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PHYTOCHEMICAL INVESTIGATION OF FRIUTS OF *DOVYALIS ABYSSINICA* AND EVALUATION OF ITS ANTIMICROBIAL ACTIVITIES

BY

BIRHANU GUTA

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JIMMA, ETHIOPIA

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A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN CHEMISTRY

BY

BIRHANU GUTA

ADVISOR: YINEBEB TARIKU (Ass.Prof.)

CO-ADVISOR: DALE ABDISSA (M.Sc.)

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JIMMA, ETHIOPIA

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Abbreviations and Acronyms

ANOVA	Analysis of variance
BDH	British Drug House
DCM	Dichloromethane
DMSO	Dimethylsulfoxide
MIC	Minimum Inhibitory Concentration
MHB	Mueller Hinton Broth
SI	Selectivity Index
HPLC	High Performance Liquid Chromatography
IR	Infra-Red
NMR	Nuclear Magnetic Resonance Spectroscopy
^{1}H-NMR	Proton-Nuclear Magnetic Resonance Spectroscopy
^{13}C-NMR	C-13 Nuclear Magnetic Resonance Spectroscopy
DEPT-135	Distortion less Enhancement by polarization
CC	Column chromatography
TLC	Thin Layer Chromatography
WHO	World Health Organization
UV	Ultraviolet

Abstract

Dovyalis abyssinica is a medicinal plant known in Ethiopia and some African countries for its edible fruit and use in traditional management of gonorrhea, brucellosis, tooth problems and mastitis in animals. The main objective of this study was to isolation and characterization of bioactive compounds from the fruits. The dried was subjected to extraction with petroleum ether, chloroform, acetone, and methanol sequentially by cold maceration method. The crude extracts obtained were then evaluated for antimicrobial activities on four bacterial strains (*S. aureus, E. coli, P. aeruginosa* and *B.cereus)* and two fungal strains (*Fusarium spp. and S. cerevisiae*) using agar diffusion assay. The most activity guided then subjected to column chromatographic separation on silica gel using increasing gradient of petroleum ether in ethyl acetate. The isolated compounds were then characterized on the bases of observed spectroscopic data (^{1}H-NMR, ^{13}C-NMR and DEPT-135) and comparison with literature. Extraction gave 11.99%, 3.5%, 2.43% & 1.67% for methanol, acetone, and chloroform and petroleum ether respectively. The extracts had a zone of inhibition 7.0-21.0 mm on tested strained. Fungal strains were more susceptible than bacteria and the chloroform extracts have shown the highest activity. Out of 254 fractions 76-89 and 108-140 gave pure compounds DA-1 (27.9 mg) and DA-2 (20.1 mg) respectively by washing with hexane and further purification on sephadex. The compounds were also characterized to be *β-sitosterol* and Catechol respectively. These compounds were not reported before from fruits of the study plant. Antimicrobial activity of Compound-**2** (DA-2) was higher than Compound-**1**(DA-1). Thus, the observed antimicrobial activities of crude extracts and isolated compounds justify the traditional use of the plant for treatment of different microbial diseases.

Key words: *Dovyalis abssinica* fruits, Extraction, Isolation, Antimicrobial activity, *β-sitosterol*, *Catechol*

1. Introduction

1.1. Background of the study

The history of medicine is an account of mankind's effort to deal with human illnesses. In prehistoric times, people obtained medicine for their ailments from their environments, particularly from plants [1]. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [2]. Since time immemorial, different parts of medicinal herbs have been used to cure specific ailments [3]. Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases [4]. Many of these natural products have been shown to present interesting biological and pharmacological activities and are used as chemotherapeutic agents or serve as the starting point in the development of modern medicines [5].

However, the potential of higher plants as a source for new drugs is still largely unexplored [6]. The traditional system of using medicinal plants for curing many diseases dates back to the age of Rig Veda. Many microbial diseases can be cured by medicinal plants without any side effects and economic issues [7]. Multidrug resistance towards antibiotics and their related effects has an added effect to pursue the use of natural drugs [8].

Today, as much as 80% of the people in the world depend on traditional medicine as primary health care [9]. However, there is need to investigate such plants to understand their properties and safety [10].

Ethiopia is the home of many plant species that are commonly used in many disease treatment by local healers as majority of people in Ethiopia depend on traditional medicine that involve use of plant parts of medicinal plant. Some of the factors attributed for these facts are accessibility, affordability as compared to modern drugs, socio-cultural background and their effectiveness against a number of health problems [11]. One of medicinal plants practiced by societies are *D. abyssinica* A. Rich. Which is a shrub that belongs to *family Salicaceae*. It is native to and common in the forests of Eastern Africa countries including Ethiopia, Kenya and Uganda [3].

Therefore, the study were done to investigate phytochemical constituent and evaluation of antimicrobial activities of the *Dovyalis Abyssinica* fruit.

1.2. Statement of the Problem

In Ethiopia and other countries most people uses *Dovyalis abyssinica* for medicinal purposes.

Similarly, the surrounding community of the study area uses its fruit for stomachache disease, rabies, gonorrhoea, bilharzias, and fever. There is also a traditional claim that the fruits promote wound healing. However, there is no more effort to isolate compounds that justifies most of the traditional claims and responsible for the biological activities observed. Therefore, the main objective of the present study was to evaluate the antimicrobial activity, isolate and characterize the fruits of *Dovyalis abyssinica.*

Moreover the study was expected to address to the following research questions.

1. Which solvent crude extract is more active on selected bacteria and fungi?
2. What is the yield of crude extract? Increase or decrease from non-polar to polar solvent?
3. Is potency of the crude extracts to increase or decrease upon purification?
4. What phytochemical constituents were present in the more active crude extract?

1.3 Objectives

1.3.1 General objective

- To investigate phytochemical constituent and evaluate the antimicrobial activity of fruits of *Dovyalis abyssinica.*

1.3.2 Specific objectives

i. To isolate phytochemical constituents of the most active crude extract using column chromatography.

ii. To elucidate structure of isolated compounds based on their observed spectroscopic data (^{1}H-NMR, ^{13}C-NMR and DEPT-135) and comparison with literature.

iii. To evaluate antimicrobial activity of the crude extracts and isolated compounds of *Dovyalis abssinica* fruits on bacterial and fungal strains selected.

1.4 Significance of the study

- The study has the following significance:-
  - Provide information about antimicrobial effects of isolated compound and crude extracts of *Dovyalis abyssinica* fruits.
  - It gives the information about the chemical profile of fruit of *Dovyalis abyssinica*.
  - It puts base line information for other researchers who wish to carry out further study on similar plant.

2 Review of related literature

2.1. Botanical Description of the study Plant

I. The family Flacourtiaceae

Flacourtiaceae has long been recognized as a *family* having a highly variable and controversial circumscription [12]. Recently, cyanogenic tribes of *Flacourtiaceae* were separated in the family Achariaceae, and the Non cyanogenic tribes, including *Dovyalis* and *Homalium*, were united with *Salicaceae* [13].

