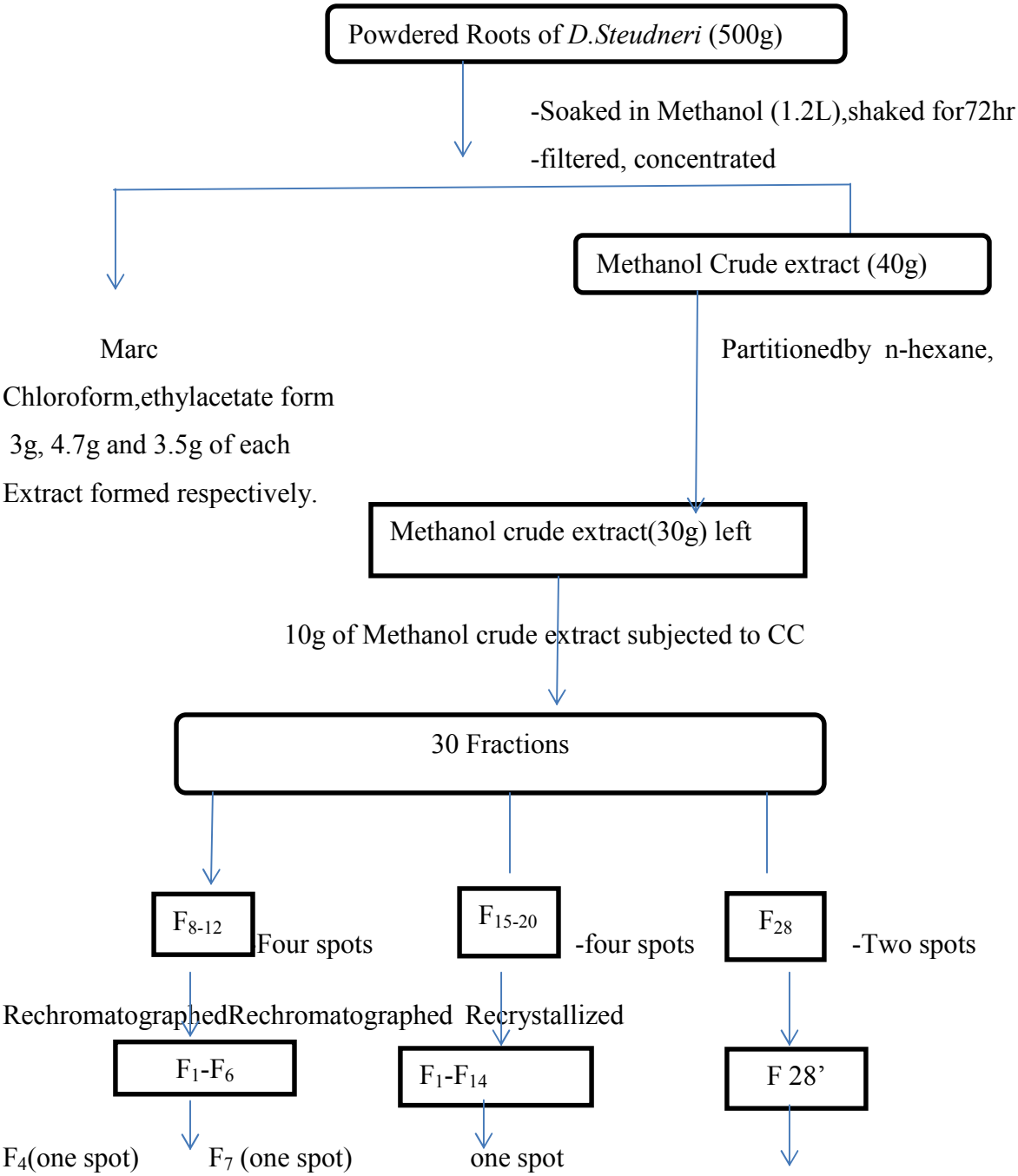
Table 3. Fractions that were isolated from F15-20 using chloroform/methanol solvent system

Solvent system	Ratio	fractions	Solvent system	Ratio	fractions
chloroform/methanol	6:4	F_1	chloroform/methanol	6:4	F_8
chloroform/methanol	6:4	F_2	chloroform/methanol	6:4	F_9
chloroform/methanol	6:4	F_3	chloroform/methanol	6:4	F_{10}
chloroform/methanol	6:4	F_4	chloroform/methanol	6:4	F_{11}
chloroform/methanol	6:4	F_5	chloroform/methanol	6:4	F_{12}
chloroform/methanol	6:4	F_6	chloroform/methanol	6:4	F_{13}
chloroform/methanol	6:4	F_7	chloroform/methanol	6:4	F_{14}

Fraction F_7 was found to have a promising spot on TLC with 15mg and compound 2 was formed.

The overall procedures used from extraction to isolation are summarized below (scheme 1) .

Scheme 1. Extraction and isolation of compounds from the roots of *D. steudneri*



3.7. PhytochemicalScreening

Phytochemical screening is very important for the identification and screening of the bioactive components such asflavonoids, phenols, alkaloids, tannins, terpenoids, steroids and glycosides. It is used for qualitative identification of the classes of metabolites which are available in the roots of *D. steudneri* extracts.

Test for flavonoids

To crude extract(0.5g), 10 mL of ethyl acetate was added and heated over a steam bath for 3 min. The mixture was filtered ,and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution and a yellow color was observed [41].

Test for Terpenoids

Each crude powder (0.5 g) was separately dissolved in 5 mL of methanol. 2mL of the extract was treated with 1mL of 2, 4-dinitrophenyl hydrazine dissolved in 100 mL of 2M HCl. A yellow-orange color was observed [41].

Test for Phenols

Each of crude extract (0.5 g) wasplaced in a different test tube and treated with a few drops of 2% of $FeCl_3$,green color was observed [42].

Test for Steroids

Each crude extracts (0.5 g)was dissolved in 5 mL of methanol. 1 mL of the extract was treated with 0.5 mL of acetic acid anhydride and cooled in ice. This was mixed with 0.5 mL of chloroform and 1 mL of concentrated sulphuric acid was then added carefully by means of a pipette. At the separations level of the two liquids, a reddish-brown ring was formed[42].

Test for Glycosides

Crude extract (0.5g)was dissolved separately in 5 mL of methanol. 10 mL of 50% HCl was added to 2 mL of each extract in test tubes. The mixtures were heated in a boiling water bath for 30 min. 5 mL of Fehling's solution was added and the mixtures were boiled for 5 min and a brick red precipitate was formed [43].

Test for Alkaloids

Crude extracts (0.5g)was defatted with 5% ethyl ether for 15 min. The defatted sample was extracted for 20 min with 5 mL of aqueous HCl on a boiling water bath. The resulting mixture was centrifuged for 10 min at 3000 rpm. 1 mLof the filtrate was treated with few drops of Mayer's reagent and a second 1 mL with Dragendroff's reagent and turbidity was observed [44].

Test for Tannins

Each crude extract (0.5g) was boiled in 10 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added to give brownish green or a blue black color [45].

3.8. Studying of Antibacterial Activities

The anti-bacterial activity of methanol extract of the roots of *D.steudneri*was done *in vitro* using the American Test Culture Collection (ATCC) bacterial strains. Four bacterial strains were obtained from Oromia Public Health Research, Capacity Building and Quality Assurance Laboratory Center, Adama, Ethiopia. Three Gram-negative bacterial strains (*Escherichia coli* ATCC 25922, *Klebsempneumonin*, *Proteus mirabilis* ATCC 25923) and one gram-positive (*Staphylococcus aureus* ATCC 25923) were selected for the study. The antibacterial activities were determined using agar disc diffusion method against different strains of bacteria [46].

3.8.1 Media preparation

Mueller Hinton agar was prepared by dissolving the solid media in distilled water. The solution was then sterilized in autoclave at 121°C for 15 minutes, cooled then poured in Petri dishes. The solution was then left to solidify.

3.8.2 Inoculation and incubation

Antibacterial activity was done on Mueller Hinton agar. 1.5 mg/mL concentrations of the sample were prepared. 6 mm-diameter wells were cut from the agar using a sterile cork-borer and both concentrations of the sample were placed in the wells. The Petri dish was then placed in an incubator for 24 hours at 37°C. At the end of incubation period, the inhibition diameter was measured and expressed in millimeters. 3 drops of each bacterial suspension were applied on the Petri dish to compare with 1 drop of chloroform applied on it. Ciprofloxacinwas used as a positive control group reference drug. Chloroform was used as a negative control group. Control for each bacterial strain, Ciprofloxacinand chloroform before sample action was at 6 mm. Antibacterial activity was determined by measuring the inhibition zone diameter (mm) against each test organism.

3.9. Procedure of Structural Elucidation of Isolated Compounds

The structures of the isolated compounds were elucidated based on data obtained from physical properties determination such as melting points and spectroscopic methods including IR and NMR. In some cases the spectral data was compared with the literature value reported for the same compounds.

4. RESULTS AND DISCUSSION

4.1. Percentage Yields of the Crude Extracts

The yield of extracts were calculated on dry weight basis using the formula,

$$\% \text{ extract yield } = \frac{\text{weight of dry extract}}{\text{Weight of sample}} x\ 100\%$$

The ground roots of *D. steudneri* were extracted with MeOH, n-hexane, chloroform and ethyl acetate to afford 40g (8%), 3 g (0.6%), 4.7 g (0.94%) and 3.5g (0.7%) respectively.