II. The genus Dovyalis

The genus *Dovyalis* comprise 11 species [14] including *Dovyalis zeyheri, Dovyalis keniensis , Dovyalis longispina, Dovyalis macrocalyx, Dovyalis hebecarpa, Dovyalis caffra* and *Dovyalis abyssinica* A. Rich, known as African gooseberry. Ceylon gooseberry is another species (*Dovyalis hebecarpa*) native to India and Sri Lanka, and Florida gooseberry is a natural cross of *Dovyalis hebecarpa* and *Dovyalis abyssinica* [3].They are frequently found in Central Africa, Mesoamerica, and the northern part of South America. The plant is commonly known as the Kei apple in most parts of the world. It is related botanically to the ramontchi or Governor's plum *(Flacourtia Ramonchi*) and to the Ceylon gooseberry *(Dovyalis hebecarpa*). The genus *Dovyalis* were known in older literature under the name *Aberia* (e.g*. Aberia caffra,* etc.). All of these fruiting plants belong to the *Flacourtiaceae* or *Placourtia family* [15].

III. Dovyalis abyssinica A. Rich

It is commonly called African gooseberry which is native to Africa [14] and locally known as "*Koshim*" in Amharic and *''koshommii''* in Afan Oromo, belongs to the small genus *Dovyalis* and family *Flacourtiaceae*. It occur naturally from Ethiopia, Eritrea and Somalia in the North through Kenya and Tanzania to Malawi in the South. Grows in upland rain forest, dry evergreen forest, on river banks and sometimes in more open wood land [16]. It is a spiny evergreen shrub or tree, up to 5m height, with a rounded crown [17]. The bark is ash grey, almost always supporting lichens. Branches armed with stout spines, up to 1½ cm long. The branchlets are covered with numerous dotted pores (lenticels). Leaves are oval to obovate, up to 5-7 cm long and 3 cm wide with arounded tip, edges unevenly rounded. It is shiny, dark green, with reddish stalks and veins. Flowers are unisexual, yellow-green or greenish without petals, 5-7 mm long. Female flowers are single or in 2-3 flowered fascicles whereas male flowers occur in clusters, with 40-60 stamens [15].

2.2 Uses of *Dovyalis* Species

2.2.1 Nutritional role

The fruit *Dovyalis abyssinica* very acidic and are eaten raw or after making jam or jelly. The fruit is also added to porridge as a flavoring. Roots and stem are good for making soup. Fruits are rich source of vitamin C, amino acids, poly phenols and other antioxidants. The leaves provide fodder for livestock, primarily goats and sheep. Flowers attract bees

a) **b)**

Fig.1 Pictures of the *Dovyalis Abyssinica* plant (**a**) and fruit part(**b**) taken from its natural habitats (Source : Birhanu's personal photo collection)

2.2.2 Traditional Medicinal role of *Dovyalis abssinica*

Medicinal values of *Dovyalis spp.* has been reported in several literature both for human and animal illness. These plants have been in use for traditional management of dysentery, diarrheic diseases, flatulence, and general debility and mosquito repellent activities [2], trypanosomes in humans and animals [18, 19].The roots also have medical properties with alleged effect on gonorrhea, bilharzias, stomach-ache and fever. The roots and thorns are used in African traditional medicine to treat amenorrhea and chest pain [2]. *D. caffra* and other *Dovyalis species* are used to treat pain in rheumatic fever and rheumatism [20]. Leaves are used traditionally to treat gonorrhea, brucellosis and teeth problems in humans and mastitis in animals [21].

2.3 Scientific studies carried on *Dovyalis species*

2.3.1 **Phytochemical constituents of the genus *Dovyalis***

Phytochemical information on the genus is sparse and mostly related to its role as asource of food. Studies reported on cyclopentenyl fatty acids [20] and tannins [21] constituents of the genus. Fruits of *Dovyalis caffra* have been investigated for their composition of pectin and amino acids [22], and for the antioxidant activity of the polyphenols present in the fruit juice [23]. Although alkaloids are generally uncommon in this family, two alkaloids have been identified in *D. caffra* [24]. Moreover, the presence of a new classes of spermidine-type alkaloids, dovyalicins A-D, in the leaves of *D. macrocalyx*, with dovyalicin A as the main alkaloid [25]. The photochemical investigation of *D. macrocalyx*, *D. abyssinica*, and *D. hebecarpa* reported the presence of two new dovyalicin-type alkaloids, dovyalicin E and dovyalicin F(**3**) along with the previously described Dovyalicin A [8]. In addition, a new Phenolglucoside, 4-hydroxytremulacin **(4**), 1, 2-cyclohexanediol glucoside (5), methyl 1-hydroxy-6-oxocyclohex-2-enecarboxylate (**6**) and tremulacin (**7**) were also reported from the study plant [14] and identified Itoside A and (**3**) from leaves of *D. caffra* and stem bark of *D.Zeyheri* [14, 26].

Higher contents of anthocyanins and carotenoids were found in dovyalis, 42.0 and 6.6 mg/100 g, respectively, as compared to tamarillo fruits with 8.5 and 4.4 mg/100 g. Although these fruits belong to different families, delphinidin 3-rutinoside (**8**) and beta-cryptoxanthin (**9**) were found to be, respectively, the major anthocyanin and carotenoid in both fruits. In addition, considering the qualitative and quantitative composition of pigments in these fruits, the anthocyanins were the pigments that most contributed to the red-violet color of *dovyalis* fruit [27].

2.3.2 **Biological activity of the *genus Dovyalis***

Qualitative phytochemical analysis of the crude extracts of *Dovyalis abbyssinica* for the tests of phytochemicals as alkaloids, saponins, flavonoids and tannins were done and show positive result for the extracts of the plant [28]. Alkaloids are well known Phytochemicals especially for their broad pharmacological activities including antibacterial and antifungal. The biological function of alkaloids and their derivatives are very important and are used in analgesic, antispasmodic and bactericidal activities [29].

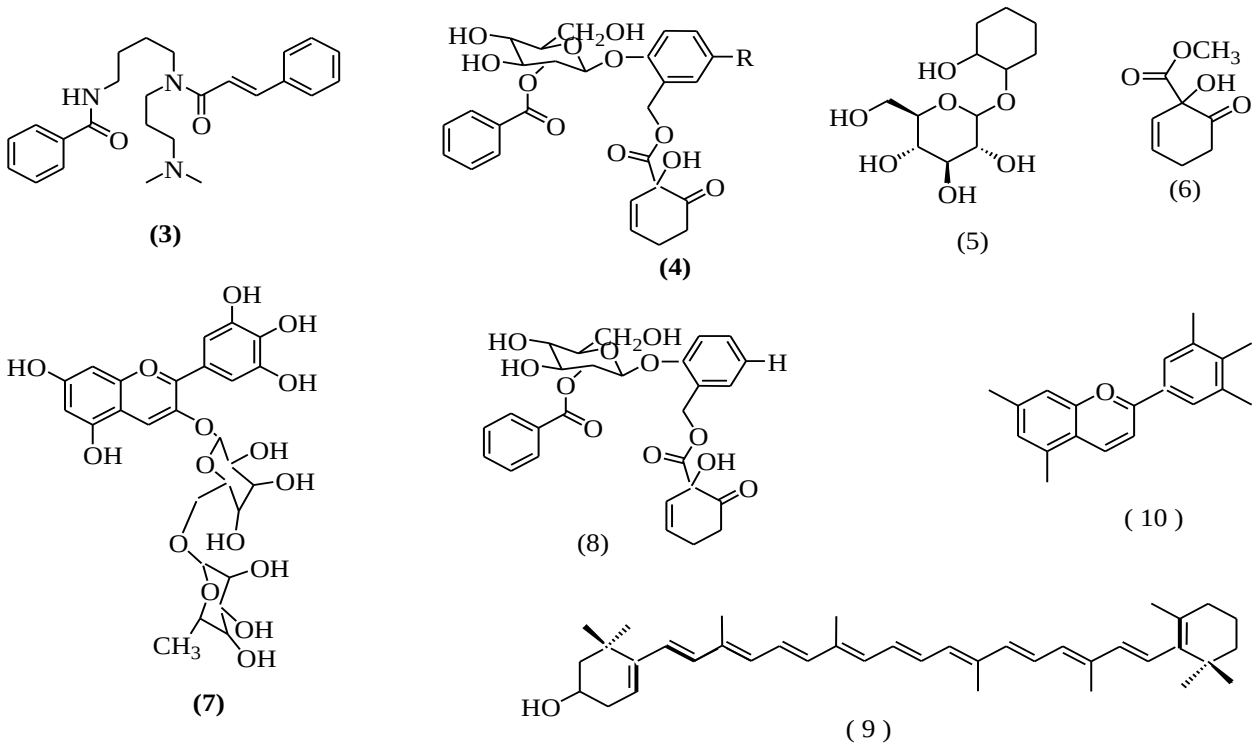

Fig.2 Chemical structure of selected Secondary metabolities isolated from plants of genus *Dovyalis*