Table 4. Amounts of the crude extracts obtained from each extract

Types of extracts	Mass of the crude extracts	% yield
methanol	40g	8
n-hexane	3g	0.6
chloroform	4.7g	0.94
Ethyl acetate	3.5g	0.7

4.2. Phytochemical Analysis of Crude Extracts

Phytochemical screening of n-hexane, chloroform, ethyl acetate and methanol crude extracts of *D. steudneri* roots revealed the presence of chemical components as shown in Table 5. Secondary metabolites such as alkaloids, saponins,terpenoids, glycosides, steroids, and tanninswere screened in the extracts of *D. steudneri*. The study revealed that n-hexane roots extract of*D.steudneri* contains terpenoid, saponins, and steroid. On the other hand, chloroform extract contains alkaloid, terpenoids and steroid. Ethyl acetate extract contains terpenoids, tannins and steroid. Methanol extract contains alkaloids,saponins,terpenoids,tannin, steroid and glycoside (Table 5).The medicinal value of plants lies in some chemical substances or secondary products that have definite physiological functions in the human body and which may help in protection against various diseases. Plants contain many active compounds such as alkaloids,

steroids, tannins, glycosides,terpenoid,steroid and tannins that are deposited in their specific parts such as leaves, bark and root, etc.

The use of some plants for medicinal purpose, in the traditional treatment of diseases is due to the presence of secondary metabolites [47], which supports the use of *D.steudneri*for the treatment of several diseases by local herbalists or traditional healers.

Table 5 . Phytochemical analysis of n-hexane, chloroform, ethyl acetate and methanol crude extracts.

S.No	Phytochemical	Crude extracts			
		n-hexane	Chloroform	Ethyl acetate	Methanol
1	Alkaloids	-	+	-	+
2	Saponin	+	-	-	+
3	Terpenoids	+	+	+	+
4	flavanoids	-	-	-	+
5	Tannin	-	-	+	+
6	phenol	-	-	-	-
7	steroid	+	+	+	+
8	glycoside	-	-	+	+

Key: (+) presence, (-) absence

4.3. Structural Elucidation of Isolated Compounds

4.3.1 Characterization of compound 1

Compound 1 was obtained as reddish solid (53mg) and its melting point was 209-211^{o}C.TLC showed R_f. 0.73 with methanol:water (4:6) solvent system as a mobile phase. After recrystallization a cleaner spot was observed on TLC (Figure 5).

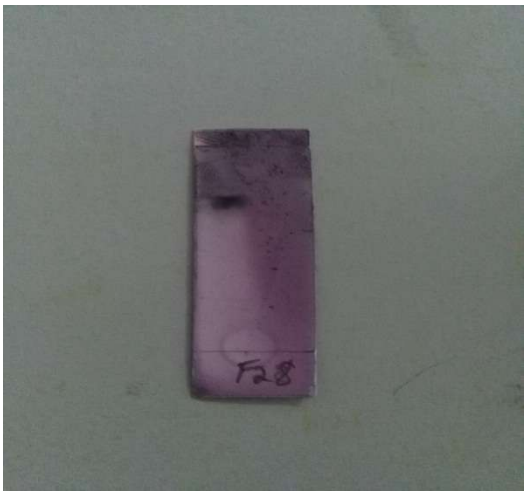


Figure 5.TLC profile of compound 1

Compound 1was soluble in waterthat showed a characteristic colour change from colourless to intense black on TLC plate upon dipping in vanillin in H_2SO_4 and heating for a few minutes.

The IR (KBr plate) spectrum of Compound 1(Appendix 1) showed, a broad absorption band at 2500-3353cm^{-1}that is indicative of O-H stretching of a carboxylic acid group. The strong absorption band at 3353 cm^{-1} showed the presence of the N-Hstretching.The strong absorption band at 2949 cm^{-1} showed the presence of the C-Hstretching The absorption band at 1639 cm^{-1} showed the presence of the C=O stretching. The absorption band at 1409 cm^{-1} showed the presence of C-Hbending.

The ^{13}H-NMR spectrum (Appendix 2) in $D_2O$of compound **1** showed the characteristic of imino acid at region between δH 2.42 to 3.92. The doublet of doublet signal centred at δH3.92 (1H) accounted to methine protons on a four membered ring adjacent to carbonyl carbon. The multiplet signal observed at δH 2.42 (1H) and 2.66 (1H) is accounted to a

diasterotopicmethylene protons adjacent to methine protons. The multiplet signal centred at δH 3.8 (2H) is accounted to methylene protons adjacent to a diasterotopic methylene protons .

The ^{13}C-NMR spectrum (Appendix 3) in $D_2O$of compound **1**, analysed with the aid of DEPT-135 (Appendix 4) in D_2Oshowed a total of four carbon atoms signals, which can be attributable to a four membered monocyclic ring composed of one carbonyl (carboxylic acid), onemethine (CH), and two methylene (CH_2) groups. This structural type was evident from the^{13}C- NMR spectrum, which contained aliphatic carbon signal of two CH_2groups at δ_C23.2 and 42.2,onemethinecarbon signal (CH) at δ_C58.9 and a carboxylic acid signal at δ_C173.8. The NMR spectral data of Compound **1** was found in agreement with the NMR spectral data of azetidine-2-carboxylic acidreported in the literature[49] (Table 6). Based on the NMR data, the structure of the compound **1**waswproposed to be azetidine-2-carboxylic acid(Figure 6). This compound was hitherto unreported from *D.steudneri.*But,this compound was a natural product isolated from the leaves of *Convallariamajalie*[48]. Reports also shows that this compound is widely distributed especially in higher plants such as *M .canadense, R. japonica, L.muscari, L.spicata, D.fragrance,D.sanderiana*and so on [48].

Table 6. Comparison of ^{1}H –NMR and ^{13}C-NMR data of (400 MHz, D_2O) spectral data of compound 1 and literature reported for azetidine-2-carboxylic acid [49].

Carbon position	^{13}C-NMR data of Comp.1	^{1}H -NMR data of Comp.1	DEPT-135 data of Comp.1	^{13}C-NMR data reported[49]	^{1}H -NMR data reported[49]	remark
1	59.1	3.92 (dd, *1H* ,J=9.6,9.6Hz)	59.1	58.9	4.07 (dd,1H J=9.4,9.4Hz)	CH
2	23.3	2.66 (*m*, 1H), 2.42 (*m*, 1H)	23.2	23.2	2.65 (m,1H) 2.37 (m,1H)	CH_2
3	42.8	3.8(m,2H)	42.2	42.2	3.89(m,1H)	CH_2
4	174.1	-	-	173.8	-	C(quaternary)

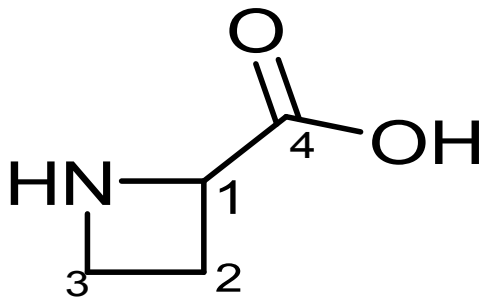


Figure 6 .Proposed structure of compound 1

4.3.2 Characterization of compound 2

Compound 2 was obtained as reddish-yellow solid (15 mg) and its TLC showed one spot at R_f value 0.65 with ethyl acetate: methanol (8:2) solvent system as a mobile phase.The compound compound 2was soluble in acetone-d_6 that showed a characteristic colour change from colourless to intense blue on TLC plate upon dipping in UV-Lamp .

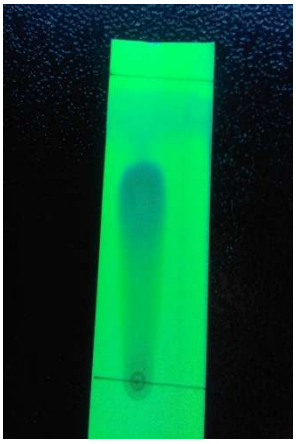


Figure 7.TLC profile of compound 2

In the IR (KBr) spectrum of Compound **2**(Appendix 5), the absorption band at 3381 cm^{-1}showed O-H stretching that indicated the presence of a hydroxyl group (alcohol functional group). The absorption band at 2913 cm^{-1} showed the presence of the C-H stretching. The absorption band at 1684 cm^{-1} showed the presence of the stretching of conjugated C=O group. Whereas the absorption band at 1510 cm^{-1}indicated the presence of aromatic ring. The absorption band at 1349 cm^{-1} showed the presence of C-C stretching.

The ^{1}H NMR spectrum of the compound 2(Appendix 6) has showed highly deshielded signal at δ_H 12.43 for hydroxyl protons.TheH-2' and H-6' proton signalsobserved at δ_H8.1 gave doublet(2H,J=9.2Hz).The H-3' and H-5' proton signals were observed at δ_H *6.9* as a doublet(2H,J=8.8Hz). The H-6 and H-8 protons were observed at δ_H 6.27(1H,d,J=2Hz) and δ 6.5(1H,d,J=1.6Hz), respectively. The proton signal appeared at δ_H 5.25(1H,d,J=6.4Hz) was assigned to the anomeric proton (1",2",3",4",5") of aβ-glucose.

The ^{13}C-NMR spectrum (Appendix 7) in acetone-$d_6$of compound 2, analysed with DEPT-135 (Appendix 8) in acetone-$d_6$showed a well resolved resonance of 21 carbon atoms. From the spectral data elevenmethine (CH) were observed. In addition, the spectral data indicated the presence of nine quaternary carbon, one methylene (CH_2) and one carbonyl(CO). Also anomeric carbon peak is observed atδc103.1. The complete ^{13}C-NMR data is given in Table 7.Besides to the observed relationships between ^{13}C-NMR and DEPT-135, ^{13}C-NMR spectral data of the proposed structure of compound **2** was found to agree with the reported literature spectral data of ^{13}C-NMR of kaempferol 3-O-β-glucopyranoside [50] (Table7). Based on the NMR data, the structure of the compound **2** was given as shown in Figure 8.

Table 7. Comparison of ^{13}C-NMR and ^{1}H-NMR (400 MHz, acetone-d_6) spectral data of compound 2 and literature reported for kaempferol 3-O-β-glucopyranoside [51].