Table 1: Biological activities of phytochemicals from *Dovyalis species*

N^{o}	Phytochemical investigation	Biological activity	Structure	Reference
1	Anthocyanins and carotenoids	Anti-oxidant activity	(6)	[27]
2	Saponins	wound healing and bleeding treatment		[30]
3	Flavonoids	preventing oxidative cell damage and carcinogenesis	(9)	[31]
4	Pectin and amino acids	the antioxidant activity of the polyphenols	(1)	[24]
5	Alkaloids	antibacterial and antifungal , analgesic,, antispasmodic activities	(2)	[30]

3. Materials and Methods

3.1 Chemicals

The following chemicals were used during the crude extraction, isolation of bioactive compounds and evaluation of its biological activities. Methanol, Acetone, Chloroform, Petroleum ether and ethyl acetate, were used as organic solvent. Iodine for detection of spots on TLC, silica gel (60-120mm mesh size) was also used. Dimethyl sulfoxide (DMSO), Mueller Hinton agar and nutrient broth as culture were used for antibacterial control for this study. Gentamicin and Miconazole were used as control drugs. All the chemicals and reagents used were of reagent grade.

3.2 Apparatus and Equipment

Materials utilized during this study also include; Rota vapors (Labo Rota 4000,Heidolph Inistrument), TLC plates, weighing balances, glass columns for column chromatography and UV-254 and 365 nm (UV-tec) chamber for detection of spots on TLC were used for the study. Spectral recording (^{1}H-NMR, ^{13}C-NMR and DEPT-135) were done using Bruker 400 MHz NMR Spectrometer. The spectroscopic analysis was carried out at Addis Ababa University.

3.3 Collection and preparation of Plant Materials

Fresh fruit of *Dovyalis abyssinica* was collected during June, 2018 from home garden at Bishoftu town, Ada'a Berga District in eastern shewa, Oromia Regional State, Ethiopia which was around 50 Km from Addis Ababa. The collected plant material was shade dried at room temperature and grounded into smaller pieces by manual grinder.

3.4 Extraction of the study Plant

The air dried plant material was then sequentially extracted with (petroleum ether, chloroform, acetone and methanol) each three times for 72 hr with frequent agitation. The extracts were then screened for their antimicrobial activity.

3.5 Isolation of pure Compounds

The chloroform extract showed better antibacterial activity and was selected for further isolation of the bioactive compounds. The chloroform crude extract (20.9 g) was adsorbed on 20 g silica gel and applied on column chromatography packed with silica gel. The column was eluted with petroleum ether, with increasing gradient of ethyl acetate.

3.6 Antimicrobial activity evaluation

The crude extracts and isolated compounds were evaluated for *in vitro* antibacterial activities against four bacterial strains *Escherichia coli* (ATCC25922), *B.aureus(10876), Pseudomonas aeruginosa* (27853) and *Staphylococcus aureus* (ATCC25923) and the antifungal activities against two fungus strains (*Fusarium* spp and *S.Cerviceas*). The bacterial and fungal strains were all obtained from Microbiology laboratory, Biology Department, Jimma University. Agar disk diffusion method was used to evaluate the antibacterial and antifungal activities of both crude extract and isolated compounds on nutrient agar. The antimicrobial activity test was done using disc diffusion method standard procedures [32-33]. Briefly, the stock cultures were maintained on the nutrient agar slants which were stored at 40°C. Agar cultures of the test microorganisms were prepared according to manufacture instruction. The test solutions were prepared by dissolving 0.2g ratio of plant extracts to achieve final stock concentrations of 200 mg/mL in DMSO. Freshly, grown liquid culture of the test pathogens solution of having similar turbidity were seeded over the Mueller-Hinton Agar medium with sterile swab. Sterile Whatman flter paper discs (6 mm) were soaked with stock solution of the extract then placed over the seeded plates. The plates were then inverted and incubated at 37^0C for 24 hr. After the incubation period, the plates were observed for clearance zone formation around the disks which indicates positive antibacterial activities of the respective plant extracts. The clear zones formed around each disk were measured in millimeter.

4. Result and discusion

4.1 Extraction data

Extraction of dried and powdered fruits of *Dovyalis abyssinica* (860 g) was carried by cold maceration technique using four different solvents (petroleum ether, chloroform, acetone and methanol) sequentially with increasing polarity for 72 hrs in each solvent with continuous shaking at room temperature. The Percentage yields of crude extracts obtained are given in table 2 below.

Table 2. Percentage yield of crude Extracts

Crude extract type	Mass of crude extract	% yield
petroleum ether	14.35	1.67
Chloroform	20.90	2.43
Acetone	29.86	3.50
Methanol	103.17	11.99

Percent yield of extract increase from non-polar extract to polar extract indicating the domination of polar compounds than non-polar with in the study plant fruit.

4.2 Compounds isolated

The crude chloroform extract (20.9 g) adsorbed on 20 g activated silica gel was used for fractionation on silica gel (60-120 mesh) column eluted with petroleum ether with increasing gradient of ethyl acetate which afforded 254 fractions of 30-40 mL each. Each fraction was analyzed with TLC and visualized under UV light at 254 nm and then by exposure to iodine vapor. The fraction that showed the same TLC development profiles (color and Rf) were combined and concentrated to dryness under reduced pressure using rotary evaporator. Accordingly, fractions 76-89 (12% EtOAc in petroleum ether) were combined and purified with hexane to give compound-**1** (27.9 mg) labeled as DA-1 which was colorless amorphous solid. Similarly, fractions 108-140 (18% EtOAc in petroleum ether) were combined and purified by sephadex LH-20 (column size: 80 cm length and 4 cm diameter) (with 1:1 of chloroform in methanol) to give 34 fractions (1-34) amongst which fraction 34 gave compound-**2** (20.1 mg) labeled as DA-2 which was brown amorphous solid.

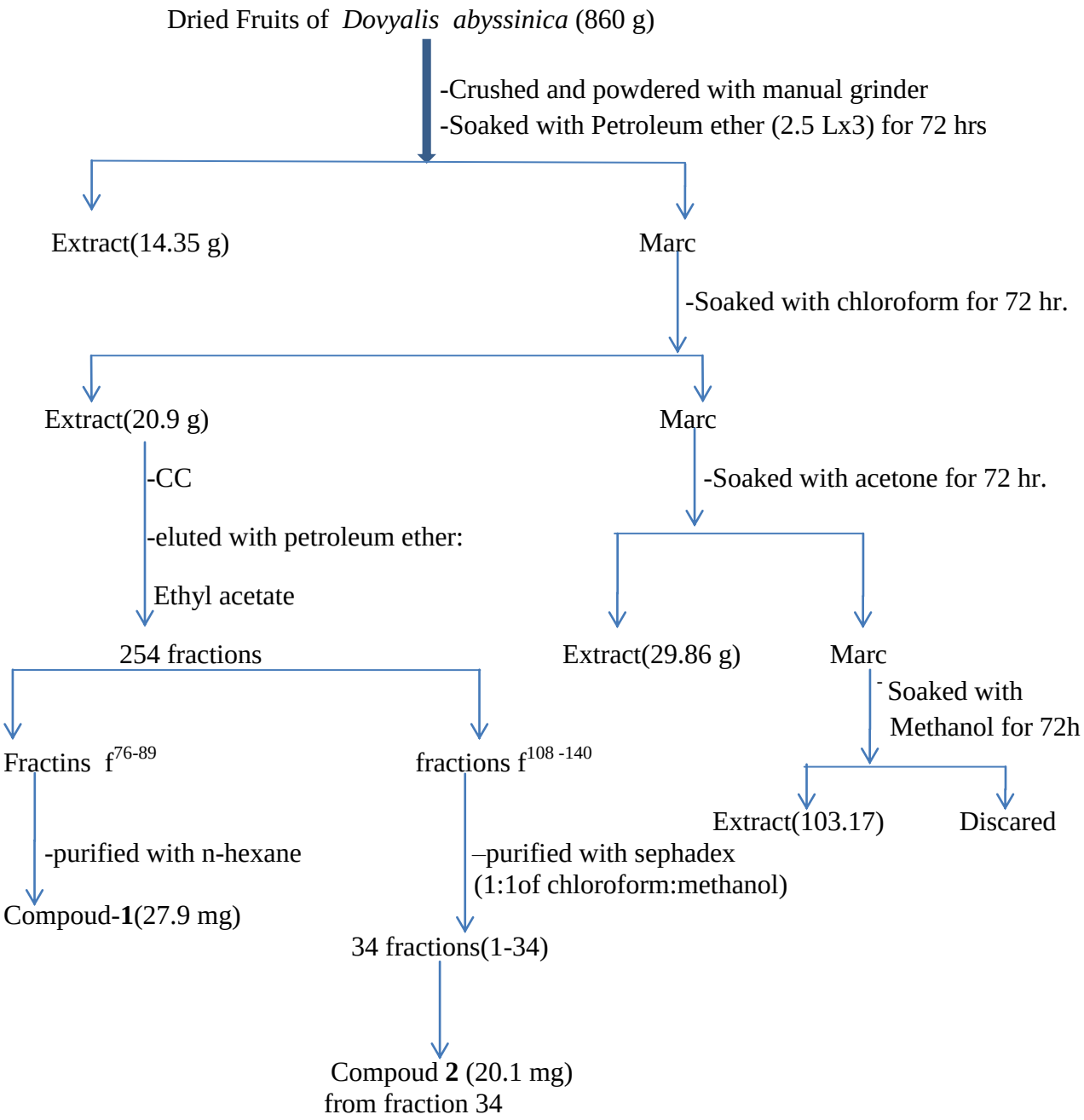

Figure 3. A scheme showing isolation and fraction of compounds from *Dovyalis abyssinica* fruits Chloroform extract.

The volume of each solvent used for extraction was (2.5L x 3 x 24 hrs)

4.3 **Characterization of Compounds isolated**

Column chromatographic separation of *Dovyalis abyssinica* fruit crude chloroform extract has led to the isolation of two compounds labeled as DA-1 & DA-2. Characterization of these compounds was carried on the bases of spectroscopic data (^{1}H-NMR, ^{13}C-NMR and DEPT-135) obtained as described below.

i. **Characterization of compound 1 (DA-1**)

Compound 1 was isolated as colourless amorphous solid (R_f = 0.80 in 75 % petroleum ether in ethyl acetate). ^{1}H-NMR spectral data of DA-1**(Appendix 1)** displayed six signals at δ 0.70(H-18), 0.82 (H-26), 0.84 (H-27), 0.86 (H-29), 1.03 (H-19) and 0.93 (H-21) in ppm which corresponds to the H-atoms attached to CH_3 group. The compound also has revealed one multiplet proton at δ 3.55 ppm (1H, C-3H) indicated the presence of oxygenated proton. The signal at δ 5.37 ppm (1H, C-6H), (1H, *t*) belonged to olefinic hydrogen . The ^{13}C-NMR and DEPT-135 spectrum showed 29 signals **(Appendix 2 & 3)**. The ^{13}C-NMR spectrum **(Appendix 2)** showed two downfield signals at δ 140.76 (C-5) and 121.74 (C-6) ppm which belong to the endocyclic carbon-carbon double bond. A signal at δ 71.8 represents the C-3 that is bonded to hydroxyl group.

According to the DEPT-135 spectra **(Appendix 3)** the peaks at δ 42.32, 39.78, 37.26, 33.94, 31.66, 31.6, 23.06, 28.26, 26.06, 24.31 and 21.09 represented the methylene (CH_2) groups. Absence of peaks at δ 140.76 (C-5), 36.51(C-10) and 42.31(C-13) ppm in the DEPT-135 spectrum originally observed in the ^{13}C-NMR spectrum also confirm the presence of quaternary carbon atoms. Based on spectroscopic data and comparison with the reported literature [32], the compound was proposed to be a steroid(17-(5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[α]phenanthren-3-ol (trivial name *β-sitosterol*) (**Figure 4**). The NMR data of Compound-**1** (DA-1) and that of *β-sitosterol* are given in **Table 3.**

Table 3. ^{1}H-NMR, ^{13}C-NMR and DEPT-135 data of Cpd-1 with reported data of *β-sitosterol*.

position	^{13}C-NMR Of Cpd 1	Reported ^{13}C-NMR of *β-sitosterol* [35]	^{1}H-NMR of Cpd 1	Reported ^{1}H-NMR of *β-sitosterol* [35]	DEPT-135 of Cpd 1	Inference
1	37.3	37.3			37.3	CH_2
2	31.6	31.7			31.6	CH_2
3	71.8	71.8	3.55 (1H, *m*)	3.53 (1H, *m*)	71.8	CH
4	42.3	42.2			42.2	CH_2
5	140.8	140.8				C
6	121.7	121.7	5.37 (1H, *t*)	5.36 (1H, *t*)	121.8	CH
7	31.7	31.8			31.7	CH_2
8	31.9	31.9			31.9	CH
9	50.1	50.1			50.1	CH
10	36.5	36.5				C
11	21.1	21.1			21.1	CH_2
12	39.8	39.8			39.8	CH_2
13	42.3	42.3				C
14	56.8	56.9			56.8	CH
15	24.3	24.4			24.3	CH_2
16	28.3	28.2			28.3	CH_2
17	56.1	56.1			56.1	CH
18	11.9	11.9	0.70 (3H, *s*)	0.69 (3H, *s*)	11.9	CH_3
19	19.4	19.4	1.03 (3H, *s*)	1.03 (3H, *s*)	19.4	CH_3
20	36.2	36.2			36.2	CH
21	18.8	18.8	0.93(3H, *d*)	0.93 (3H, *d*)	18.8	CH_3
22	34.0	34			33.9	CH_2
23	26.1	26.1			26.1	CH_2
24	45.8	45.9			45.8	CH
25	29.7	29.1			29.7	CH
26	19.8	19.8	0.82 (3H, *d*)	0.85 (3H, *d*)	19.8	CH_3
27	19.0	19.0	0.84 (3H, *d*)	0.83 (3H, *d*)	19.0	CH_3
28	23.1	23.1			23.1	CH_2
29	12.0	12.1	0.86 (3H, *t*)	0.87 (3H, *t*)	12.0	CH_3