Carbon position	^{13}C-NMR datacomp.2	^{1}H-NMR datacomp.2	DEPT-135 data comp.2	^{13}C-NMR data reported	^{1}H-NMRdata reported	Remark
2	157.8		-	157.4		C(quaternary)
3	134.3		-	134.3		C(quaternary)
4	178.1		-	178.4		C(quaternary)
5	161.9		-	161.9		C(quaternary)
6	98.8	6.27d	98.8	98.7	6.20d	CH
7	164..5		-	164.9		C(quaternary)
8	93.8	6.5d	93.8	93.6	6.4d	CH
9	157.8		-	157.9		C(quaternary)
10	104.5		-	104.6		C(quaternary)
1’	121.3		-	121.6		C(quaternary)
2’	131.1	8.1d	131.1	131.1	8.05d	CH
3’	115.09	6.9d	115.09	114.9	6.88d	CH
4’	160.2		-	160.4		C(quaternary)
5’	115.3	6.9d	115.3	114.9	6.88d	CH
6’	131.1	8.1d	131.1	131.1	8.05d	CH
1”	103.1	5.25d	103.08	102.9	5.26d	CH
2”	74.5	5.25d	74.5	74.6	5.26d	CH
3”	76.1	5.25d	76.1	76.9	5.26d	CH
4”	70.6	5.25d	70.6	70.2	5.26d	CH
5”	77.01	5.25d	77.8	77.3	5.26d	CH
6”	61.7	3.57m	61.6	61.5	3.52dd,3.69dd	CH_2

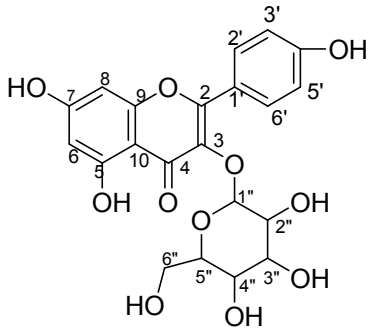


Figure 8. Proposed structure of compound 2

4.3.3Characterization of compound 3

Compound 3 was obtained as gummy substance (14 mg) and soluble in $CDCl_3$ that showed a characteristic colour change from colourless to intense pink on TLC plate upon dipping in vanillin-H_2SO_4 and heating for a few minutes.

Figure 9.TLC profile of compound 3

In the IR ($CDCl_3$) spectrum of the compound 3 (Appendix 9), the absorption band at 3421cm^{-1}showed the O-H stretching that indicated the presence of a hydroxyl group. The strong absorption band at 2922 cm^{-1} showed the presence of the C-H stretching, due to the stretching vibrations of aliphatic -CH_3groups.The bands at 2843cm^{-1} indicate C-H stretching of methylene groups. The band at 1714 cm^{-1} indicated the presence of carbonyl group of carboxylic acid. The absorption band at 1465 cm^{-1}could be attributed to the presence of C-Hbending. The band of medium intensity at 1373 cm^{-1} indicates C-O stretching of carbonyl carbon of carboxylic acid.

Analysis of the ^{1}H-NMR ($CDCl_3$, 400 MHz) spectrum of compound 3(Appendix10) exhibited a triplet signal at 2.36 ppm for hydrogens adjacent to carbonyl group which are slightly deshielded. The spectrum also indicates multiplate signals at 1.67 for methylene (H-3) ppm, broad singlet at 1.27 ppm (chain) and intense triplet at 0.89 ppm for methyl hydrogens (H-16). The observed ^{1}H-NMR spectral data (Table8) of compound DS-17A was found to be consistent to that of palmitic acid reported in literature [52]

The analysis of ^{13}C-NMR spectrum of compound 3 (Appendix 11) showed signals at 178.5 (C-1) carbonyl carbon of fatty acid**s**, 33.7 (C-2), 24.7 (C-3), 29.3 (C-4), 29.4 (C-5), 29.6 (C-6 and 7), 29.2 (C-8), 29.7 (C_9-C_{12} of the chain), 29.07 (C-13), 31.9 (C-14), 22.6 (C-15) and 14.1 (C-16) ppm (Table8). The DEPT-135 spectrum of the compound 3 showed a single signal at δc 14.127 indicating the presence of one methyl carbon (appendix 11).The observed ^{13}C-NMR spectrum of Compound 3 was found to be consistent with that of the data reported for palmitic acid[52]. Moreover, the observed melting point of Compond 3(59-62.5^{0}C) was comparable to the reported melting point value of palmitic acid (i.e., 63-64°C) [52]

Table 8. ^{13}C-NMR ,^{1}H-NMR and DEPT-135 ($CDCl_3$,400MHz) data of compound 3 along with the reported NMR data of Palmitic acid

Carbon position	^{13}C-NMRdata of Comp. 3	^{1}H-NMR data of Com. 3	DEPT-135 data of Comp.3	^{13}C-NMR data reported[52]	^{1}H-NMR data reported [52]	remark
1	178.5	-	-	179	-	Carbonyl
2	33.7	2.36(2H,t,J=7.6Hz)	33.7	34.03	2.34	CH_2
3	24.7	1.67(2H,m)	24.7	24.69	1.63	CH_2
4	29.3	1.27(s)	29.3	29.3	1.27	CH_2
5	29.4	1.27(s)	29.4	29.4	1.27	CH_2
6&7	29.6	1.27(s)	29.6	29.6	1.27	CH_2
8	29.2	1.27(s)	29.2	29.2	1.27	CH_2
9-12	29.7	1.27(s)	29.7	29.7	1.27	CH_2
13	29.07	1.27(s)	29.07	29.07	1.27	CH_2
14	31.9	1.27(s)	31.9	31.9	1.27	CH_2
15	22.6	1.27(s)	22.6	22.7	1.27	CH_2
16	14.1	0.83(3H,t,J=6.8,7.2Hz)	14.1	14.1	0.88	CH_3

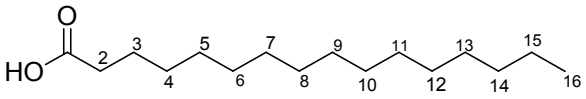


Figure 10. Proposed structure of compound 3

4.4. Antibacterial Activities of Extracts from Roots of *D.steudneri* and IsolatedCompounds

Antibacterial activity was performed with the aim of assessing the potential antibacterial activity of the extractsand isolated compounds from*D.steudneri* roots against the selected bacterial strains. The zone of inhibition was then identified for the bacterial strains that exhibited reasonable activity performed on the prepared concentrations of the extract. The inhibitory effects of *D.steudneri*extract against *E. coli, S. aureus,Klebsempneumonin and P. mirabilis*were determined. The results of zone of inhibition in diameter against tested pathogens are presented in Table 9 .

When the zone of growth inhibition values of the crude extracts are compared with each other,theactivities of methanol extracts was found to be superior against all the bacterial species.The data shows that the zones of growth inhibition values are 14,8,9,8mm against *S.aureus, E.coli, K.pneumonin*and *P.mirabilis*respectively. For *in vitro* antibacterial activity tests for the isolated pure compound 1 was evaluated. This test indicates that isolated compound 1had some activities against the bacterial test strains.The diameter of growth inhibition zone displayed on both gram positive and gram negative were good with variable degree of potency .However,compound 1have shown better anti-bacterial activity on *S.aureus* a gram positive bacteria with zone of inhibition 14mm.

Table 9. Antibacterial activity of *D.steudneri* roots crude extracts

Bacterial Strain	Gram	Diameter of zone of growth inhibition(mm) chloroform extract	ethylacetate extract	methanol extract	ciprofloxacin	chloroform extract
S.aureous	+	8	10	14	23	NI
E.coli	-	8	NI	8	21	NI
K.pneumonin	-	9	7	9	19	NI
P.mirabilis	-	NI	NI	8	24	NI

Key: NI= No inhibition, + positive, - negative

5. CONCLUSION AND RECOMMENDATIONS

5.1 .Conclusions

The dried powder of roots of *D. steudneri* was extracted with methanol,n-hexane , chloroform and ethylacetate. The TLC analysis of each extracts showed the presence of several compounds on TLC upon dipping in vanillin in H_2SO_4 and heating for a few minutes. Phytochemical screening tests has shown that *D. steudneri*roots extract contain alkaloids,flavonoids,steroids,saponin,tannin, terpenoids and glycosides. The methanol crude extract was subjected to column chromatography and yielded an imino acid, a flavonoid, and afatty acid characterized asazetidine-2-carboxylic acid (Compound 1),kaempferol 3-O-*β*-glucopyranoside **(**compound 2**)** andpalmitic acid**(**Compound 3**)**respectively**.** All of them were isolated for the first time from the roots of this plant. The chemical structures were characterized on the basis of NMR spectral analysesand comparison with reported data in literatures. The antibacterial activity evaluation of the methanol crude extract showed moderately sensitive against *S. aureus,* which support its use in traditional medicine in the treatment of wounds.

5.2. Recommendations

- Although, the compounds isolated were not the only compounds present in the plant material as evidenced from TLC analysis, future work should aim at isolating other compounds not isolated in this study and determine their antibacterial activity.
- It is recommended to carry out further works using HPLC , HPLC-MS and MS for analysis of the compounds isolated from the plant roots.
- It is also recommended that future research should be extended to cover other biological activities including antifungal, anti-cancer, antiviral, antimalarial and anti-plasmodial activity.