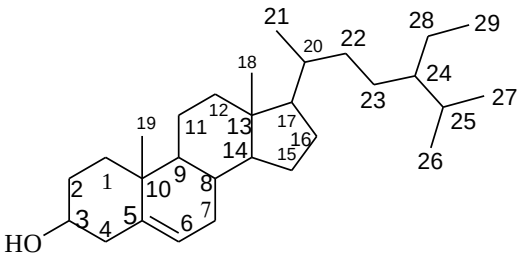

Figure 4. The proposed structure of compound-**1** (*β-Sitosterol)*

This compound was also previously characterized from Momordica charantia , *Corchorus fascicularis* Lam and fruits of Sida rhombifolan [35-37].

ii. **Characterization of compound 2 (DA-2)**.

Compound 2 was obtained as brown amorphous solid (Rf=0.60 70 % petroleum ether in ethyl acetate). ^{1}H-NMR of the compound-**2 (Appendix 4)** showed multiplet signals at δ 6.83-6.92 and a singlet at δ 5.50 which suggest the presence of two sets of ortho substituted aromatic proton bearing the same subistituent at both positions (an aromatic hydroxyl group). The ^{13}C-NMR spectrum (appendix 5) also display three signals at δ 143.5, 115.6 and 121.3 indicating three symmetrical aromatic carbons. The absence of peaks at δ 143.51 ppm in the DEPT-135 spectrum **(Appendix 6)** which was originally observed in the ^{13}C-NMR spectrum also confirm the presence of quaternary carbon atoms in the compound . The observed spectral data compound 2 was also found to be consistent with ^{1}H-NMR, ^{13}C-NMR and DEPT-135 data of 1,2-benzenediol (Catechol) reported in literature [37,46] **(Table 4).**

Table 4. ^{1}H-NMR, ^{13}C-NMR and DEPT-135 data of Compound-2 (DA-2) with reported data.

position	^{13}C-NMR of cpd 2	DEPT-135 of cpd 2	^{1}H-NMR of Cpd 2	Remark
1,2	143.5	-	-	C
3,6	121.3	121.31	6.83-6.92	CH
4,5	115.6	115.57	5.50	CH

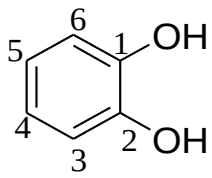

Figure 5.The proposed structure of compound-**2 (DA-2)** (*Catechol)*

Therefore, from the ^{1}H-NMR, ^{13}C-NMR and DEPT-135 Data analysis the proposed chemical structure of Compound-2 (DA-2) was IUPAC name 1,2-benzenediol (Common name *catechol*). Presence of Catechol and its derivatives (such as Urushion, Catechin, Bonediol) was reported in the Seeds, fruits and other morphological parts of various plants including Poison oak, Onion T, apples, Sacred fig tree(*Ficus religiosa*) and *Bonellia macrocarpa* [38-44,] .

4.4 Evaluation of Antimicrobial activities of the crude and Isolated compounds

All the crude extracts (200mg/ml) and the isolated compounds (DA-1 & DA- 2) (27.9 & 20.1mg/ml) were evaluated for their antimicrobial activities on four bacterial strains (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus cereus)* and two fungal strains (*Fusarium strain* and *Saccharomyces cerevisiae*) using agar disc diffusion method. The zones of growth inhibition (in mm) of each test samples are indicated in Table 5. The crude chloroform extract was found to be more active on *Bacillus cereuse* and *pseudomonas aureginousa (both 11 mm)* but with moderate effect on *E.coli* and *Fusarium strain* (both 10 mm). The methanol extract is also observed to be inactive relative to the standard drugs in all bacterial and fungal strains. Moreover compound-**2** (DA-2) was slightly more inhibitory than compound-**1** (DA-1) both on bacterial and fungal strains tested. Effect of both compounds was more pronounced on fungi than bacteria.

Table 5. Zone of inhibition of crude extracts and compounds isolated (DA-1 & DA-2) from *Dovyalis abyssinica* fruits against four bacterial strains and two fungal strains.

Test strain used		Diameter of zone of inhibition (in mm)								
		Pet.	CH	AC	ME	DA-1	DA-2	G	M	DMSO
Bacterial	*B.cereuse*	8	11	7	NA	8.5	11	19	NT	NA
	S.aureus	7	7	7	NA	9	8.5	21	NT	NA
	E.coli	8	10	8	NA	8.5	11	27	NT	NA
	Ps. aeruginosa	11	11	7	NA	11	11	21	NT	NA
Fungal	*Fusarium strain*	21	10	9	NA	10.5	12	NT	22	NA
	S.cerevisiae	12	9	NA	NA	13	16	NT	19	NA

NA: Not active and NT: Not tested PE=Petroleum ether; CH= Chloroform; AC= Aceton; ME=Methanol; G= Gentamicine; M=Miconazole

The antibacterial activity data obtained for *β*-sitosterol in this study is also comparable with literature report for the antibacterial activity of the same compound isolated different plants carried on similar bacterial strains [34, 40, 45, 46]. *β-sitosterol* is anatural micro-nutrient which is found in the cells and membranes of all oil producing plants, fruits, vegetables, grains, seeds and trees. It has been proven to be a safe, natural and effective nutritional supplement and has shown amazing potential benefits in many diverse applications [32]. Earlier experimental studies have shown it is important bioactive component exhibiting various Pharmacological properties such as antiinflamatory, antipyretic, antiarthritic, antiulcer, insulin releasing, antidiabetic, antioxidant and antistress agent [32]. 1,2-Benzenediol is an organic phenol extracted from methanol stem extracts of *Ficus religiosa*. It is also known as catechol or pyrocatechol. Literature survey showed catechol possesses anticancer (breast), antioxidant and pesticides properties [38]. Some literatures also report the antimicrobial, anti-cancer and anti-inflammatory activity of plant derived catechols [43-44, 57-58].

5. Conclusions and Recommendations

5.1 Conclusions

The present investigation revealed that the extract of *Dovyalis abyssinica* fruits have marginal antimicrobial activity which explains its use as a traditional medicine. Extraction gave 1.67%, 2.43%, 3.5% & 11.99% for petroleum ether, chloroform, acetone and methanol respectively. Column chromatographic purification of the chloroform extract has been carried out using petroleum ether and EtoAc combination as eluent. Both compounds (DA-1 and DA-2) were isolated and characterized using spectroscopic data (^{1}H-NMR, ^{13}C-NMR and DEPT-135) and by comparing the observed spectral data with literature reports. The crude exract and compounds exhibited antimicrobial properties with respect to the zone of inhibition against four bacterial strains (*S. aureus, E. coli, P. aeruginosa* and *B. cereuse)* and two fungal strains (*F. strain* and *Saccharomyces cerevisiae).* DA-2 was found to be more active than DA-1 as evidenced from zone of inhibition in mm (**Table 5).** Maximum activity was observed against *p. aureginousa* (Gram-positive) and minimum activity was noted for *E. coli* (Gram-negative) bacterial strains. Based on our result it can be concluded that the study plant fruits contain bioactive compounds that are effective against the tested bacterial and fungal strains.

5.2 Recommendation

These results also provide scientific validity and credence to the ethno medicinal use of this plant in the treatment of ailments caused by some of the pathogenic bacteria used in this study. High lights the usefulness of *Abyssinica* fruits in the treatment of bacterial and fungal infections. The observed result also confirmed that the isolated compounds are promising candidates for further antimicrobial activity tests in antimicrobial drug discovery. The compounds isolated are not the only compounds present in the study plant fruits extract as evidenced from TLC analysis. Further work should therefore, be carried out to isolate other compounds which may be more bioactive. Thus further test is recommended on large number of bacterial and fungal strains to decide their potential as candidates in development of antimicrobial drugs. And also more research should be carried out on both the crude extracts and the isolated compounds to include other antibacterial, antifungal, antimalarial and insecticidal activities.

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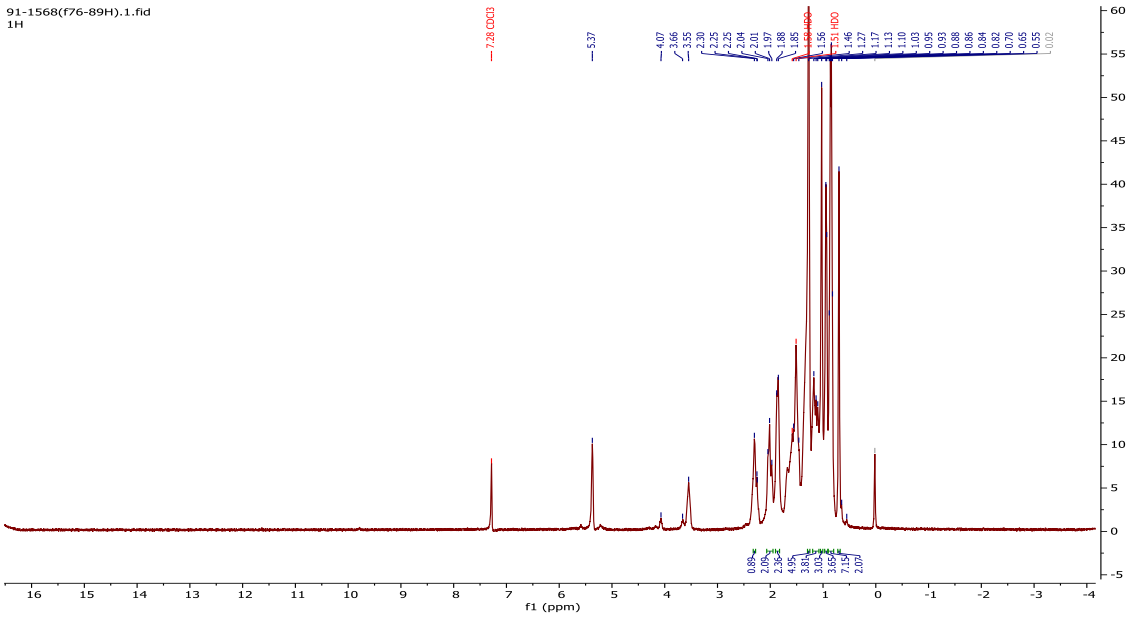
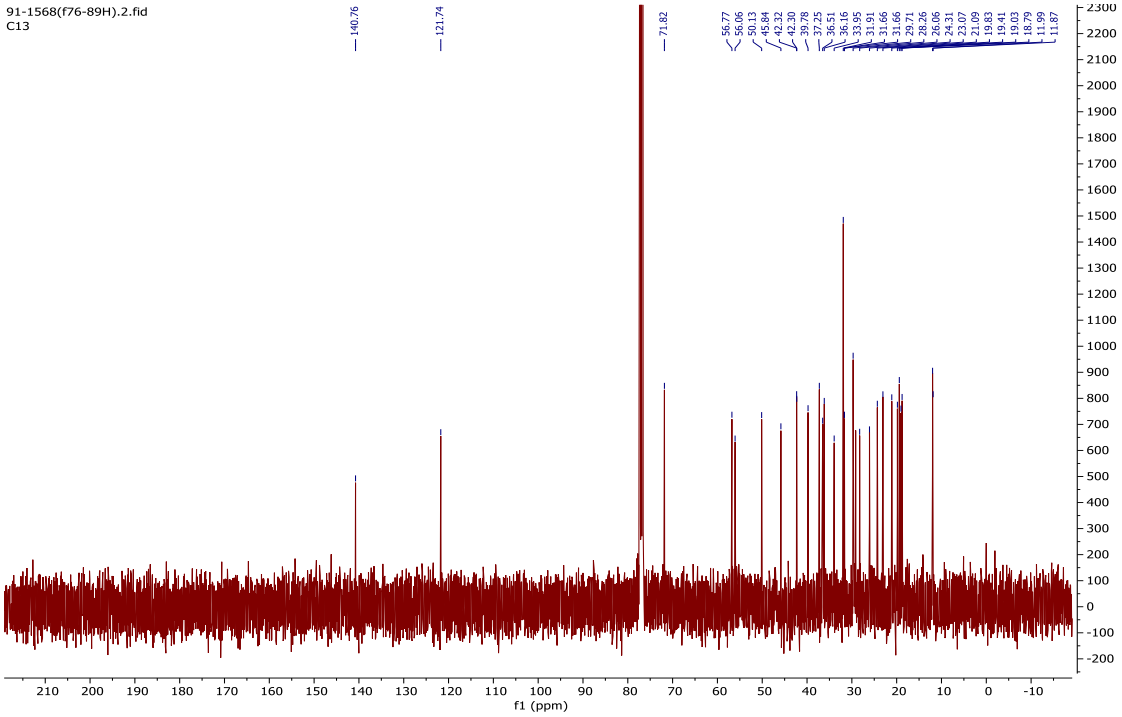
Appendices