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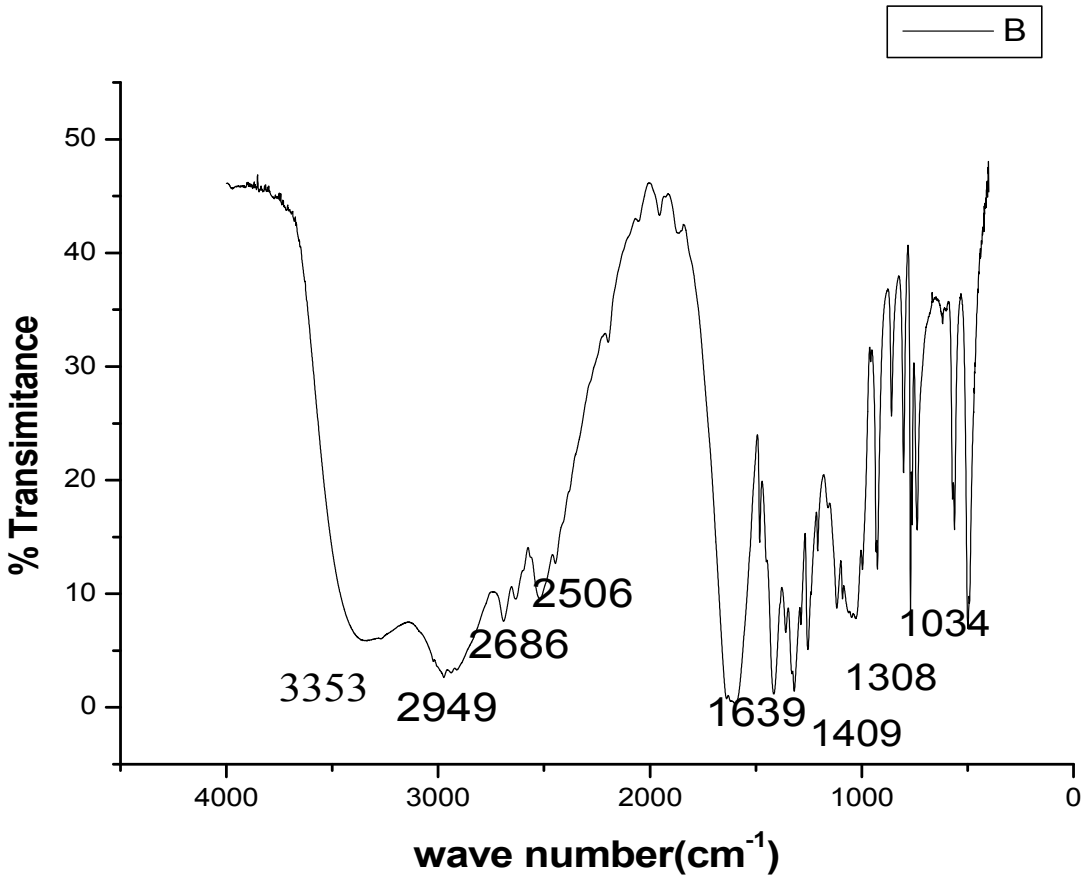
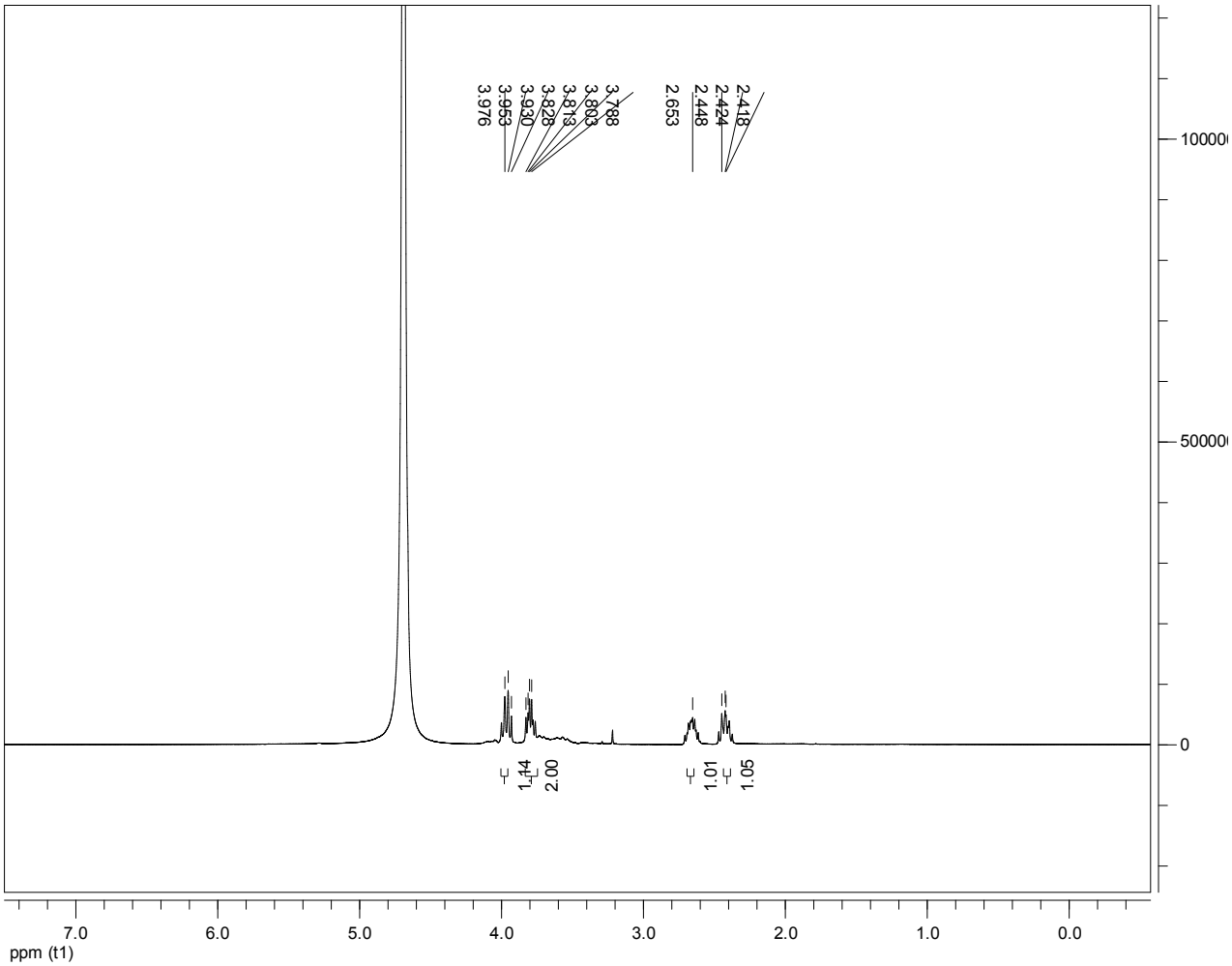
APPENDIXES

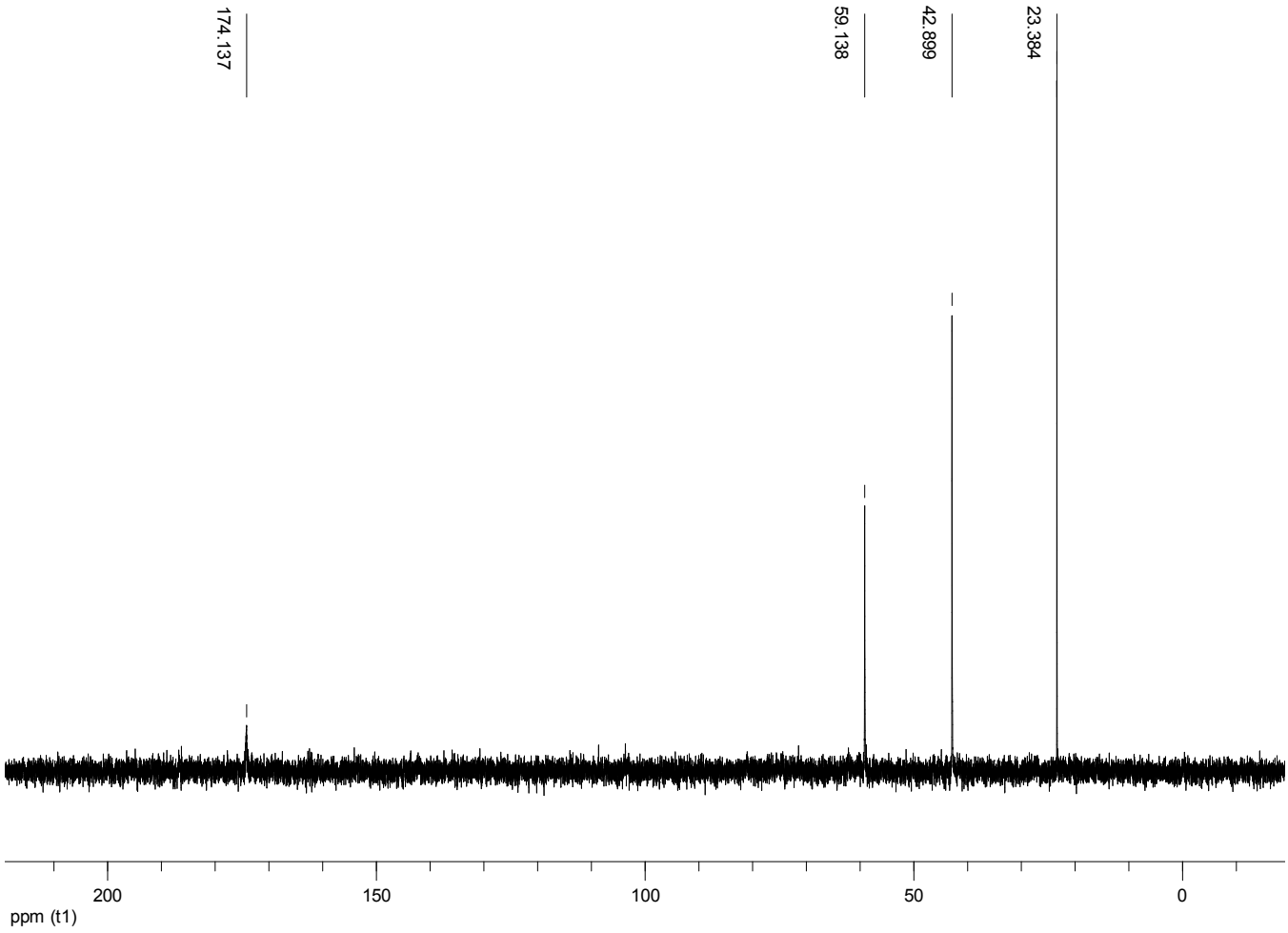
Appendix 1.IR Spectrum of compound 1



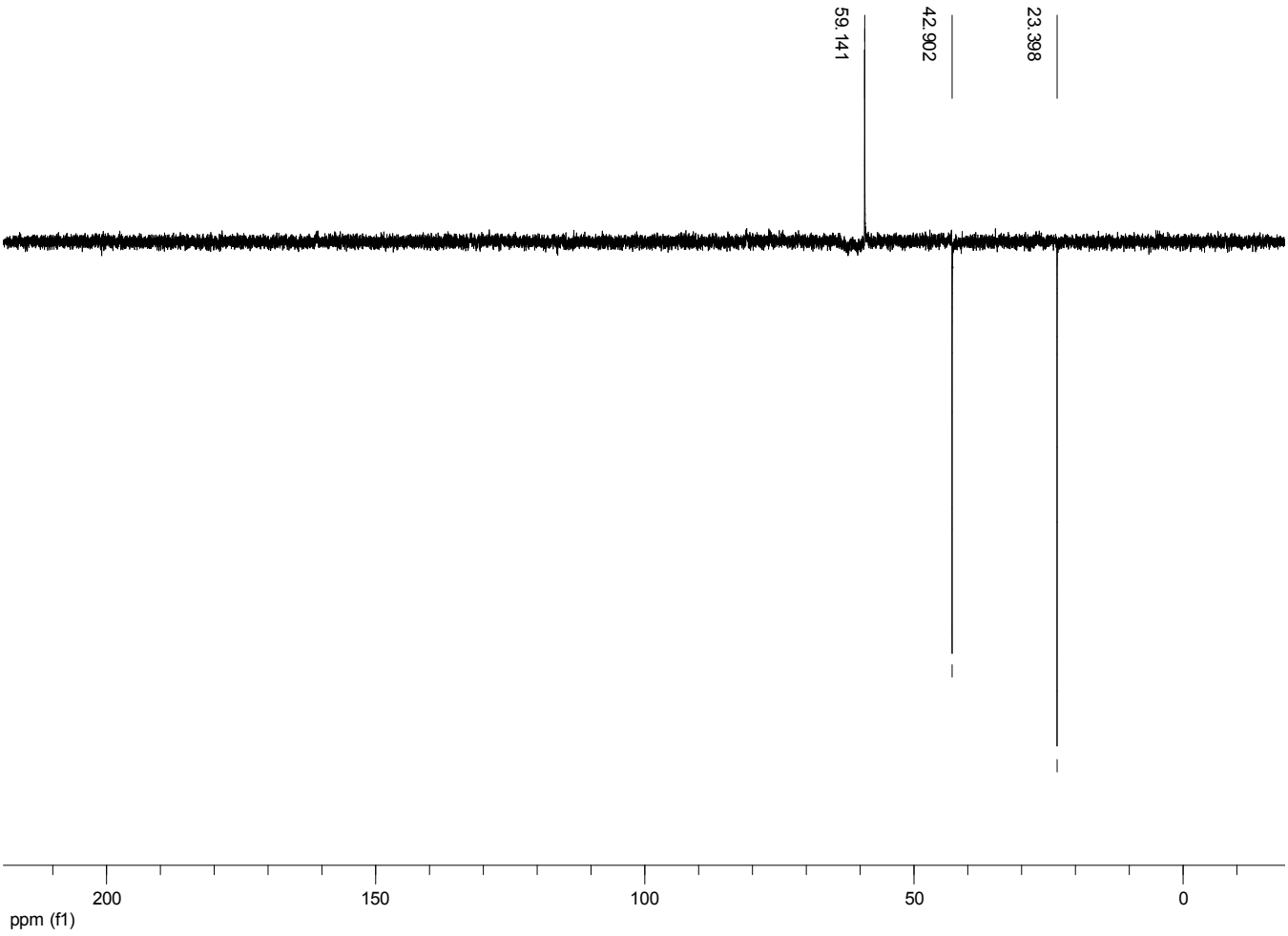
Appendix 2. ^{1}H-NMR spectrum of compound 1 in D_2O



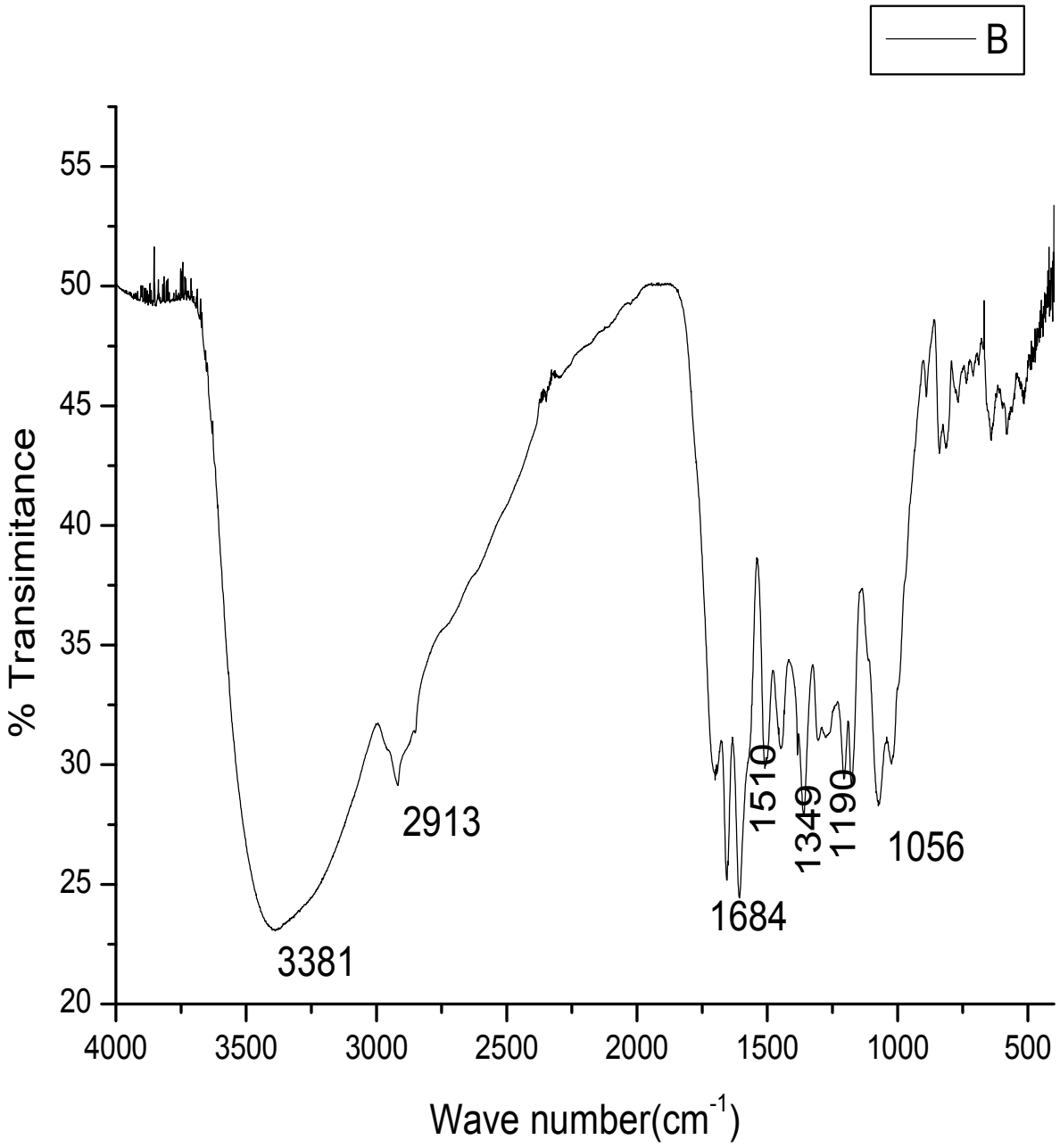
Appendix 3. ^{13}C- NMR spectrum of compound 1 in D_2O