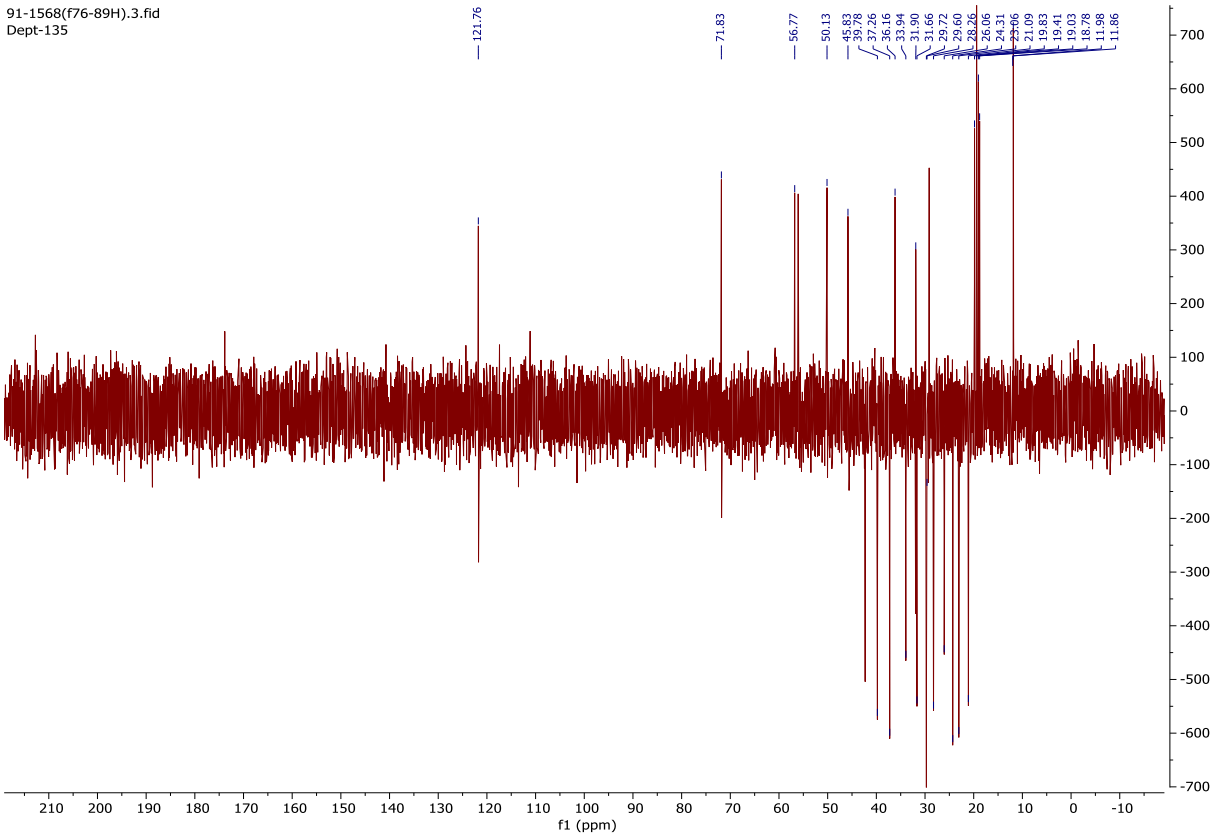
Appendix 1. ^{1}H-NMR Spectrum of Compound 1 (DA-1) in $CDCl_3$



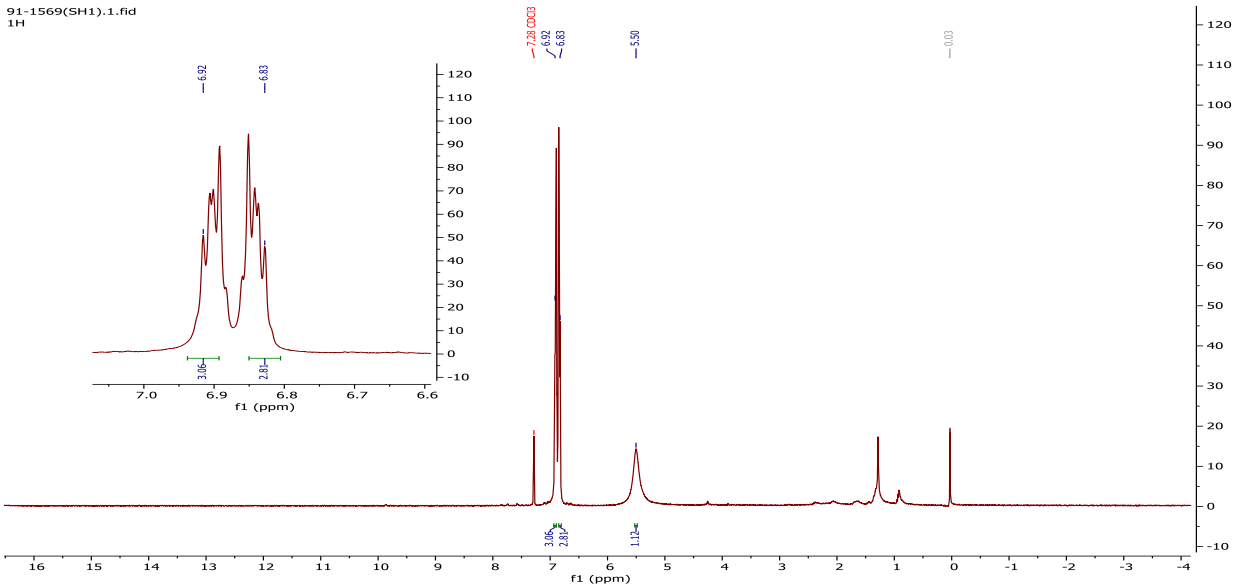
Appendix 2. ^{13}C-NMR Spectrum of Compound-1(DA-1)



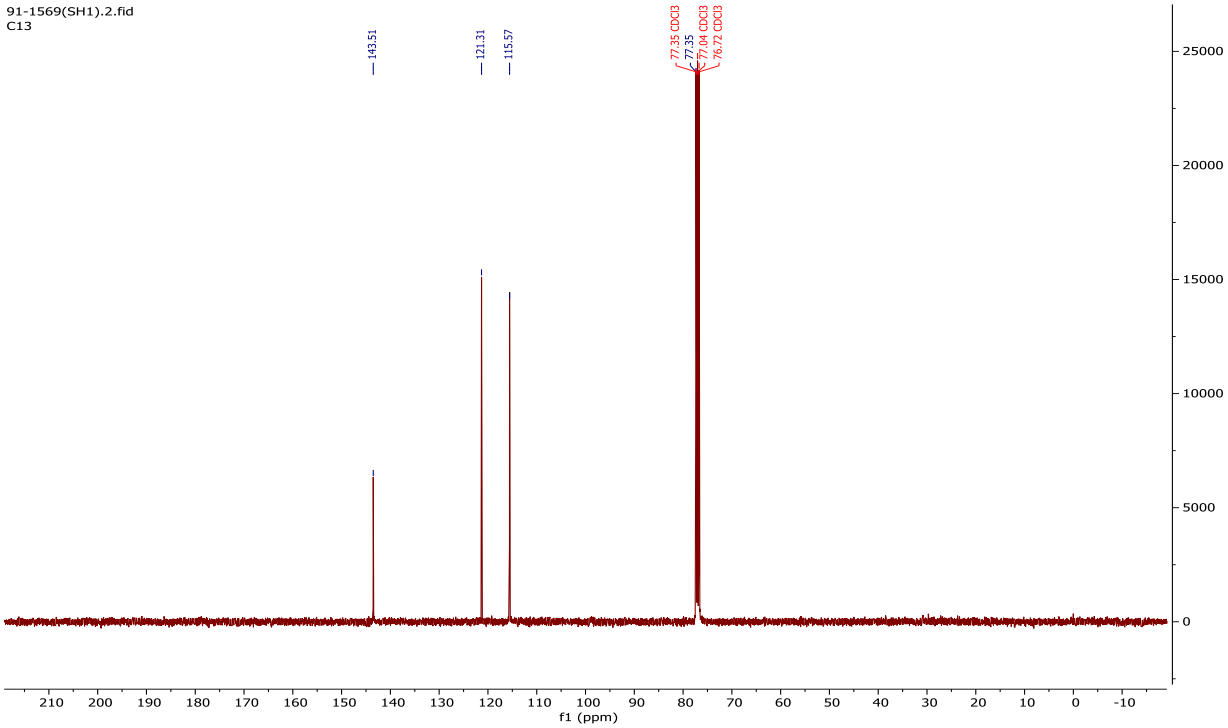
Appendix 3. DEPT-135 of Compound-1 (DA-1)