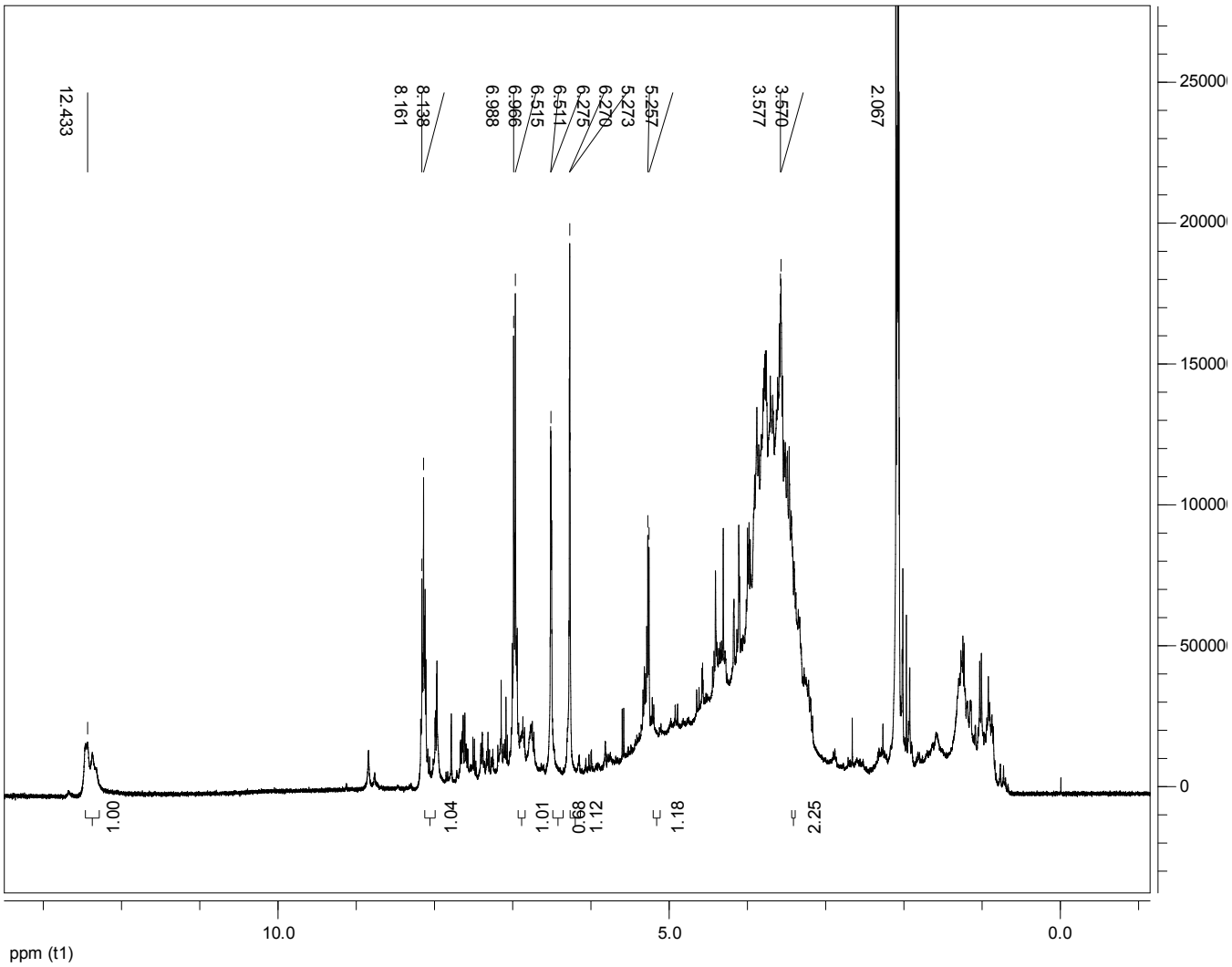
Appendix 4.DEPT-135 NMR spectrum of compound 1 in D_2O



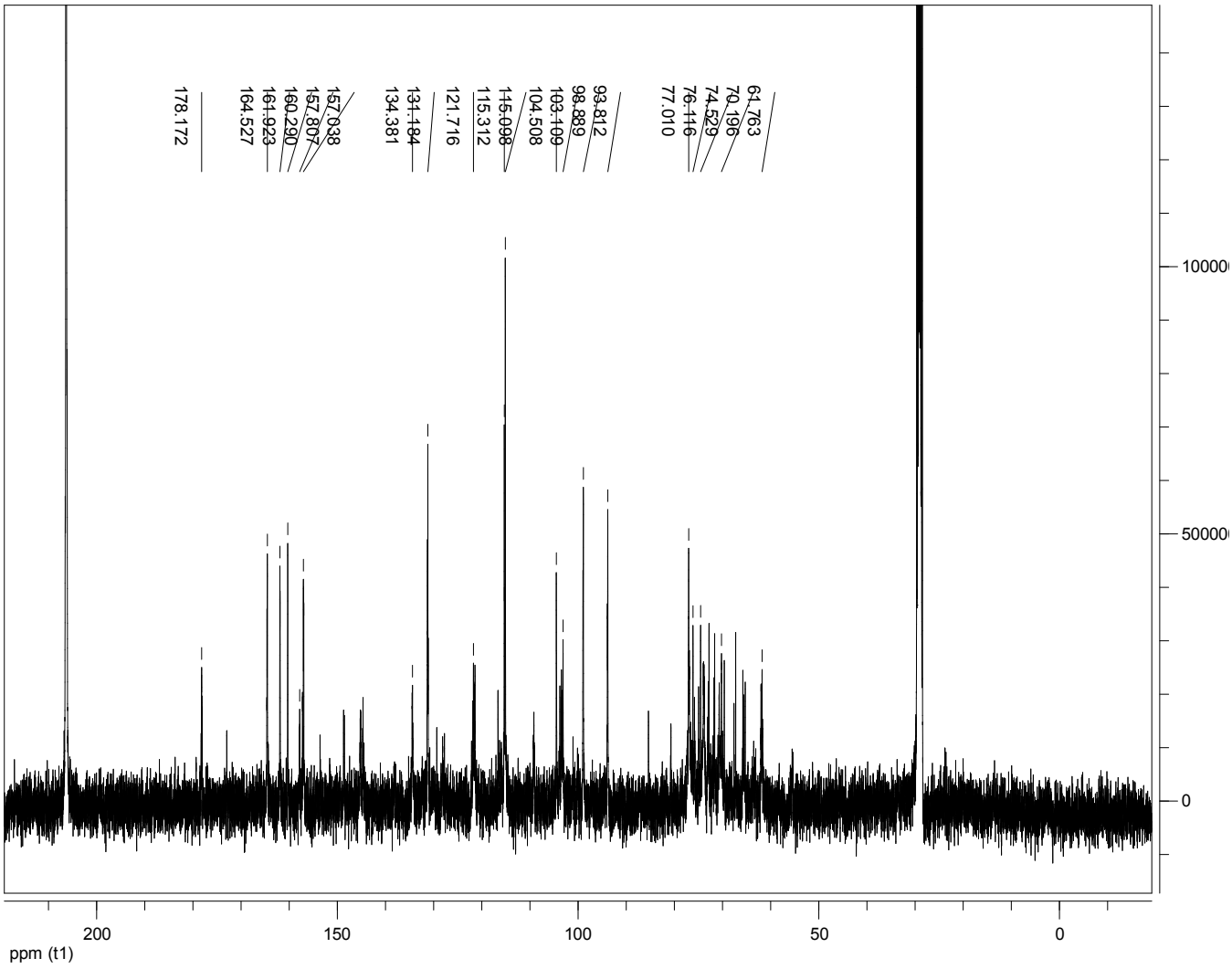
Appendix 5 .IR spectrum of compound 2



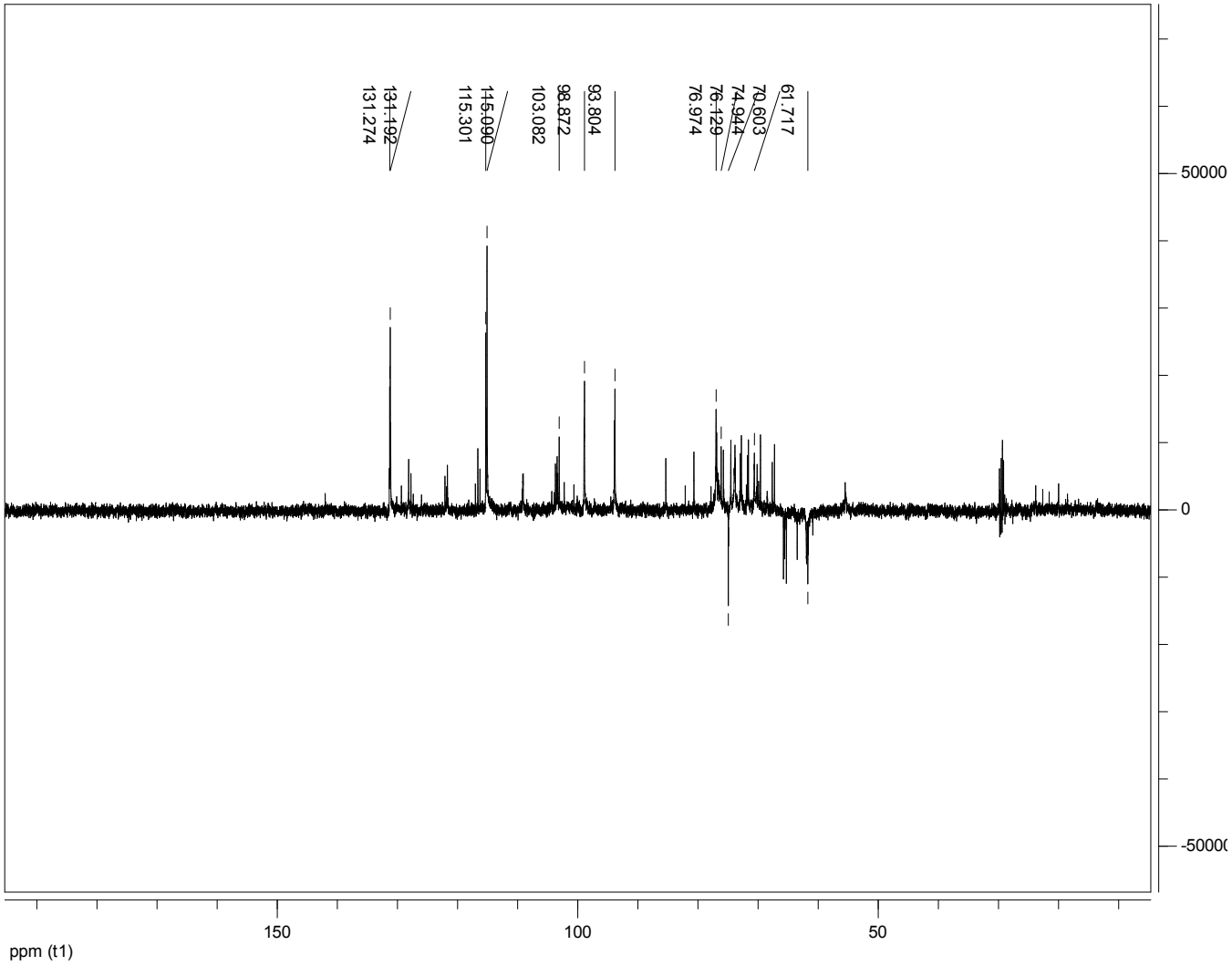
Appendix 6 .[1]H-NMR spectrum of compound 2 in acetone $-d_6$