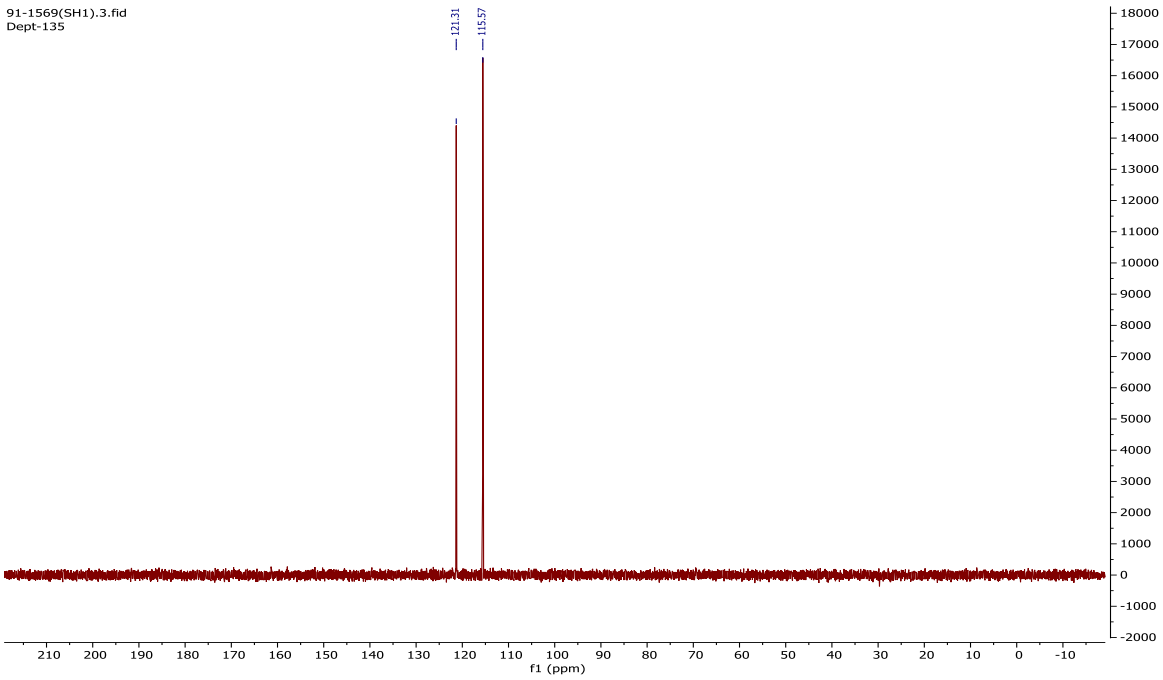
Appendix 4. ^{1}H-NMR Spectrum of compound-2 (DA-2)



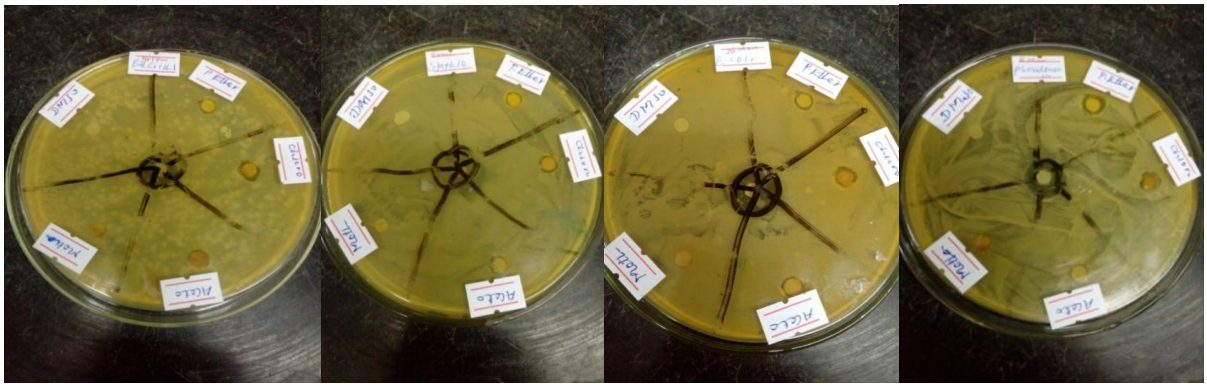
Appendix 5. ^{13}C-NMR Spectrum of compound-2 (DA-2)



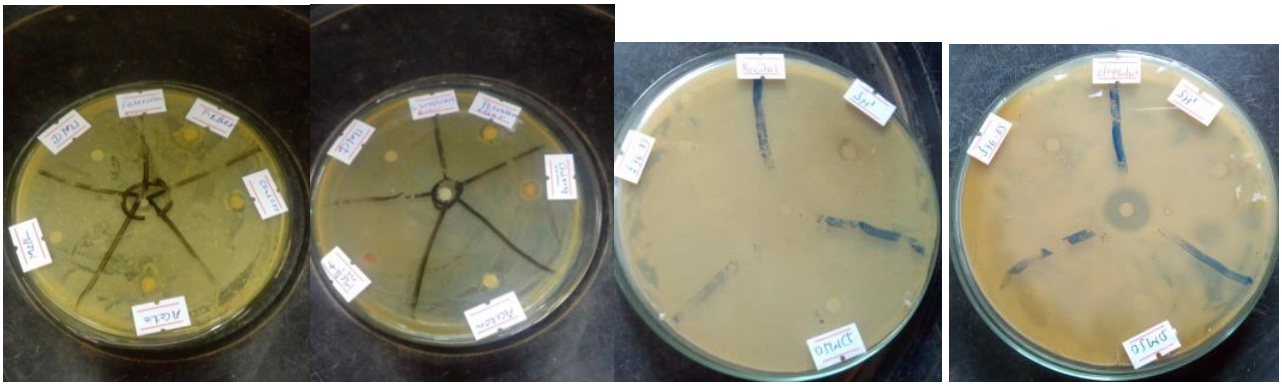
Appendix 6. DEPT-135 of Compound-2 (DA-2)



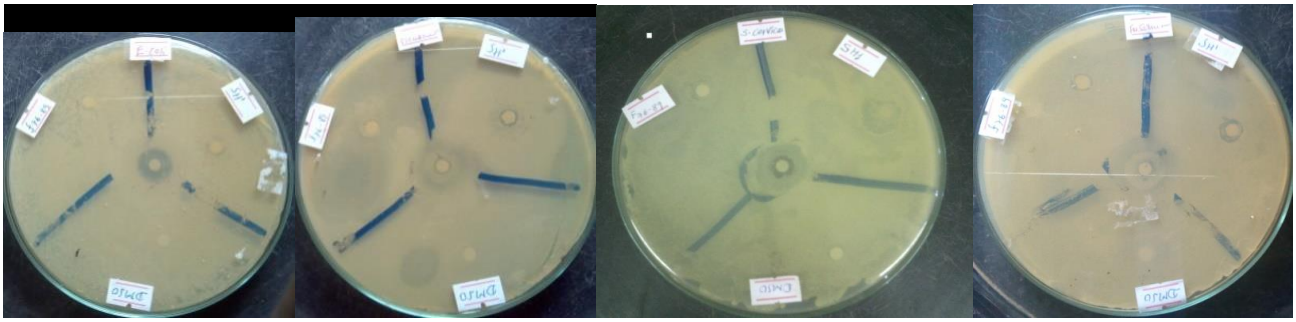
Appendix 7. Evaluation of Antimicrobial activity of Crude extracts & Isolated Compounds



B.aureus *Staphylococcus aureus* *E.coli* *P.aeruginosa*



Fusarium Strain Saccharomyces Cerevisiae *B.aureus* *Staphylococcus aureus*



E.coli *P.aeruginosa* *Saccharomyces Cerevisiae* *Fusarium Strain*