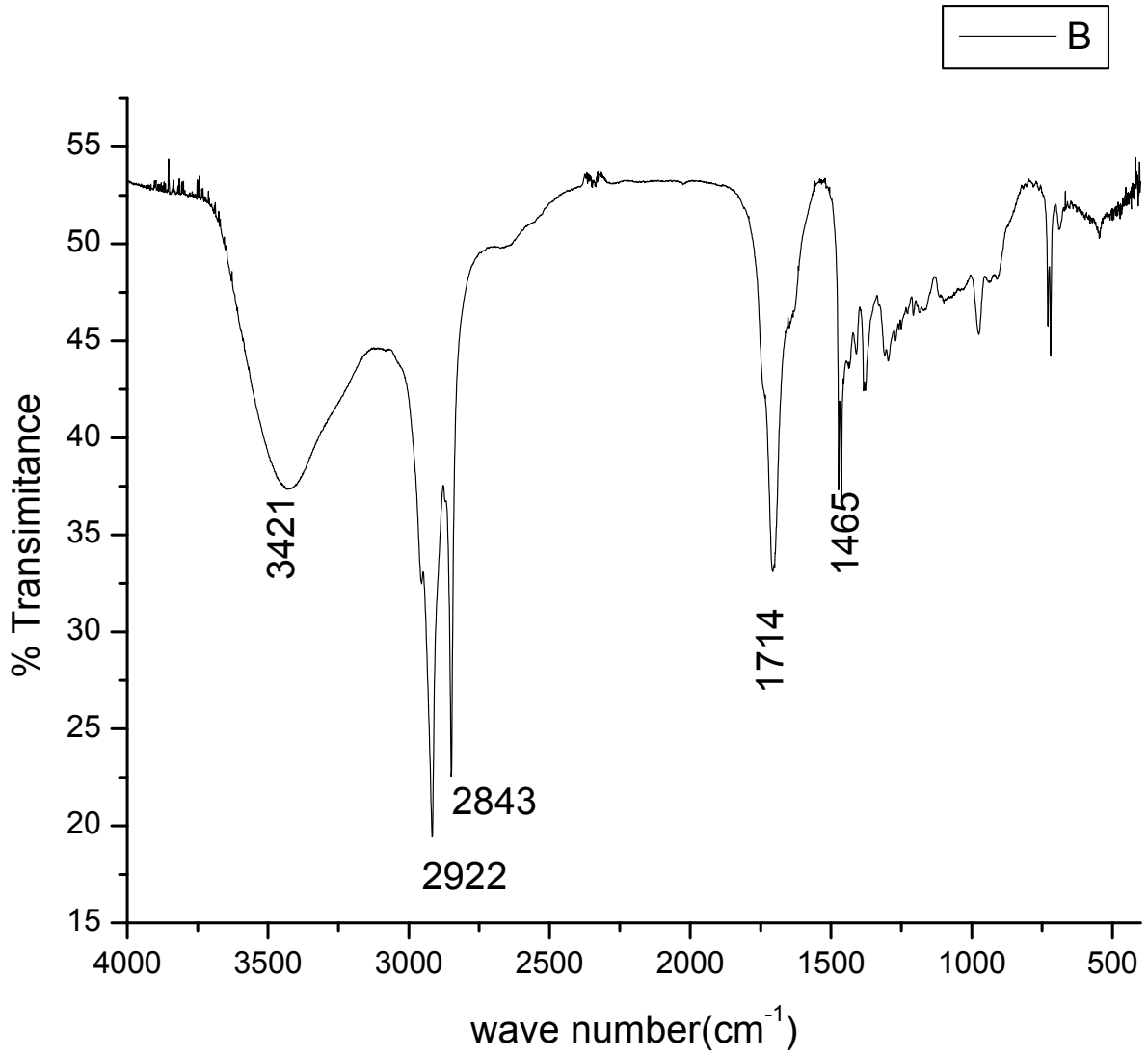
Appendix 7. ^{13}C-NMR spectrum of compound 2 in acetone-d_6



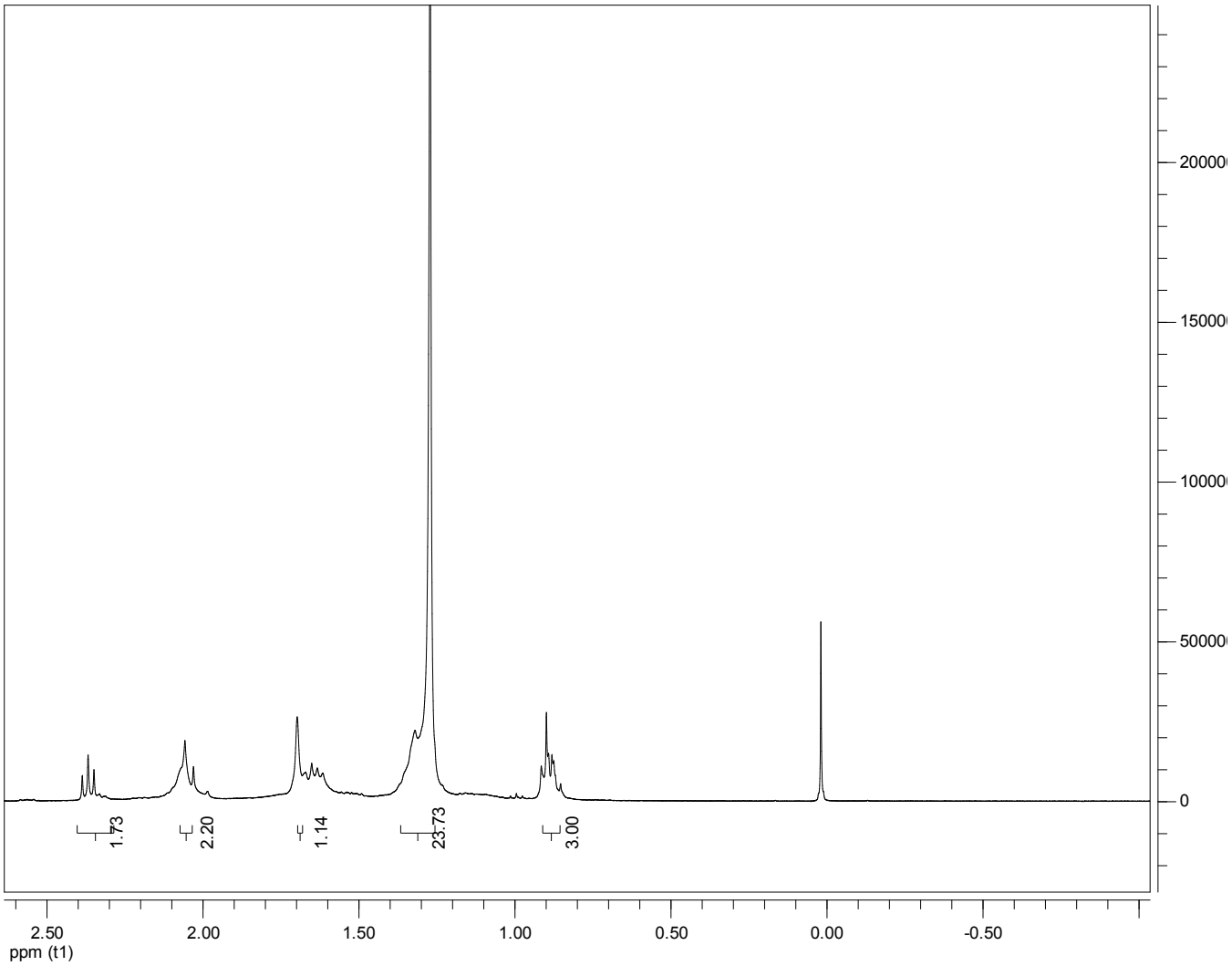
Appendix 8.DEPT-135 NMR spectrum of compound 2 in acetone-d_6



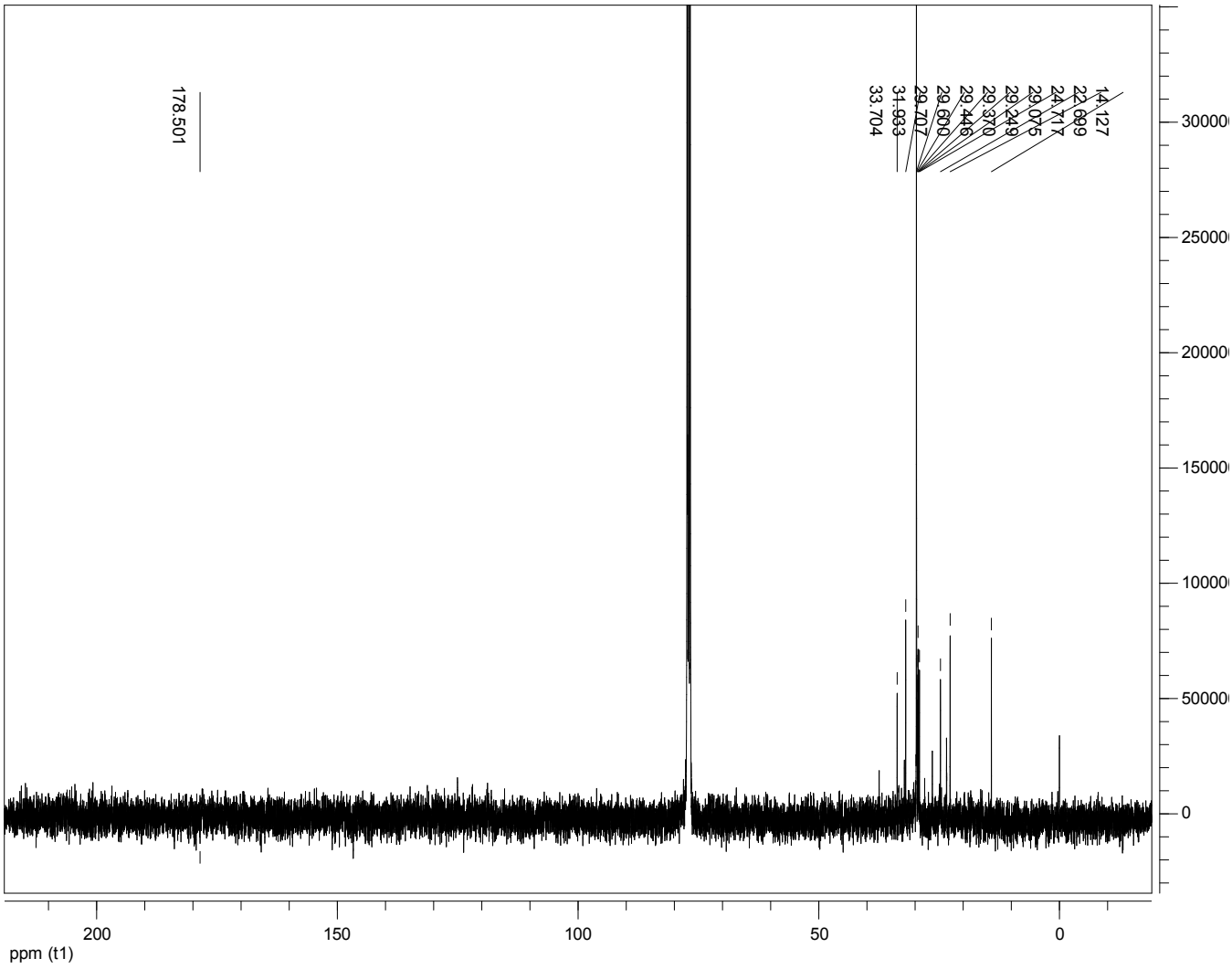
Appendix 9.IR spectrum of compound 3



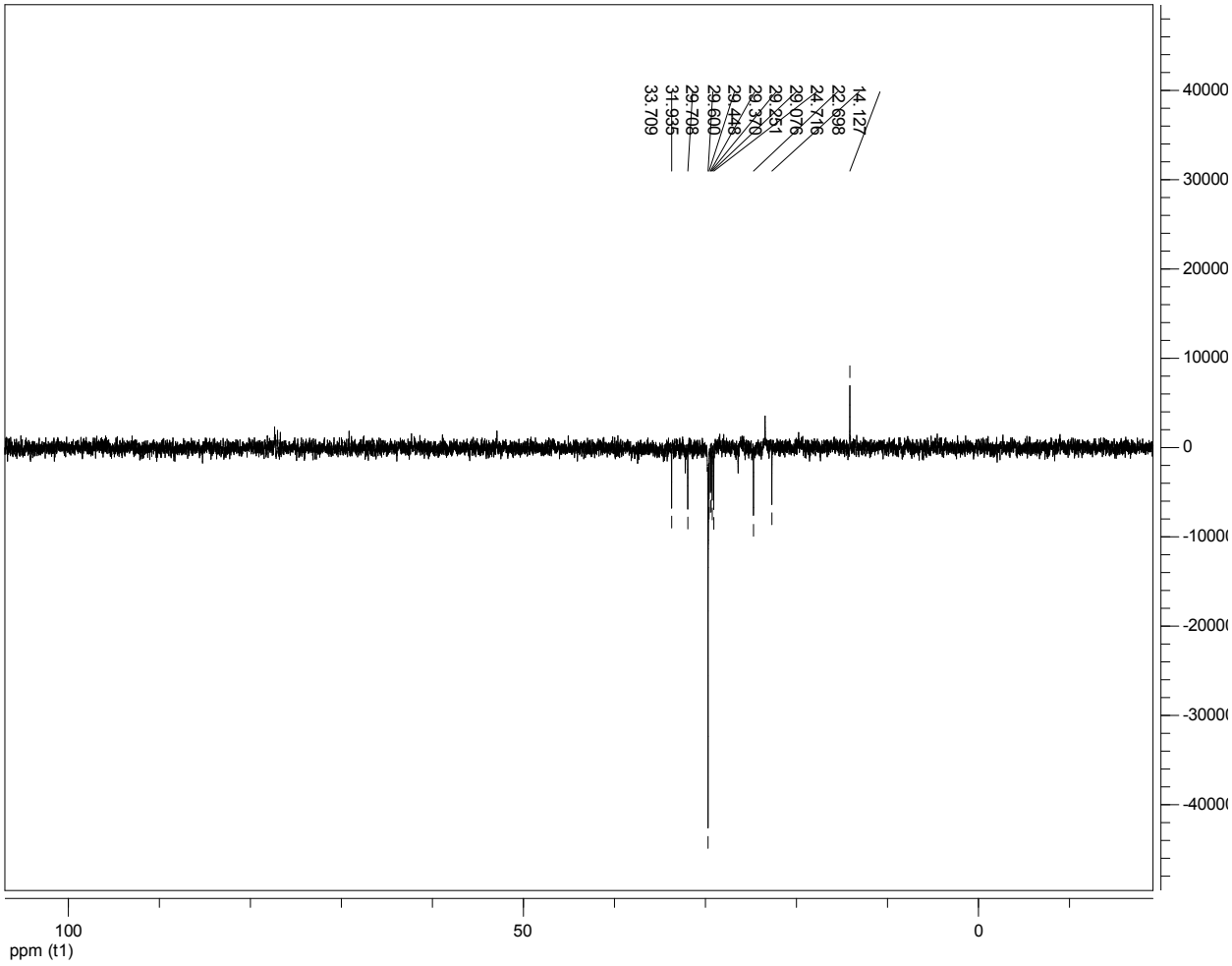
Appendix 10 .[1]H-NMR spectrum of compound 3 in $CDCl_3$



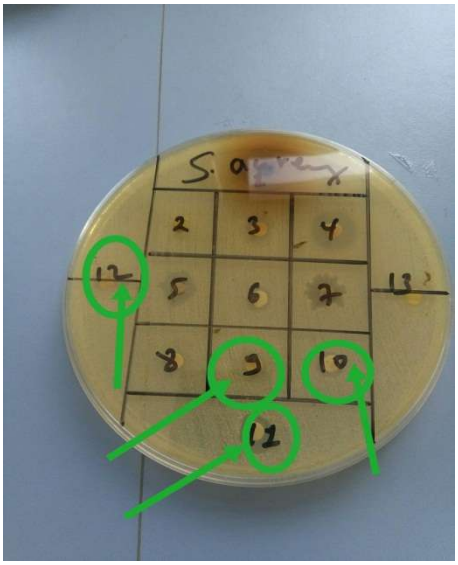
Appendix 11. ^{13}C- NMR spectrum of compound 3 in $CDCl_3$



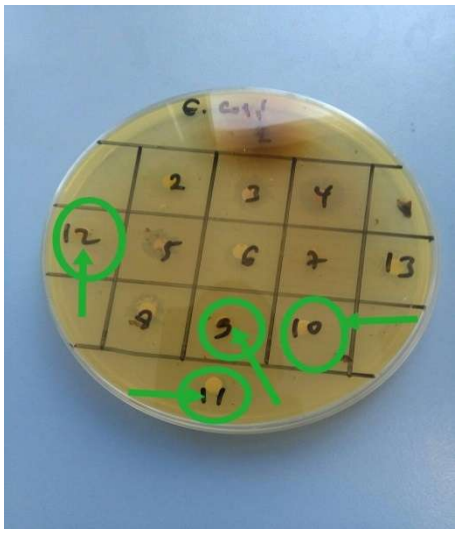
Appendix 12. DEPT-135 NMR spectrum of compound 3 in $CDCl_3$



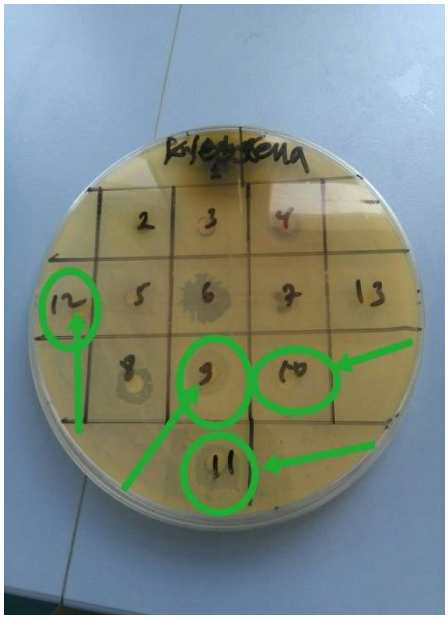
Appendix 13. Antibacterial activity of roots of *D.steudneri* extracts



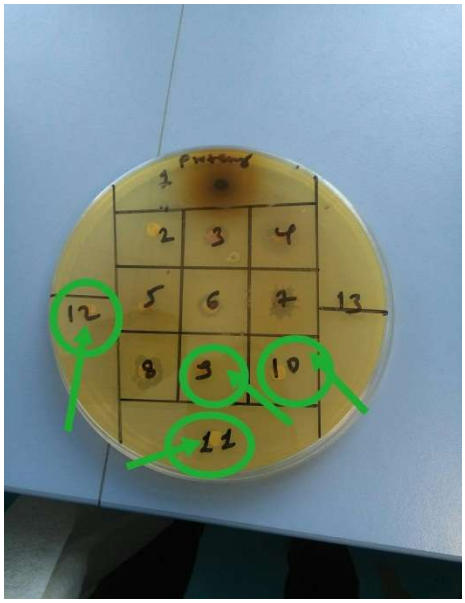
a) *D.steudneri* extracts against *S.aureus*



b) *D.steudneri* extracts against *E.coli*



b) *D.steudneri*extracts against*K.pneumonin*



d)*D.steudneri* extracts against *P.mirabilis*